Choosing Indicators: GCaMP & More

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Scientific Support Webinar Series

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Webinar Series

- Deep dive of relevant topics for success with Mightex products such as OASIS Implant, Macro, Polygon 1000
- Current and soon-to-be users, distributors, core facility management teams, etc.
- Offer opportunity to meet with Mightex scientific team members and ask questions/ learn new tips and tricks to optimize data collection and experiment success
- Meet other Mightex product users (e.g., OASIS Implant, OASIS Macro, Polygon, etc.)

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Webinar Topics

- ❑ OASIS Implant GRIN lens implantation surgery → www.mightexbio.com/mypage
- ❑ **Choosing Indicators – GCaMP & More – today!**
- ❑ Headmount surgery and behaviour optimization
- ❑ Experimental data collection and analysis using PolyScan3
- ❑ Pattern generation methods and closed loop control of the Polygon 1000

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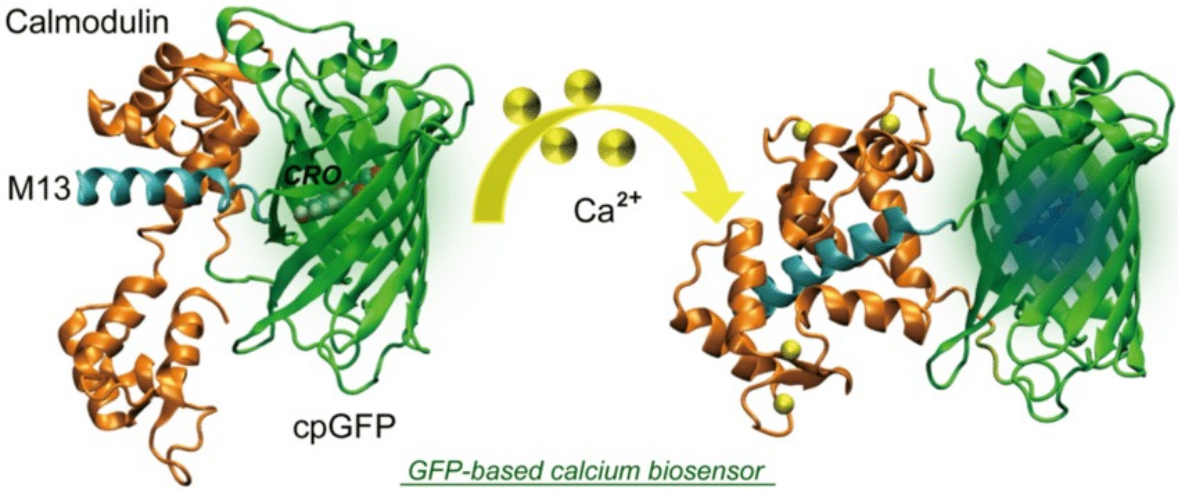
Fluorescence Imaging



- Genetically encoded fluorescent indicators (GEFIs) are used in conjunction with imaging methods to provide real-time insight into changing molecular concentrations
- Consist of fluorescent protein connected to a binding protein such that when a molecule of interest is bound it results in a dramatic change in fluorescent signal
- This can be used as a proxy measure for concentration changes
- Regional signal vs. single cell signal
  - Fiber Photometry
  - Cellular Resolution Calcium Imaging

  NB OASIS Implant can do both!

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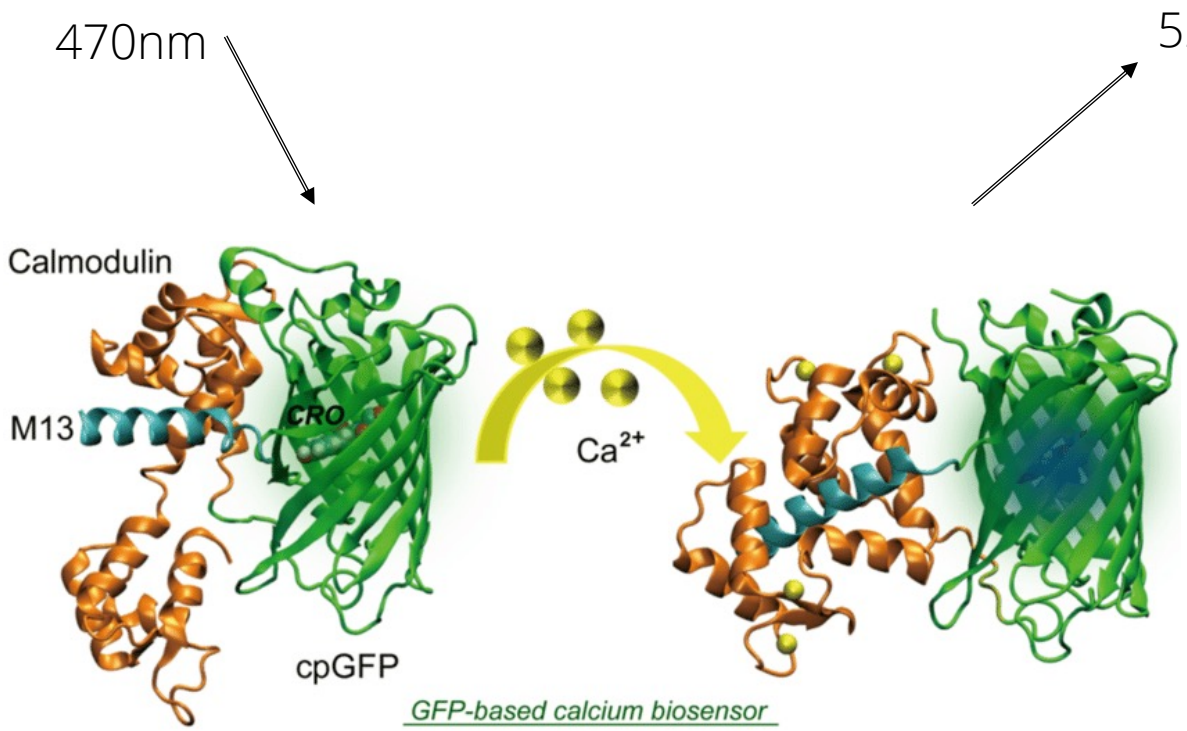
How to image fluorescent signal



- A wavelength of light must be applied in order to activate the fluorescent marker (known as the excitation wavelength)
- In the presence of the molecule of interest (e.g., Calcium) this activation allows the applied light to be photoconverted to a secondary specific wavelength known as the emission wavelength

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Viral vs. Genetically Mediated Indicator Expression

Viral Transfection:

- Transfection time
- Strong transfection (i.e., more signal to record)
- Troubleshoot/ Pilot for infusion parameters
- Most commonly used and well-established method
    - Lots of papers detailing the approach and which virus was used
    - Can also be combined with viral infusion for parallel techniques (e.g. optogenetics)
- Genetic mouse lines are needed to target a specific neuronal population of interest with a cre-recombinase/ DIO virus
    - This can limit experimental opportunities

Genetic Expression:

- Involves genetic engineering of mouse lines that carry indicator of choice within cell population of interest
- Selectivity provide by transgenic animals with promoter-driven cell-specific expression
- Typically, weaker fluorescent signal than viral mediated indicator expression
- Requires fewer surgeries and experiments can begin at a younger age (useful for adolescent research and long experimental timelines)
- Longer lasting viable indicator expression

Where possible, we recommend using a virally-mediated indicator

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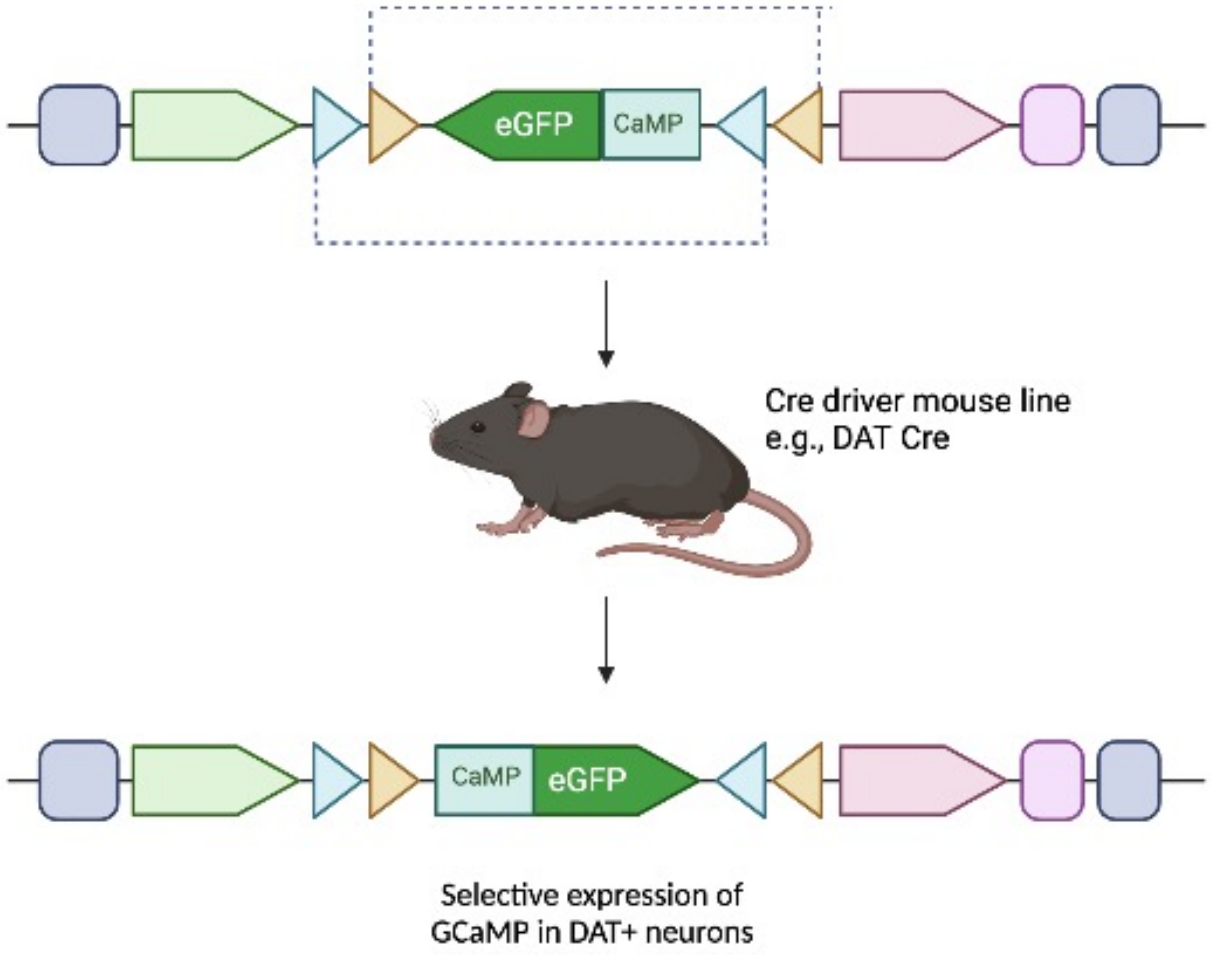
Expression selectivity – Cre dependent viruses



Types of Sensors

- In neurons, calcium channels are activated during action potentials, leading to brief increases in intracellular calcium levels (called calcium transients).
- Measuring these Ca transients provides a proxy measure of neuronal activity (i.e., action potentials)

- Calcium Indicators
  - GCaMP 6 series
    - Most popular series; used primarily for recording action potentials with fast decay times in populations with large calcium transients
  - GCaMP 7 series
    - Several-fold higher ΔF/F0 (i.e., brighter signal) and a wider range of kinetics when compared to the earlier GCaMP6 sensors
  - GCaMP 8 series
    - Improved sensitivity and speed. Compared to GCAMP7 series, the new series all have a faster rise time resulting in better temporal resolution of data collected

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Types of Sensors

GCaMP S, F, M, and X varieties:

- GCaMP F – fast spiking neurons, fast kinetics (i.e., quick rise and quick decay)
  - Spike interval of 10ms or less
- GCaMP M – neurons with medium decay kinetics (e.g., useful for discovery)
  - Spike interval of 10 -25ms
- GCaMP S – optimized for neurons with slow kinetics
  - Sike interval over 25ms
- GCaMP X – calmodulin-based calcium sensors that have been shown to cause side effects during some in-vivo uses, such as interference with the function of L-type calcium channels, nuclear accumulation, and cytotoxicity → we advise against using these versions

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Neurotransmitter Sensors

Being able to visualize neurotransmitter concentration is a vital components of understanding circuit dynamics

There is a growing number of neurotransmitter sensors that function based on the same basic concept as calcium indicators

- Dopamine sensors
  - dLight
  - GRAB DA
- Norepinephrine & Serotonin
  - GRAB NE
  - GRAB 5HT
  - iSeroSnFr
- Glutamate
  - GluSnFr
  - iGluF
- GABA
  - iGABASnFR
- ACh
  - iACHSnFR

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Voltage Sensors

Enables scientists to visualize neural activity using fluorescent activity sensors that are sensitive to changes in voltage

Much faster kinetics than calcium or neurotransmitter signals – requires faster sensors and imaging capabilites

Genetically engineered voltage sensors include:

- ASAP3
- Voltron
- QuasAR3
- SomArchon

NB voltage sensors are still largely in development – significant advances will need to happen before widespread use in place of electrophysiology

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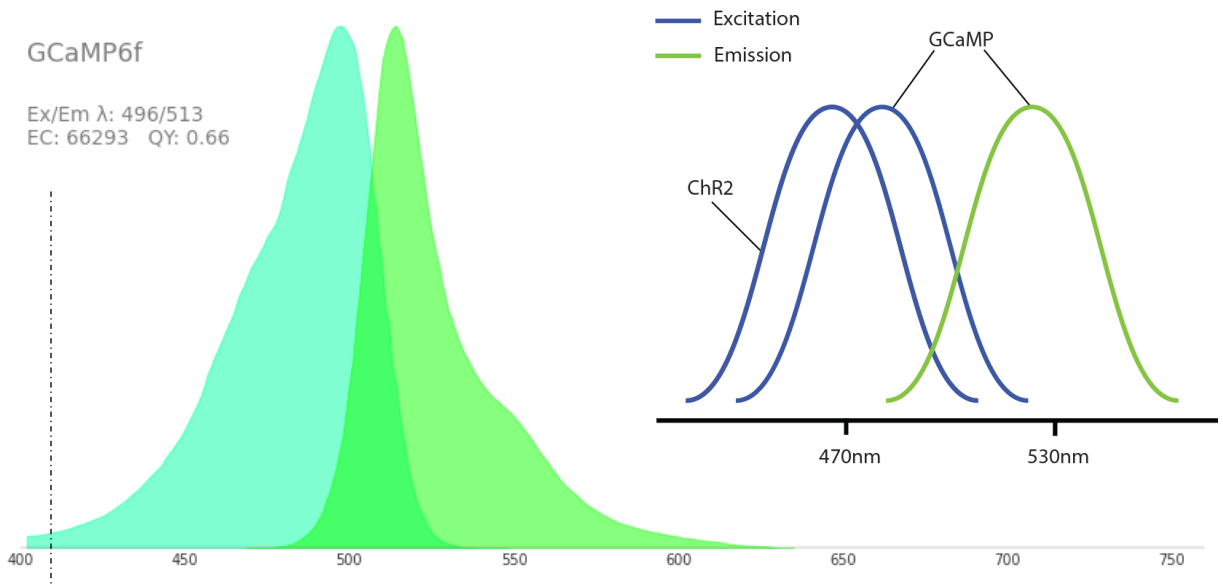
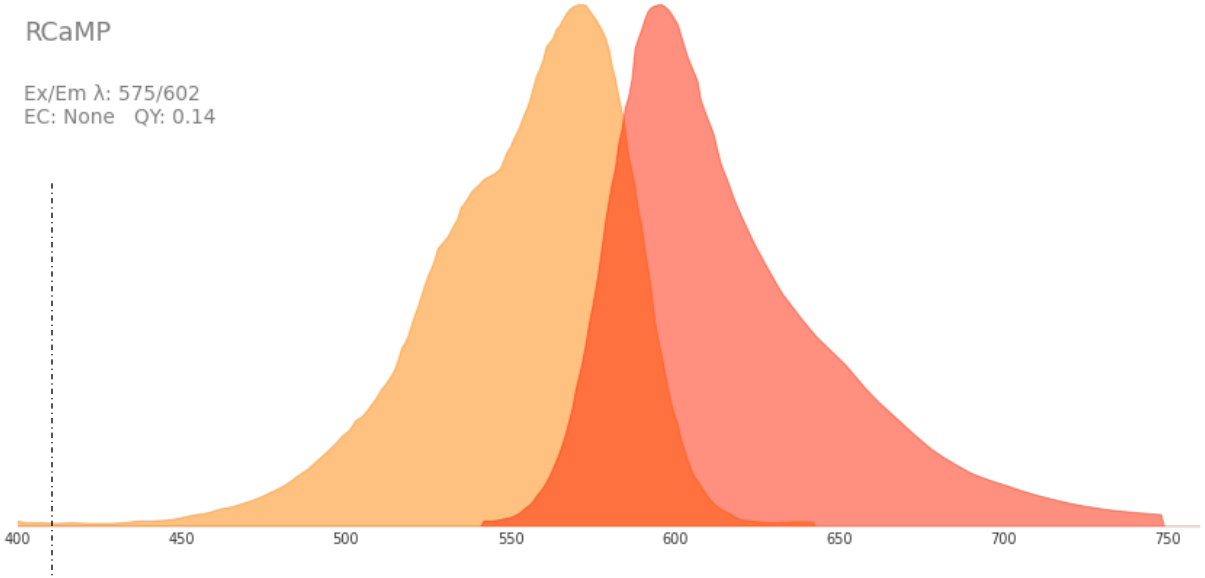
Wavelengths Matter





- Most used indicator is GCaMP
  - excitation at 470nm
  - emission at 525nm

  There are experimental conditions where these wavelengths are required for other uses and cannot be used for imaging (cross talk)

  Newer sensors have been release using different wavelengths of light:
- Novel indicator - RCaMP
  - excitation at 570nm
  - emission at 625nm

  Many experiments also require inclusion of an isosbestic control wavelength e.g., 405nm

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Factors to consider when choosing your sensor

Experimental Factors:

- Duration of experiment
- Cells of interest
- Region/ population of interest

Technical Factors:

- Do you have wavelength limitations (other techniques)
- What kind of dynamic range do you need to record (ΔF/ F %)
- Acceptable transfection time?
- Transgenic mouse line requiring a specific serotype?

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Factors to consider when planning your surgeries and experiments

Surgical Factors:

- Titer calculations
  - How concentrated in your virus and how easily does it transfect cells?
- Amount to infuse
  - Pilot (infuse varying quantities in scrub mice)
  - Refer to literature
- Where to infuse
  - Stereotaxic coordinates to ensure viral placement into your region of interest
  - How to reach? Multiple sites?
- Wait time
  - How long will you have to wait for the virus to diffuse away from the infusion site during surgery?
- What next
  - Implantation of optical ferrule? Recovery and wait for next surgery?

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Factors to consider when planning your surgeries and experiments

Experimental Factors:

- Excitation parameters
  - Frequency, duration of stimulation, intensity of light
  - Degradation of signal over time/ Photobleaching

- Do you need to run control experiments?
  - Different wavelength of light (non-excitation, isosbestic)
  - Control virus (fluorophore only, no active binding possible)

- Verification of successful viral transfection
  - Immunohistochemical assays
    - DAPI, colocalization with biomarker for neuronal population of interest (e.g., TH+ for dopamine cells)

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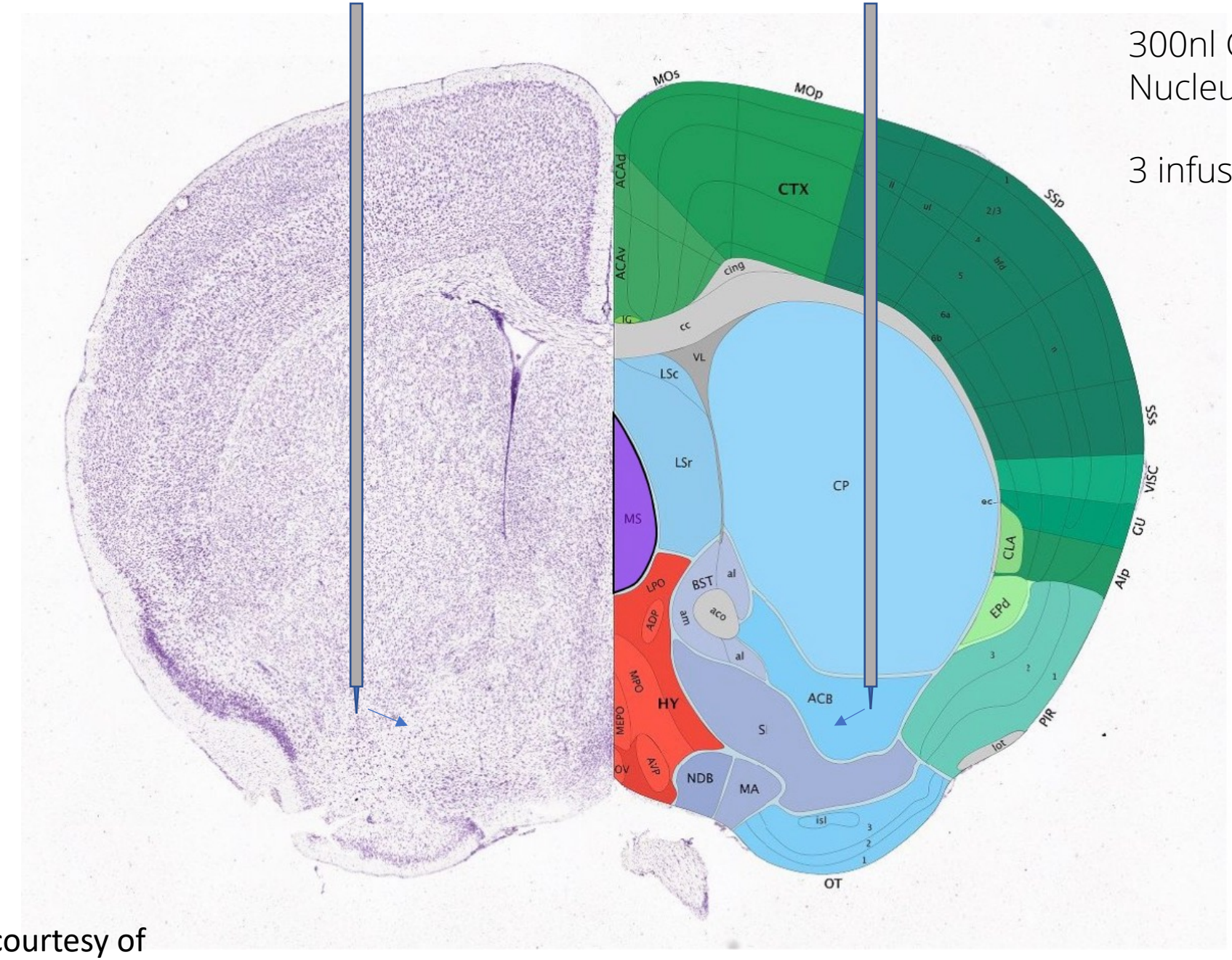
Viral Infusion Surgery Overview



300nl GCaMP7s (bilaterally)
Nucleus Accumbens (Shell)

3 infusion locations (-3.8, -3.7, -3.6)

Infusion Rate: 3uL/ min
Wait time: 5min per injection
Final wait time: 10min

Image courtesy of
Allen Brain Atlas

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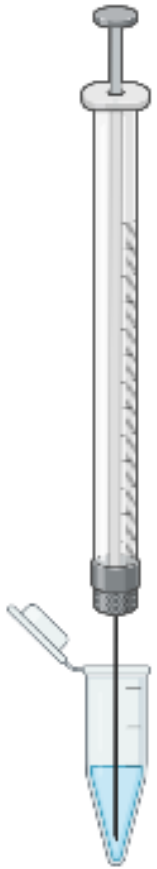
Preparing the virus



Viruses are photosensitive! Store in dark place/ cover from light

- Virus should be stored in small aliquots
  - Approx. amount of virus needed for ~4-5 surgeries (or however many you plan to do in a day)
  - Stored in sealed, labelled contained in -80C freezer
- Remove 1 aliquot for your day of surgeries
- Defrost while covered in aluminum foil
- Position aliquot underneath empty Hamilton Syringe (connected to microsyringe infusion pump)
- Slowly draw virus up into Hamilton syringe, ensuring NO air bubbles enter the syringe
  - Speed ~500nL per min
- Carefully remove aliquot and discard in appropriate biosafety waste
- Engage infusion pump to slowly push virus out until 1 droplet appears on the end of the needle tip

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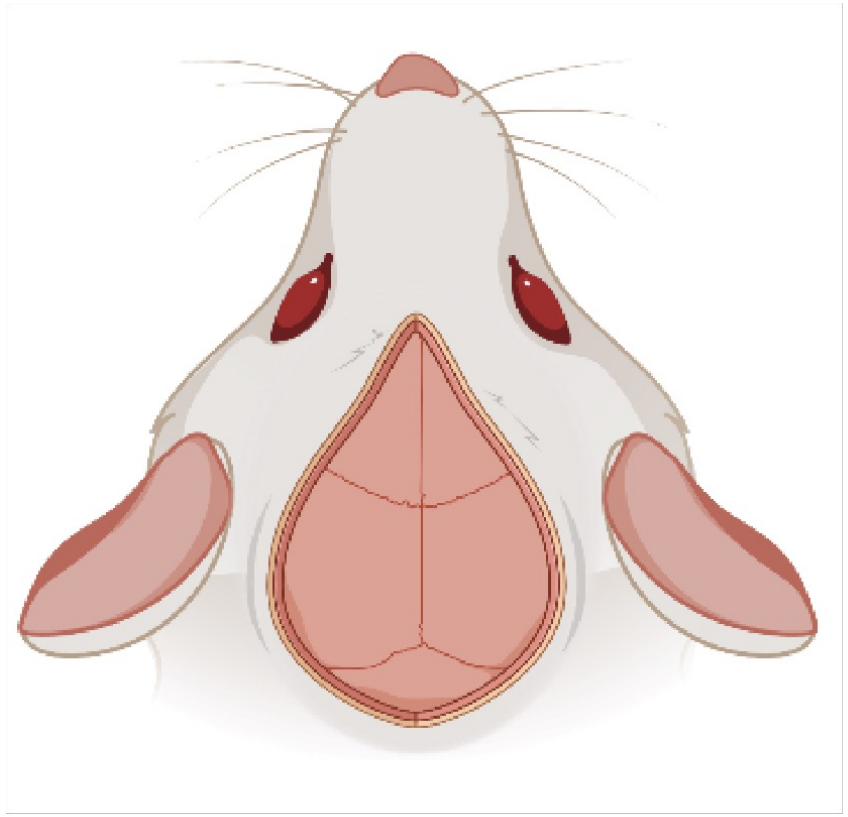
Clearing and preparing the skull



- Anaesthetize animal (isofluorane ideally)
- Head fix and level skull
- Administer IP saline and preoperative analgesic
- Remove fur from scalp (pluck, cut, shave)
- Use scalpel to make small incision line above area for infusion
- Use surgical Q tips to scrub away top layers of fascia to reveal skull
- Use hemostats to hold incision site open and reveal sutures and landmarks

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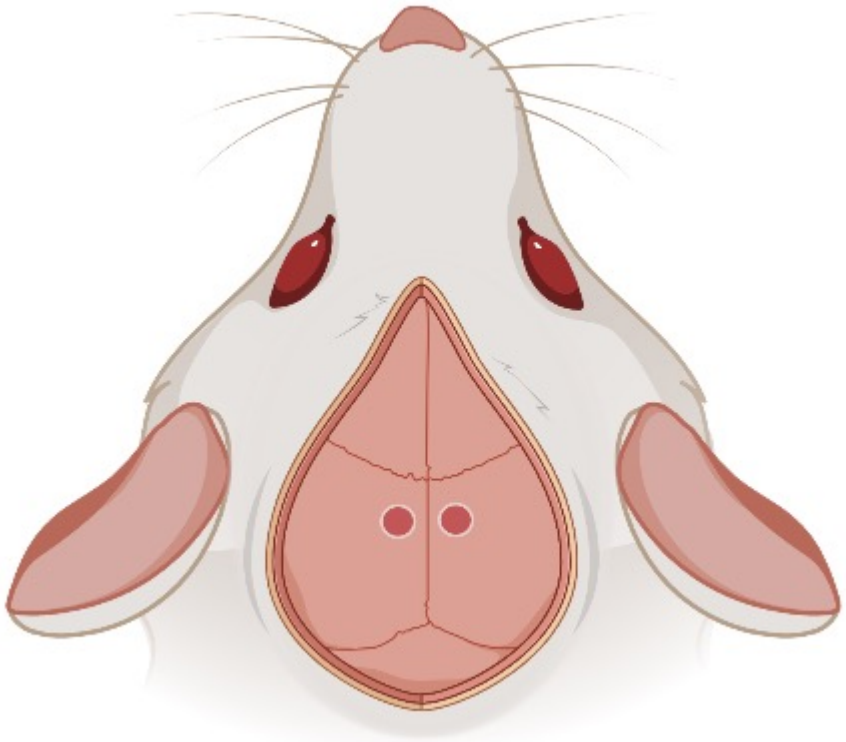
Craniotomy



- Zero coordinates at Bregma (and/or Lambda), confirm skull is level
- Attach stereotaxic or use hand-held drill with medium round drill bit (details in primer), move to above craniotomy site
- Hold 5ml syringe of saline with beveled needle in one hand and slowly drip saline onto the skull to keep it moist

- Turn on drill while in mid-air (away from the animal)
- Move slowly up and down in completely vertical direction increasing pressure on the skull to incrementally produce a hole in the skull.

- Make sure to stop when you see the wetness and pale pink of the meninges
- Remove drill and flush craniotomy with saline and dry with corner of Kim Wipe
- Be careful of hitting sutures or vasculature. If you find blood is pooling, stop drilling and stem bleeding to clear the visual field before continuing

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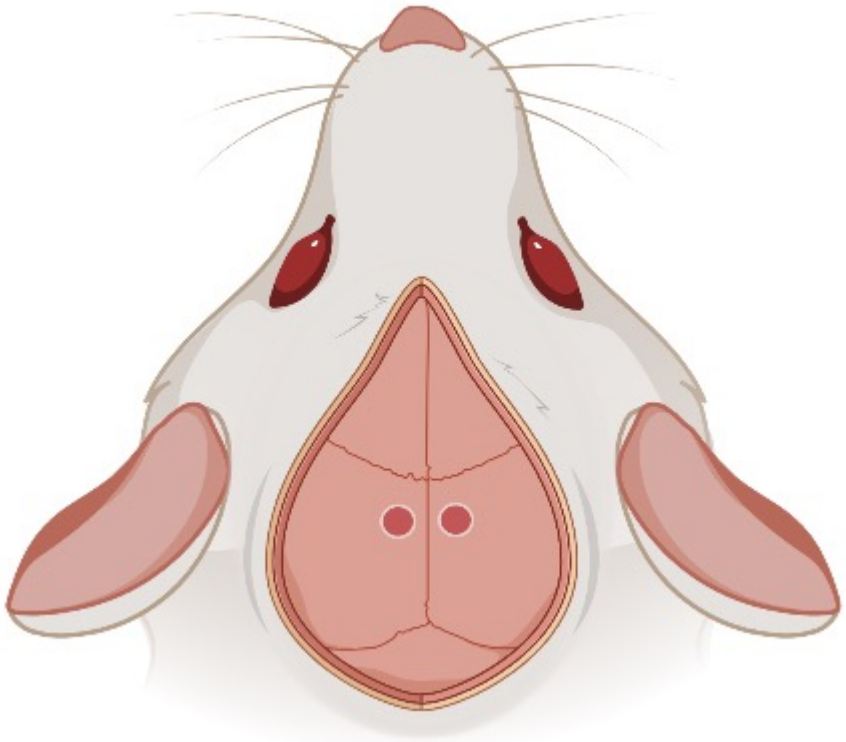
Viral Infusion



- Using a bevelled needle tip, gentle break through the dura
- Make tiny flicking motions on the edge of the craniotomy away from the center to clear any debris
- Attach Hamilton syringe to stereotax and position centrally above first craniotomy site
- Ensure that the bevel of the needle is facing the direction you want the infusion to be
- Slowly lower the Hamilton syringe so that the tip of the needle enters the craniotomy and the surface of the brain. Slower lower and retract 100microns 3 times to create the beginning of the needle tract.
- Raise the syringe and eject virus until 1 tiny droplet is beaded on the bevel of the needle. Wipe away using a surgical Q tip.
- Carefully lower the primed syringe to the surface of the brain and then zero your stereotaxic DV coordinates.
- Lower to the lowest DV coordinates you will be infusing virus at in smooth and precise motions. Overshoot by 50microns an then retract to your desired DV

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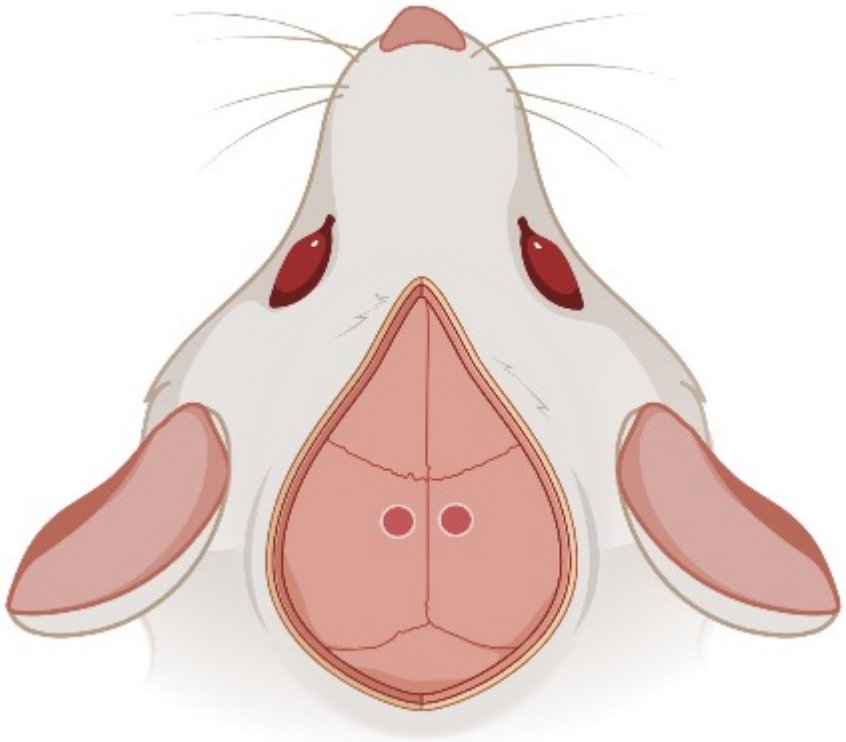
Viral Infusion



- Note the virus volume in syringe at beginning and time of infusions
- Begin your first infusion
- Wait for the appropriate rest period before moving slowly to your next DV coordinate and repeat
- Note volume at end of each infusion

- Once you have competed all of the infusions within this hemisphere, retract slowly to 500microns above your final infusion site and wait for an additional 10minutes before removing the syringe from the brain

- Double check the syringe by ejecting virus until one small bead in present on the needle bevel to confirm patency of the syringe. Repeat for second hemisphere

- Remove syringe, clean skull, and close fascia and incision with surgical sutures
- Cover with antibiotic gel and provide post operative care as per AUP

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Post-operative Care

- 3 days of analgesic (e.g., meloxicam i.p.)
- Monitor suture opening/weight loss/ signs of stress for 7 days
- Antibiotics if needed or part of your AUP
- Recovery 4-6 days

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Summary

- There are numerous fluorescent indicators available for in vivo imaging of calcium and neurotransmitter fluctuations
- GCaMP is the most common Ca imaging indicator and there are numerous series and types to choose between
- The type of experiments you are conducting determines the type of indicator you should use

- Viral transfection vs. genetic encoding of indicators

- Viral Infusion Surgery
  - Prepare virus
  - Prepare skull/ Craniotomy
  - Infuse Virus (wait times important)
  - Close and Recovery

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Available Resources

- Surgery Primer – will be available on MyPage (www.mightexbio.com/mypage)
- Webinar recordings

- Calcium Imaging Guide
- OASIS Implant product focused webinars via website and MyPage
- Future webinars
- Support team – contact us!

Webinar on headmount surgery (next!) – April 14th!

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Thank you!

Webinar and primer will be available:

www.mightexbio.com/mypage

Further questions: Catherine.Thomas@mightex.com

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