※

$$\begin{tikzcd} z \arrow[r] & x \arrow[r] & y \\ & u \arrow[u] \arrow[ur] & \end{tikzcd}$$

x: independent variable
y: dependent variable
u: covariates
z: instrumental variable (IV)

- Structural equation between x & y:

$$Y = X\beta + U \quad (\mathrm{cov}(x,u) \neq 0) \quad ①$$

$$\hat{\beta} = \frac{\mathrm{cov}(x,y)}{\mathrm{var}(x)} = \frac{\mathrm{cov}(x, x\beta + u)}{\mathrm{var}(x)}$$

$$= \frac{\mathrm{cov}(x, x\beta)}{\mathrm{var}(x)} + \frac{\mathrm{cov}(x,u)}{\mathrm{var}(x)}$$

$$= \beta^* + \frac{\mathrm{cov}(x,u)}{\mathrm{var}(x)} \quad ②$$

The real causal effect we want to estimate

bias, since $\mathrm{cov}(x,u) \neq 0$

Matrix form of $\hat{\beta}$:

$$\hat{\beta} = (X^TX)^{-1}X^TY = (X^TX)^{-1}X^T(X\beta + U)$$

$$= (X^TX^{-1})X^TX\beta + (X^TX)^{-1}X^TU$$

$$= \beta^* + \underbrace{(X^TX^{-1})^{-1}X^TU}_{\text{bias}} \quad ③$$

- Two-stage least square approach to estimate unbiased β (β^*).

↳ Stage 1:

- $X = Z\gamma + V \quad (\mathrm{cov}(z,u)=0, \mathrm{cov}(z,v)=0)$ ④

$$\Rightarrow \hat{\gamma} = (Z^TZ)^{-1}Z^TX \quad ⑤$$

$$\hat{X} = Z\hat{\gamma} = Z(Z^TZ)^{-1}Z^TX \triangleq P_Z X \quad ⑥$$

(use IV to predict x)

↳ Stage 2:

- $Y = \hat{X}\beta + E \quad (E \neq U) \ (\mathrm{cov}(\hat{X}, E) = 0)$.

$$\Rightarrow \hat{\beta}_{2SLS} = (\hat{X}^T\hat{X})^{-1}\hat{X}^TY$$

$$\overset{⑥}{=} (X^TP_Z^TP_ZX)^{-1}X^TP_Z^TY$$

$$= (X^TP_ZX)^{-1}X^TP_Z^TY \quad ⑦$$

$$\left\{ \begin{aligned} P_Z^TP_Z &= (Z(Z^TZ)^{-1}Z^T)^T(Z(Z^TZ)^{-1}Z^T) \\ &= Z(Z^TZ)^{-1}Z^TZ(Z^TZ)^{-1}Z^T \\ &= Z(Z^TZ)^{-1}Z^T \\ &= P_Z \end{aligned} \right\}$$

IV estimator:

$$☆ \ \hat{\beta}_{IV} = (X^TP_ZX)^{-1}X^TP_Z^TY \quad ⑧$$

where $P_Z = Z(Z^TZ)^{-1}Z^T$

Except for 2SLS method, it can also be proved from generalized method of moments (not shown).

From 2SLS approach, it is easy to see

$$Y = Z\hat{\gamma}\beta + E$$

$$\hat{\beta}_{2LS} = \frac{dy/dz}{\hat{\gamma}} = \frac{dy}{dz} \Big/ \frac{dx}{dz} \quad ⑨$$

- The Wald estimator:

when z is binary. ($P(z=1) = p$).

$$\mathrm{cov}(Y, z) = \mathbb{E}[Yz] - \mathbb{E}[Y]\mathbb{E}[z]$$

$$= p\,\mathbb{E}[Y \mid z=1] - (p\,\mathbb{E}[Y \mid z=1] + (1-p)\,\mathbb{E}[Y \mid z=0]) \cdot p$$

$$= p\,\mathbb{E}[Y \mid z=1] - p^2\,\mathbb{E}[Y \mid z=1] - p(1-p)\,\mathbb{E}[Y \mid z=0]$$

$$= p(1-p)(\mathbb{E}[Y \mid z=1] - \mathbb{E}[Y \mid z=0]) \quad ⑩$$

Similarly, $\mathrm{cov}(X, z) = p(1-p)(\mathbb{E}[X \mid z=1] - \mathbb{E}[X \mid z=0])$.

$$\hat{\beta}_{IV} = \frac{dy}{dz} \Big/ \frac{dx}{dz} = \frac{\mathrm{cov}(Y,z)}{\mathrm{var}(z)} \Big/ \frac{\mathrm{cov}(X,z)}{\mathrm{var}(z)}$$

$$= \frac{\mathrm{cov}(Y,z)}{\mathrm{cov}(X,z)} = \frac{\mathbb{E}[Y \mid z=1] - \mathbb{E}[Y \mid z=0]}{\mathbb{E}[X \mid z=1] - \mathbb{E}[X \mid z=0]} \quad ⑪$$

※ IV approach applied in Lopperod et al. (2018).

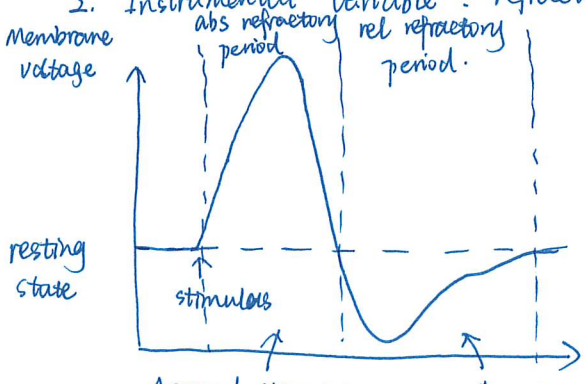
1. Optogenetics:
   - use light to stimulate some neurons (S_1) (by light-sensitive ion channels).
   - these stimulated neurons will cause other neurons (S_2) to fire.

   ⇒ we can study connections among neurons.

   challenges:
   - hard to stimulate a single neuron.
   - network states also affect both S_1 and S_2

   ↑ covariates issue.

2. Instrumental variable: refractory state. (absolute).



A second stimulus (no matter how strong) will NOT excite the neuron.

A stronger than normal stimulus is needed to elicit neuronal excitation.

Whether neuron S_1 is in absolute refractory state or not can only affect the firing of S_2 (y) through the firing of S_1 (x).

3. IV approach

$$\begin{tikzcd} z \arrow[r] & x \arrow[r] & y \\ & u \arrow[u] \arrow[ur] & \end{tikzcd}$$

x: spike times of pre synaptic neurons (S_1) in M trials

y: spike times of postsynaptic neurons (S_2) in M trials.

z: whether presynaptic neuron is in absolute refractory state ($z=1$) or not ($z=0$).

According to the Wald estimator,

$$\hat{\beta}_{IV} = \frac{\bar{y} - \bar{y}_r}{\bar{x} - \bar{x}_r} \propto \bar{y} - \bar{y}_r \quad (12)$$

$\bar{y}$: average # trials where successfully stimulating S_1 resulted in a response in S_2.

$\bar{y}_r$: average # trials where an unsuccessful stimulation of S_1 resulted in a response of S_2.

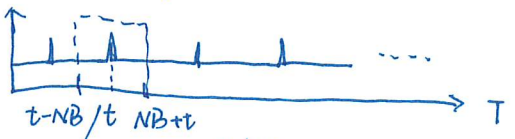
$\bar{x}$: successful stimulations of S_1

$\bar{x}_r$: unsuccessful stimulations of S_2.

※ Competing method 1: cross-correlation histogram (CCH) in Lepperod et al. (2018)

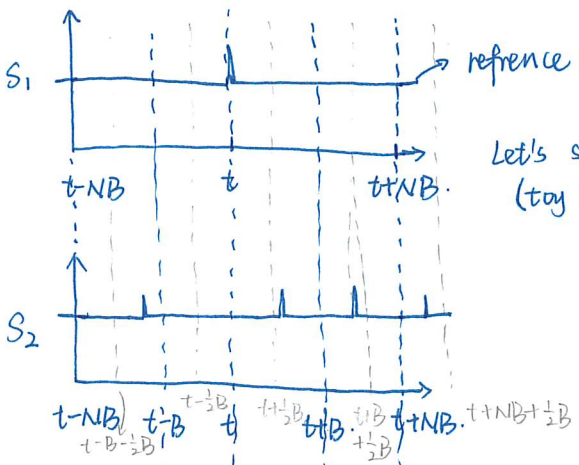
1. CCH

spike trains of presynaptic neuron S_1:

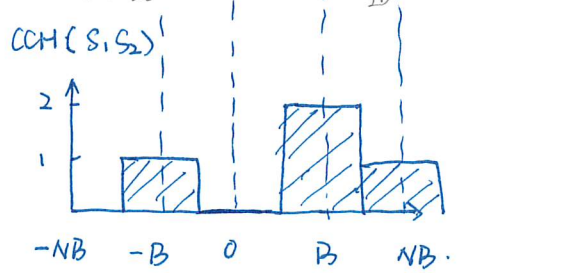


N: # bins

B: time resolution, i.e. length of each bin



Let's say $N=2$ (toy example)



2. Connections estimated using CCH.

(1) Assume # spikes in a bin follows Poisson distribution.

$$p(\#\text{spikes in } m\text{-th bin} \geq C \mid \lambda(m)) = 1 - \sum_{k=0}^{C-1} \frac{e^{-\lambda(m)} \lambda(m)^k}{k!} \quad (13)$$

Since we want to study the effects of S_1 on S_2, the peaks of S_2 in positive-lag direction need to be larger than the peaks in negative-lag direction. (i.e. continuity correction).

After correction:

$$p(C \text{ or more} \mid \lambda(m)) = 1 - \sum_{k=0}^{C-1} \frac{e^{-\lambda(m)} \lambda(m)^k}{k!} - \frac{1}{2} \cdot \frac{e^{-\lambda(m)} \lambda(m)^C}{C!}$$

(2) H_0: the two spike trains are uncorrelated at a precise temporal resolution (i.e. B).
(Null hypothesis)

steps to test H_0:

a. compute raw CCH

b. compute a predictor CCH → similar concept to α-level

c. estimate the probability to obtain the raw CCH given the predictor CCH.

(3) The convolution method to compute predictor CCH (i.e. estimate $\lambda(m)$ in (1)).

In this paper, $\lambda(m)$ is the raw CCH convolved with a hollow Gaussian kernel at bin m.

(4) The connection weight: spike transmission probability. (English et. al. 2017).

$$P_{trans} = \frac{1}{n_{pre}} \sum_{m=3ms}^{6ms} CCH(m) - \lambda(m)$$

n_{pre}: # spikes detected in the presynaptic neuron.

※ Competing method 2: Logistic regression.

1. Pick a 4ms window for both pre- & post-synaptic neurons, with a lateney relative to stimulation time of 0 and $T_{syn}+D$ ms respectively

T_{syn}: synaptic intergration time constant (1ms).

D: synaptic delay (1.5ms)

2. Estimate the probability $p(y=1 \mid x)$ by fitting:

$$y = \begin{cases} 1 & \text{if } \beta_0 + \beta_1 x + u > 0 \\ 0 & \text{else} \end{cases}$$

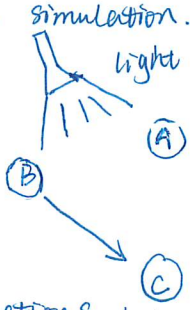
x: presynaptic neuron's time window contains a spike or not

y: postsynaptic neuron's time window contains a spike or not.

3. Synaptic weight : logit function

$$p(x) = \frac{1}{1+e^{-(\beta_0+\beta_1 x)}}$$

※ 3-neuron simulation.



light will stimulate A & B directly (covariate)

Assume B can cause C firing, but A can't.

1. Connections between neurons by CCH

CCH among neurons: (Fig 2(b))

- strong correlation between A&B
- significant: B&C, A&C

Connetivity estimated from CCH: (Dashed lines in Fig. 3b & 3c)

B-C : similar to ground truth.

A-C : much higher than ground truth.

2. Connections by IV (solid lines in Fig 3b & 3c)

B-C , A-C : both are close to ground truth.

※ Large-scale simulated networks.

1. Setup

- 1250 randomly connected leaky integrate and fire (LIF) neurons.
  - ↳ 250 had inhibitory synapses.
  - ↳ 800 excitatory neurons for stimulation.
  - ↳ For weight estimates, 100 stimulated neurons & 100 non-stimulated neurons were selected from the excitatory population.
  - ↳ 3 different amounts of relative inhibition. $g = 9.9, 4.4, 3.0$

g determines the connection strength of inhibitory synapse relative to the connection strength of excitatory synapse.

$$J_{in} = g\, J_{ex}$$

$J_{(\cdot)}$: post synaptic current. (PSC) amplitude

(P5)

$J_{(\cdot)}$ will determine the post synaptic current:

$$I^i_{syn}(t) = \sum_{j \in N(i)} J_j\, \alpha(t - t_j - D)$$

where $N(i)$ are the incoming ~~neurons~~ synapses on neuron i; t_j is an incoming spike through synapse j; D is the delay of a synapse.

$\alpha(\cdot)$ function is given by:

$$\tau_{syn}\, \alpha(t) = t e^{-\frac{t}{\tau_{syn}}} H(t)$$

where τ_{syn} is the synaptic integration time constant; $H(\cdot)$ is the Heaviside step function.

Then the leaky integrate-and-fire (LIF) model is given by:

$$\frac{dV_m^i}{dt} = -\frac{(V_m^i - E_L)}{\tau_m} + \frac{I^i_{syn}(t)}{C_m}$$

V_m^i — membrane potential of neuron i

E_L — leaky potential

(when V_m^i reaches a threshold, an action potential is emitted, and V_m^i will be reset to E_L)

τ_m — membrane time constant.

C_m — membrane capacitance.

2. Results.

(1) Mean square error (relative to ground truth) of CCH approach is much higher than that of IV approach. (Fig 4c & 4d, dashed lines VS solid lines)

$\boxed{\text{Remark}}$: MSE increases as hit rate increases. (Fig 4d)

It is because high hit rate happens when stimulation intensity is strong. That will cause correlated refractory times and lower the statistical power.

(2) · False positives & false negatives.

a. false positives of CCH estimator & logistic regress estimator are both higher than IV estimator (Fig 5a).

b. false negatives of CCH estimator & IV estimator are at similar level (logistic regression estimator has no FN since its FPR is 100%). (Fig 5b)

(P6)

(3) ~~Fod~~ Linear fit between ground truth and the estimators.

a. Good linear fit between IV estimator and ground truth ($R^2 = 0.77$) (Fig 5c)

b. Poor CCH estimation ($R^2 = 0.01$)
CCH only estimates stimulation strength (see the color bar) (Fig. 5d).

※ Hippocampal spike recordings (real data)

1. Setup.
- CA1 pyramid neurons of 2 mice
- whether a unit is optogenetically stimulated or not?
  → compare # spikes during each stimulation VS # spikes in the same interval but 2s before the stimulation.
- 17 putative presynaptic units & 86 putative postsynaptic units are considered.

2. Results.
- IV estimates & CCH estimates are somewhat correlated (Fig 6a, dashed line, $r = 0.34$)
- IV estimates & CCH estimates are different (Fig 6b)
  ↓
  The IV estimates error bars do not overlap with linear fit in many cases.

※ Discussion.

1. Make sure the refractory period is a good instrumental variable.
Refractory period can be affected by the network activity.

2. Randomness of refractory times is important for IV approach.
Even if neuron's refractory states are strongly correlated during normal network operations, there can be ways of randomizing ~~refractoring~~ refractoriness, e.g. use a task or situation where neurons are as uncorrelated as possible.

3. Main problem of optogenetics: non-local
Need ways to make the stimulation effect more locally.

4. Very weak stimulation cannot be used for causal inference. Although weak stimulation mainly elicit spikes in very close-by neurons, it will still affect the membrane potential of many neurons further away. Therefore, it does not help ~~for~~ with the confounding issue.