Learning Pupillometry

From Theory to Analyses

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University of
Konstanz

Agenda

Day 1: Theory & Experimental Design

What is pupillometry? What can pupil size tell us?
Why and how do we measure pupil size?

Experimental design considerations
e.g., potential confounds, simulating pupil data, pre-existing datasets

Day 2: Pre-processing & Analyses

Hands-on coding workshop covering a variety of potentially useful analyses, from condition-averaging the pupil dilation response, to analyzing the dynamics of single-trial pupil time series.

Agenda

Day 1: Theory & Experimental Design

What is pupillometry?
What is the pupil?
Why does the pupil appear black?
What are the neural underpinnings of pupil changes?
Why does the pupil change size?
What are the visual and cognitive functions of changes in pupil size?
How fast can the pupil move?
How long does the pupil take to respond?
Is the pupil subject to voluntary control?

How do we measure pupil size?

Experimental design considerations

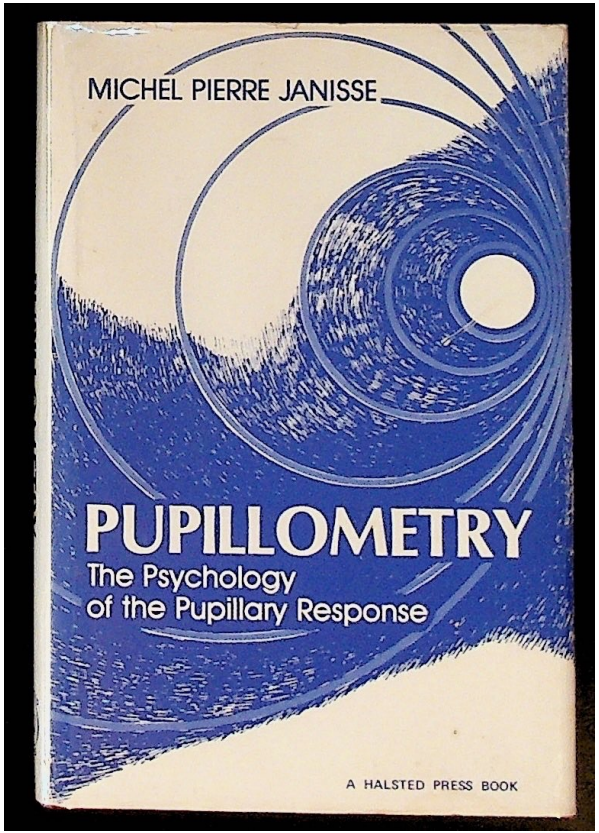
What is pupillometry?

Generally, pupillometry refers to the measurement of the pupil(s), or the study of the pupil(s)

Within experimental psychology, pupillometry has come to be associated with measuring attention and/or cognitive effort

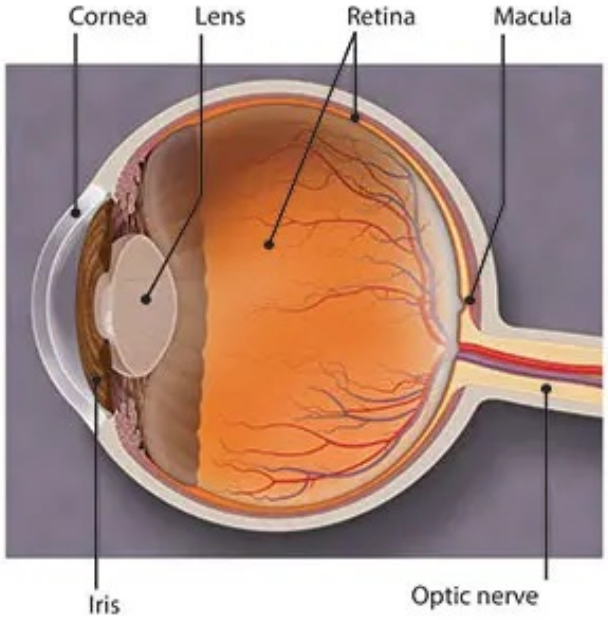
"[. . .] has more advantages than disadvantages than most other dependent measures of behaviour"

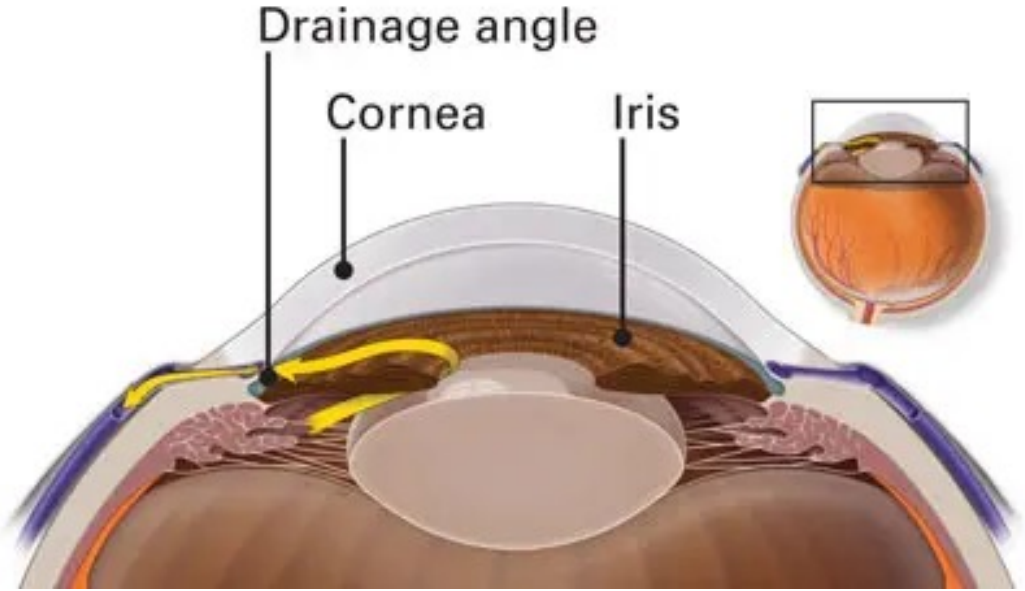
- Janisse, *Pupillometry*



What is the pupil?

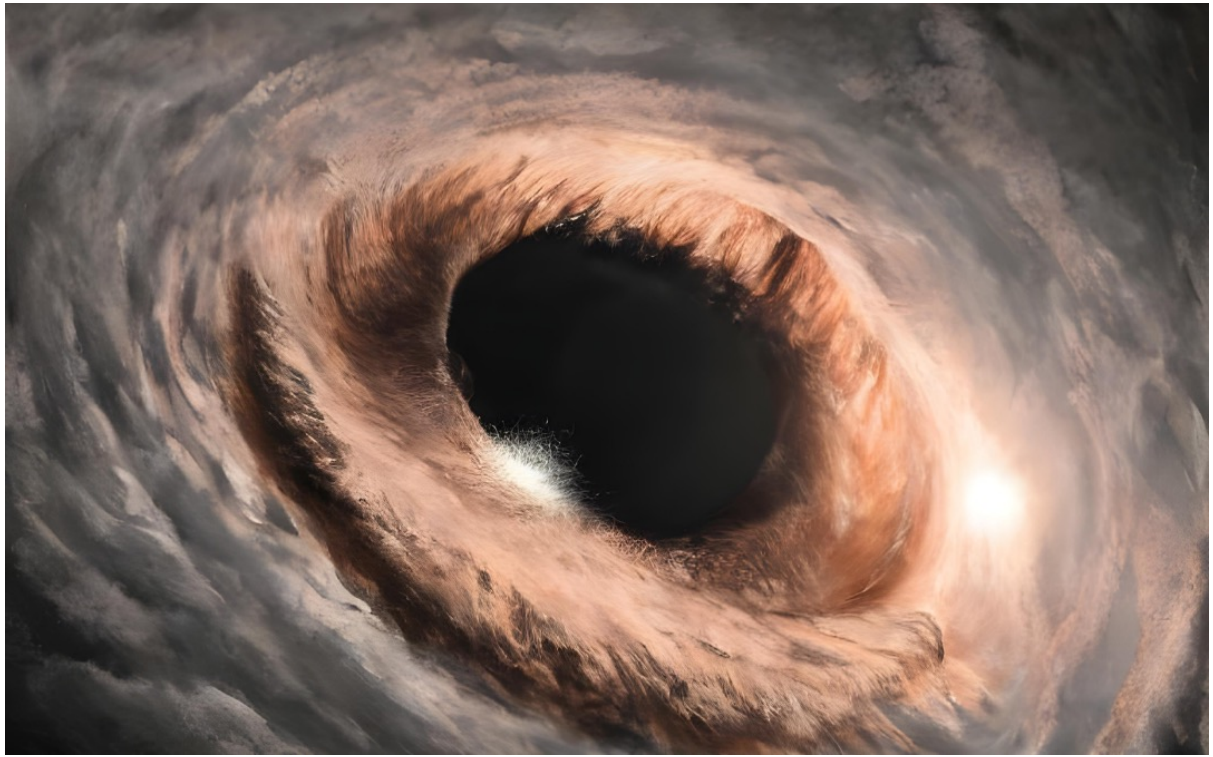
Hole in center of iris!





https://www.aao.org/eye-health/anatomy/parts-of-eye

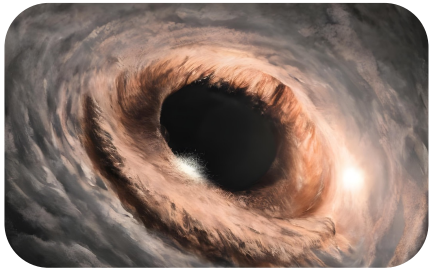
Why does the pupil appear as a black hole?



https://www.ifa.hawaii.edu/2023/02/first-observational-evidence-linking-black-holes-to-dark-energy/

Why does the pupil appear as a black hole?

Light entering eye is absorbed by tissues in the eye (e.g., retina)



Dilated eye exam

https://www.youtube.com/watch?v=M6IlOKXlCqs

Red eye in photos

https://en.wikipedia.org/wiki/Red-eye_effect#/media/File:BoldRedEye.JPG

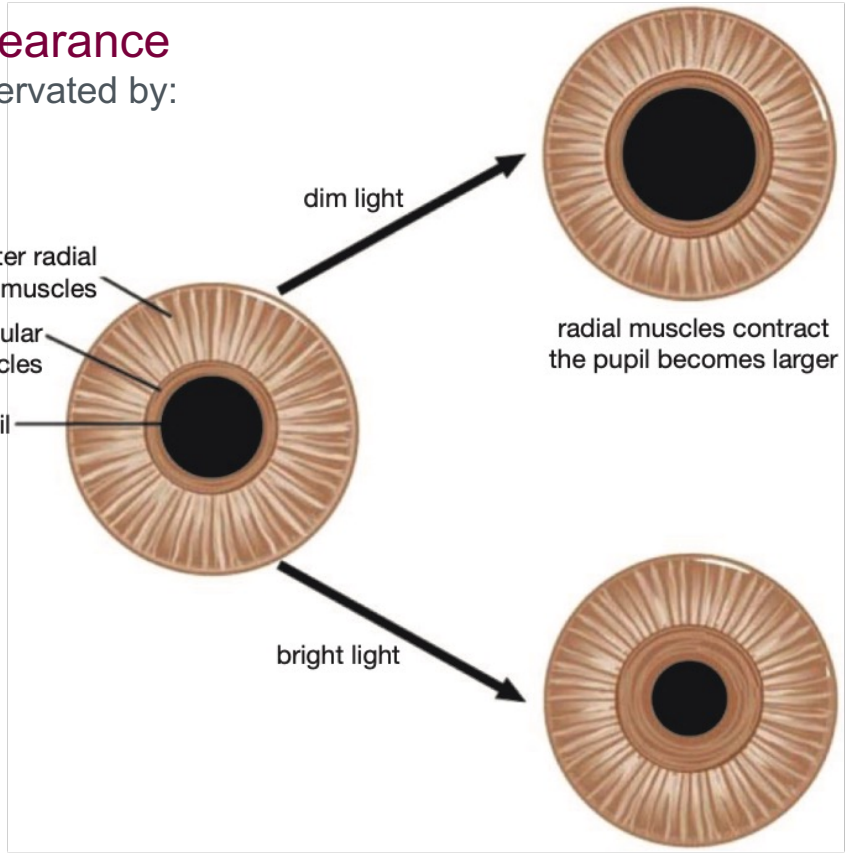
Iris muscles alter pupil appearance

Radial vs. circular muscles are innervated by:

Sympathetic
Nervous System
- superior cervical ganglion

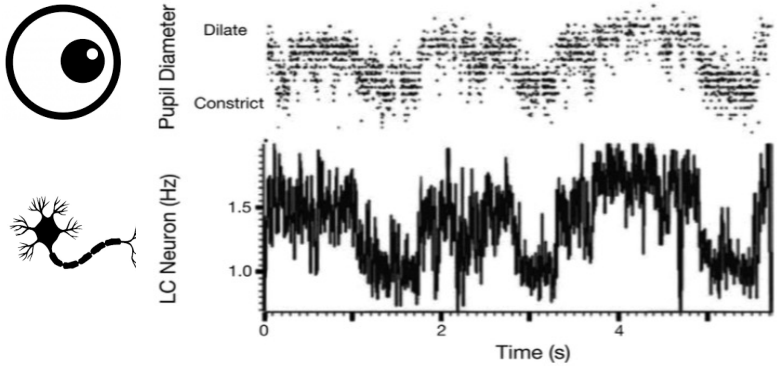
Parasympathetic
Nervous System
- Edinger–Westphal nucleus

BUT. Lots of interactions



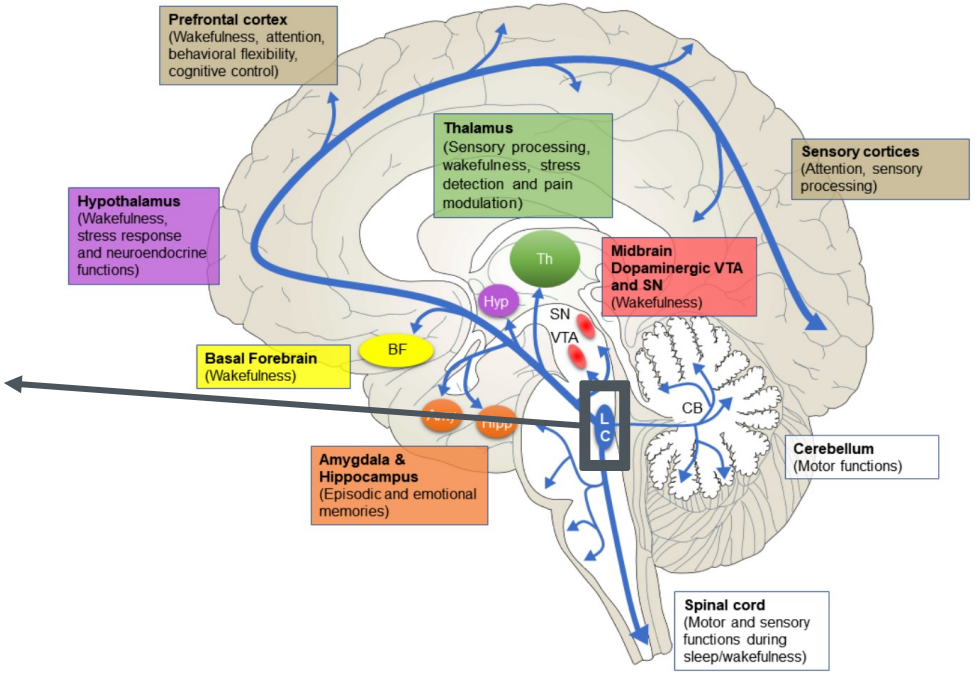
https://www.brainscape.com/flashcards/b31-the-nervous-system-eye-11341118/packs/20053479

The pupil as an index of Locus Coeruleus / Noradrenergic activity



(Rajkowski et al., 1993)

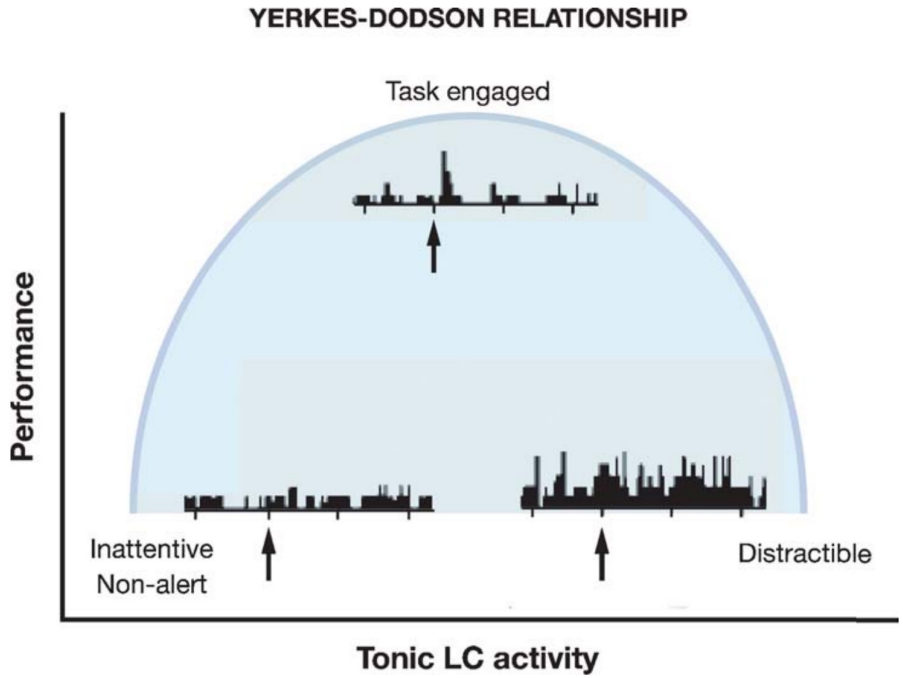
Norepinephrine system

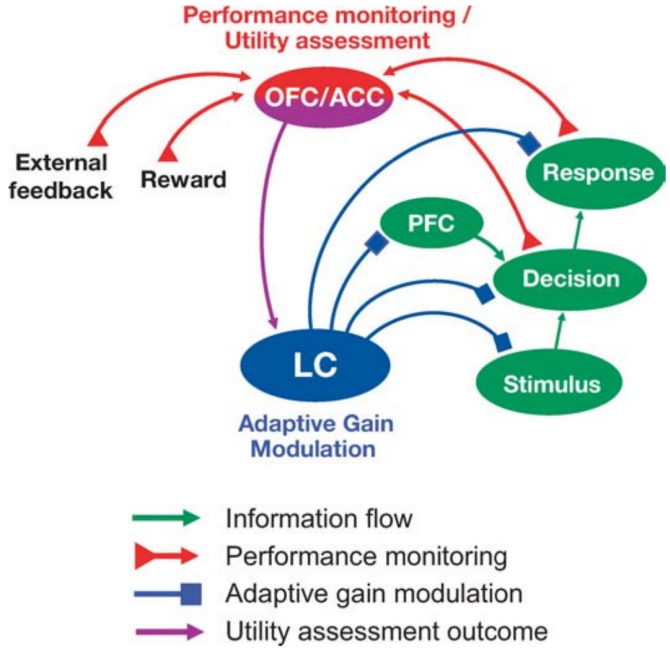


(Bari, Chokshi, Schmidt, 2020)

BUT. Lots of interactions

LC-NA activity & Adaptive Gain Theory

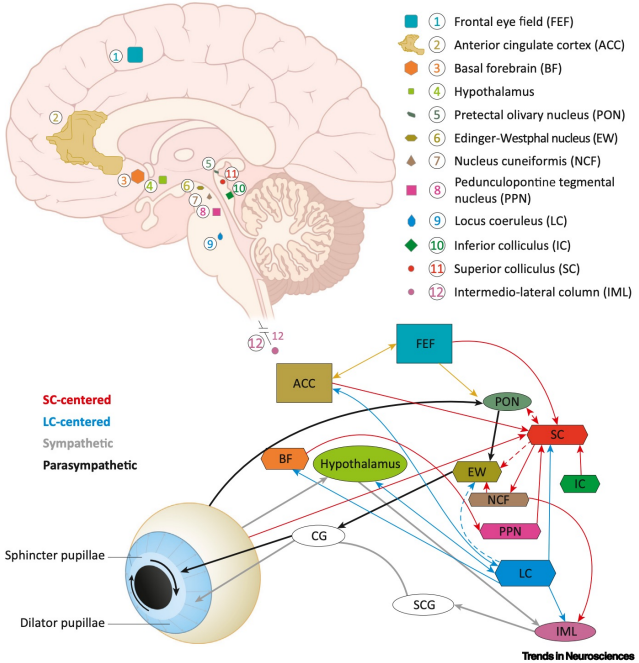




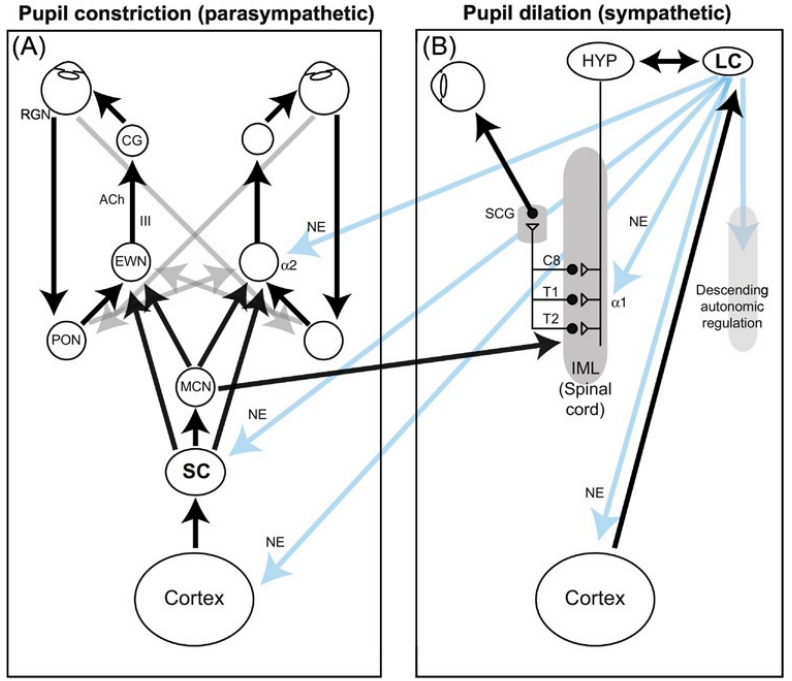
Aston-Jones & Cohen, 2005

Additional neuromodulatory and neuroanatomical influences on pupil size

Cholinergic (Reimer et al., 2016); **Serotonergic** (Schmid et al., 2015); **Dopaminergic** (de Gee et al., 2014)



Strauch et al., 2022



Joshi & Gold, 2020

Why does the pupil change size?

Many reasons!

One recently proposed taxonomy (Strauch et al., 2022):

Low level

- Light level
- Focal distance

Intermediate level

- Alerting & Orienting

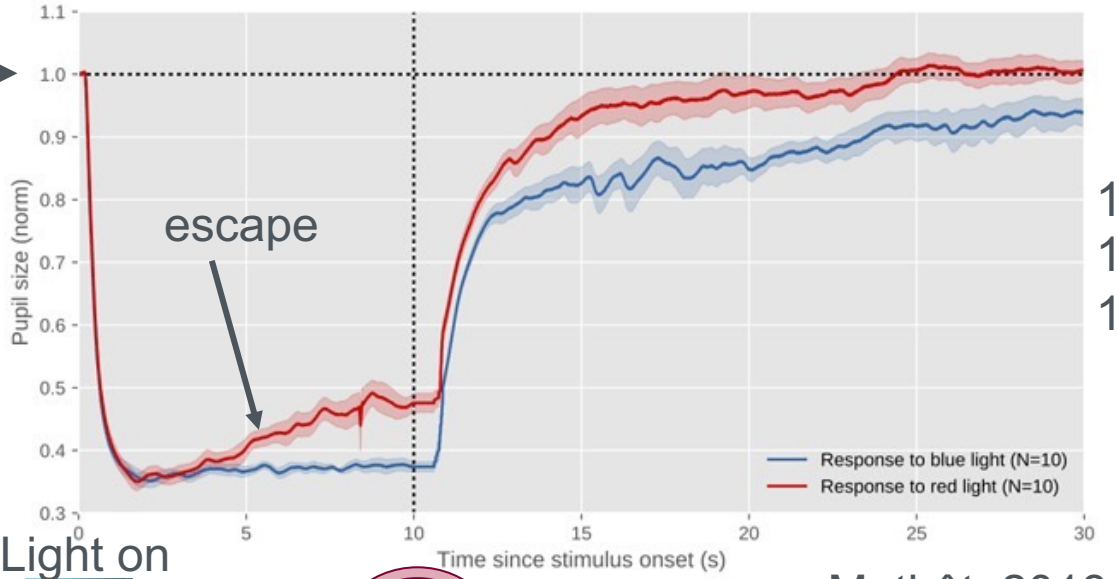
High level

- Executive control

Why does the pupil change size?

Pupillary light reflex
- Constriction of pupil in response to light
  - Sequence: constriction to minimum, escape (or not), return to original size

Baseline pupil size



1 participant
10 trials red
10 trials blue

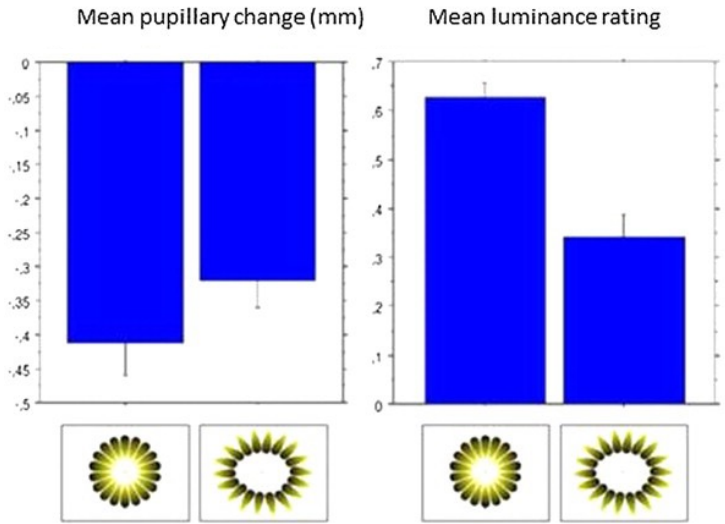
Light on

Mathôt, 2018

Why does the pupil change size?

Pupillary light “reflex”

- Sensitive to top-down influences (e.g., attention, processing, interpretation)
  - **Perceived brightness**

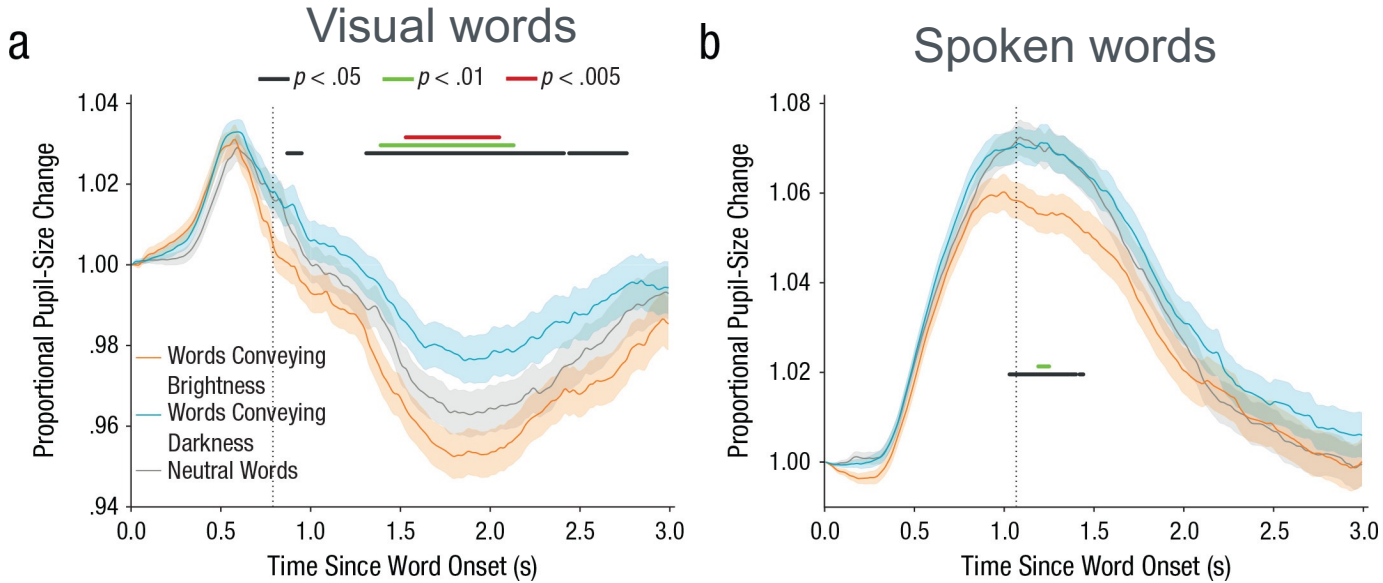


Laeng & Endestad, 2012

Why does the pupil change size?

Pupillary light “reflex”

- Sensitive to top-down influences (e.g., attention, processing, interpretation)
  - **Words conveying brightness**

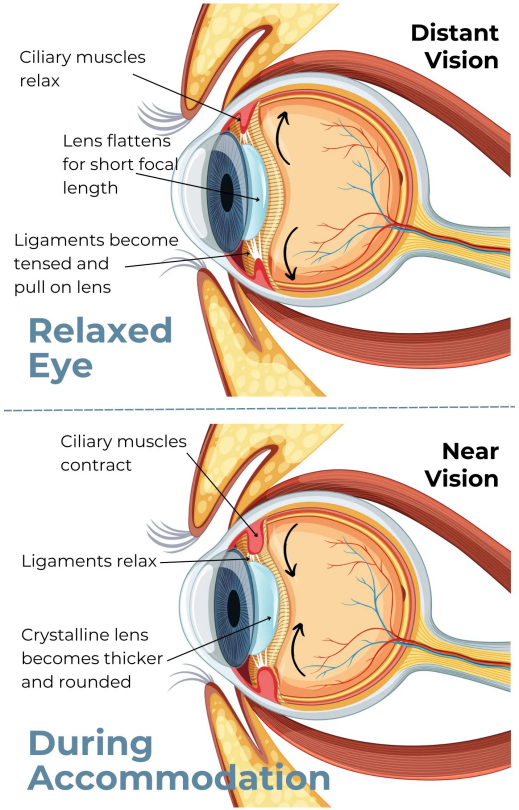


Mathôt et al., 2018

Why does the pupil change size?

Learn about other pupillary reflexes here:
https://eyewiki.aao.org/Reflexes_and_the_Eye

- **Dark reflex:** dilation response to darkness
- **Ciliospinal reflex:** dilation response to noxious stimuli
- **Near accommodation:** constriction response when focusing on close objection



https://www.accuvision.co.uk/glossary/accommodation/

What are the visual functions of changes in pupil size?

Dilation

- Better visual detection in dim environments
  - See e.g., Franke et al., 2022
- Help with adaptation from bright to dark (when rods/cones still adapting slowly)

Constriction

- Increase depth of field / visual sharpness (small pupil = small lens = less visual distortion)

Why does the pupil change size?

Cognitive or psychosensory reasons:

- Mental effort
- Attention
- Uncertainty, decision-making
- Surprise, salience, prediction error
- Sexual or emotional arousal
- Response preparation, motor activity
- Fatigue, time-on-task
- Imagined arousal, effort, brightness
- Memory, familiarity

Sometimes pre-conscious effects!

Learn more: https://link.springer.com/article/10.3758/s13428-023-02098-1/tables/1
Fink et al., 2023

Why does the pupil change size?

Cognitive process	Key papers	Key findings
Mental effort	Kahneman and Beatty (1966); Kahnemann and Beatty (1967); Johnson (1971); Kramer et al. (2013)	Pupil size sensitive to variations in effort (greater effort, greater pupil size)
Attention (general)	Kahneman (1973); Hoeks and Levelt (1993); Iriki et al. (1996); Smallwood et al. (2011); Wierda et al. (2012)	Pupil signal indexes changes in attention over time. Pupil time course can be modeled via attentional pulses. Pupil exhibits greater spontaneous fluctuation when attention is decoupled from task
Attention (spatial)	Mathôt et al. (2013); Binda et al. (2013); Naber et al. (2013a)	When focusing attention on a visual object (even covertly), the pupil adjusts to the objects' brightness
Uncertainty, decision-making	Friedman et al. (1973); Einhäuser et al. (2008); Jepma and Nieuwenhuis (2011); Laeng et al. (2012); de Gee et al. (2014); Urai et al. (2017); Kawaguchi et al. (2018); Colizoli et al. (2018a)	Pupil diameter increases prior to perceptual shifts and decision-making. Negative relationship between pupil size and decision confidence
Surprise, salience, orienting, prediction error	Beatty (1982a); Preuschoff et al. (2011); Wang et al. (2014); Fink et al. (2018); Alamia et al. (2019)	Pupil dilation response to surprising / alerting / salient stimuli, even when below perceptual threshold or unconscious and irrelevant to the task. Positive relationship between pupil size and prediction error
Sexual or emotional arousal	Hess and Polt (1960); Bradley et al. (2008)	Greater arousal correlated with greater pupil size. Heart rate and skin conductance also correlated with pupil activity
Response preparation, motor activity	Einhäuser et al. (2008); Reimer et al. (2014); McCloy et al. (2016)	Making a motor response (e.g., a button press) increases pupil dilation, which begins prior to the motor response. In mice, fluctuations in pupil size correlated with locomotion activity
Fatigue, task performance	Lowenstein et al. (1963); Beatty (1982a); Aston-Jones and Cohen (2005); Murphy et al. (2011); Eldar et al. (2013); McGinley et al. (2015); Knapen et al. (2016)	Performance on a task follows an inverse U-shaped function, with optimal performance at intermediate pupil sizes. Tonic pupil size decreases with time-on-task. Pupil size and behavior are correlated with changes in neural gain
Imagined arousal, effort, or brightness	Whipple et al. (1992); Laeng and Sulutvedt (2014); Sulutvedt et al. (2018); Kang and Banaji (2020)	Even during imagery or visual illusions, the pupil follows the same dilation patterns as observed during naturalistic conditions
Memory and familiarity	Võ et al. (2008); Kafkas and Montaldi (2011); Naber et al. (2013b); Papesh et al. (2012); Gomes et al. (2021)	Greater pupil size for greater familiarity; however, false alarms also produce pupil dilation (i.e., the pupil may not distinguish accurate familiarity, rather participants' belief). Greater pupil size at encoding predicts greater retrieval success. cf. Beukema et al. (2019) and "Surprise" section above

What are the more general functions of changes in pupil size?

Why is it that “higher” level processes like mental effort should be connected with the neural systems that control a light reflex, all indexed by pupil size?

Your thoughts?

https://PollEv.com/laurenfink203

What are the more general functions of changes in pupil size?

Why is it that “higher” level processes like mental effort should be connected with the neural systems that control a light reflex, all indexed by pupil size?

Possible answers:

1. Whenever the ascending arousal system – of which the noradrenergic LC is a key center – becomes active (e.g., because of cognitive or affective processing) the pupil dilates in proportion of the LC activation (e.g., Alnæs et al., 2014)

2. Active vision / nervous system, as a whole, should prime itself for an optimal response
   - Increased pupil size (due to load) might act as a compensatory mechanism for making sure that important changes in the environment are not missed

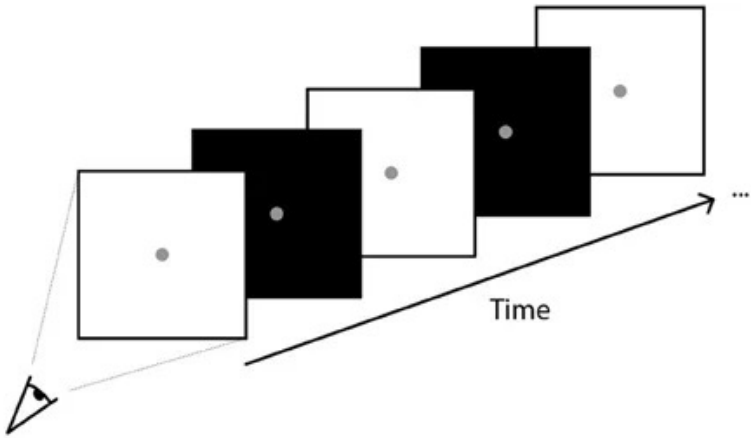
How fast can the pupil move?

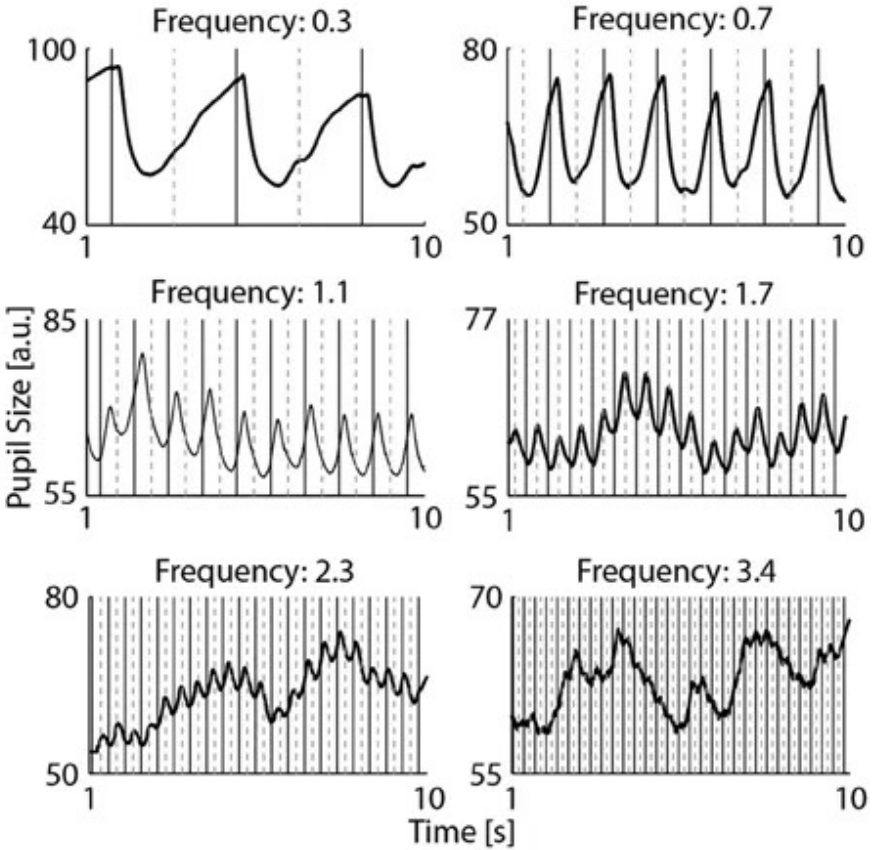
What is the fastest frequency we might care about in pupil data?

https://PollEv.com/laurenfink203

How fast can the pupil move?

Flashing screen black and white at different frequencies

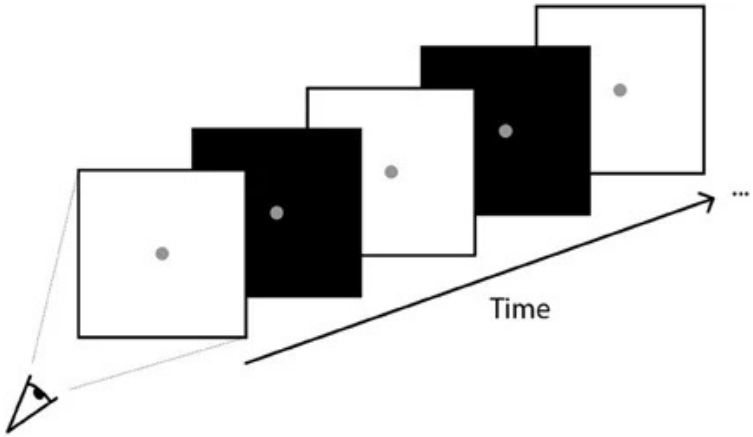


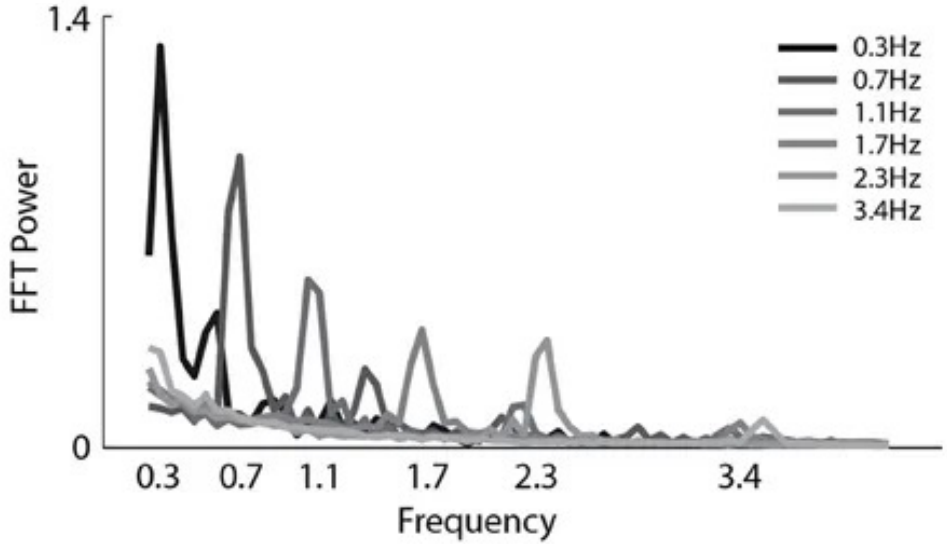


Naber et al., 2013

How fast can the pupil move?

Flashing screen black and white
at different frequencies





Answer: Not much faster than 3 Hz

Naber et al., 2013

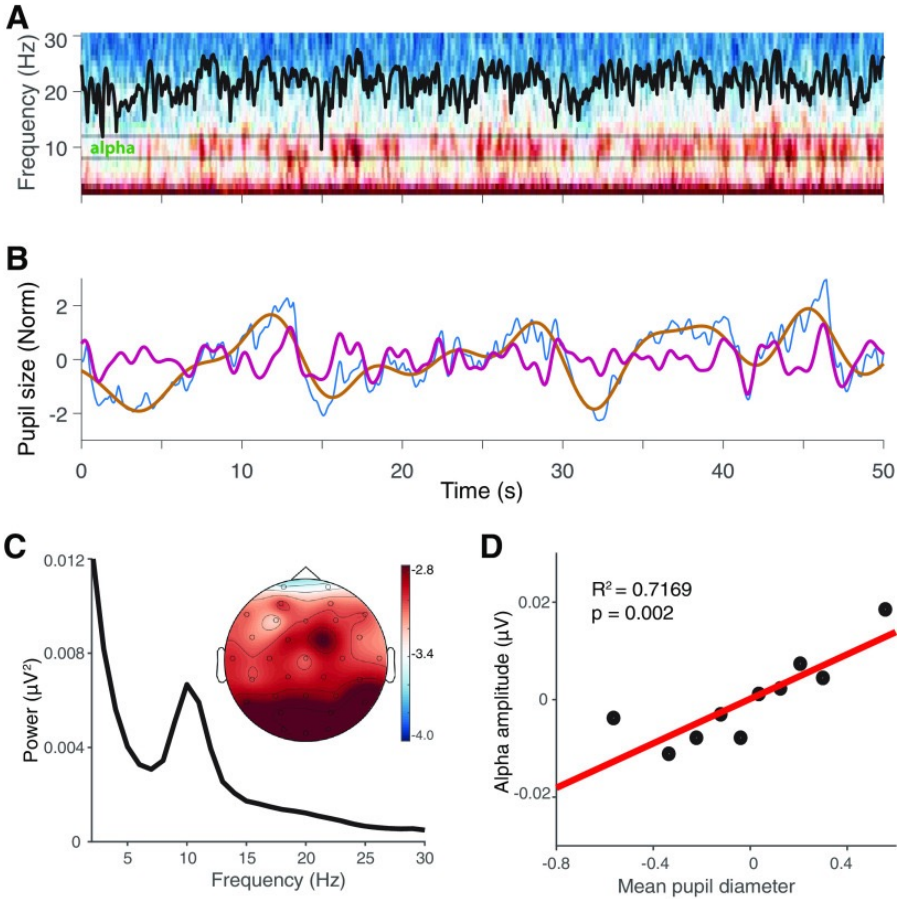
“Low” vs. “High” Frequency activity

Montefusco-Siegmund et al., 2022

High: 0.2 and 1 Hz

Low: 0.05 and 0.2 Hz

High-frequency pupil activity and alpha power covary

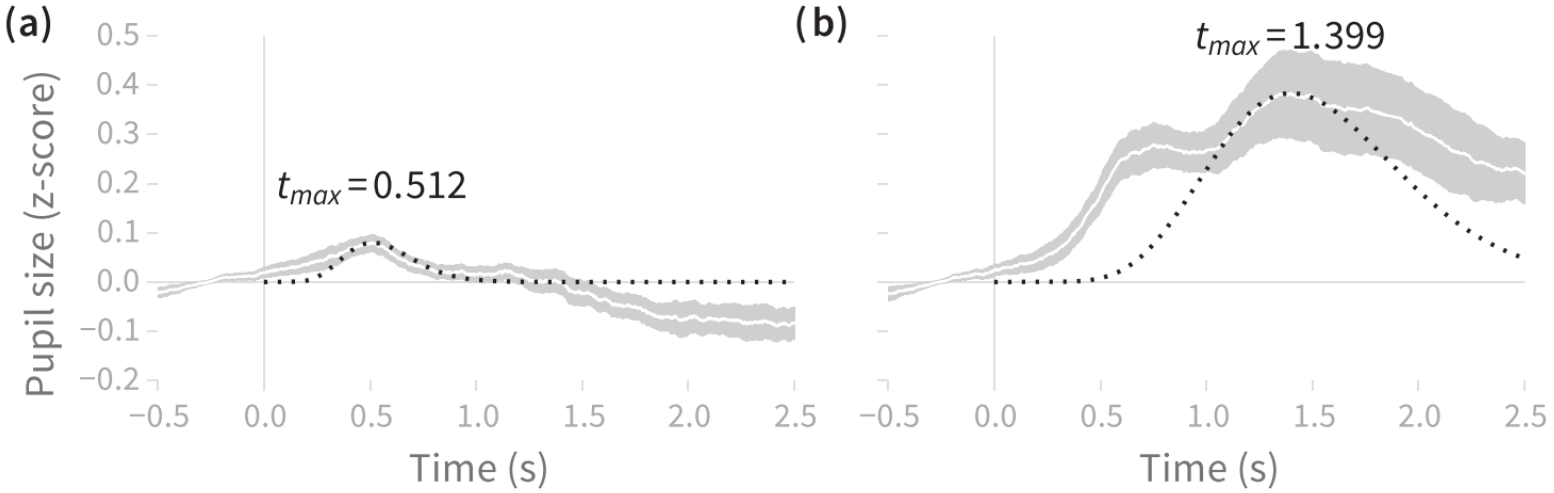


How long does the pupil take to respond?

Hoeks & Levelt, 1993; McCloy et al., 2016

Pupillary Response Function $\mathrm{PRF} = t^n e^{\left(-\frac{nt}{t_{max}}\right)}$

t = delay until pupil size returns to basline
n = number of neural signaling steps between impulse and pupil response
t_max = pupil response peak latency

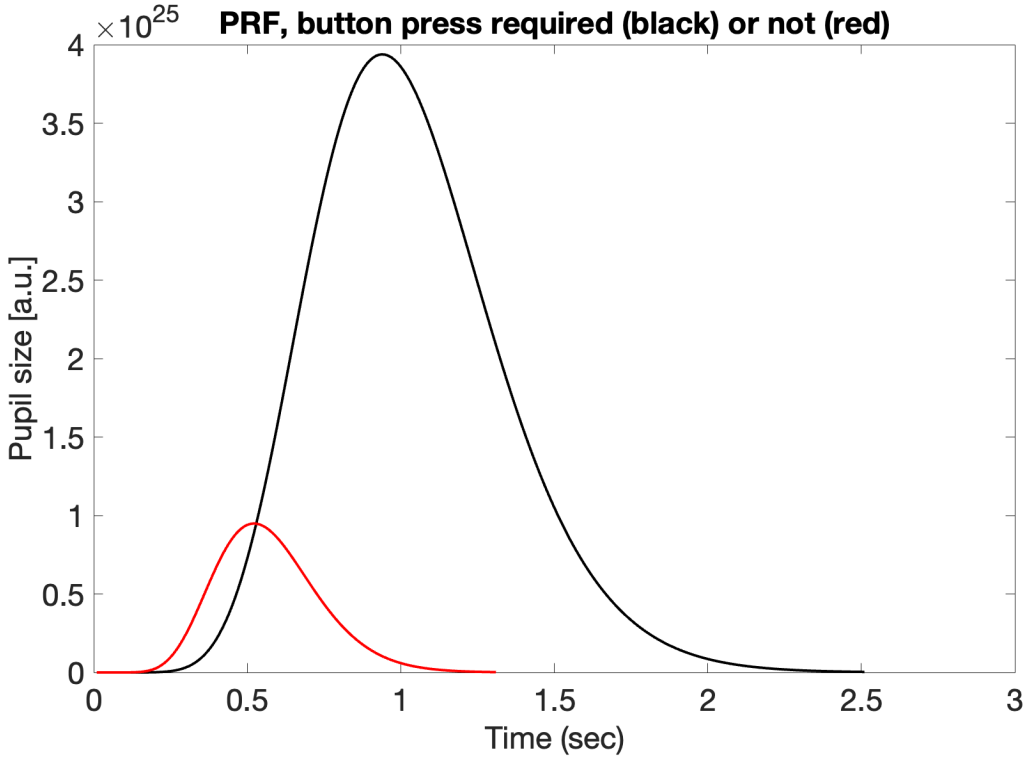


How long does the pupil take to respond?

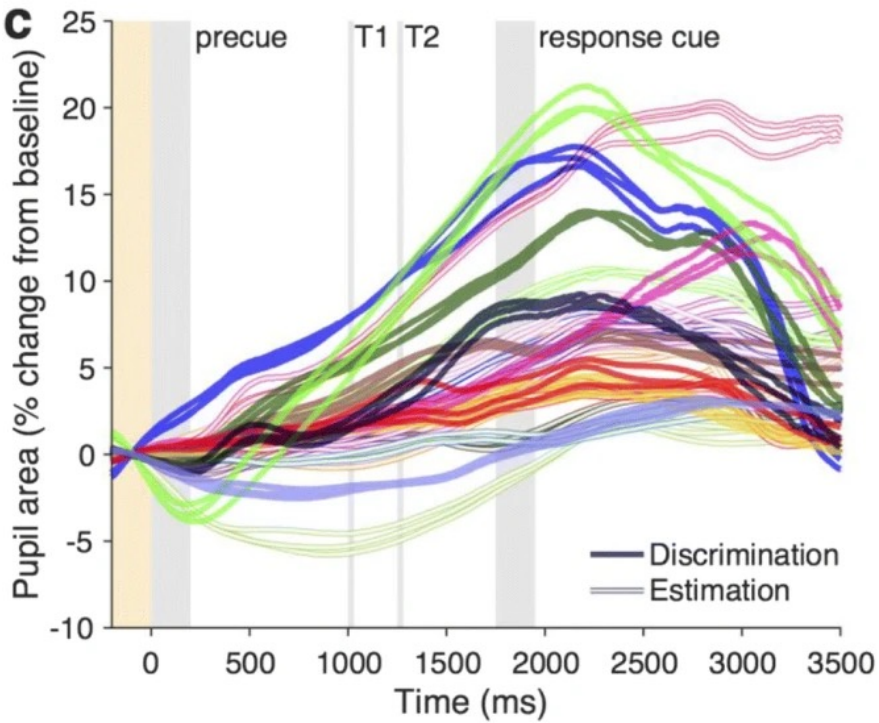
$$\mathrm{PRF} = t^n e^{\left(-\frac{\mathrm{nt}}{t_{\max}}\right)}$$

It depends on:

- Button press requirement
- Stimulus intensity
- Cognitive state
- Individual differences



Individual differences in pupillary response function



$$PRF = t^n e^{\left(-\frac{nt}{t_{max}}\right)}$$

Recommend fitting individual response function

t_max varies widely across individuals, but consistent within an individual

https://github.com/jacobaparker/PRET

Denison et al., 2020

Is the pupil subject to voluntary control?

https://PollEv.com/laurenfink203

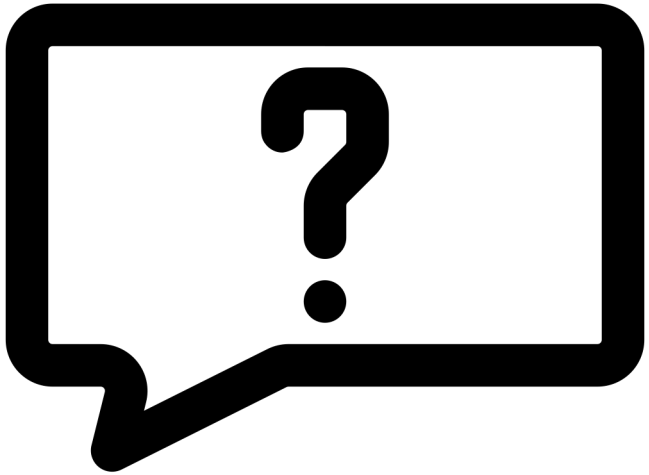
Is the pupil subject to voluntary control?

Generally, the answer is thought to be **NO**
- And that is one of the core advantages of this method!

If people claim to have control over pupil size, it is usually through *indirect* strategies
- Imagining something bright
- Imagining something arousing
- Change in focus / vergence (accommodation reflex)

HOWEVER:
See case study in Eberhardt et al. (2021)

Questions?

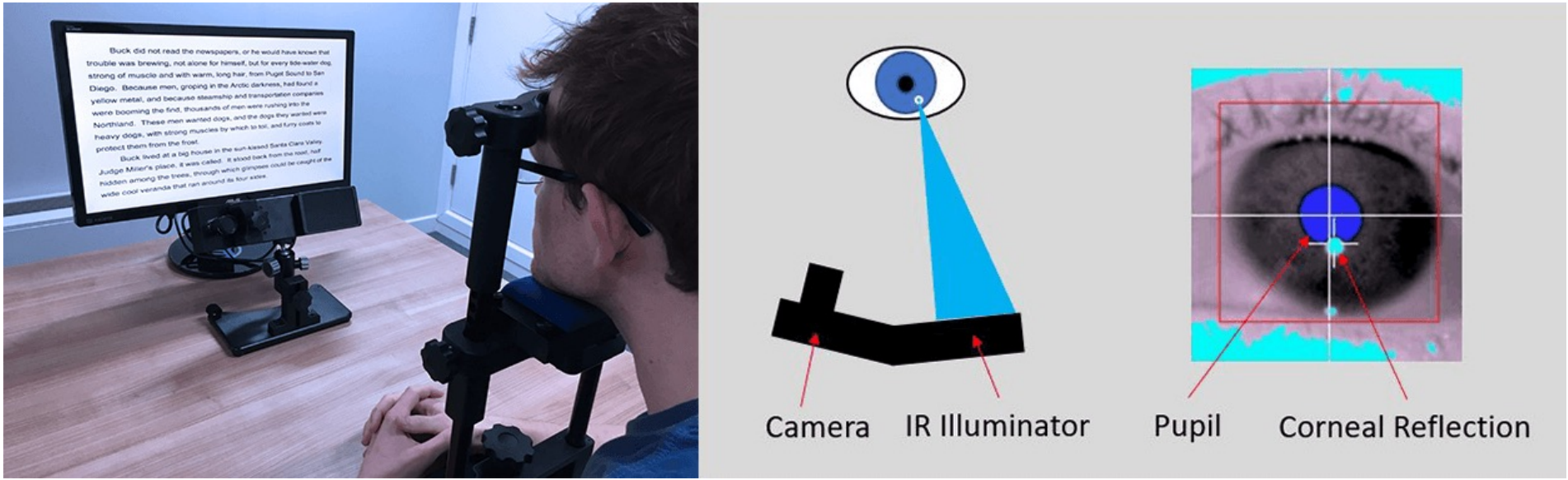


Measuring pupil size

How do we measure pupil size?

Feature based	Appearance Based
External light source reflections -> Eye features -> 3D Eye model	2D Images -> Machine Learning Model
Infrared cameras (commercial eye trackers)	Off-the-shelf camera
High accuracy More restrictive More expensive	Lower accuracy Wide range of applications Cheaper

How do we measure pupil size?



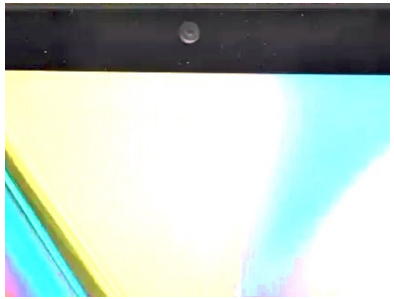
https://www.sr-research.com/about-eye-tracking/

Cameras can be “remote” or “head-mounted”; Proprietary or open-source

Remote camera

https://www.sr-research.com/eyelink-1000-plus/

https://www.amazon.ca/100fps-Infrared-Webcamera-Android-Windows/dp/B07PPN7TXQ



https://www.laptopmag.com/features/im-a-laptop-reviewer-and-webcams-suck-but-these-3-laptops-have-the-best-ones

Head-mounted camera(s)

https://pupil-labs.com/products/neon/shop

https://pupil-labs.com/products/neon/shop

https://www.tobii.com/products/eye-trackers/wearables/tobii-pro-glasses-3

Hardware considerations

Remote vs. Head-Mounted

What is your use case?

- Stimuli on screen or in natural environments?
- Seated and relatively stationary or standing and moving?

Feature or appearance-based

What is your use case?

- What is accuracy required?
- What is your budget?

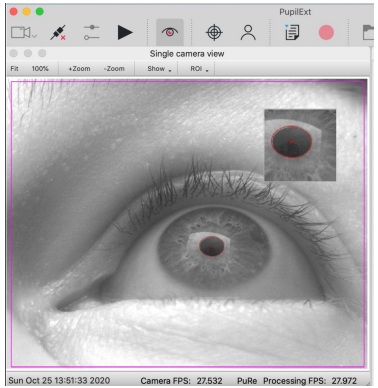
~35000 EUR

https://www.sr-research.com/eyelink-1000-plus/

~6000 EUR

https://pupil-labs.com/products/neon/shop

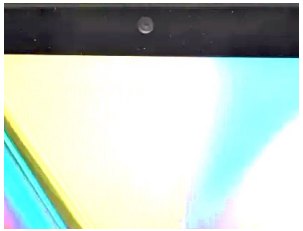
BYOC
Bring your own camera/ computer



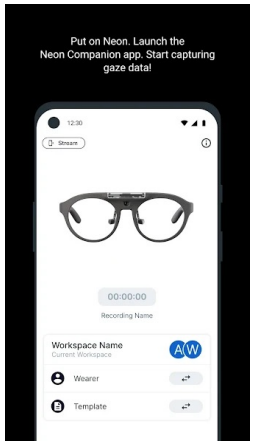
https://www.frontiersin.org/journals/neuroscience/articles/10.3389/fnins.2021.676220/full

Over-arching hardware / software workflow

1. Eye-tracking hardware

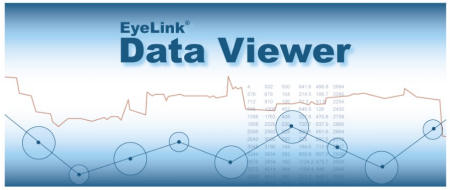


2. Data Collection software



PsychoPy

3. Data Analysis software



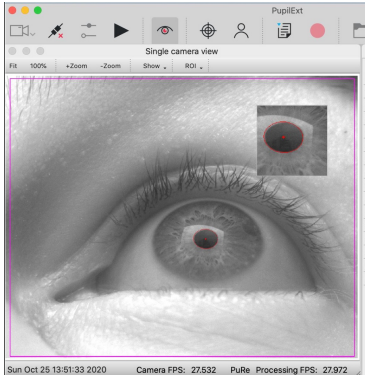
FOSS or custom

gazeR (Geller et al., 2020)
pupillometryR (Forbes, 2020)
PyTrack (Ghose et al., 2020)
PuPl (Kinley & Levy, 2021)
CHAP (Hershman et al., 2019)

[Data Collection & Analysis] Free & Open-Source Software

PupilEXT

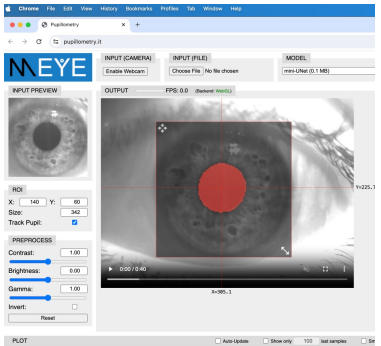
Zandi, B., Lode, M., Herzog, A., Sakas, G., & Khanh, T. Q. (2021). PupilEXT: Flexible open-source platform for high-resolution pupillometry in vision research. *Frontiers in neuroscience*, *15*, 603.



- Highly accurate, **in-lab** solution (bring your own high-res, high Fs camera)
- Choose from pupil detection algorithms (Starburst, Swirski, ExCuSe, ElSe, PuRe and PuReST)
- https://github.com/openPupil/Open-PupilEXT

MEYE

Mazziotti, R., Carrara, F., Viglione, A., Lupori, L., Verde, L. L., Benedetto, A., ... & Pizzorusso, T. (2021). MEYE: web app for translational and real-time pupillometry. *eneuro*, *8*(5).

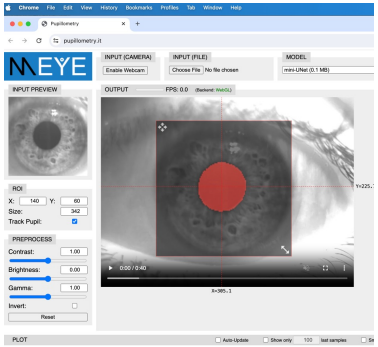


- **Webcam (BYO IR cam), browser-based (OS-independent)** solution
- Modern laptop Intel(R) Core(TM) i7-9750H 2.60GHz CPU and an Intel(R) UHD Graphics 630 GPU can process up to 28 frames/s (fps).
- https://github.com/fabiocarrara/meye

[Data Collection & Analysis] Free & Open-Source Software

MEYE

Mazziotti, R., Carrara, F., Viglione, A., Lupori, L., Verde, L. L., Benedetto, A., ... & Pizzorusso, T. (2021). MEYE: web app for translational and real-time pupillometry. *eneuro*, *8*(5).



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"A USB IR webcam (model Walfront5k3psmv97x, Walfront) equipped with a Varifocal 6–22 mm M12 objective (catalog #149129, Sodial) was used to acquire images of the eye. The camera was equipped with six IR LEDs to illuminate the eye uniformly, optimizing contrast between the iris and the pupil. Photic stimulation was delivered using an Arduino Due (Arduino) microcontroller connected via USB to the notebook and programmed to emulate a keyboard. The Arduino emulates a keyboard (using the keyboard. h library) to send event triggers to MEYE in the form of keystroke events. The microcontroller drives a stripe of four LEDs (WS2813, WorldSemi) using the FastLED. h library, flashing bright white light for 500 ms with an interstimulus interval of 5 s (see Fig. 3A). The subject sat in front of a monitor screen (24 inches; model CF390, Samsung) at a distance of 60 cm, with the head stabilized by a chin rest and instructed to maintain fixation on a small dot presented in the center of the screen for the whole duration of the recording (57 s). A total of 10 flash stimuli have been presented through the strip of LEDs mounted above the screen."

Software considerations

How is pupil size/diameter calculated?

Do you care to know?

- If yes, need open-source software

Do you want to create your own customizations / real-time applications?

- If yes, need open-source software

How much compute power do you have / need?

Which operating system(s) is required?

Do you need dedicated support and/or actively maintained code-bases?

Possible experimental scenarios

https://www.sr-research.com/about-eye-tracking/

https://eyereply.com/en/home/research/eye-tracking/

Bishop et al., 2021

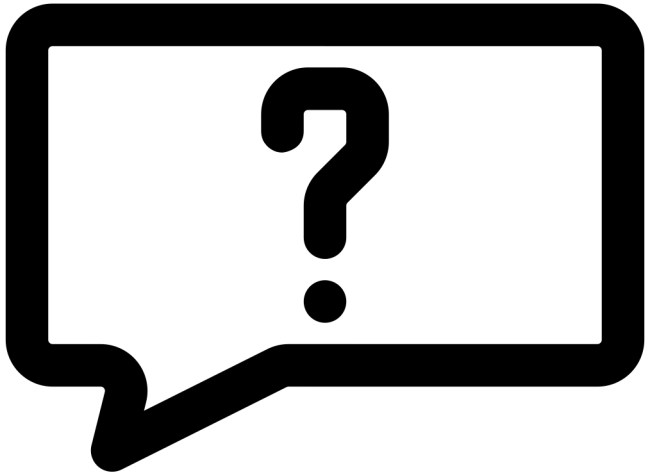
Should we do pupillometry “in the wild”?

What are the potential issues?

https://PollEv.com/laurenfink203

We can return to this question after we discuss experimental design considerations

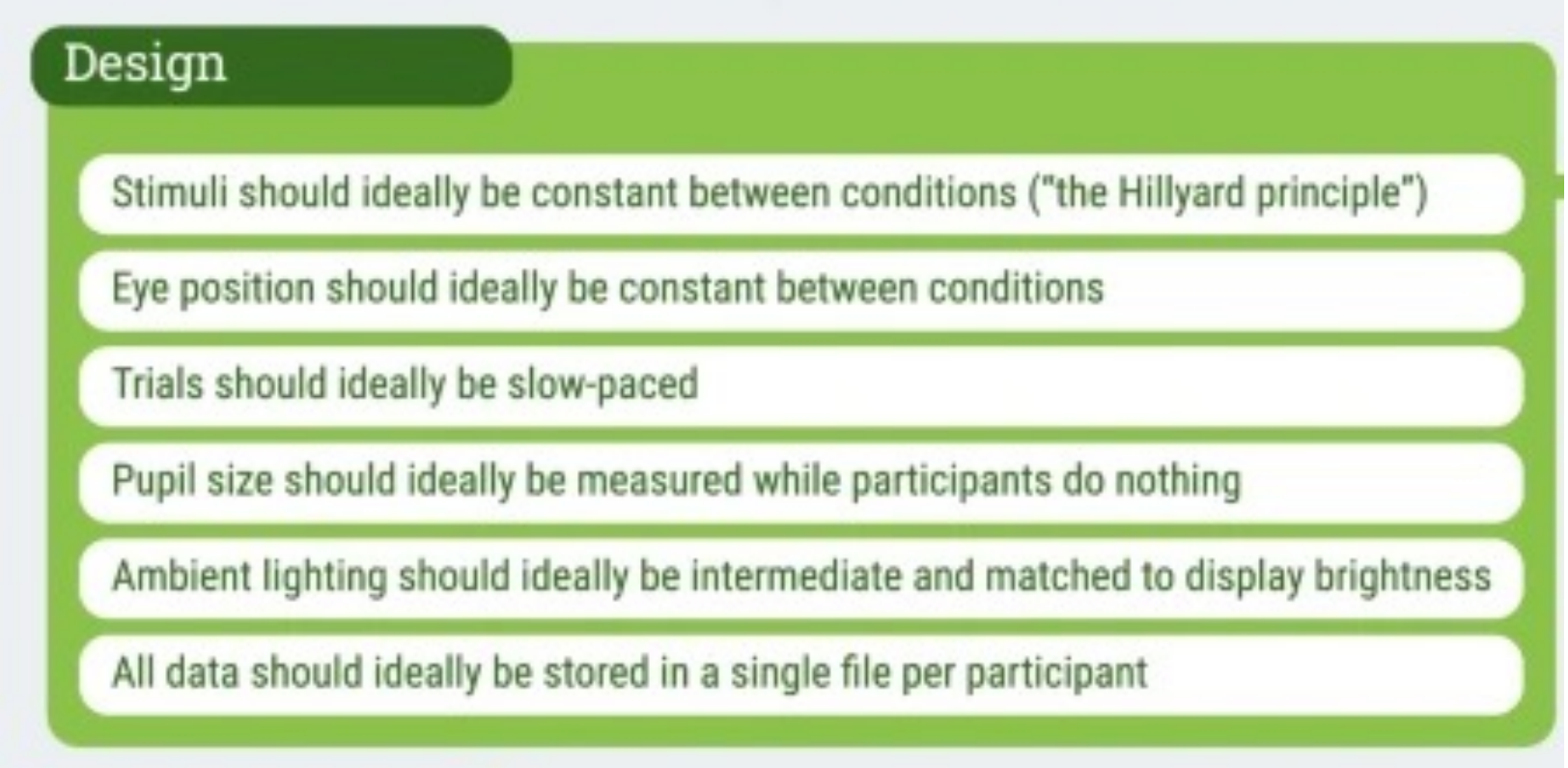
Questions?



Experimental Design Considerations

Experimental design considerations

Mathôt & Vilotijević, 2022



Experimental design considerations

Mathôt & Vilotijević, 2022

Stimuli should ideally be constant between conditions ("the Hillyard principle")

Basic idea is to vary the cognitive process or state (not stimuli)

- Unless of course you are interested in understanding the effects of different stimulus features!

Examples visual features that affect pupil size:

- Brightness
- Motion
- Color
- Rhythm (Naber et al., 2013)

Example auditory features that affect pupil size

- Intensity (Wang & Munoz, 2015)
- Rhythm (Fink et al., 2018; Damsma, 2017)
- Timbre (e.g., voice vs. cello; Akça et al. 2022)

Experimental design considerations

Mathôt & Vilotijević, 2022

Eye position should ideally be constant between conditions

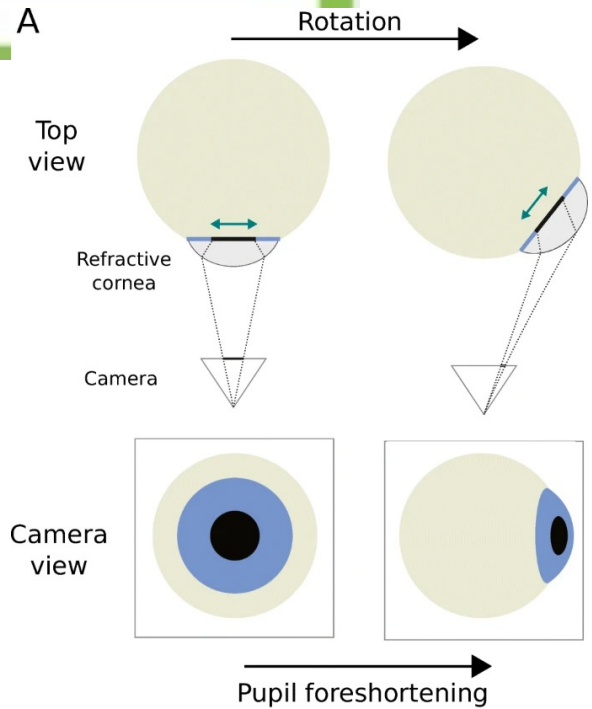
Why? Eye movements affect measured pupil size

Pupil Foreshortening Error

There are options to correct, but depends on type of eye-tracker used and task demands.

But also (beyond measurement issues):

- Eye movements and blinks really do alter pupil size
- Certain eye positions may really affect pupil size (e.g., due to greater effort to look there)



Petersch & Dierkes, 2022

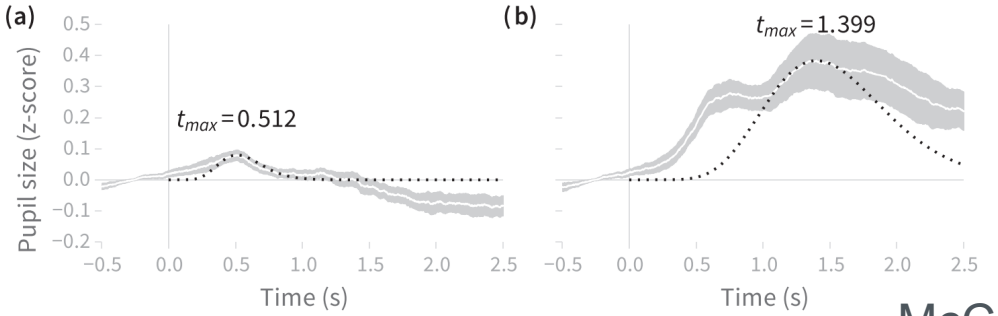
Experimental design considerations

Mathôt & Vilotijević, 2022

Trials should ideally be slow-paced

We have already talked about modeling the pupillary response function

RECALL



McCloy et al., 2016

Experimental design considerations: Inter-stimulus-Intervals

How much time do you need between stimuli / trials?

Consider what we learned about physiological limits of changes in pupil size

Consider what we learned about PRF
- Individual differences
- Button press required or not
  - Alters time til max dilation

https://PollEv.com/laurenfink203

Experimental design considerations

Trials should ideally be slow-paced — Mathôt & Vilotijević, 2022

We can actually simulate how long our trials or inter-stimulus intervals should be!

https://tinyurl.com/PRFCONV

Mathôt & Vilotijević, 2022

Pupil size should ideally be measured while participants do nothing

Say you are comparing two conditions which may differ in cognitive load

You are interested in the effect of load on pupil size

If participants are required to make a button press, we already know it will affect their pupil size and response latency

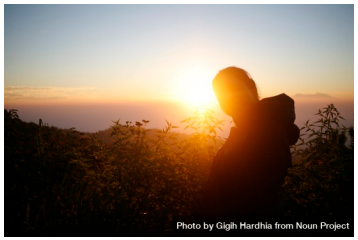
What could you do?

- Require passive viewing post-stimulus presentation, but pre-decision (still might be subject to motor preparation effects)
- Measure pre-target pupil size (but maybe not the effect of interest?)

Experimental design considerations

Mathôt & Vilotijević, 2022

Ambient lighting should ideally be intermediate and matched to display brightness



Dynamic range of pupil = 2-8 millimeters

- If pupil already at dilation max (little room to see dilation effects)
- If pupil already at constriction minimum (little room to see constriction effects)

2mm

Ideal to conduct experiments at intermediate pupil size

8mm

Experimental design considerations

Mathôt & Vilotijević, 2022

Ambient lighting should ideally be intermediate and matched to display brightness

Also avoid large differences between room lighting and stimulus display

https://higginsvisioncare.com/how-to-prevent-digital-eye-strain/

“Discomfort glare”

Experimental design considerations: **Do you even need to collect data?**

Collecting data is:

- Costly
- Time-consuming
- Environmentally unfriendly
- Not possible for some researchers (e.g., no access to specialized equipment or funding)

Can your research question be answered with a pre-existing dataset?

https://PollEv.com/laurenfink203

Pre-registering new experiment or analysis

"Preregistration is the practice of **documenting your research plan** at the beginning of your study and storing that plan in a **read-only public repository"** https://help.osf.io/article/145-preregistration

Have you ever pre-registered a study?

https://PollEv.com/laurenfink203

Benefits of pre-registration:

- Increase discoverability of research
- Reduce unintentional false positive inflation
- Distinguish exploratory from confirmatory analyses

"Pre-registration is a plan not a prison"
https://www.cos.io/blog/preregistration-plan-not-prison

Experimental design considerations: Sample size, # of trials, etc.

How many participants do you need? How many trials do you need?

There is no generic ”right” answer to these questions.
It depends on:

- Type of participant(s), task, experimental design and planned analysis (e.g., 2x2 ANOVA, time series analysis), etc. etc.

Strategy:

1. Look for other studies similar to the one you want to do!
   - What are the reported effect sizes? What sample size did they need for such an effect?

2. Follow general guidelines w/r/t to your final planned statistical model(s); see e.g., Brysbaert, 2019 (https://journalofcognition.org/articles/10.5334/joc.72)

Homework before tomorrow (optional)

Find a pre-existing, publicly available dataset that interests you!

General repositories:
OpenNeuro.org
Open Science Framework (osf.io),
Google Dataset Search (https://datasetsearch.research.google.com/)

Specific papers (non-exhaustive!):
Grenzebach et al. (2021); Bishop et al. (2021); Pajkossy and Racsmány (2019); Kooijman et al. (2021); Winter et al. (2021); Mathôt et al. (2017); Scheepers et al. (2016); Urai (2016); Pelagatti et al. (2020); Lehmann et al. (2019); Chapman and Hallowell (2020); Rozado (2019); Wahn et al. (2016); Nakakoga et al. (2020); Kucewicz (2021); Colizoli et al. (2018b); Moeller et al. (2021); Pavlov et al. (2021); Gee et al. (2017b); Zhao et al. (2020); Lee et al. (2019, 2021); Ribeiro and Castelo-Branco (2021); Clewett et al. (2019); Hanke et al. (2016); Bianco et al. (2021); Madore (2020); Keung (2020); Keitel et al. (2021)

What are you most hoping to learn tomorrow?

https://PollEv.com/laurenfink203

What is your preferred programming language?

https://PollEv.com/laurenfink203

What is your main take-away from today?
Did you learn something new?

https://PollEv.com/laurenfink203

Thank you!!

Questions?

Contact:
finkl1@mcmaster.ca

Learning Pupillometry

From Theory to Analyses

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School of Computational Science & Engineering
Center for Advanced Research in Experimental
& Applied Linguistics (ARiEAL)

University of
Konstanz

Agenda

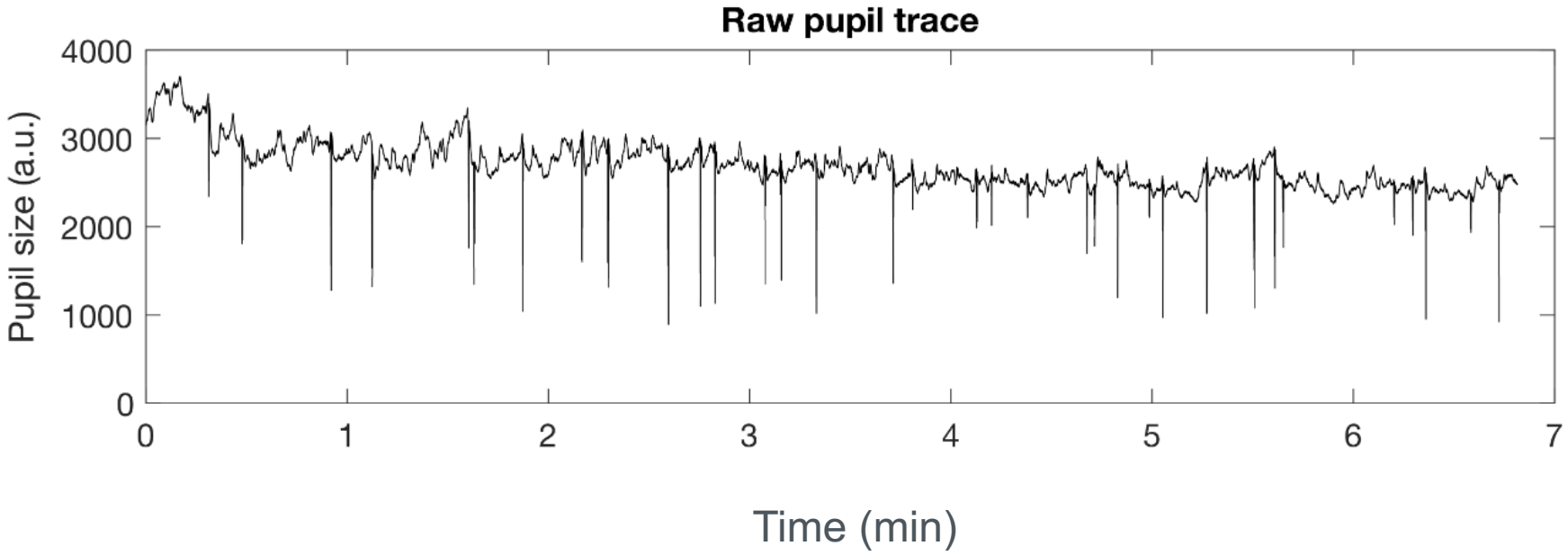
Day 2: Pre-processing & Analyses

Hands-on coding workshop covering a variety of potentially useful analyses, from condition-averaging the pupil dilation response, to analyzing the dynamics of single-trial pupil time series.

- Pre-processing pupil data
- Condition averaged analyses
- The importance of data visualization
- Time series analyses & signal-to-signal approaches

What does raw pupil data look like?

What are these spikes?



Pre-processing pupil data

How should we get rid of them?

Step 1. Find them
Step 2. Set them to NaN
Step 3. Interpolate them (necessary depending on analysis)

Pre-processing pupil data

How should we get rid of them?

Step 1. Find them
→ How?

Step 2. Set them to NaN

Step 3. Interpolate them (necessary depending on analysis)
→ How?

Pre-processing pupil data

Some eye-trackers will return a “flag” to specify “valid” pupil data

It is important to understand what that means in the context of your own eye-tracker

timestamp [ns]	pupil diameter [mm]	is valid
1709835084722379215	4.61620569229126	1
1709835084727379215	4.652669906616211	1
1709835084732504215	4.679261207580566	1
1709835084737504215	4.6961588859558105	0
1709835084742504215	4.659944534301758	0
1709835084747504215	4.728400230407715	0
1709835084752504215	4.780755043029785	.
1709835084757503215	4.703244686126709	.
1709835084762504215	4.659246444702148	.
1709835084767503215	4.67100191116333	
1709835084772504215	4.679454803466797	
1709835084777509215	4.677417278289795	

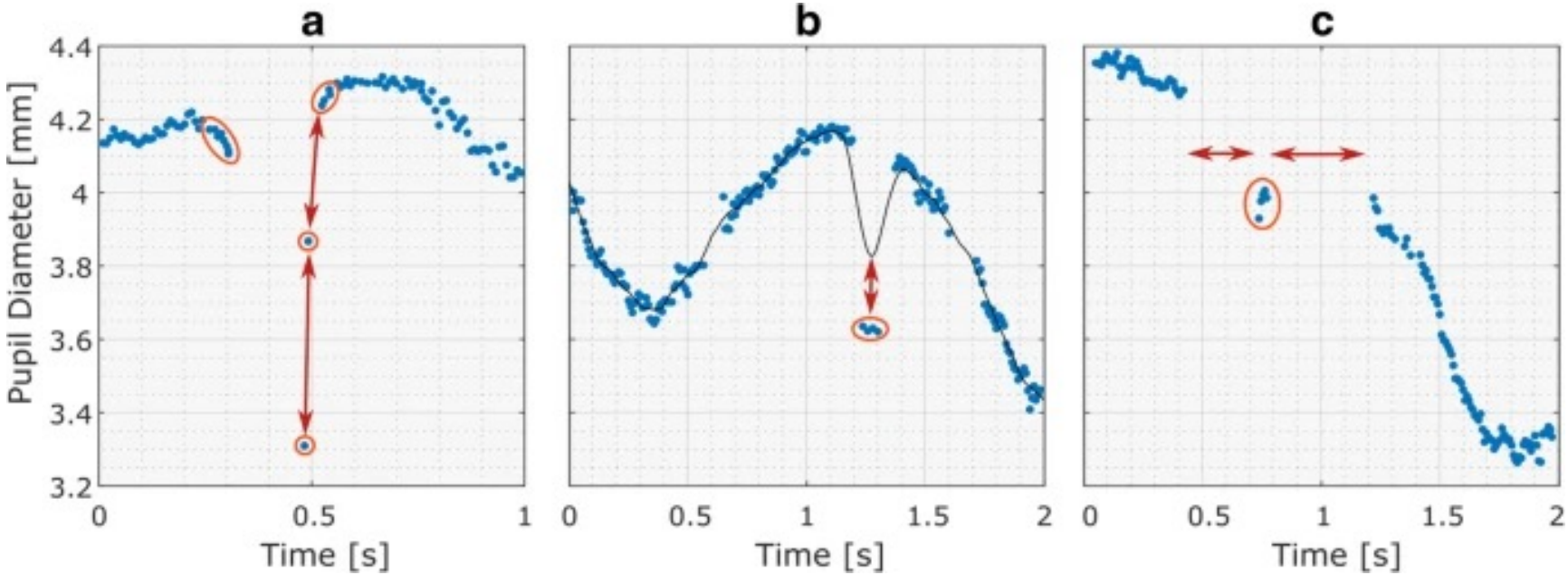
is blink
is saccade
gaze x
gaze y

Great for helping you find problematic data!

Pre-processing pupil data

What about other potentially problematic data points?

Speed outliers | Trend line deviations | Temporally isolated samples



Kret & Sjak-Shie (2019)

Pre-processing pupil data

How should we get rid of them?

Step 1. Find them
→ How?

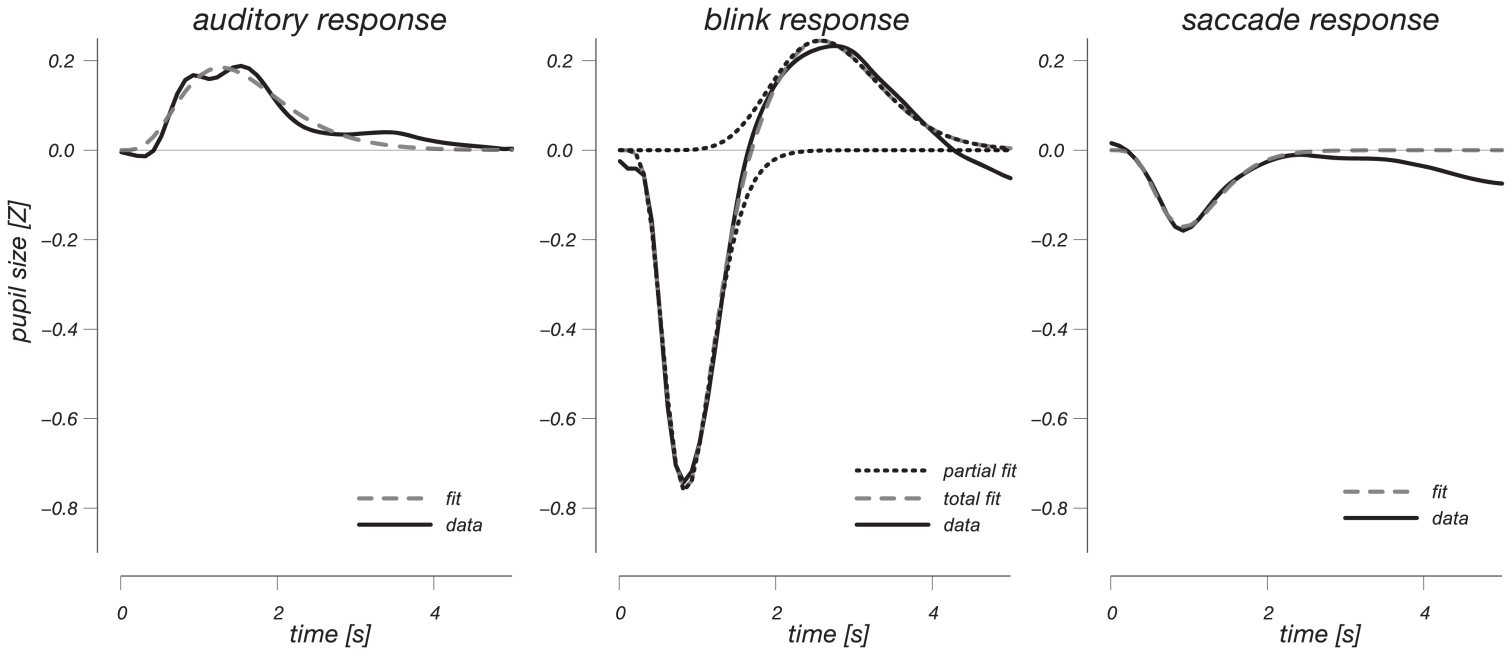
Multiple open-source algorithms for doing this.

gazeR (Geller et al., 2020)
pupillometryR (Forbes, 2020)
PyTrack (Ghose et al., 2020)
Mathôt and Vilotijević (2022)
PuPl (Kinley & Levy, 2021)
CHAP (Hershman et al., 2019)

1. Don't re-invent the wheel
2. Would be great if the field moved towards more standardized tools (it is!)

Pre-processing pupil data

Previous steps take care of blinks. But is it enough? What about saccades?



Knapen et al., 2016

Quick side note: Blinks

Types: Voluntary, Reflexive, **Spontaneous**

Function: Protect and hydrate eyes (4 blinks per min; Doane, 1980)

Why blink more often than necessary? (15-20 per minute avg)

Lose 150-400msec of information each blink = roughly 44 min of each day

Function: Provide attentional breaks

Activate default mode network (Nakano et al., 2013; Nakano, 2015)

Used as index of:

Striatal dopamine (Disrupted in clinical conditions involving dopamine; c.f. dopamine debate: Jongkees and Colzato, 2016 vs. Sescousse et al., 2018; Dang et al., 2017)

Attention / effort / cognitive load / information chunking / fatigue

(Cummins, 2012; Nakano & Kitizawa, 2010; Orchard & Stern, 1991; Hall, 1945; Bauer et al., 1987; Johns, 2003)

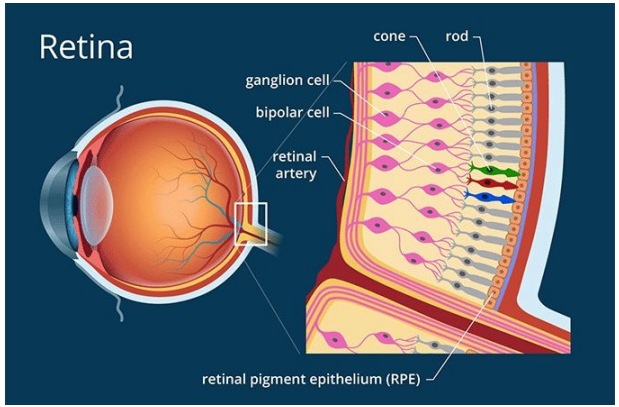
- e.g., occur at pauses / turns in speech; increase when tired; decrease with load

Quick side note: Eye movements

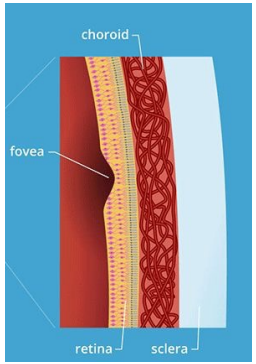
Fixations: to foveate target
Average duration = 150-300 ms
→ fixational eye movements (e.g., microsaccades)
→ To maintain foveation on target

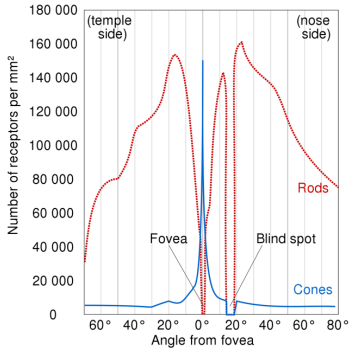
Saccades: to change point of fixation
- Average duration = 20-40ms
- Linear relationship between saccade amplitude and duration
- (smaller saccades = shorter dur)

Smooth pursuit: to maintain foveation on moving target



Retina comprised of light-sensitive cells (rods and cones), which send info to brain





Fovea has highest density of cones (responsible for color and fine detail)

https://www.allaboutvision.com/resources/retina.htm & .. eye-anatomy/fovea/

Quick side note: Why you might want to record data at a higher sampling rate

Recall from yesterday: the pupil is slow → ~ 3 Hz

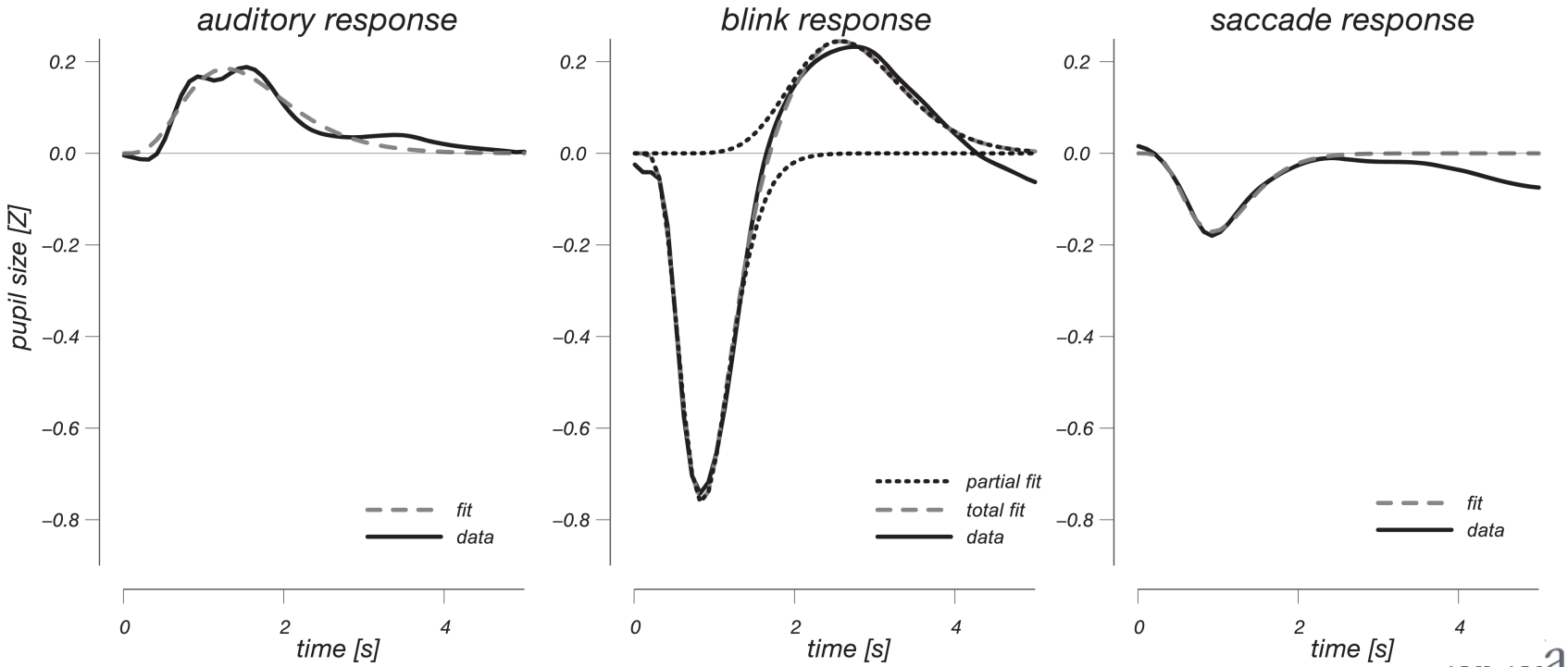
Why do we often record eye-tracking data at higher sampling rates?

- To be able to accurately identify saccades (and microsaccades)!

Pre-processing pupil data

Blink avg. duration = 150-400ms

Saccade avg. duration = 20-40ms



Pre-processing pupil data

So, should you model (individually, for each subject, the pupillary response to blinks and saccades?

Practically, speaking I think it would be easier to rather design your experiment well. Blink and saccade effects will only confound your results if they systematically differ between the conditions you are comparing.

→ It would be easier to quickly analyze blink and saccade data by condition, than to fit individual response functions and correcting pupil data

→ Why?

→ Many observations of blinks and saccades are necessary to form an accurate model. Blinks are sparse events. You might not be able to fit a good model from your data.

Pre-processing pupil data

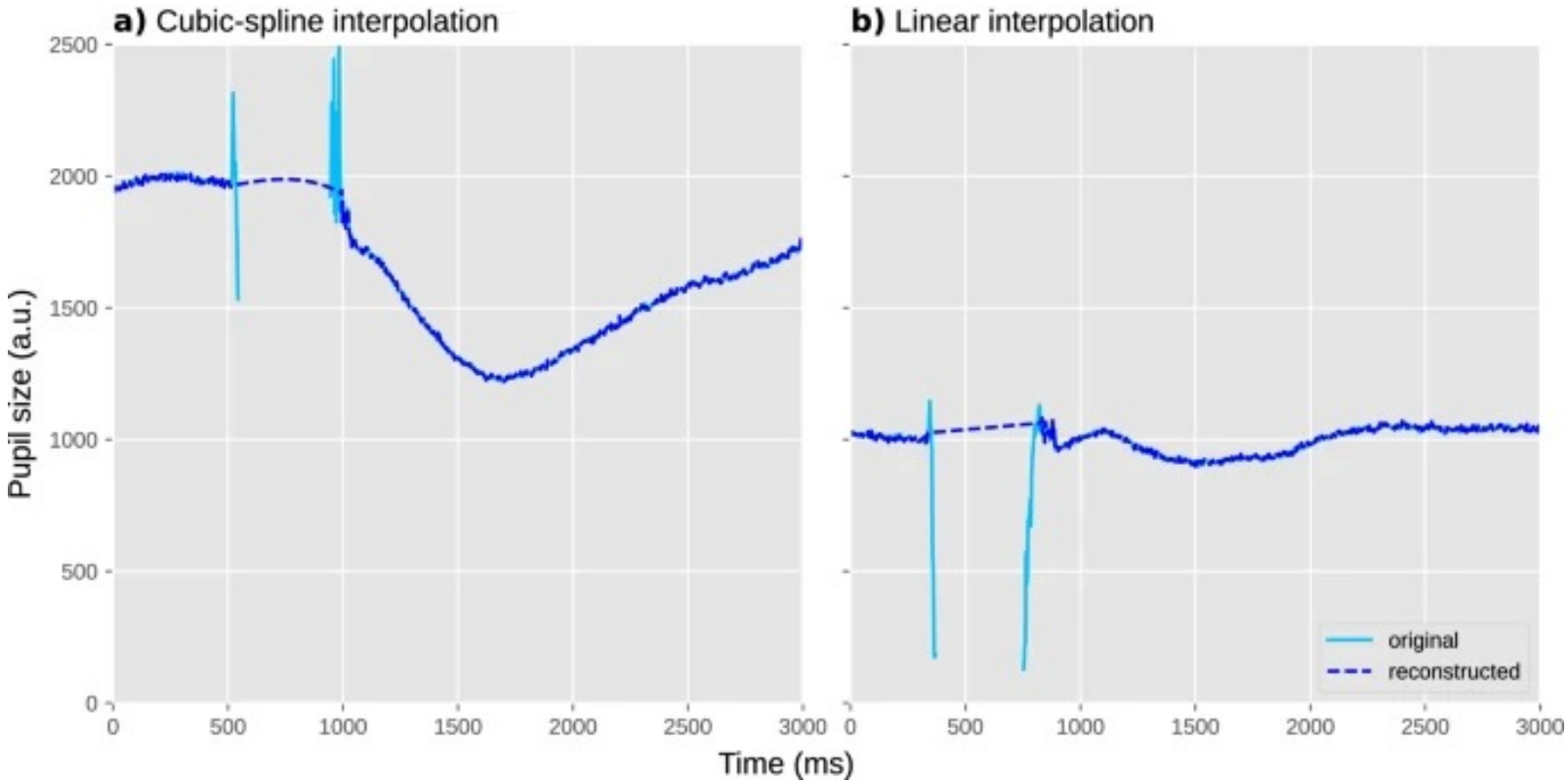
How should we get rid of them?

Step 1. Find them
→ How?

Step 2. Set them to NaN

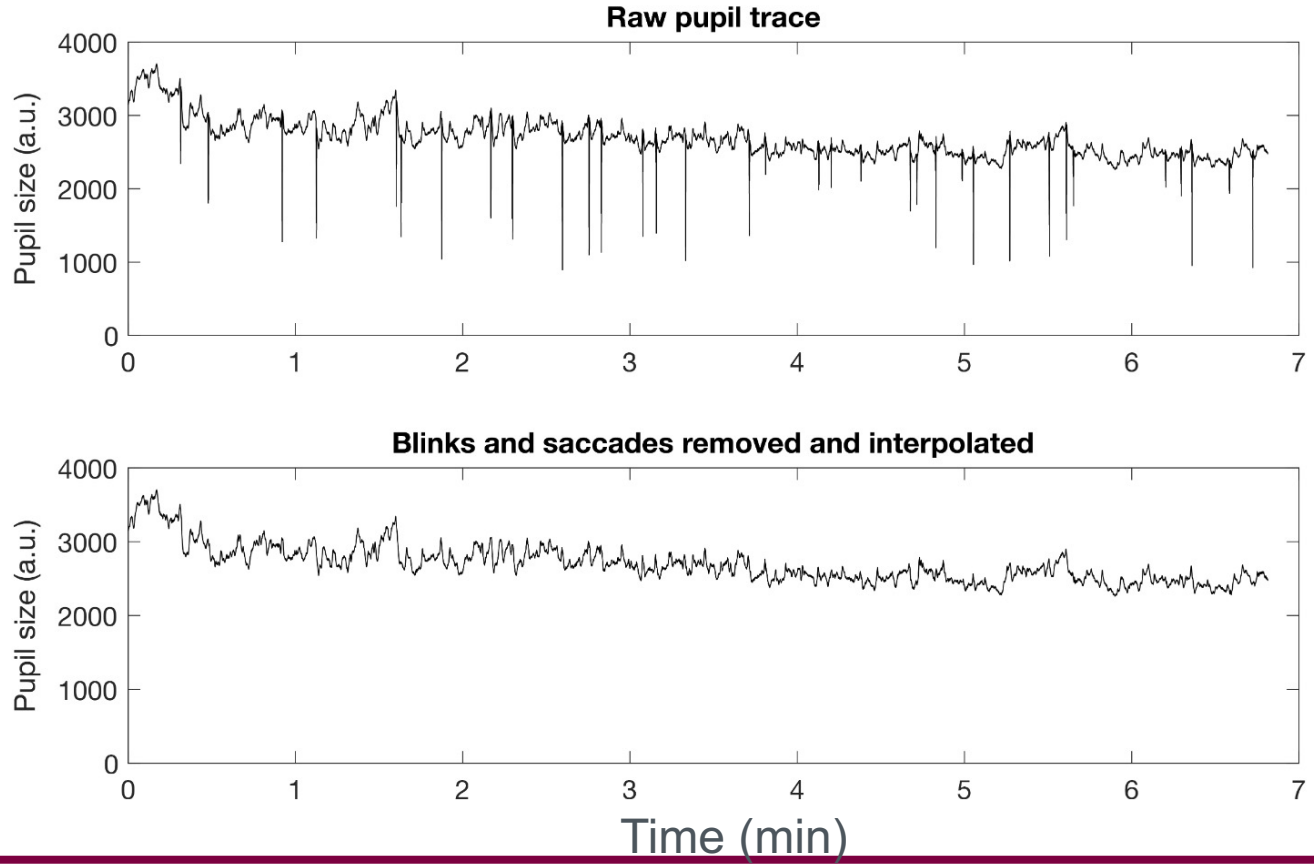
Step 3. Interpolate them (necessary depending on analysis)
→ How?
  → Linear or spline interpolation
→ Why?
  → Missing data reduces statistical power; might create edge artifacts in plots
  → Time series analyses require evenly sampled, continuous data

Pre-processing pupil data: **Interpolation**



Mathôt & Vilotijević, 2022

Pre-processing pupil data: **After interpolation**



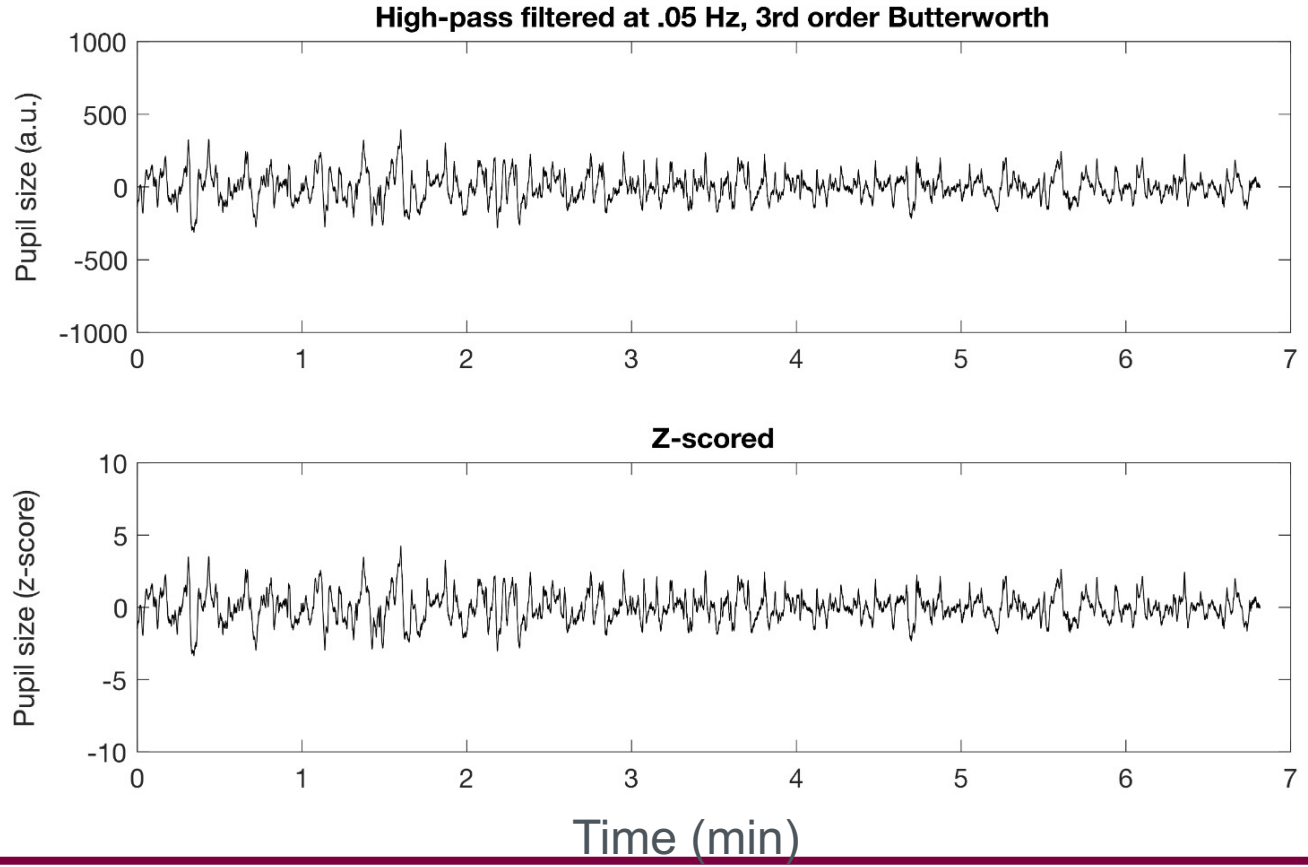
Fink et al., 2018

Pre-processing pupil data: **Transformations**

Possibly:

- **Convert** from arbitrary units to millimeters
  - Re: yesterday's question:
    - Support page (need log in credential): https://www.sr-research.com/support/thread-154.html
- **Downsample**
  - Might want more highly sampled data for:
    - Looking at light reflex (quick constriction)
    - Smoother-looking figures
    - 100 Hz is more than enough!
- **Filter**
  - Which frequencies do you care about?
  - Are you going to do analyses over longer time scales?
  - Do you need to? (can introduce artefacts, autocorrelation)

Pre-processing pupil data: **Transformations**



Pre-processing pupil data: **Transformations**

Possibly:

- **Normalization**

$$x' = \frac{x - \bar{x}}{S(x)}$$

(e.g., Fink et al., 2018)

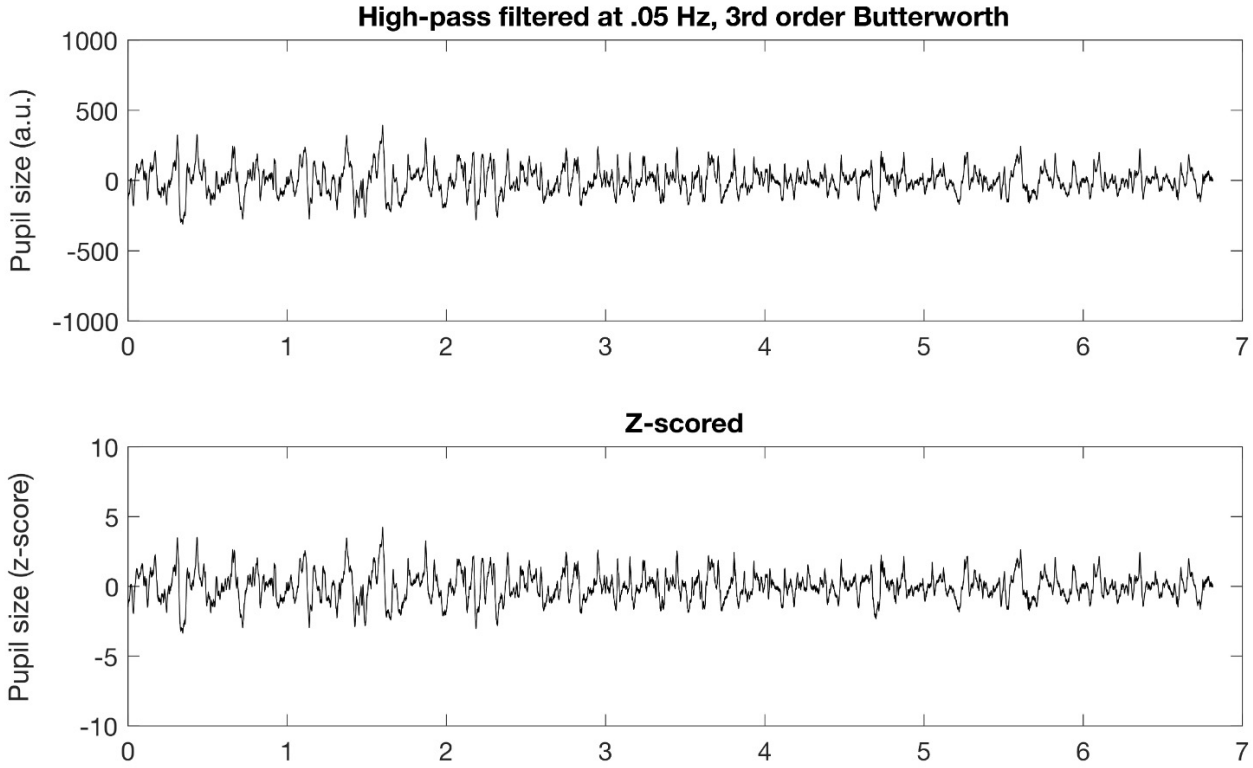
$$x' = \frac{x - x_{\min}}{x_{\max} - x_{\min}} * 100$$

(e.g., Piccado et al., 2010)

Note that normalization puts pupil data in relative units (not millimeters!)

- **Baseline correction**
  - Often used in epoch-based analyses
  - Note that correction results in negative relationship between baseline and epoch pupil dilation (Mathôt & Vilotijević, 2022)

Pre-processing pupil data: **Transformations**



Fink et al., 2018

Pre-processing pupil data: **Transformations**

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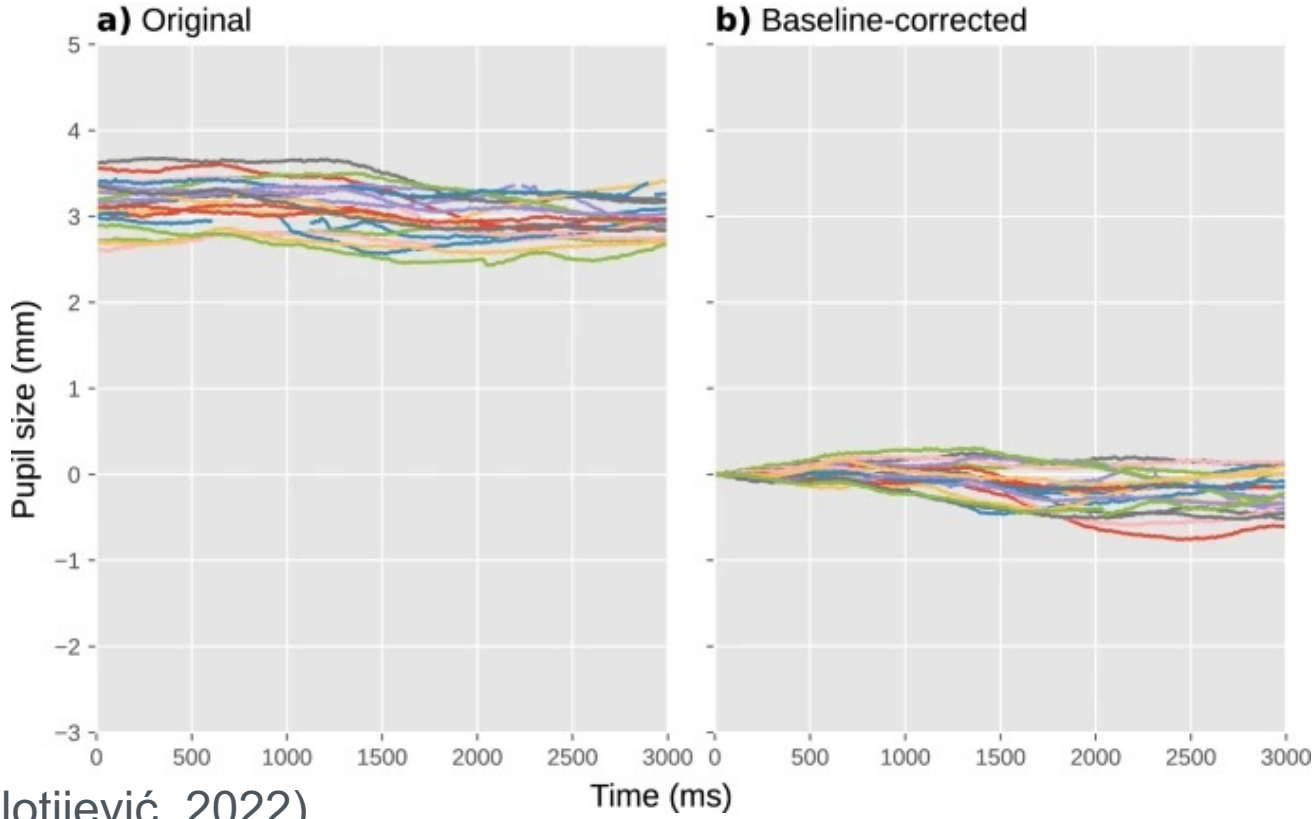
Pre-processing pupil data: **Transformations**



(Mathôt & Vilotijević, 2022)

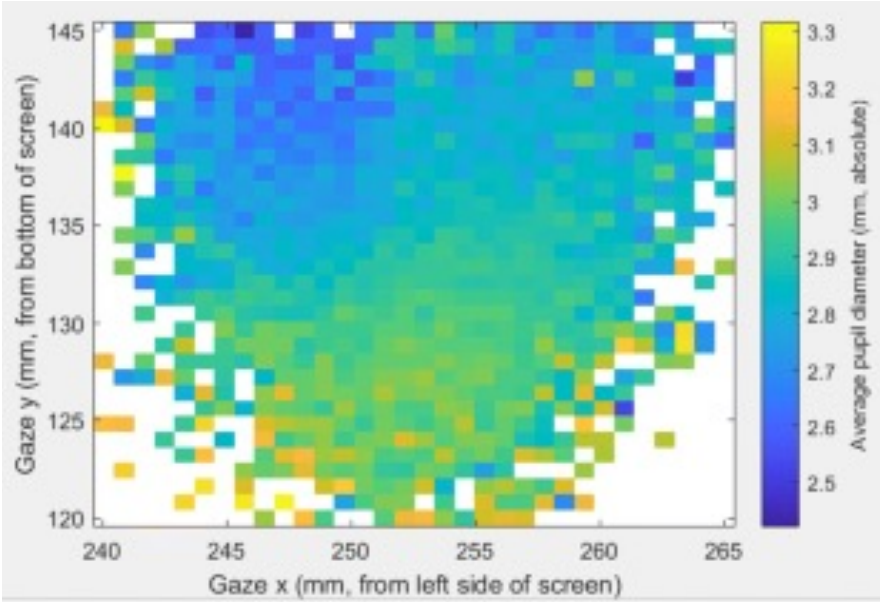
Pre-processing pupil data: **Gaze-correcting pupil size**

This would only be necessary if participants were not fixating centrally during your task

Toolboxes exist!

See Kinley & Levy (2022)

Pupil size (color) across screen location



Pre-processing pupil data: **Summary & Recommendations**

Think about what steps are necessary for your research context

Consider pre-registering pre-processing plan
- Not covered today: how will outlying participants and trials be determined (if at all?)

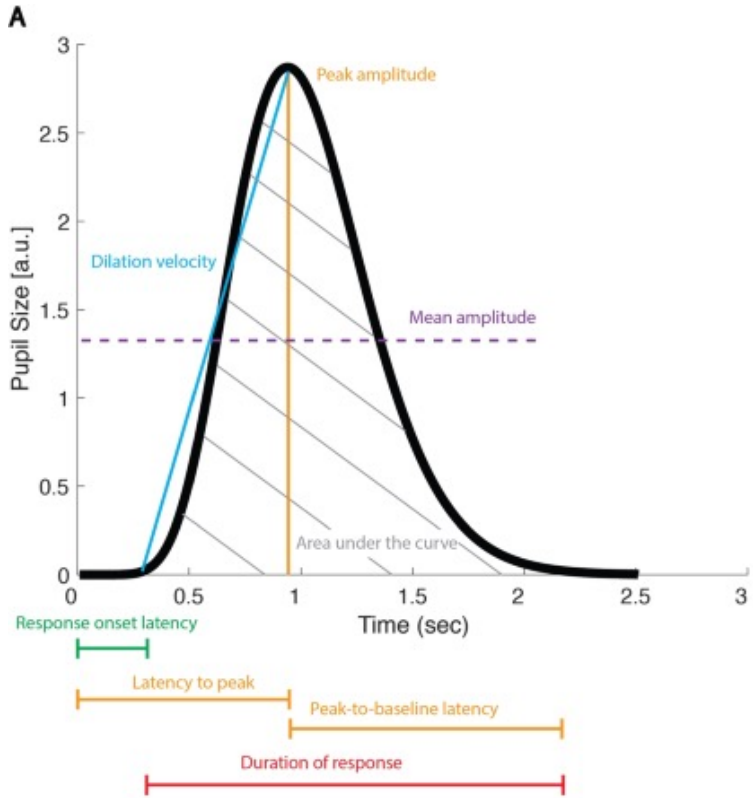
Be sure to visualize data for each participant (some participants might have unexpectedly noisy signals or e.g., blink dynamics)

Visually check for discontinuities (artefacts) after data cleaning

Make sure you understand how any pre-processing steps affect your final interpretation of what the pupil signal represents

Condition-averaged analyses

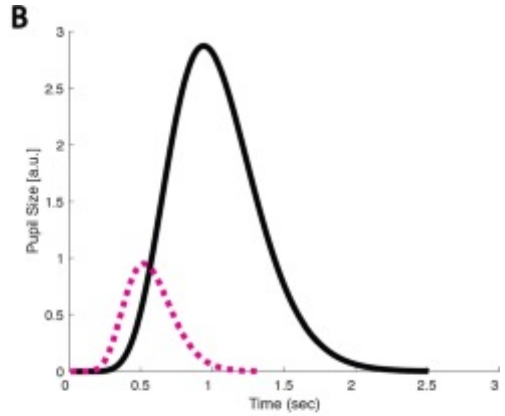
Condition averaged analyses: **Potential metrics of interest**



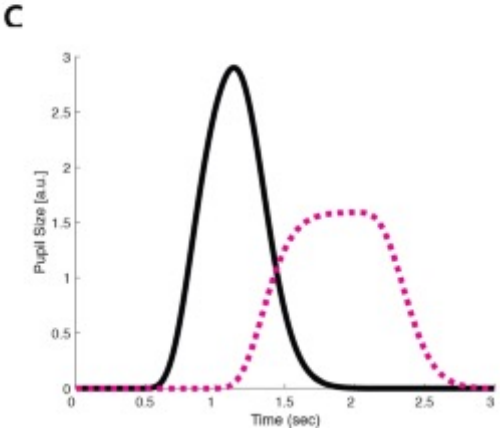
Fink et al., 2023

Condition averaged analyses

Same slope, different mean



Same mean, different slope and onset latency



Fink et al., 2023

Let’s play with some data!

Pretend these data are already pre-processed perfectly.
Your task is to print summary statistics and plot pupil size by condition

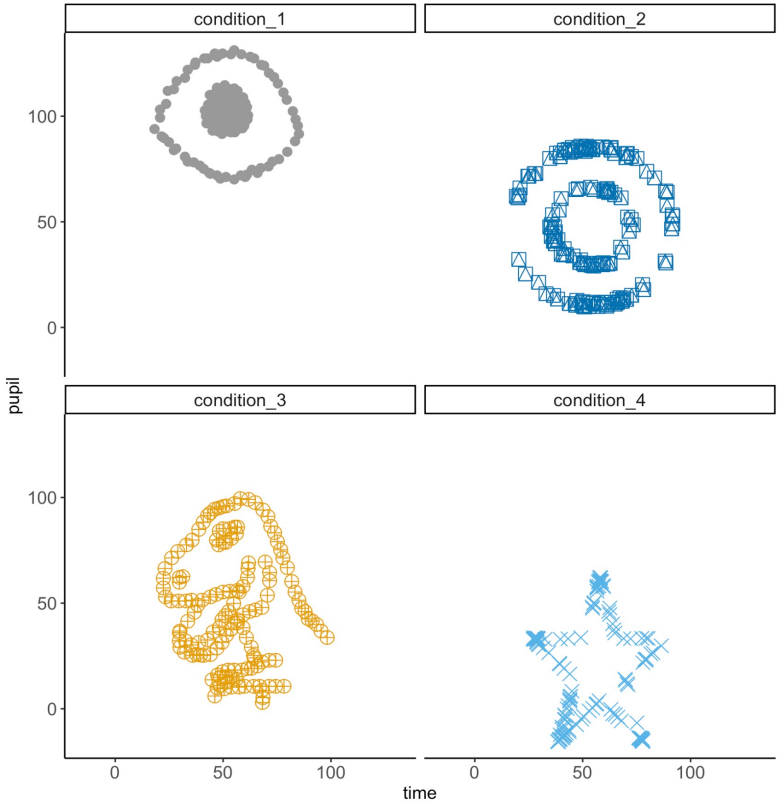
Here are the data:
https://beatlab.mcmaster.ca/assets/pupilTutorial/KonstanzWorkshop_pupilData_byCondition.csv

Let’s play with some data!

Possible approach provided here:

https://beatlab.mcmaster.ca/assets/pupilTutorial/KonstanzPupilWorkshop.html

Why should you visualize data?

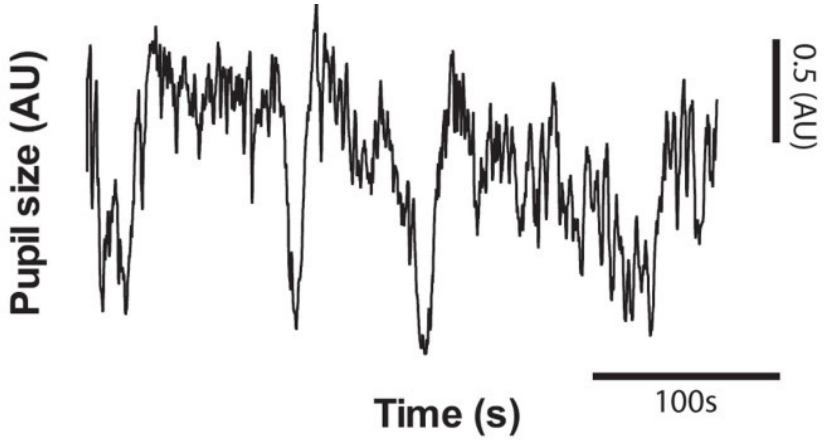


To make sure
you aren't looking at noise!

Why should you visualize data?

To make sure you aren't looking at noise!

What would actual pupil data look like?

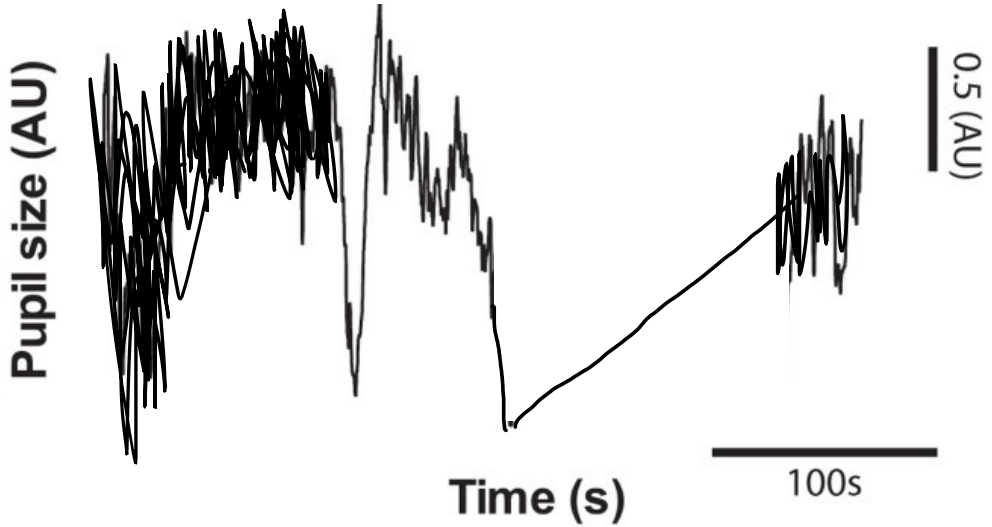


Zenon, 2017

Why should you visualize data?

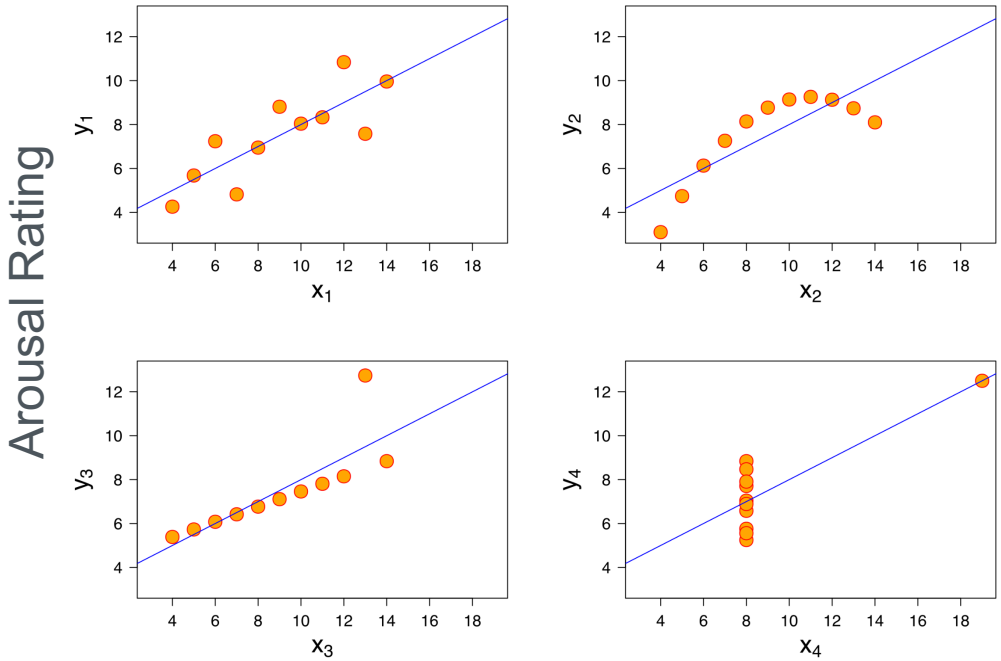
To make sure you aren't looking at noise!

What would noise look like?



Why should you visualize data?

To understand what statistical tests and models are appropriate!



https://en.wikipedia.org/wiki/Anscombe%27s_quartet

The mapping between data types and visualizations

(not all visualizations are appropriate!)

What options exist?

https://python-graph-gallery.com/

https://r-graph-gallery.com/

How do you choose?

https://www.data-to-viz.com/#explore

Caveats.. (every plot has pros and cons! Some more than others..)

The spaghetti chart

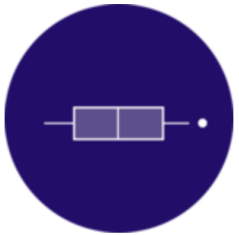
A line graph with too many lines becomes unreadable: it is called a spaghetti graph.

Pie chart

The human eye is bad at reading angles. See how to replace the most criticized chart ever.

Play with histogram bin size

Always try different bin sizes when you build a histogram, it can lead to different insights.



Do boxplots hide information?

Boxplots are a great way to summarize a distribution but hide the sample size and their distribution.

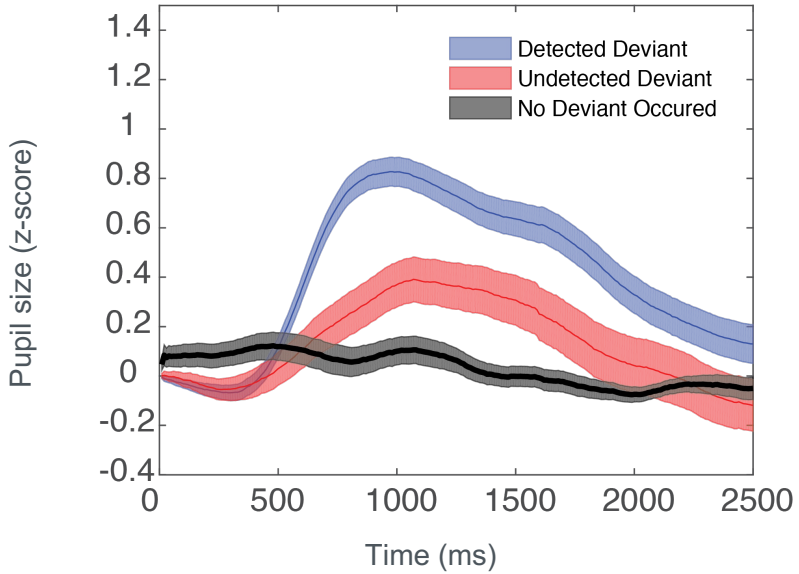
https://www.data-to-viz.com/caveats.html

Summary: Condition-averaged analyses

Even if only interested in mean pupil size, please plot your data!

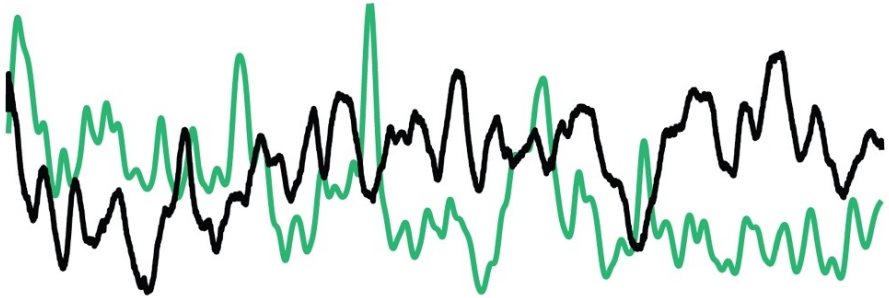
All the tools you need are available!

- gazeR (Geller et al., 2020)
- pupillometryR (Forbes, 2020)
- PyTrack (Ghose et al., 2020)
- Mathôt and Vilotijević (2022)
- PuPl (Kinley & Levy, 2021)
- CHAP (Hershman et al., 2019)



Time Series Analyses & Signal-to-Signal Approaches

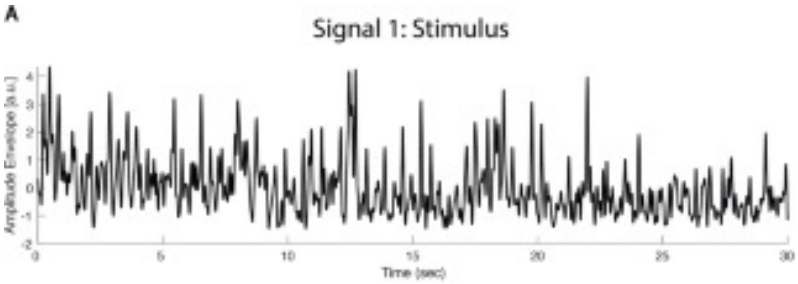
Time series analyses & signal-to-signal approaches

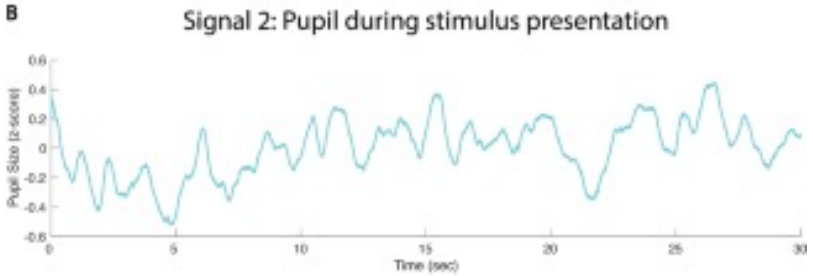


Why?

1. Lots of information is lost when averaging over time
2. We might want to know something about the relationship between the pupil and some other signal
    - Other signal could be e.g.,
        - Speech, music, movie, etc.
        - Another physiological signal (respiration, EKG, EEG, GSR, etc.)
3. We might learn something new!

Time series analyses & signal-to-signal approaches

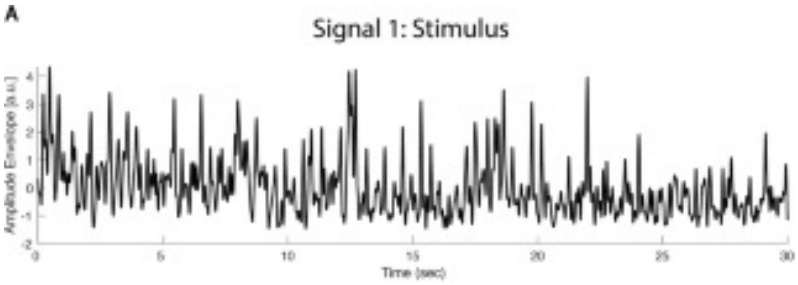


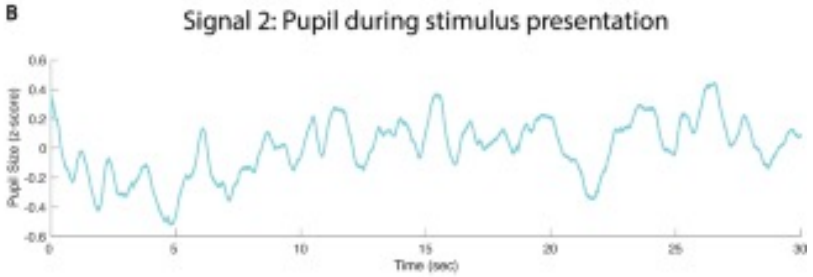


What is the temporal relationship between these two signals?
How far behind is the pupil?
How much variance in the pupil data does the stimulus data explain?

(a mean won't work)

Time series analyses & signal-to-signal approaches

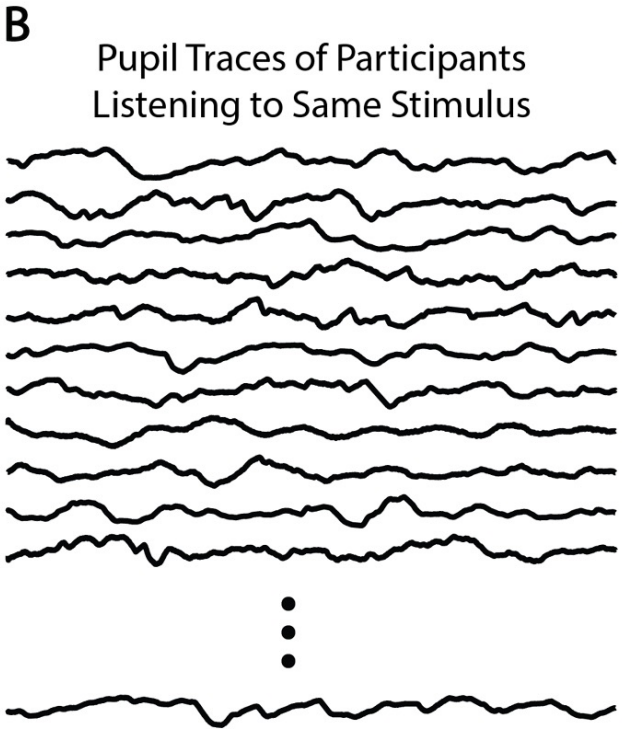




What is the temporal relationship between these two signal?
How far behind is the pupil?
→ Lag correlation

How much variance in the pupil data does the stimulus data explain?
→ Correlation
→ Regression

Time series analyses & signal-to-signal approaches



What if you have multiple traces and you want to know at which time points all participants respond similarly?

(a mean won't work)

Inter-Subject Correlation (ISC)

Shown to reflect stimulus processing, attentional engagement; e.g., ISC while watching educational video predicted test scores (Madsen et al., 2021, PNAS)

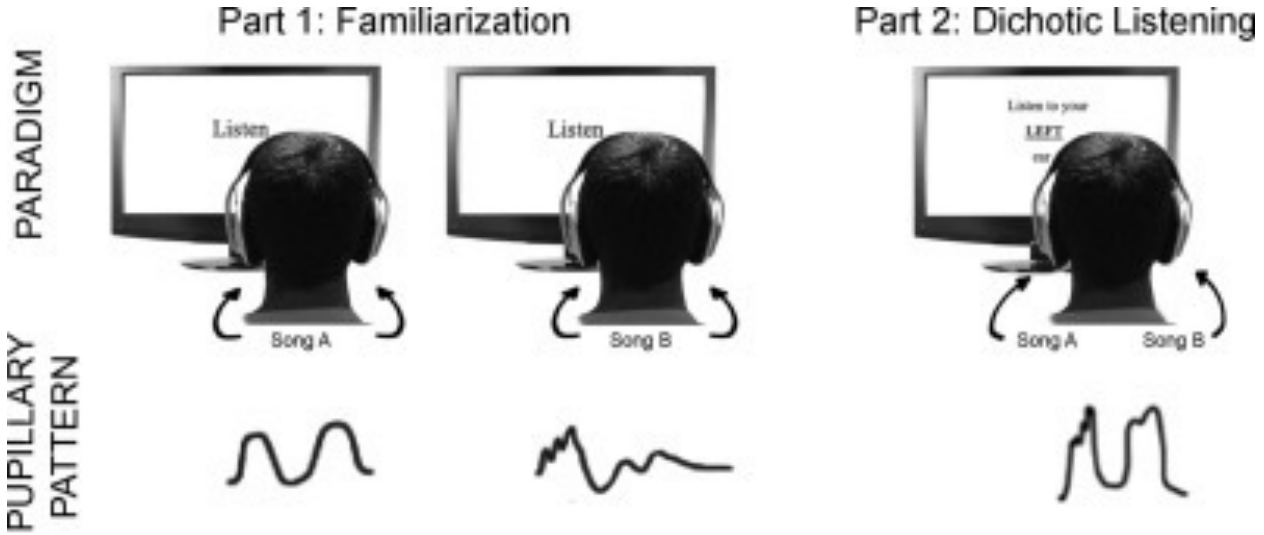
Time series analyses & signal-to-signal approaches

Can we decode which stimulus the person is listening to during dichotic presentation?

(a mean won't work)

Kang & Wheatley, 2015)

Time series analyses & signal-to-signal approaches

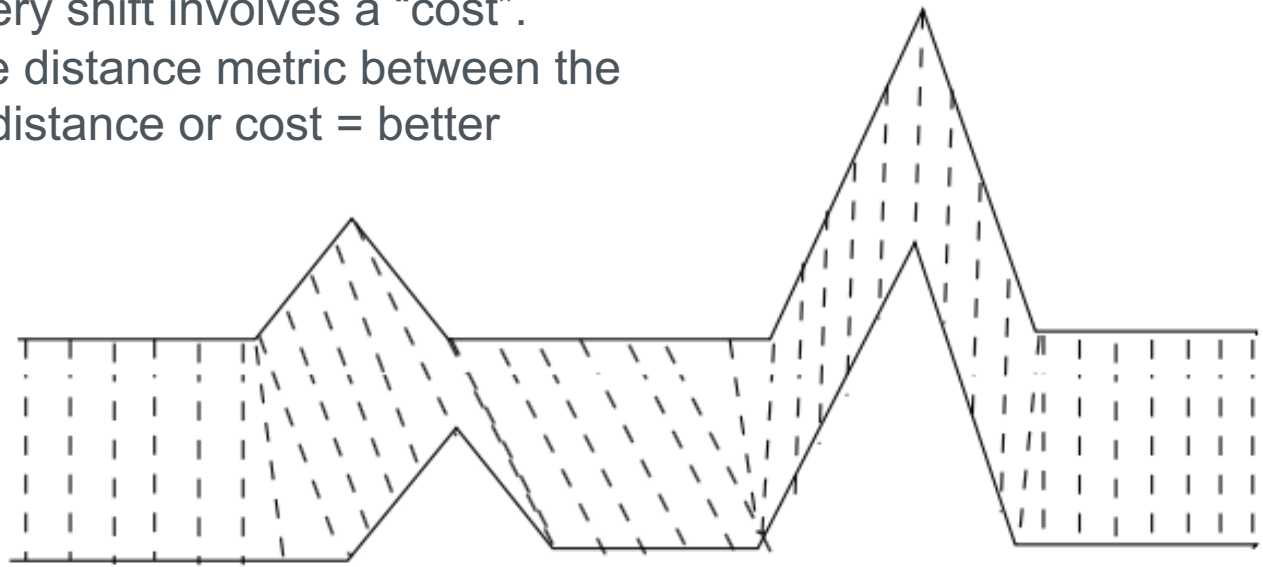


Use Dynamic Time Warping to figure out which template is most like recorded signal

Kang & Wheatley, 2015)

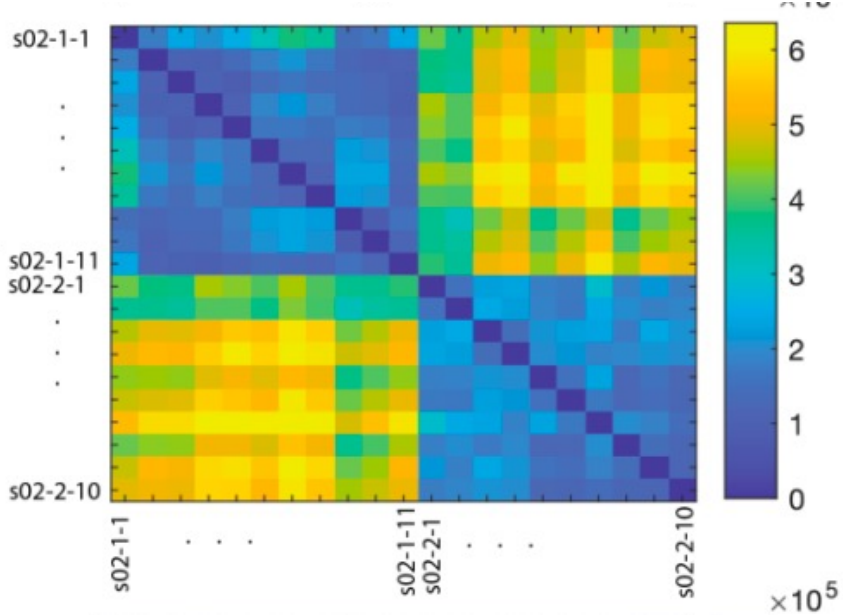
Dynamic Time Warping

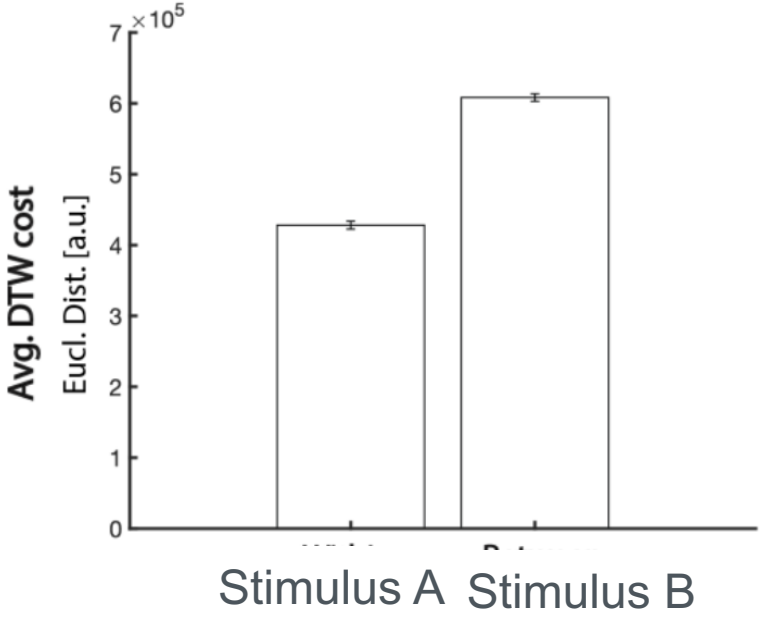
Allow for shifting of samples between signals to find best match. Every shift involves a “cost”. Ultimately use some distance metric between the two signals. Lower distance or cost = better



https://commons.wikimedia.org/w/index.php?curid=41617143

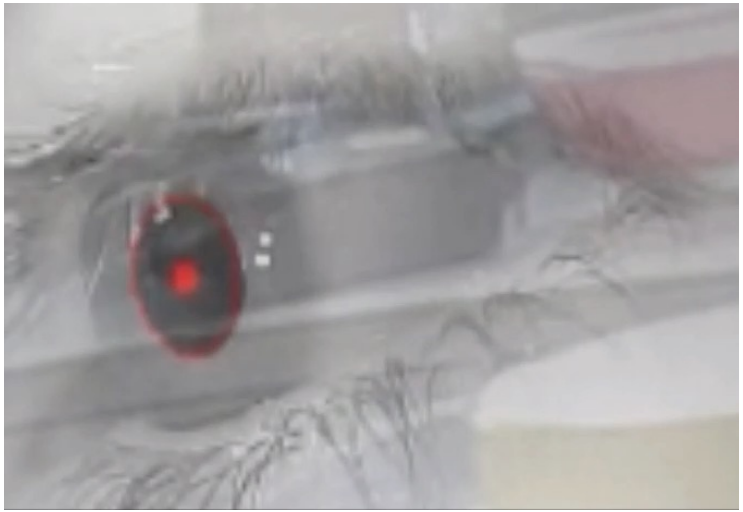
Dynamic Time Warping





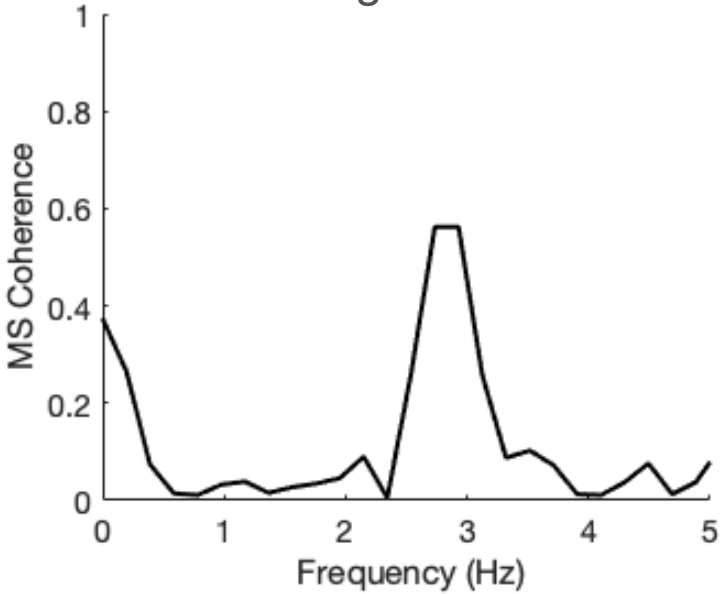
What if you want to know how synchronized two signals are?

(a mean won't work)

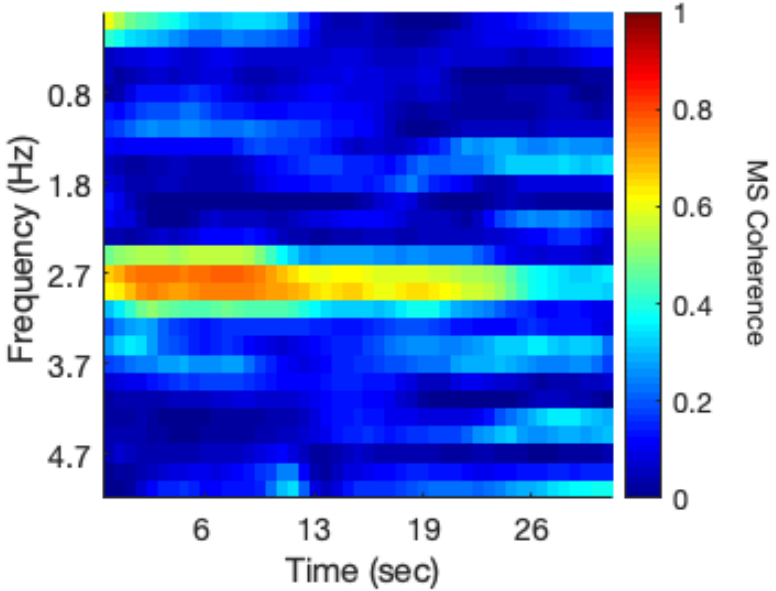


Phase Clustering

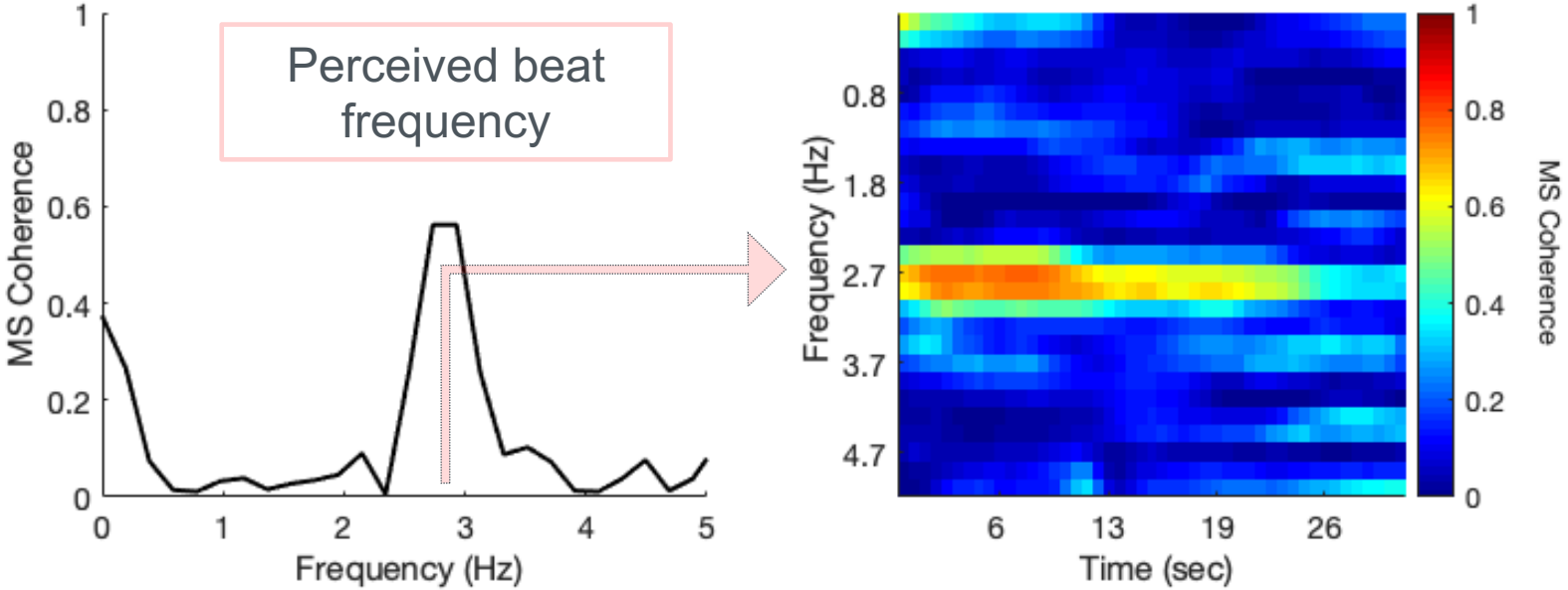
Averaged over time



Time Resolved

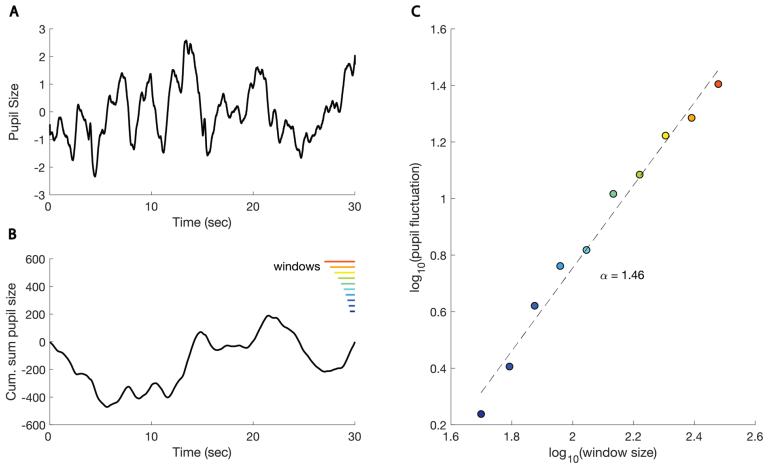


Phase Clustering

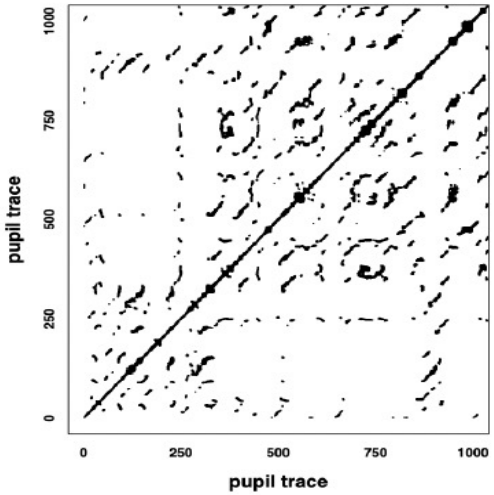


Take-away: **Novel analytic approaches → Novel insights**

Detrended Fluctuation Analysis
(e.g., do long range temporal correlations increase or decrease during certain states?)



Recurrence-based analyses
(e.g., do patterns repeat in the data?)



Fink et al., 2023, *BRM*

Let’s play with some data!

Here are the data:

https://beatlab.mcmaster.ca/assets/pupilTutorial/KonstanzWorkshop_pupilStimulus_TimeSeries.csv

Let's play with some data!

Here are the data:

https://beatlab.mcmaster.ca/assets/pupilTutorial/KonstanzWorkshop_pupilStimulus_TimeSeries.csv

These data come from the full code tutorial that accompanies the paper:

Fink, L., Simola, J., Tavano, A., Lange, E., Wallot, S., & Laeng, B. (2023). From pre-processing to advanced dynamic modeling of pupil data. *Behavior Research Methods*, 1-37.

Code and more resources here: https://github.com/lkfink/pupilTutorial

Thank you!!

Questions?

Contact:
finkl1@mcmaster.ca