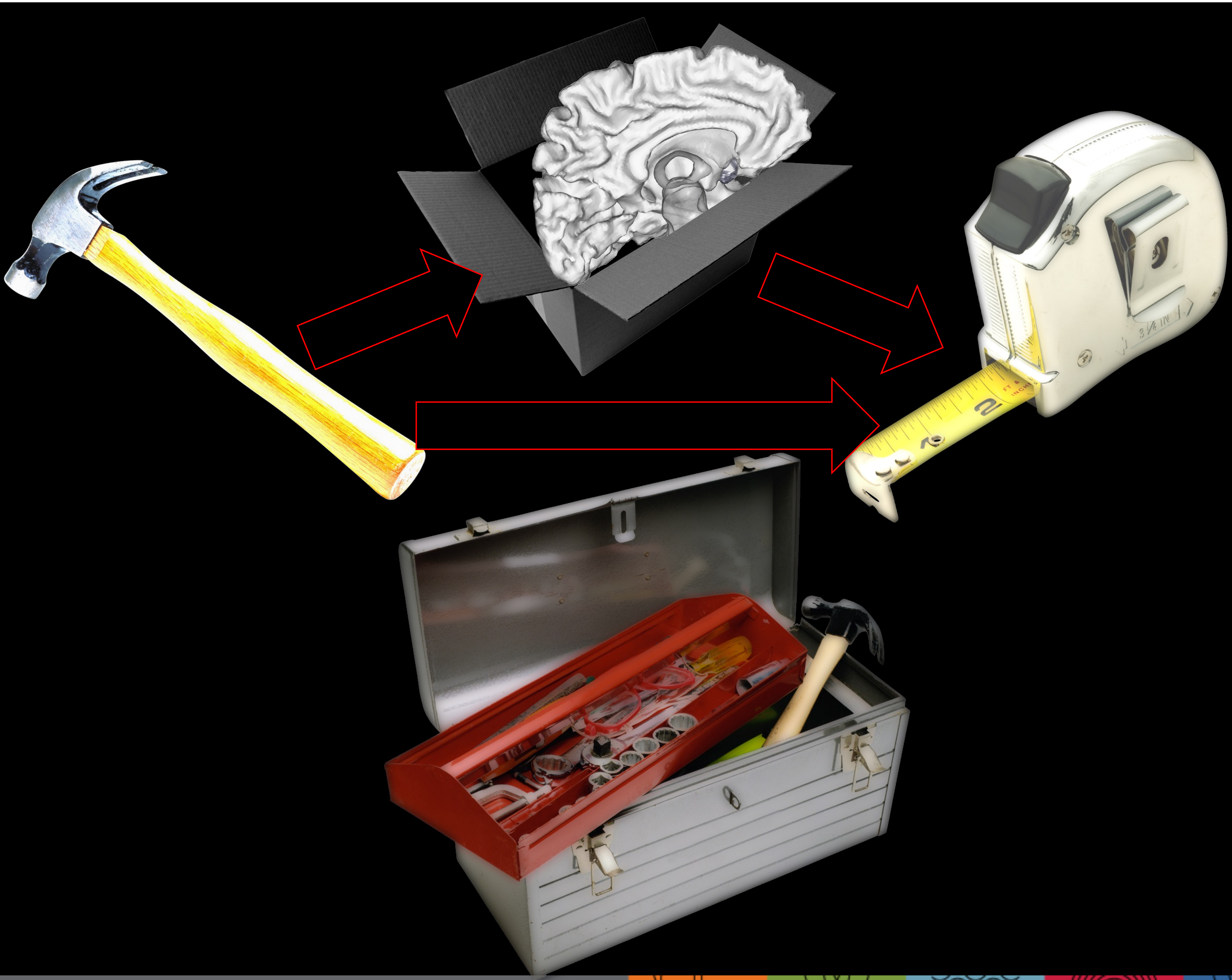
U.S. Department of Health & Human Services • National Institutes of Health

NIH National Center for Complementary and Integrative Health

Mediation analysis for fMRI-based pain assessment

Lauren Atlas

July 20, 2015



Mediation analysis for fMRI

- ✓ Assess dynamic relationships between experimental manipulation, brain, and behavior
- ✓ Voxelwise mixed effects (multilevel) path analysis
- ✓ Identify candidates for causal inference
- ✓ Effective connectivity analysis
- ✓ Identify sources of individual differences in pathway strength

Roadmap

- Introduction (Pain imaging)
- Mediation analysis for fMRI
  - Single level mediation
  - Multi-level mediation
- Brain mediators of the effects of heat on pain
- Brain mediators of expectancy effects on pain

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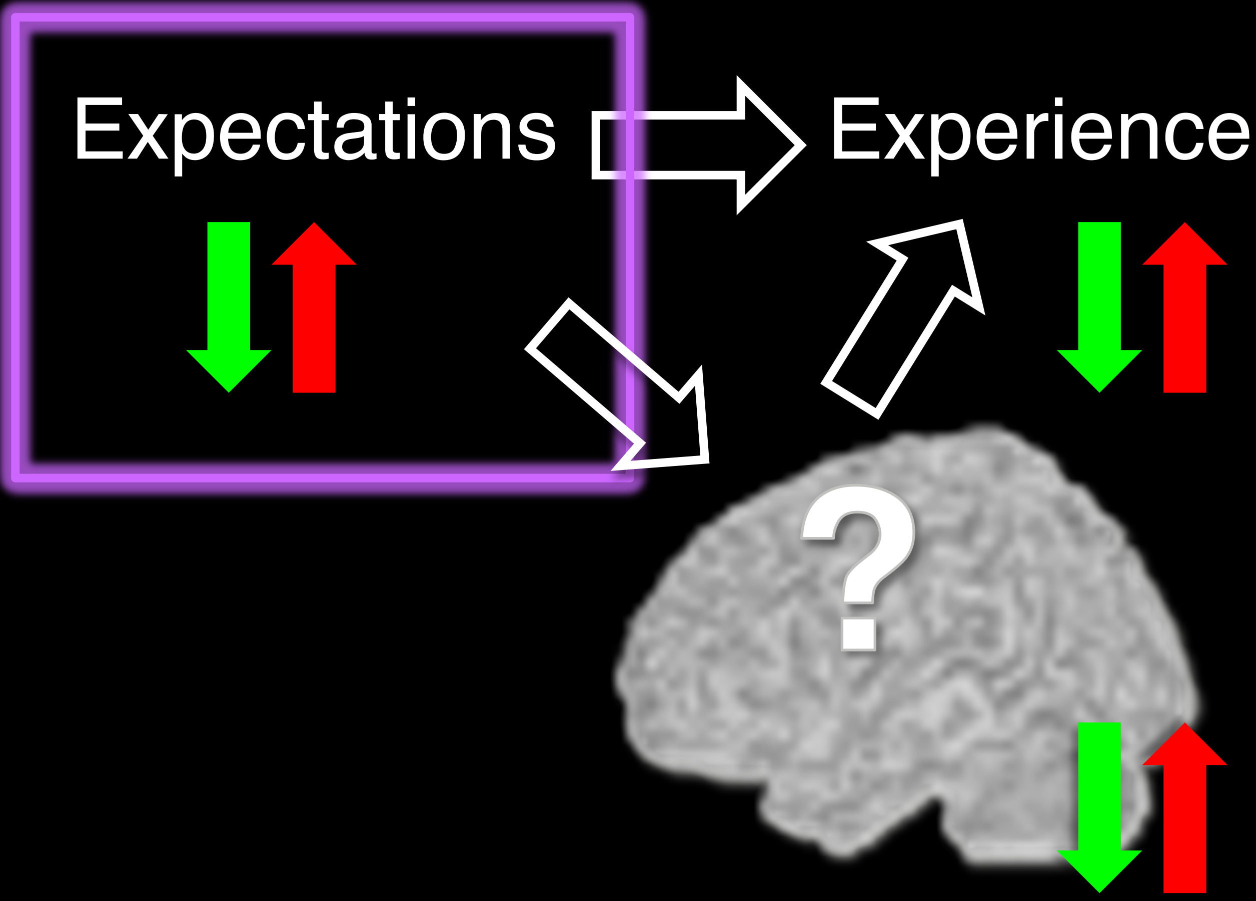
PAIN



Expectations

Experience

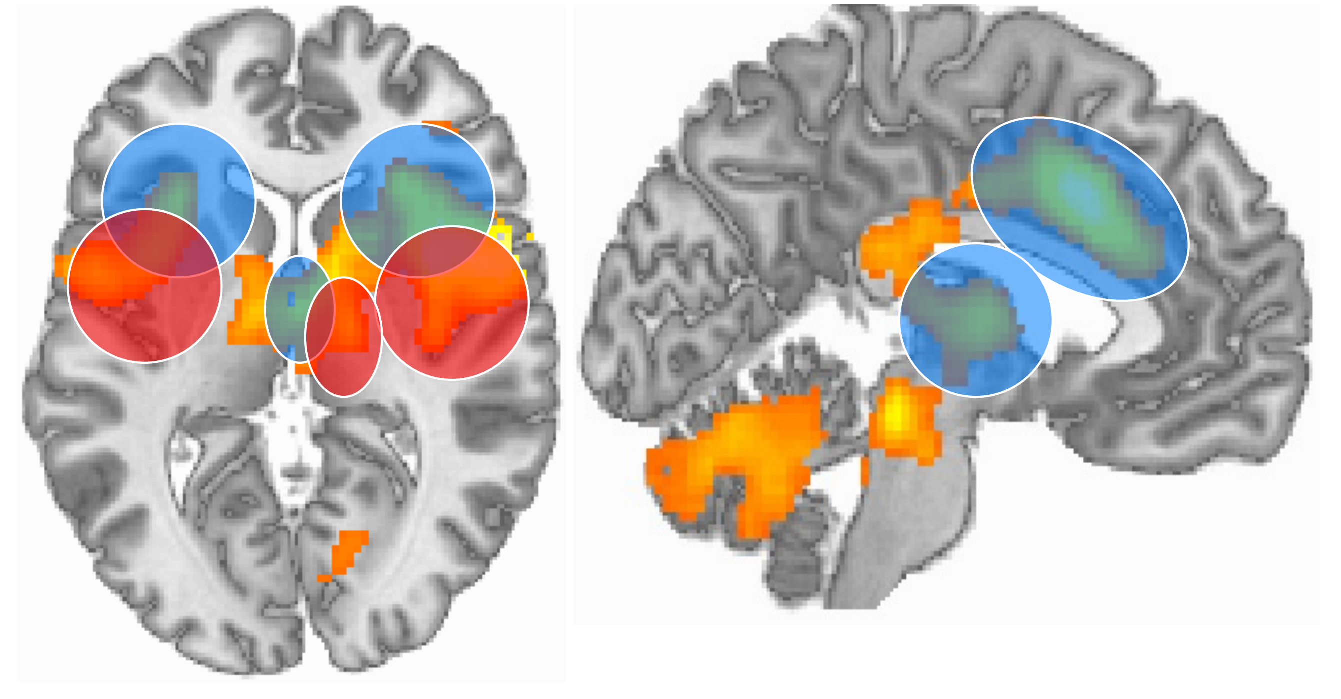
PAIN

"An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage."

–IASP Task force on taxonomy, 1994

°C
°F
50
40
30
20
10
0
10
20
30
120
100
80
60
40
32
20
0
20

“The pain matrix”



Sensation | **Affect**

Sensation	Affect
Lateral thalamus	Medial thalamus
SI	Anterior cingulate
SII	Anterior insula
Posterior Insula	

High vs Low intensity stimulation
Five studies, N = 114
FWE, p<.05

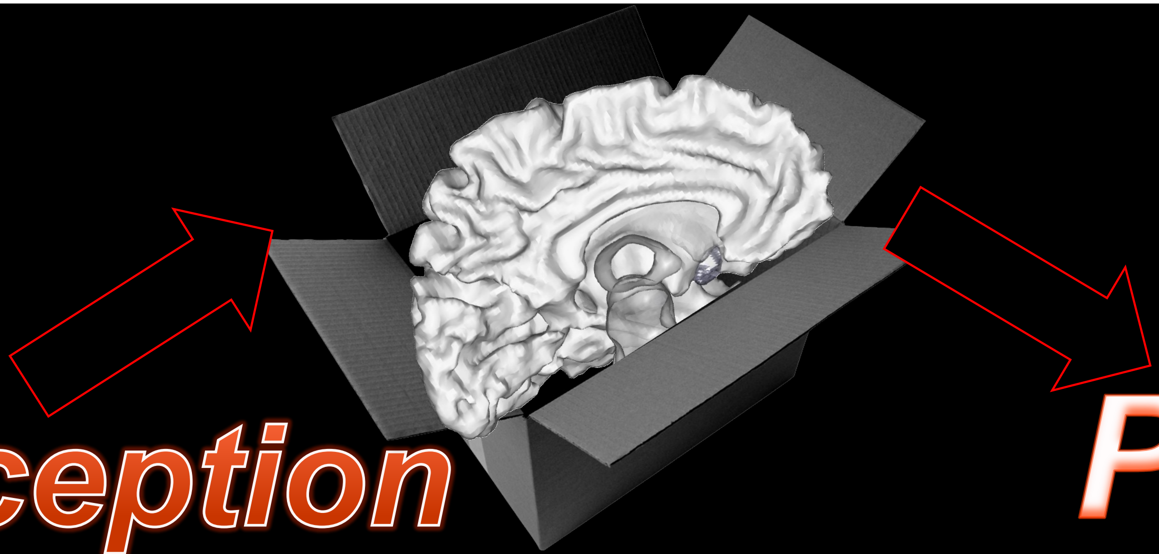
Atlas et al. (2010), *JNeurosci*

PAIN

"Pain is always subjective."

"Activity induced in the nociceptor and nociceptive pathways by a noxious stimulus is not pain, which is always a psychological state, even though we may well appreciate that pain most often has a proximate physical cause."

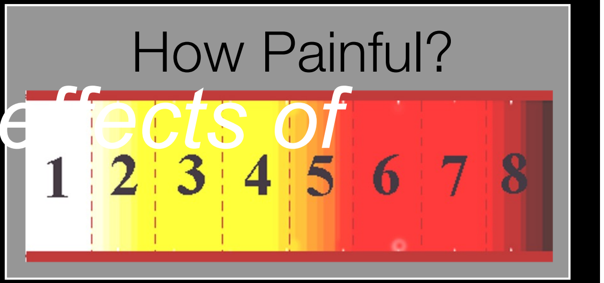
–IASP Task force on taxonomy, 1994



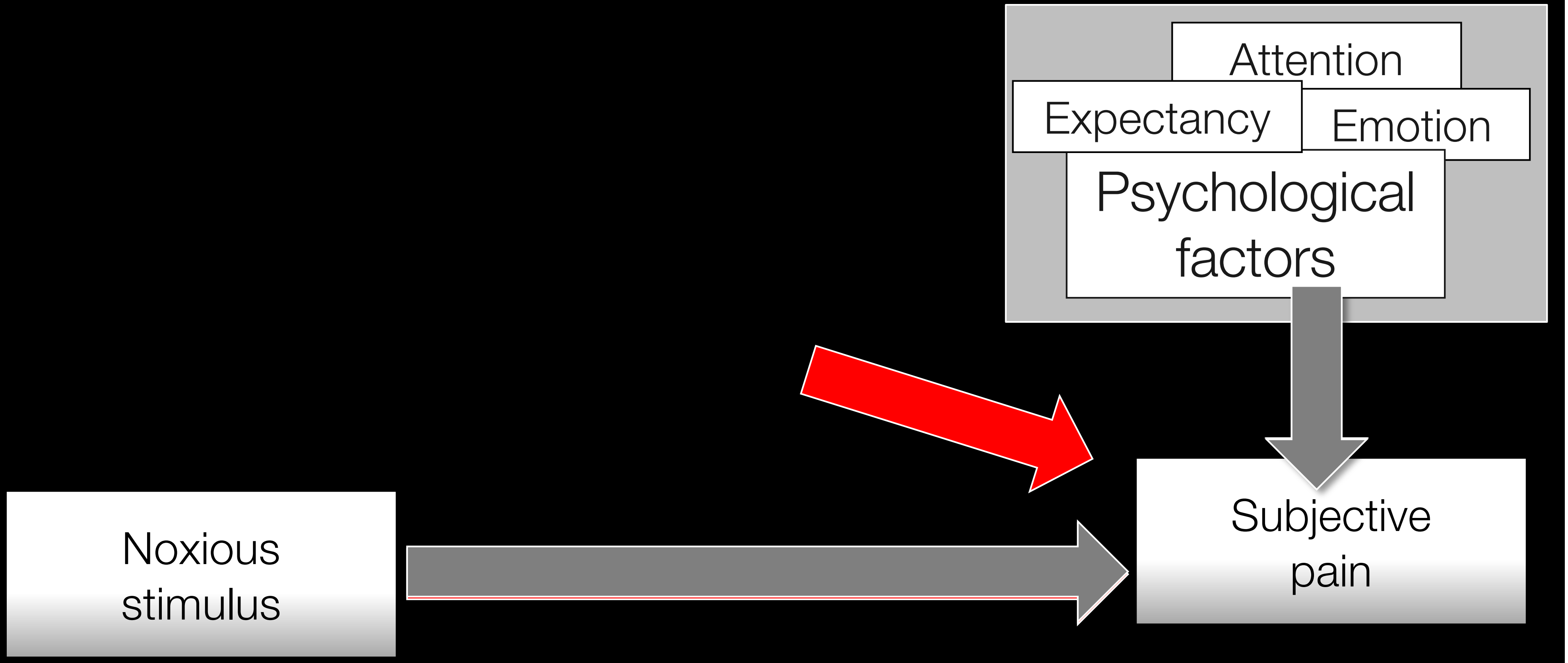
Nociception

PAIN

Noxious stimulus → Subjective pain



I. Which brain pathways mediate the effects of noxious stimuli on subjective pain?



Attention

Expectancy

Emotion

Psychological factors

Noxious stimulus

Subjective pain

I. Which brain pathways mediate the effects of noxious stimuli on subjective pain?

II. Which pathways mediate expectancy effects on pain?

Roadmap

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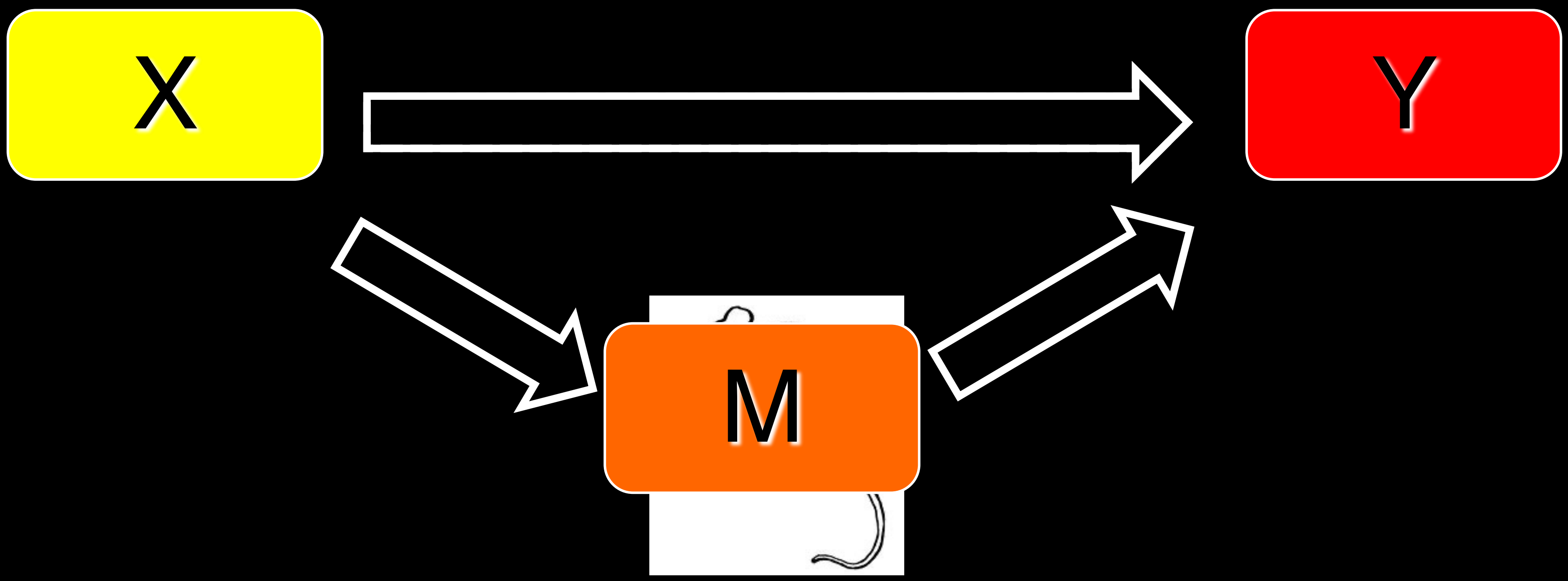
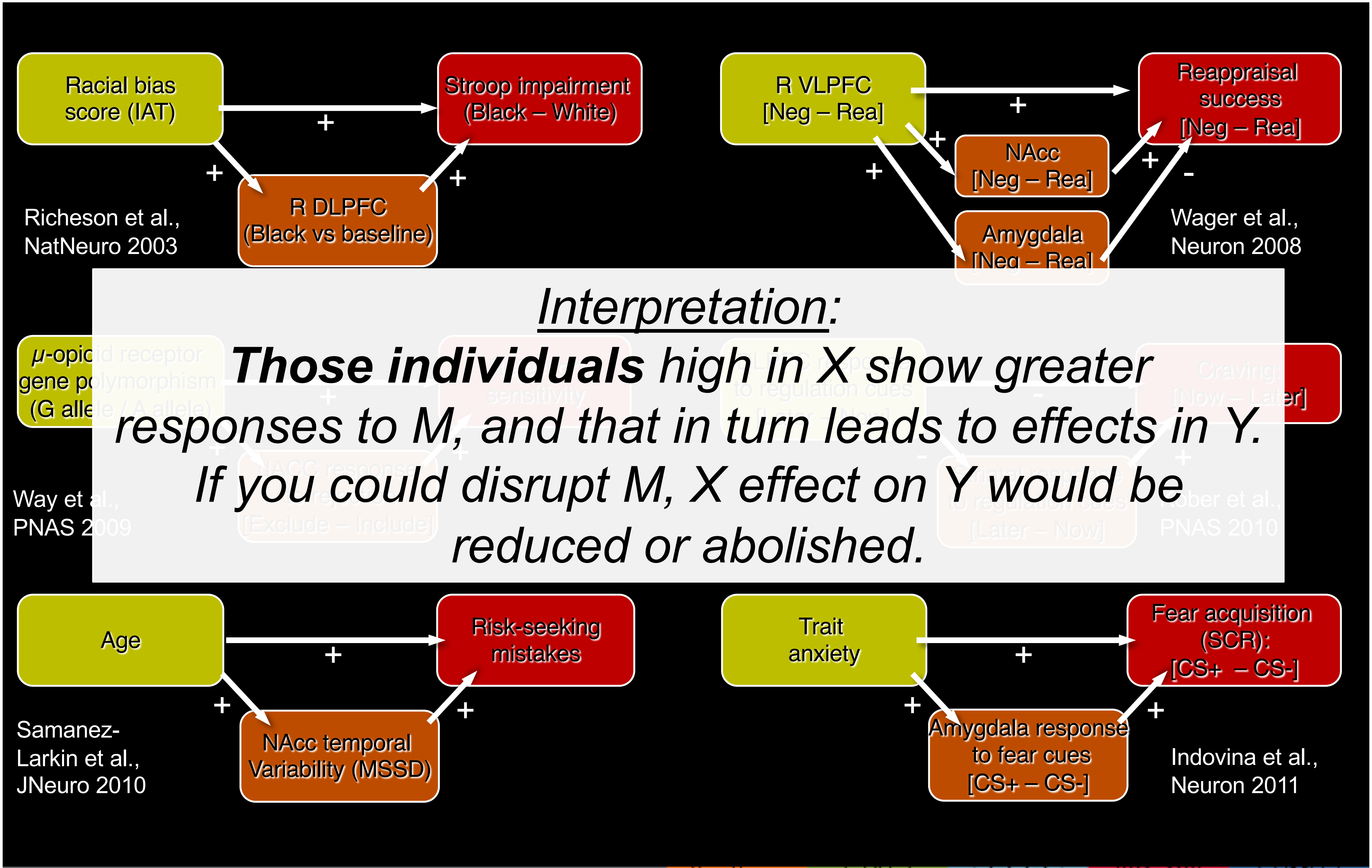
Mediation



Woodworth, 1928

*Provides a formal test of whether the relationship between input (S/**X**) and output (R/**Y**) can be explained by a mediator (M; brain activity)*

Single level mediation in neuroimaging


Racial bias score (IAT)
Stroop impairment (Black – White)
R DLPFC (Black vs baseline)
+
+
+
R VLPFC [Neg – Rea]
Reappraisal success [Neg – Rea]
NAcc [Neg – Rea]
Amygdala [Neg – Rea]
+
+
+
+
-
μ-opioid receptor gene polymorphism (G allele / A allele)
sensitivity
to regulation cues
Craving: [Now – Later]
[Exclude – Include]
to regulation cues
[Later – Now]
Age
Risk-seeking mistakes
NAcc temporal Variability (MSSD)
+
+
+
Trait anxiety
Fear acquisition (SCR): [CS+ – CS-]
Amygdala response to fear cues [CS+ – CS-]
+
+
+

Richeson et al., NatNeuro 2003

Wager et al., Neuron 2008

Way et al., PNAS 2009

Kober et al., PNAS 2010

Samanez-Larkin et al., JNeuro 2010

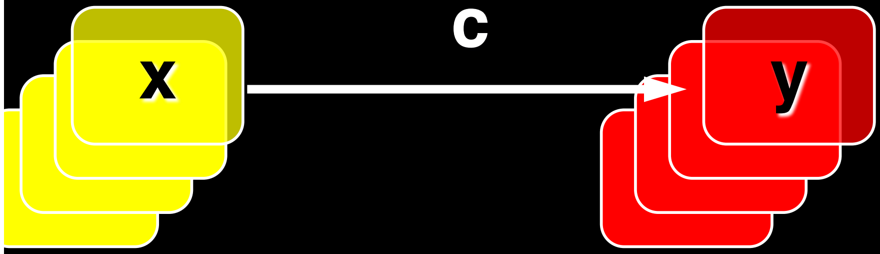
Indovina et al., Neuron 2011

__Interpretation__:

***Those individuals** high in X show greater responses to M, and that in turn leads to effects in Y. If you could disrupt M, X effect on Y would be reduced or abolished.*

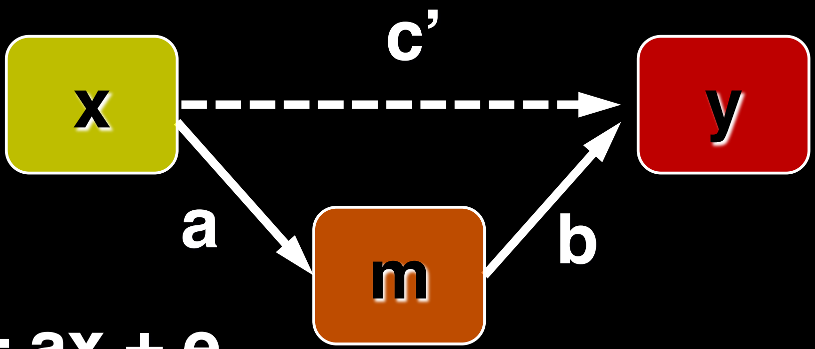
Demonstrating Mediation

Reduced model, without mediator



$$y = cx + e_y$$

Full model, with mediator



$$m = ax + e_m$$

$$y = bm + c'x + e'_y$$

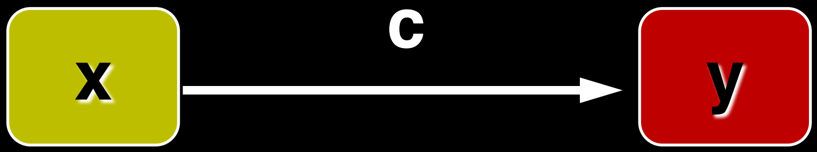
Baron and Kenny (1986) – conjunction of 3 effects:

1) ***c*** **effect: There is a relationship to be mediated**
2) ***a*** **effect: initial variable related to mediator**
3) ***b*** **effect: mediator relates to outcome, controlling for initial variable**

And, if m is a complete mediator, c' = 0

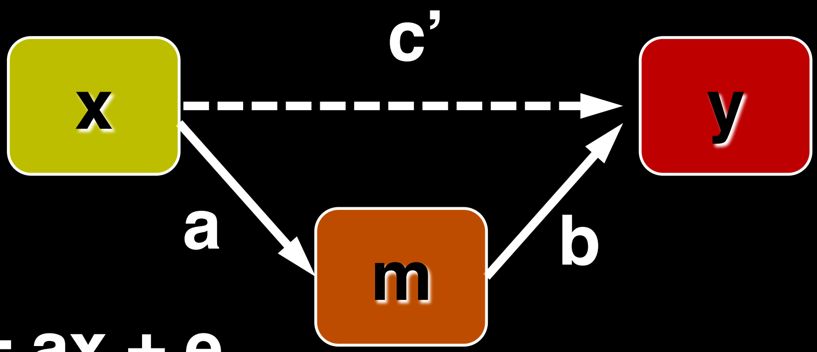
Demonstrating Mediation

Reduced model, without mediator



$$y = cx + e_y$$

Full model, with mediator



$$m = ax + e_m$$

$$y = bm + c'x + e'_y$$

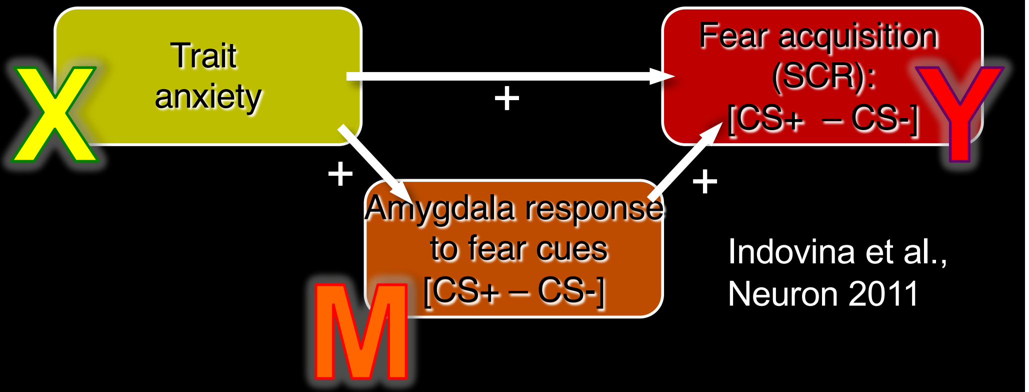
Does *m* explain some of the x-y relationship (c)?

$$c - c' = a*b$$

Counterfactual: If we were to prevent m from varying, the effect of *x* on *y* would be reduced or absent.

Interpreting mediation

- Individuals higher in [X] show greater [Y] because of changes in [M]
- The effect of [X] on [Y] can be explained by individual differences in [M]
- Group differences in [Y] are due to group differences in [M]



X

Trait anxiety

+

Fear acquisition (SCR): [CS+ – CS-]

Y

+

Amygdala response to fear cues [CS+ – CS-]

M

+

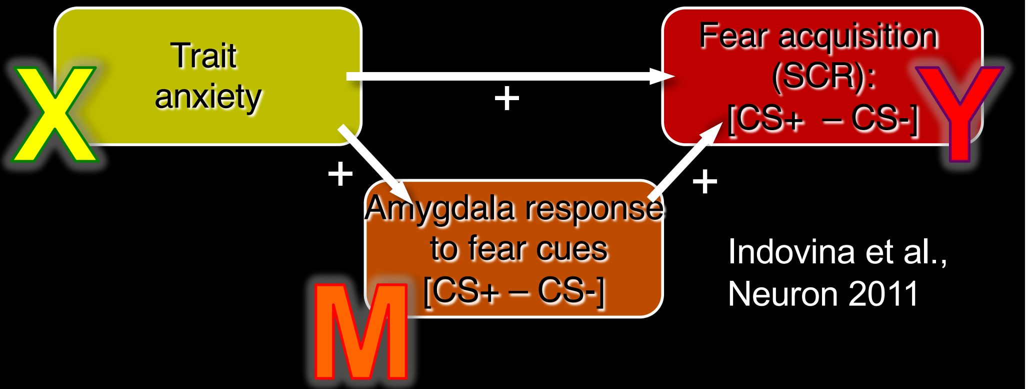
Indovina et al., Neuron 2011

Interpreting mediation

Stronger inferences about directionality if variables are randomly assigned, separated in time

Strongest inference when you can experimentally manipulate X AND M (e.g. using TMS)

- see Holland, Rubin


X

Trait anxiety

+

+

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Y

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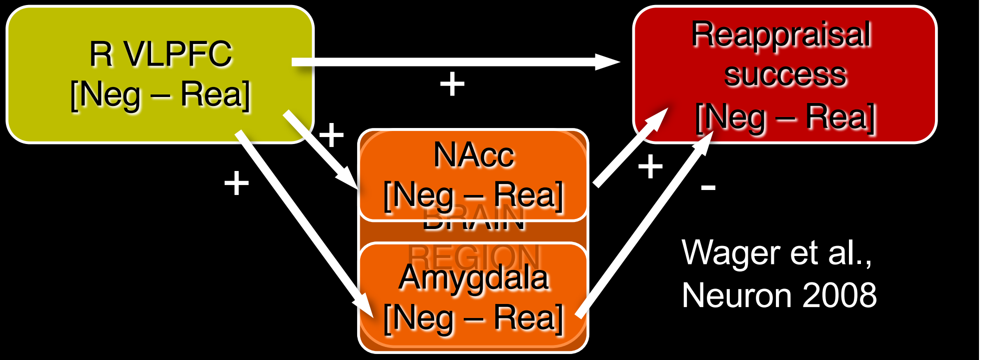
Indovina et al., Neuron 2011

Single level mediation in neuroimaging



R VLPFC
[Neg – Rea]

NAcc
[Neg – Rea]

BRAIN
REGION

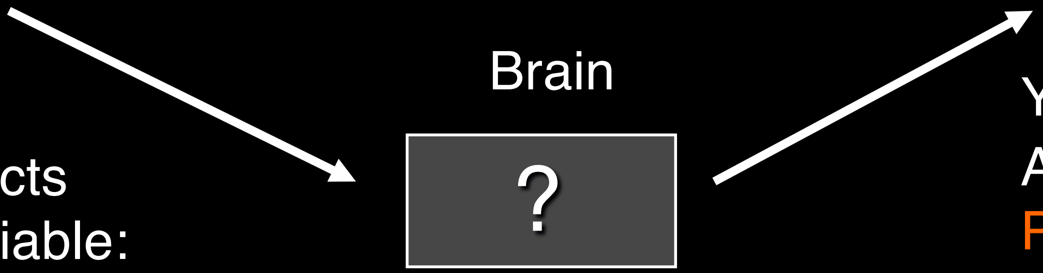
Amygdala
[Neg – Rea]

Reappraisal
success
[Neg – Rea]

Wager et al.,
Neuron 2008

Voxel-wise mediation
effect parametric
mapping (MEPM)

“Mediation Effect Parametric Mapping”



X (initial
variable)

X is…
A between-subjects
experimental variable:
Healthy controls vs
Chronic pain patients

Brain

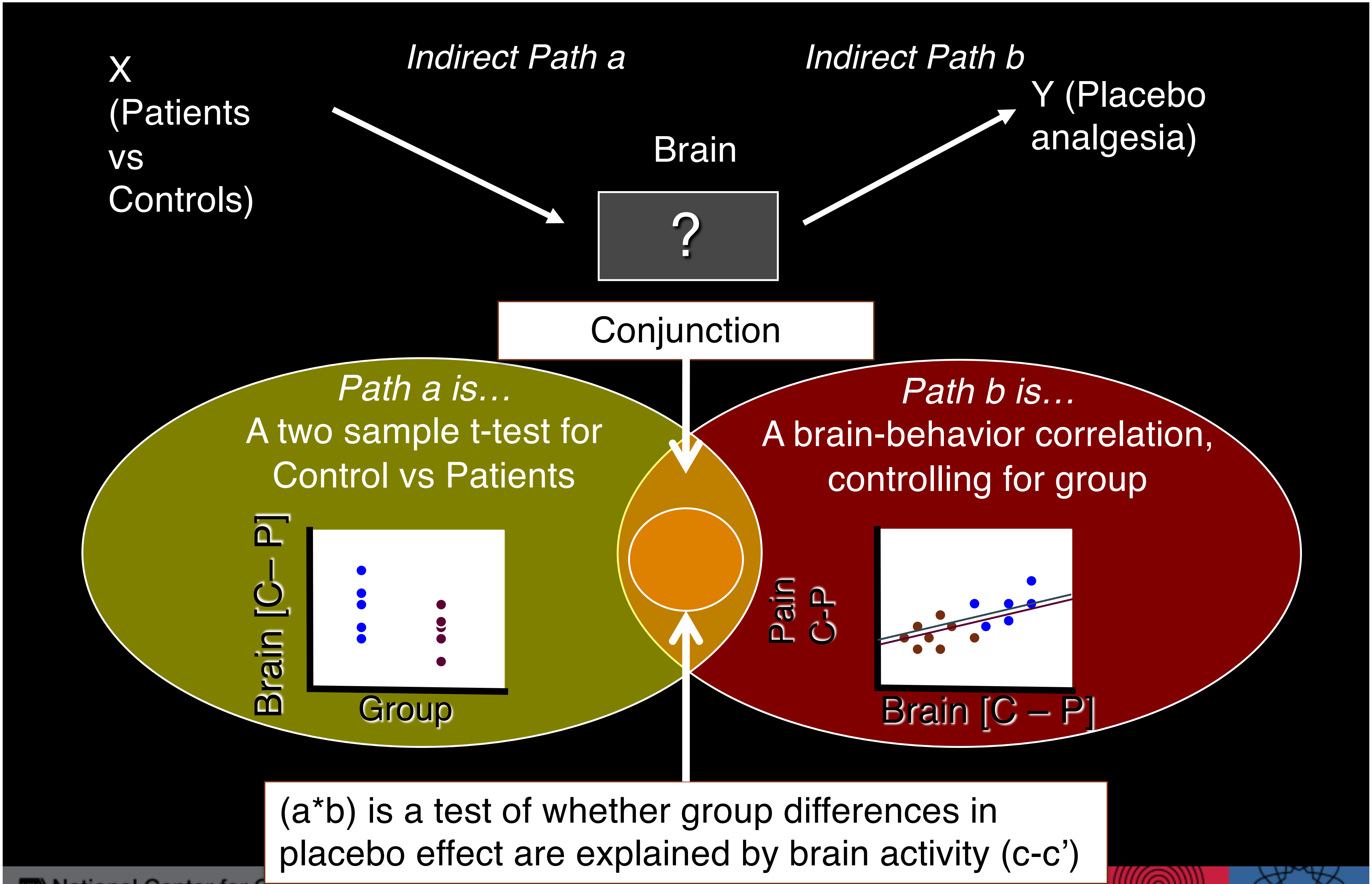
?

M is…
Contrast values from
a voxel
[Control – Placebo]

Y (outcome)

Y is…
A behavioral variable
Pain reports for
[Control – Placebo]:
Placebo analgesia

“Mediation Effect Parametric Mapping”



X (Patients vs Controls)

Indirect Path a

Brain

?

Indirect Path b

Y (Placebo analgesia)

Conjunction

Path a is…
A two sample t-test for Control vs Patients

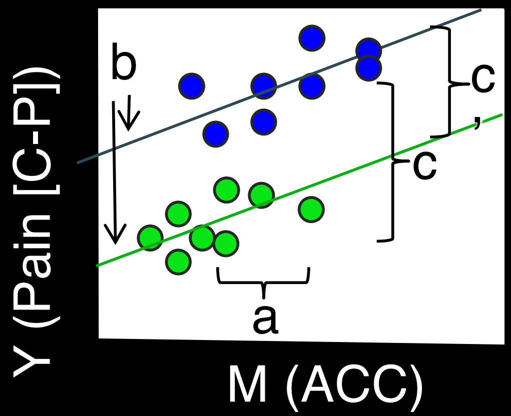
Path b is…
A brain-behavior correlation, controlling for group

(a*b) is a test of whether group differences in placebo effect are explained by brain activity (c-c’)

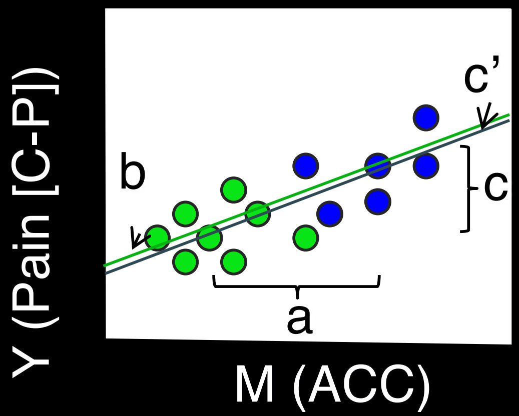
Mediation vs. Conjunction

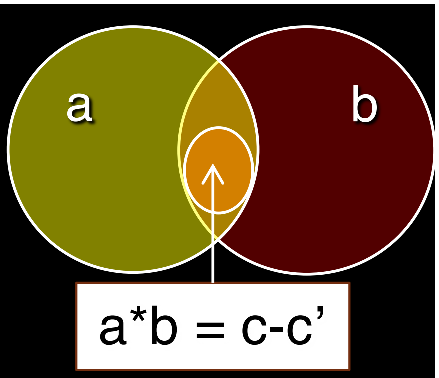
X
● Controls
● Chronic pain patients

Conjunction without mediation



Conjunction and Mediation





Path *c*: Group difference in placebo effects on pain

Path *a*: Group difference in placebo effect on ACC

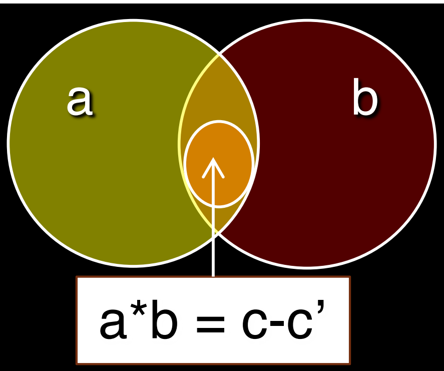
Path *b*: Brain-behavior correlation in ACC, controlling for group

Path *c'*:

Left: Group difference in placebo analgesia even when you account for path *b*: No mediation, c – c' = 0

Right: Group diffs in ACC responses entirely explain group diffs in placebo analgesia; Full mediation, c' = 0

Testing the significance of a*b



- Sobel test

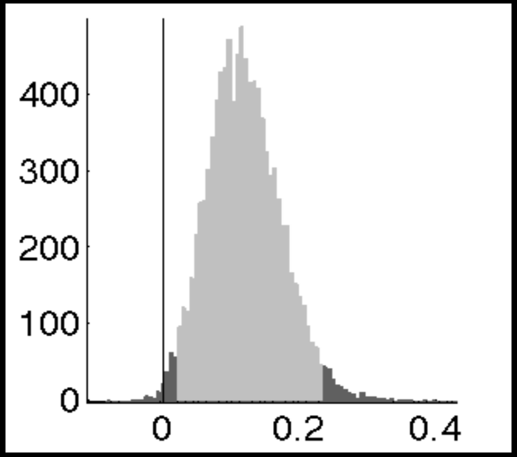
  Aroian, L. A (1944)

$$Z = \frac{ab}{b^2 se(a)^2 + a^2 se(b)^2 + se(a)^2 se(b)^2}$$

- Assumes a, b are normally distributed
- Usually conservative (p-values higher than needed)

- Bootstrap test :

Efron, 1994; Shrout & Bolger, 2002; Preacher & Hayes, 2004



Histogram of bootstrapped Indirect (a*b) effects

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Mediation analysis:

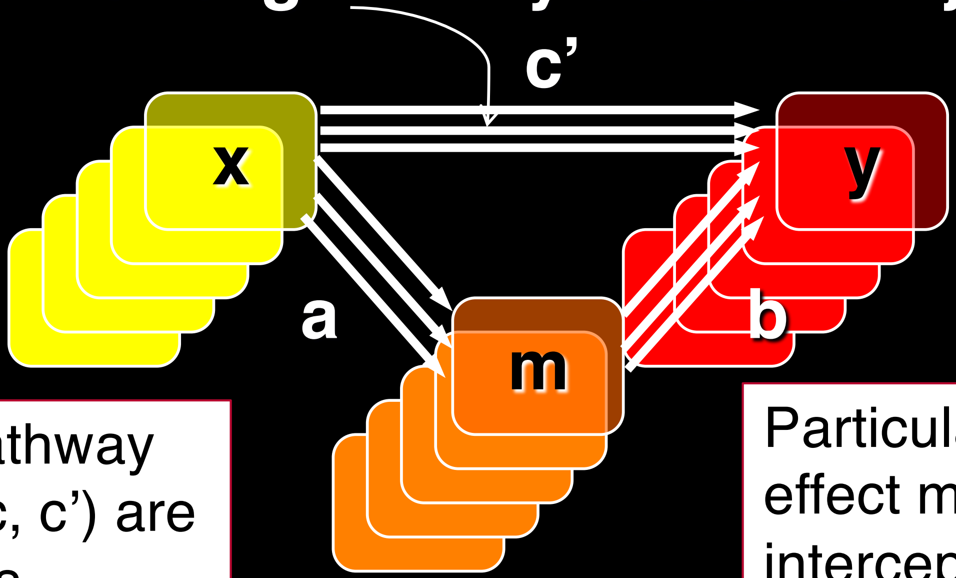
Single vs multilevel mediation

$$c - c' = a*b + \mathrm{cov}(a,b)$$

Kenny, Korchmaros, & Bolger (2003)

Rather than one value per subject model the entire timeseries, assess within-subjects effects

Path strengths vary across subjects



Observations on each variable are nested within subjects

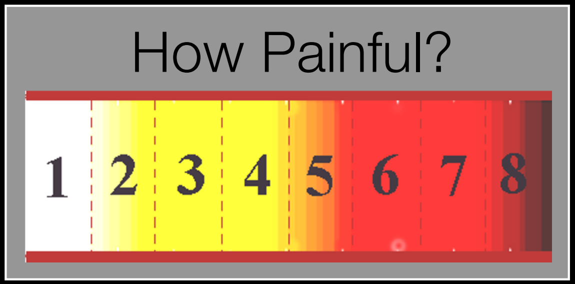
Subject-level pathway strengths (a, b, c, c') are random variables

Particular type of mixed-effect model: Random intercept, random slope model, with subject as random effect

Example: FMRI experiment

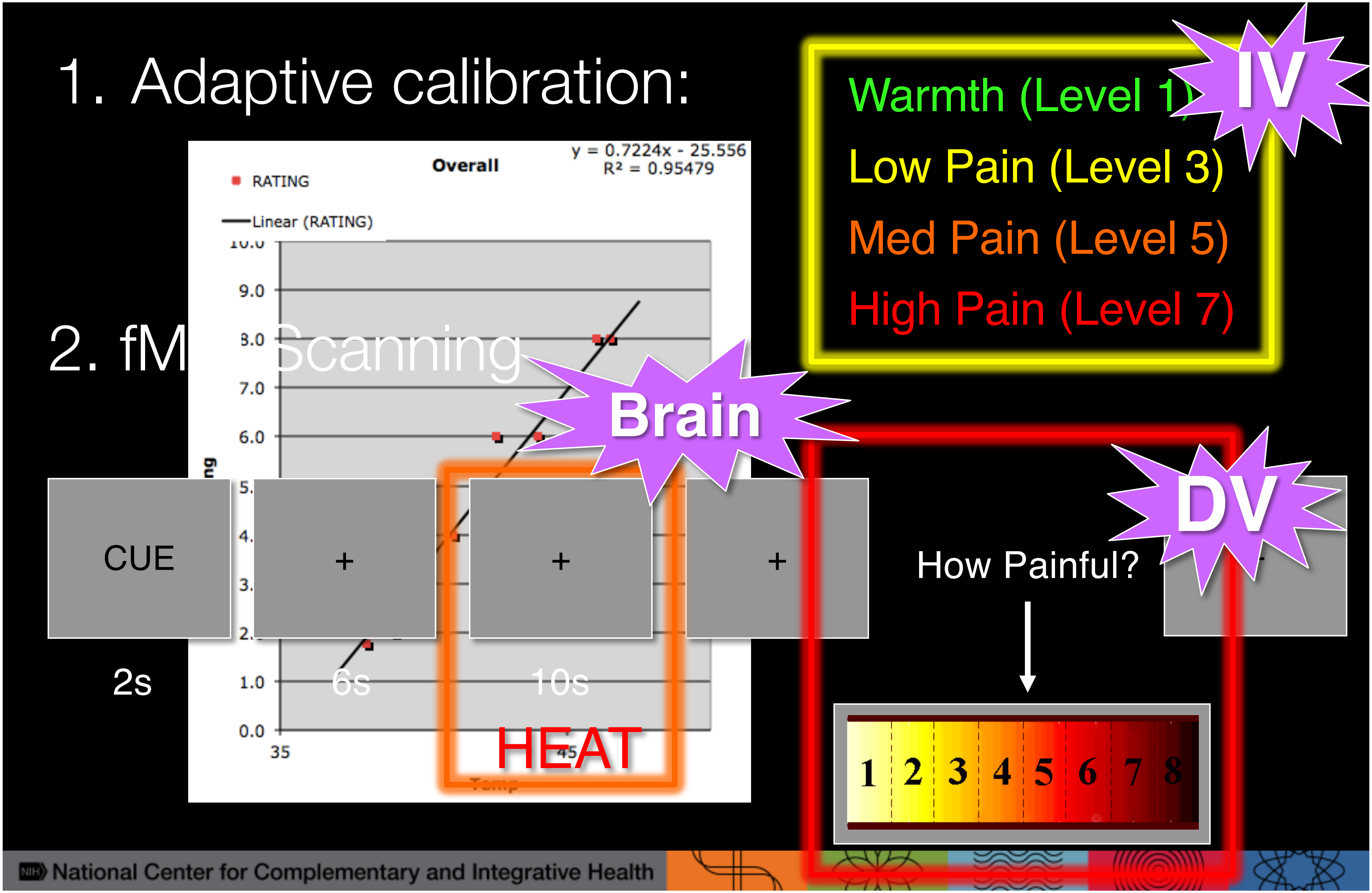
Which brain pathways mediate the effects of temperature (noxious heat) on pain?

°C
°F
50
40
30
20
10
0
10
20
30
120
100
80
60
40
32
20
0
20


How Painful?
1
2
3
4
5
6
7
8

Atlas et al., 2014, Pain

Design



1. Adaptive calibration:

2. fM Scanning

Warmth (Level 1)

Low Pain (Level 3)

Med Pain (Level 5)

High Pain (Level 7)

IV
Overall
RATING
Linear (RATING)
y = 0.7224x - 25.556
R² = 0.95479
10.0
9.0
8.0
7.0
6.0
5.
4.
3.
2.
1.0
0.0
35
45
Temp
Brain
DV
CUE
2s
+
6s
+
10s
HEAT
+
How Painful?
1
2
3
4
5
6
7
8

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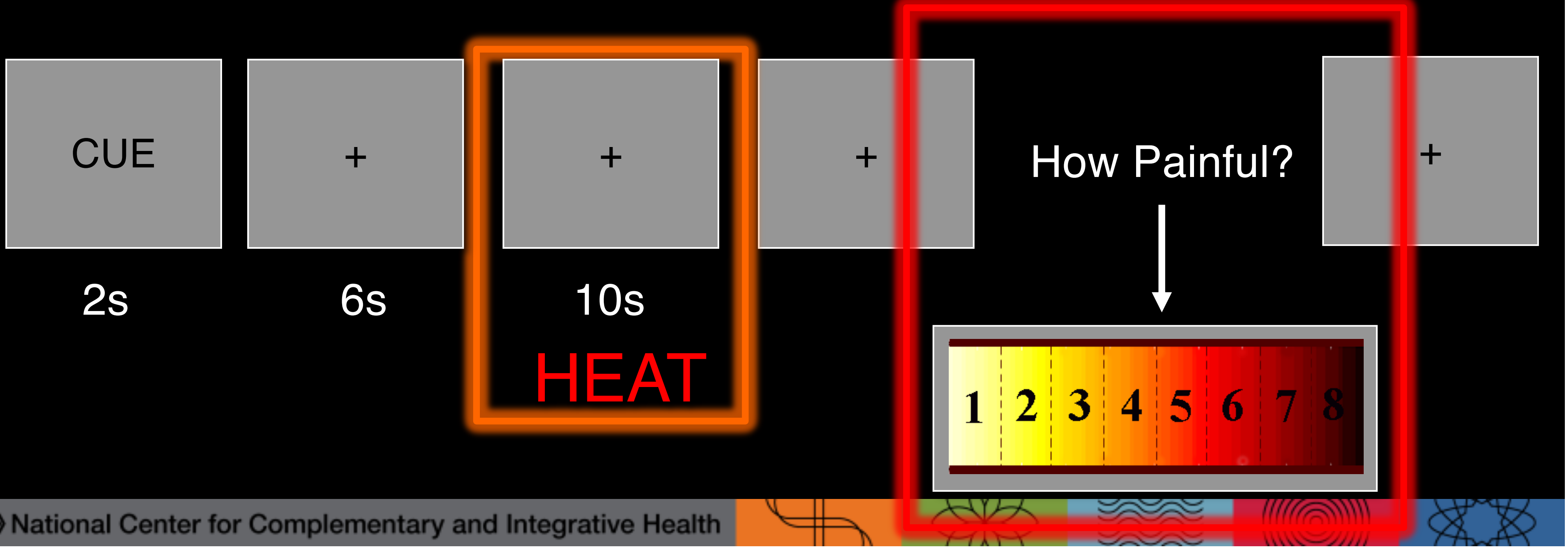
Design

1. Adaptive calibration:

- Warmth (Level 1)
- Low Pain (Level 3)
- Med Pain (Level 5)
- High Pain (Level 7)

2. fMRI Scanning

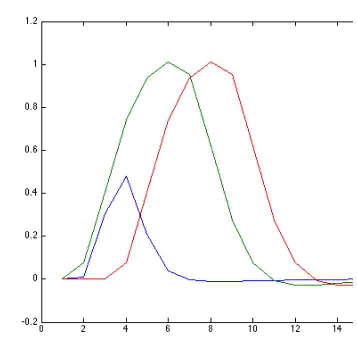

CUE
2s
+
6s
+
10s
HEAT
+
How Painful?
1
2
3
4
5
6
7
8
+

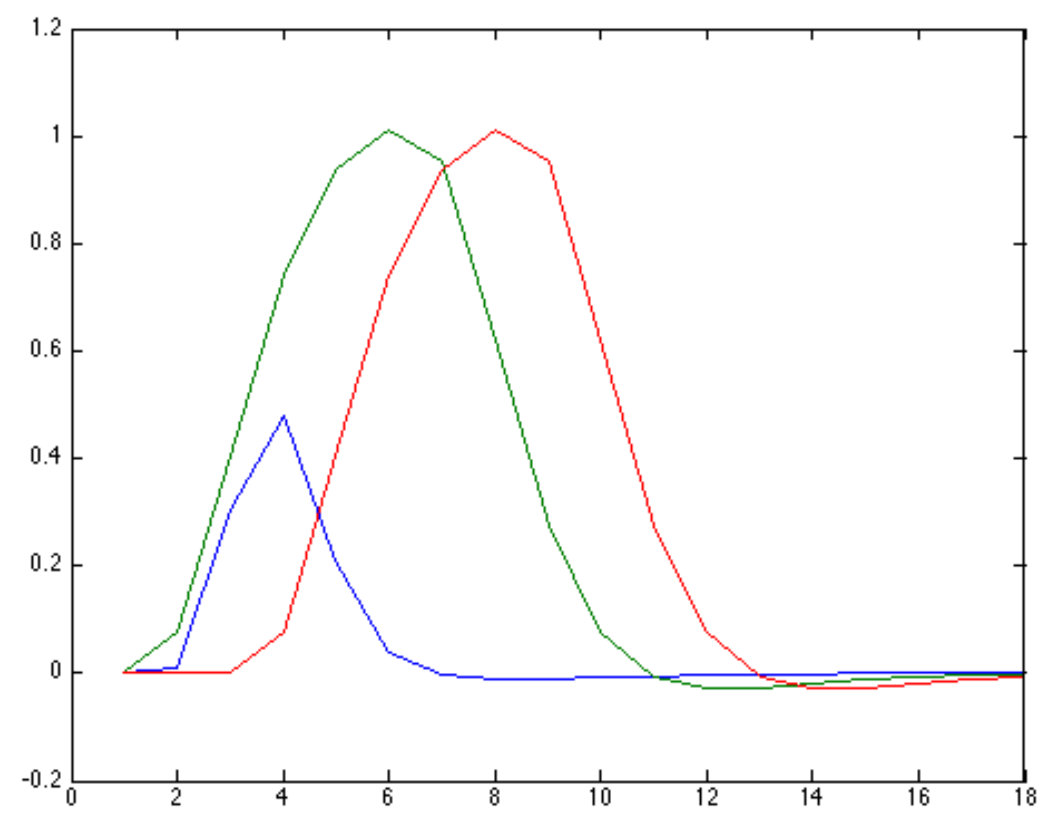
Important! # of observations

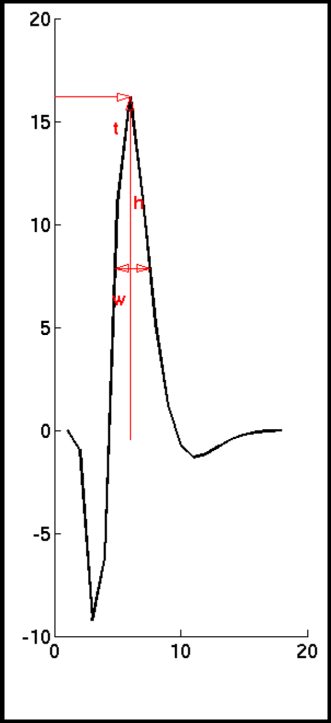
- Within each subject, need equal observations of X, M, and Y
- Easy if individual differences or 3 brain regions (connectivity)
- Here, each trial has:
    - 1 temperature (X)
    - 1 rating (Y)
    - ~10 seconds of pain, plus HRF!

Voxelwise single trial analysis

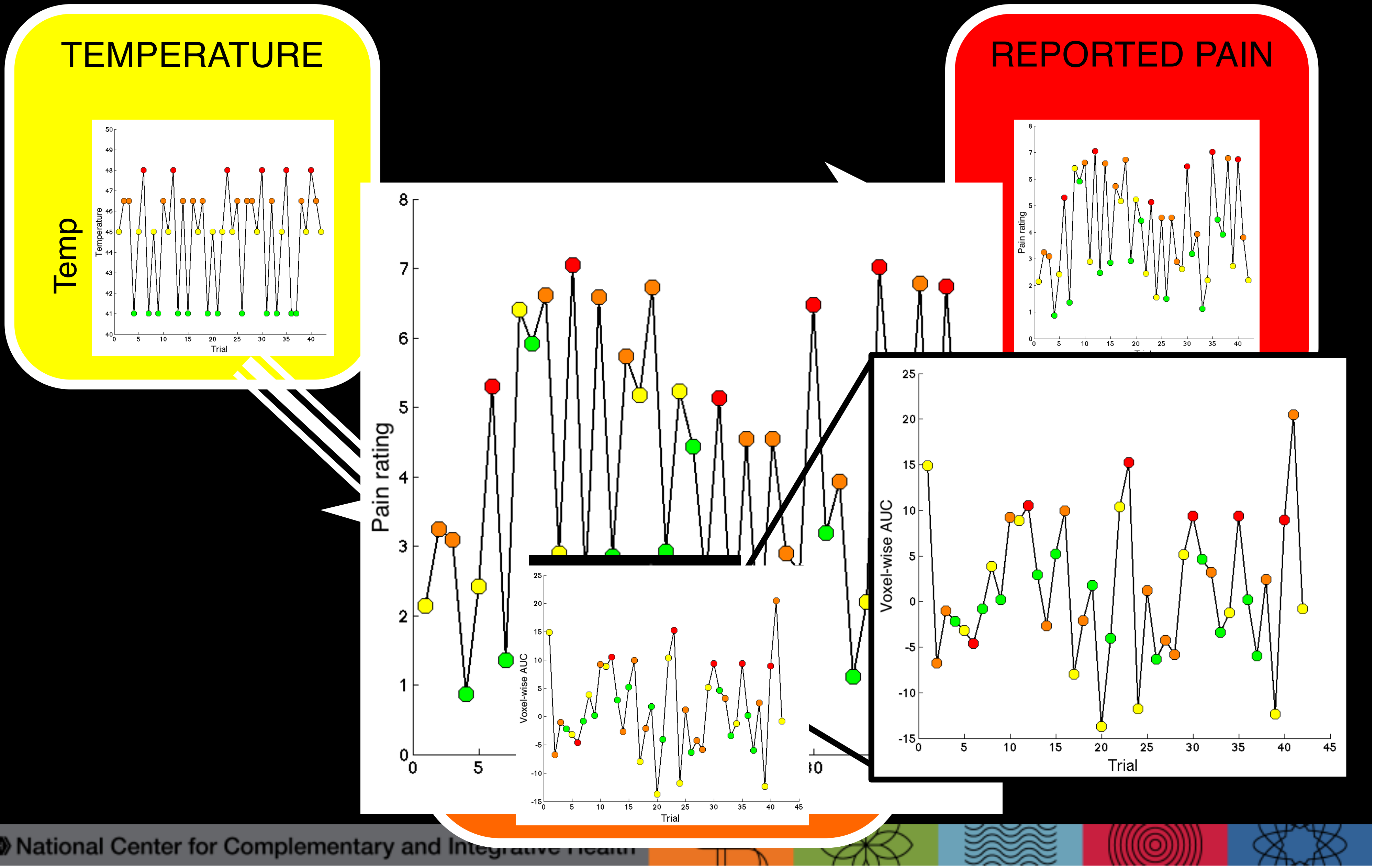
1. Fit basis functions trial-by-trial
2. Es
   an
3. Us
   an



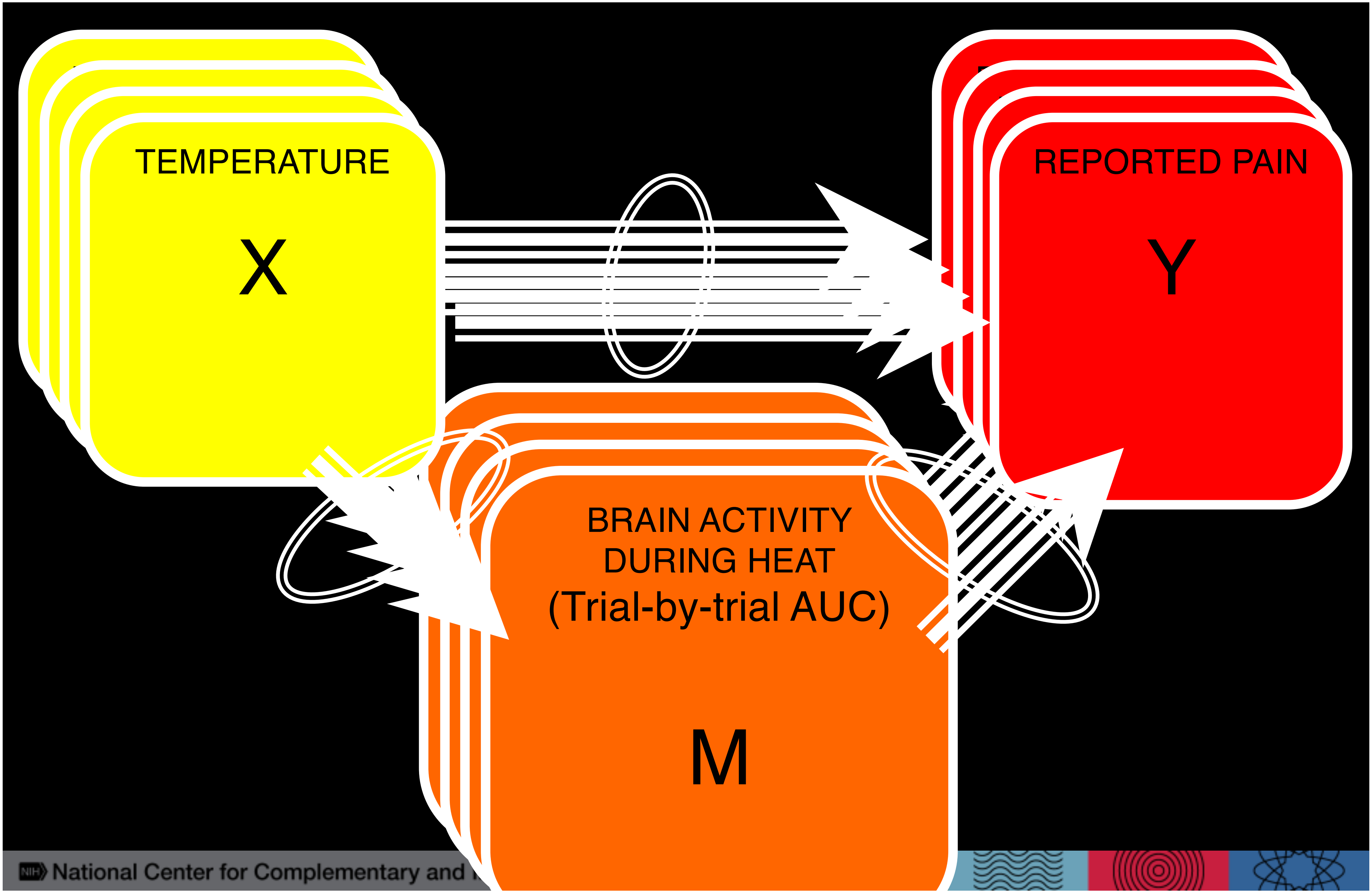




Mediation model



Mediation model



TEMPERATURE

X

REPORTED PAIN

Y

BRAIN ACTIVITY
DURING HEAT
(Trial-by-trial AUC)

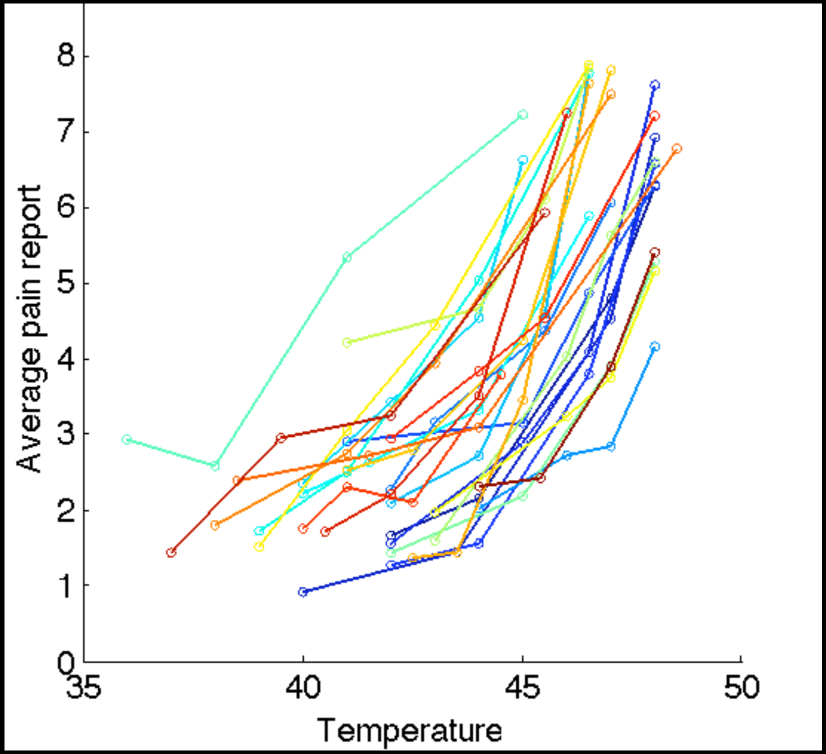
M

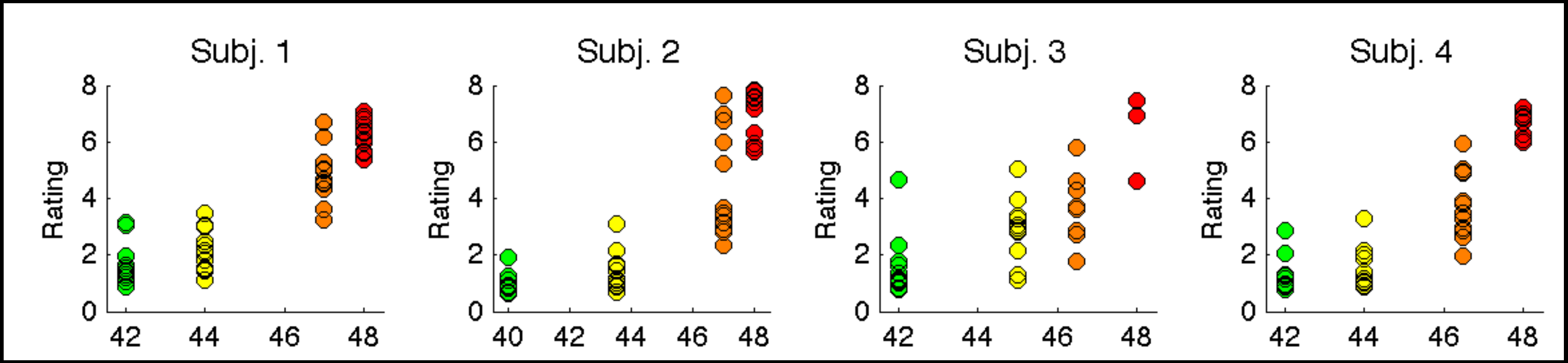
National Center for Complementary and

RESULTS

Atlas et al., 2010, *JNeurosci*

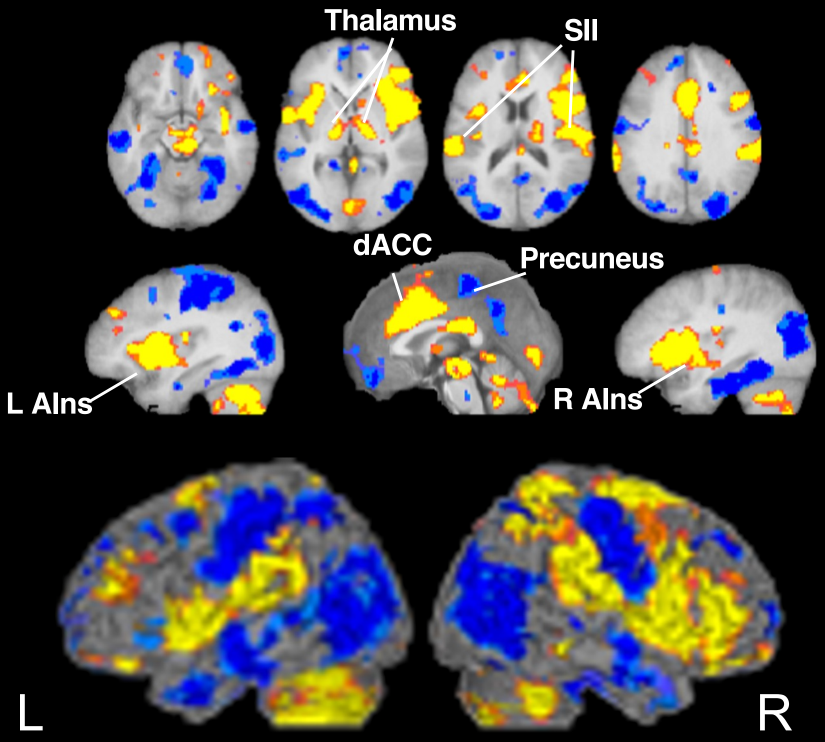
Temp → Path c → **Pain**





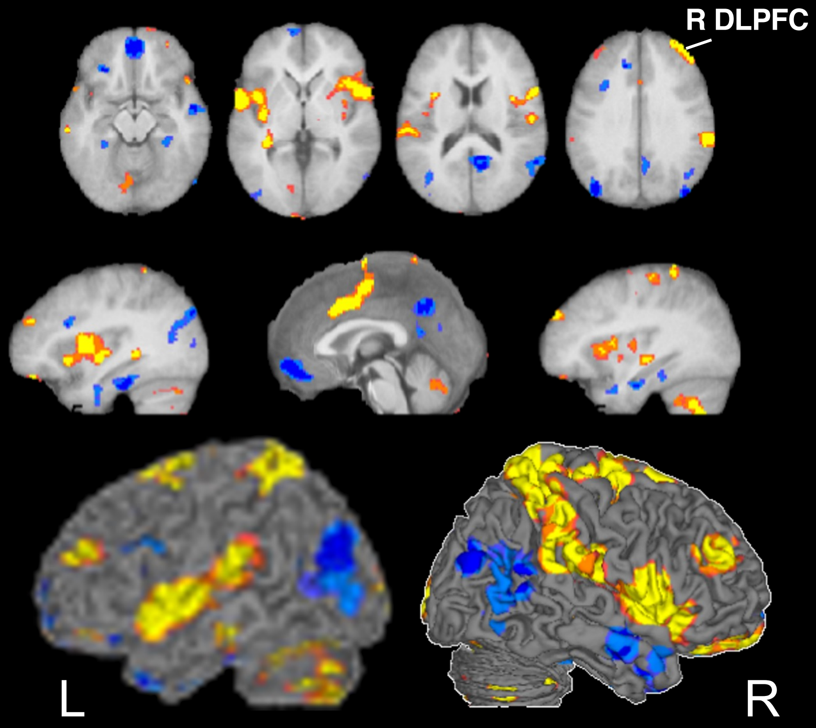
Path a

Temp → *Brain*


Thalamus
SII
dACC
Precuneus
L AIns
R AIns
L
R

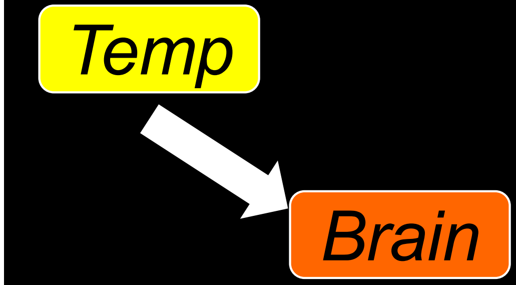
Path b

Brain → *Pain*



	+	-
p < .001	🟨	🟦
p < .005	🟧	🟦
p < .01	🟥	🟦

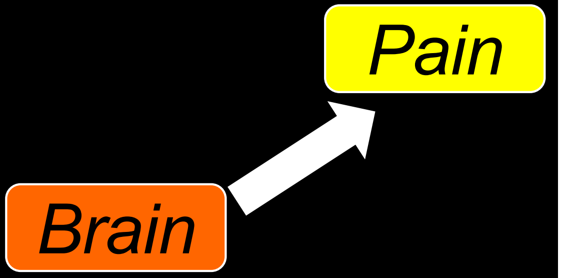
Path a



Temp

Brain

Path b

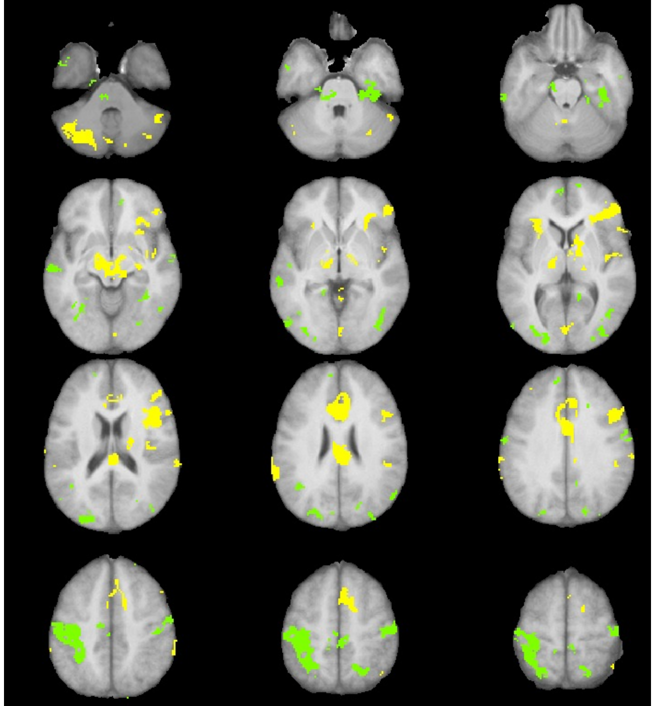


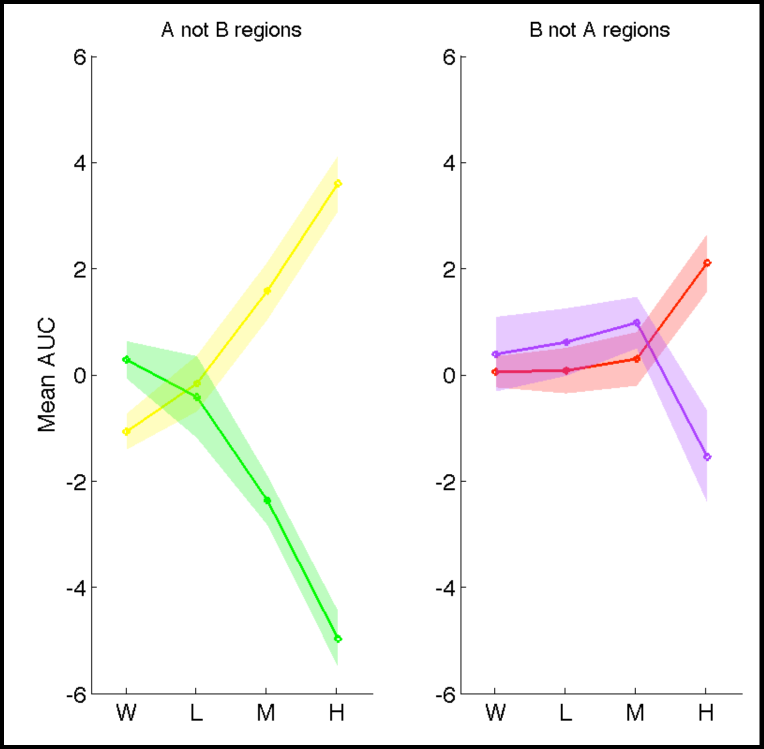
Brain

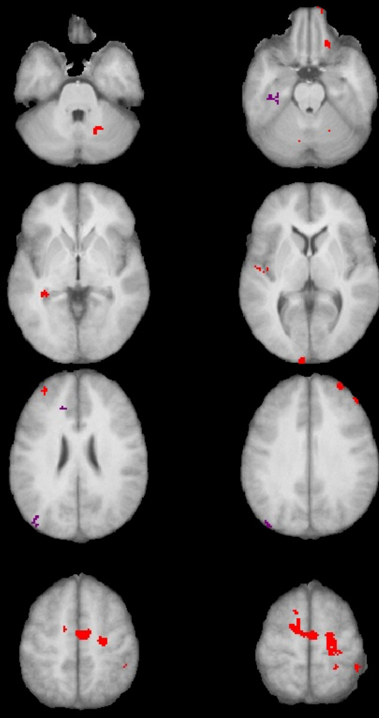
Pain

🟨 🟩 Path *a* (temperature), not *b*

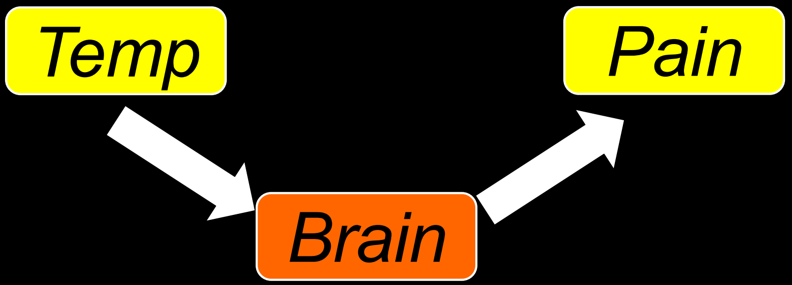
🟥 🟪 Path *b* (pain report), not *a*







	+	-
p < .001	🟨	🟦
p < .005	🟧	🟦
p < .01	🟥	🟦

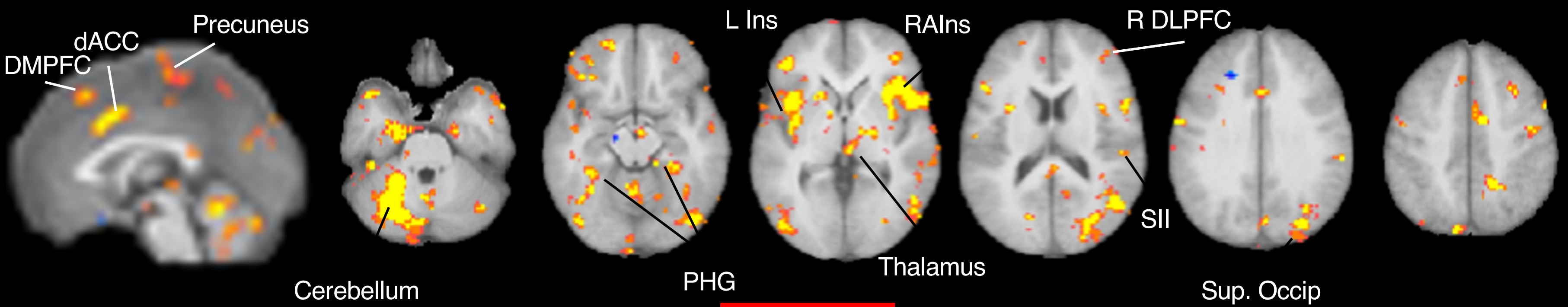


Temp

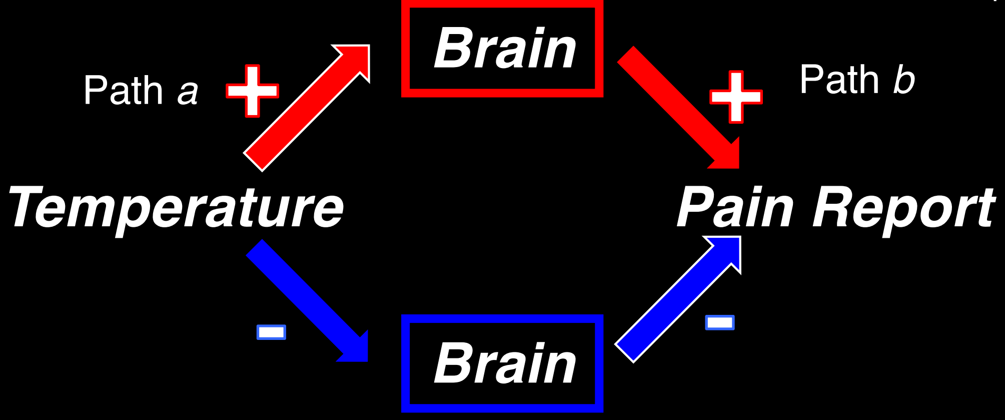
Brain

Pain

	+	-
p < .001	🟨	🟦
p < .005	🟧	🟦
p < .01	🟥	🟪



DMPFC
dACC
Precuneus
Cerebellum
PHG
L Ins
RAIns
Thalamus
R DLPFC
SII
Sup. Occip



Path *a* **+**

Brain

Path *b* **+**

Temperature

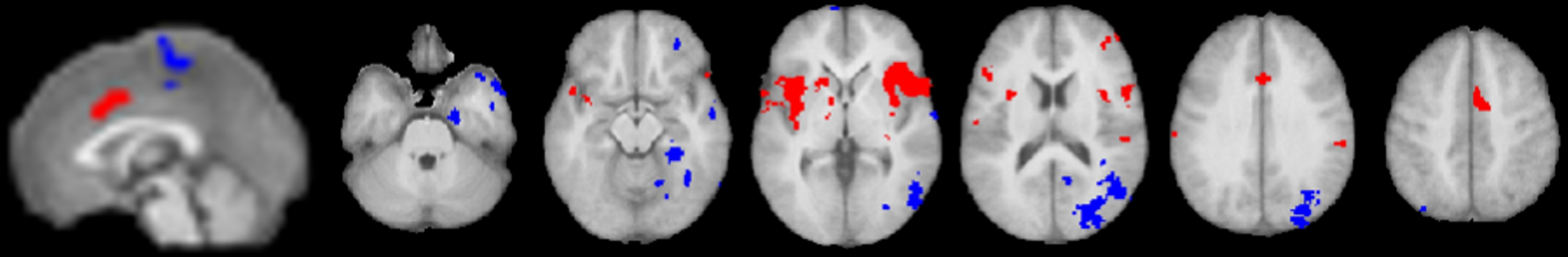
Pain Report

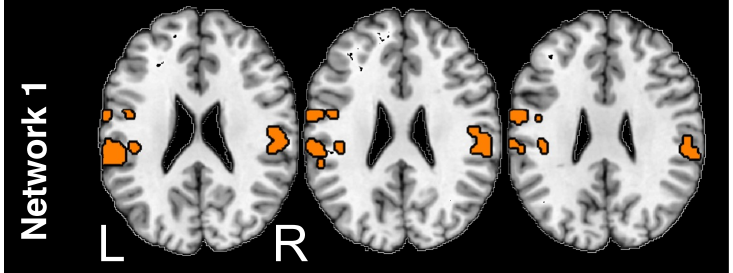
-

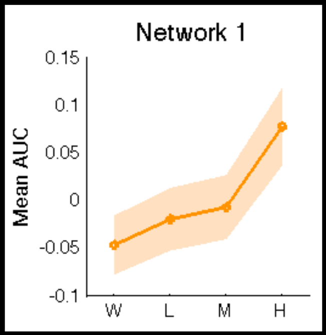
Brain

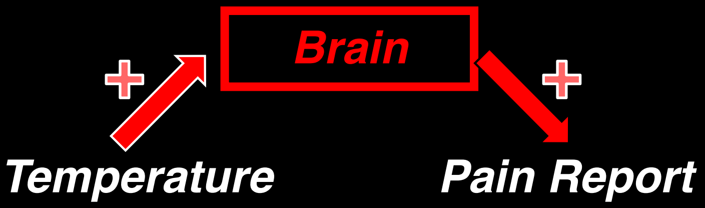
-

Consistent mediators (significant Path a and Path b effects in group)




Network 1
L
R



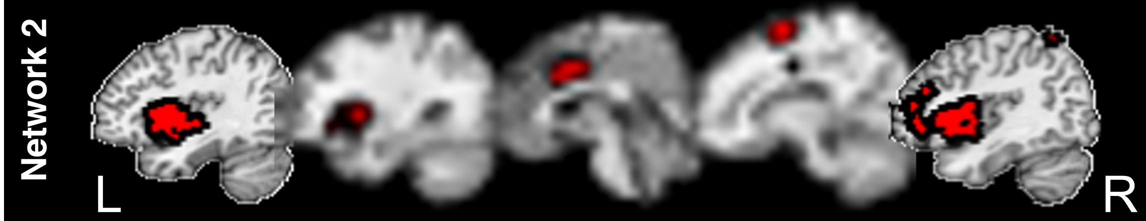

Brain

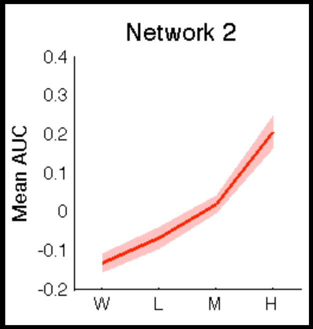
+

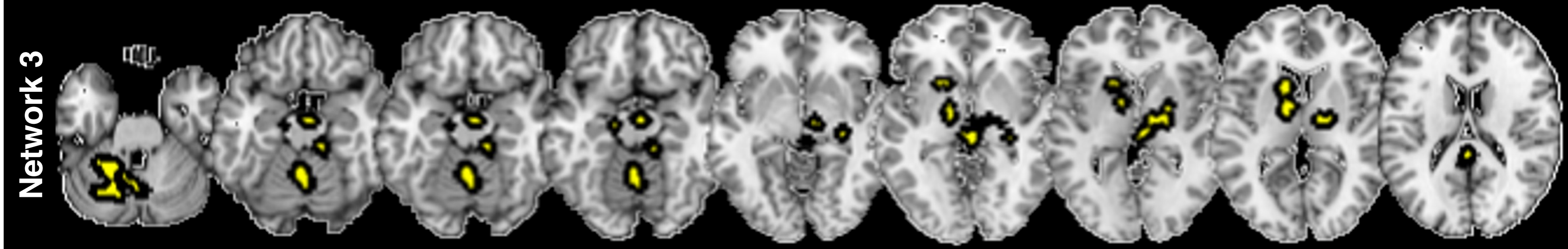
+

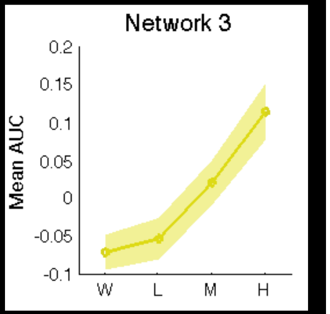
Temperature

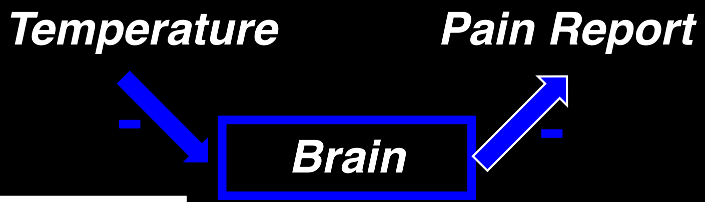
Pain Report


Network 2
L
R







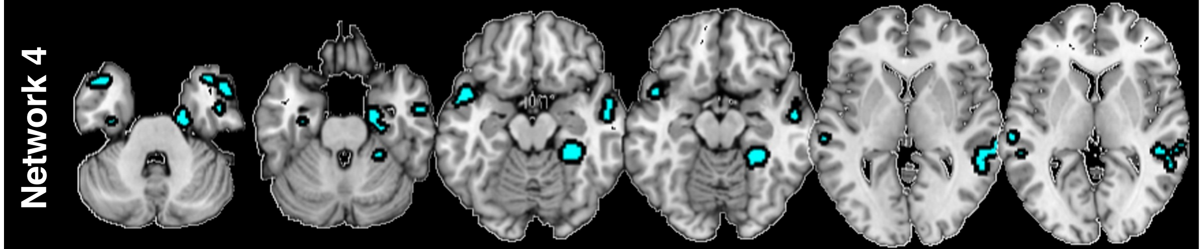

Temperature

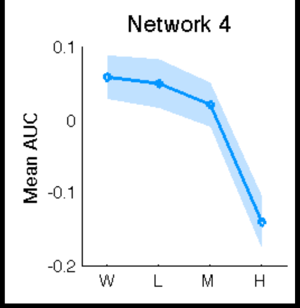
Pain Report

-

Brain

-





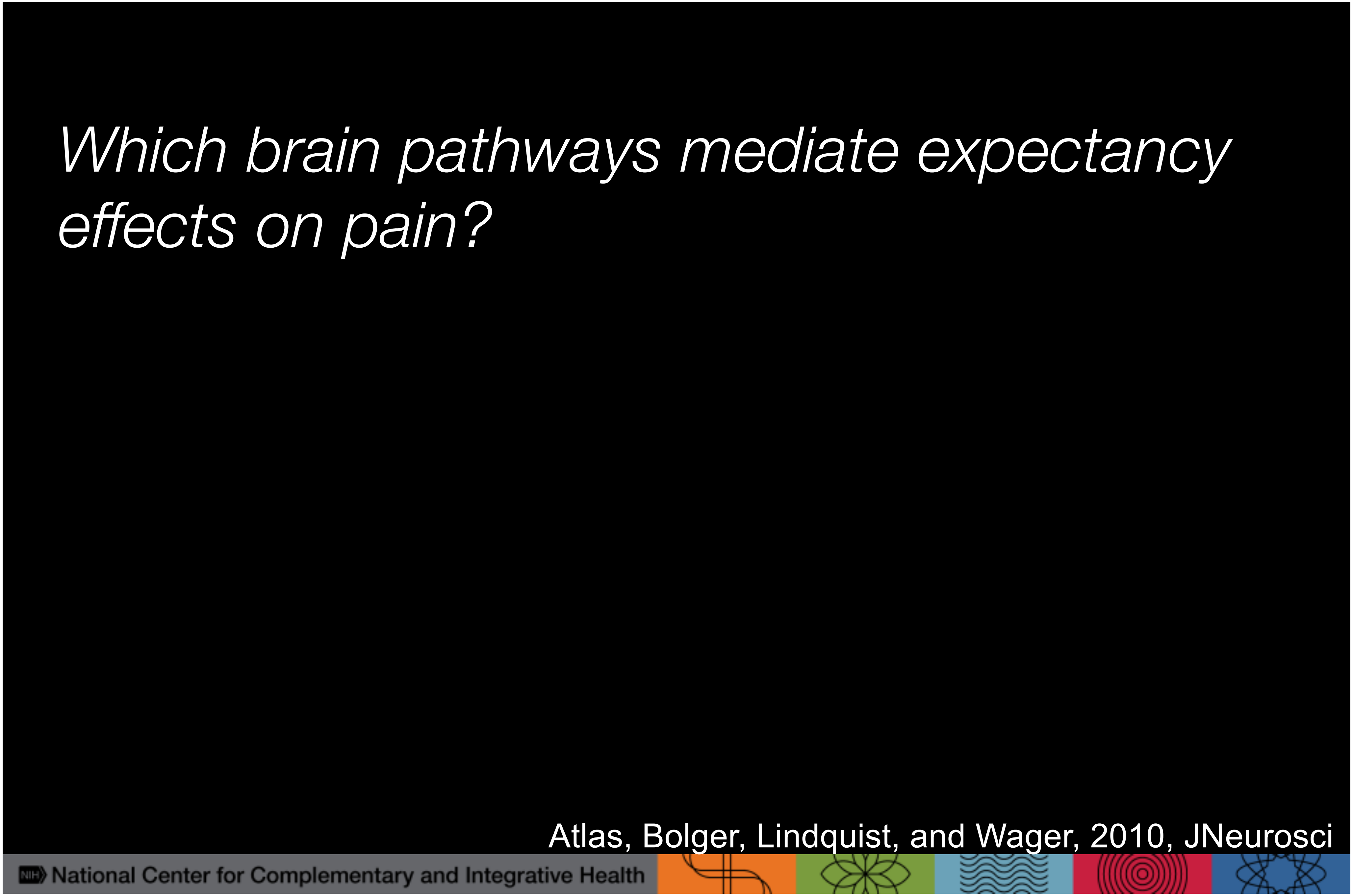
Study 1 Summary

Pain is generated by a combination of independent networks

- Increases with temp + increases predict pain (e.g. SII, “salience network”)
- Decreases with temp + decreases predict pain (e.g. DMN)
- Suppression effects / negative mediators (DMPFC, mOFC)
- Pain-related without responding to temperature (e.g. DLPFC, DMPFC, OFC)

Mediation can help identify regions that link objective stimulus with subjective response

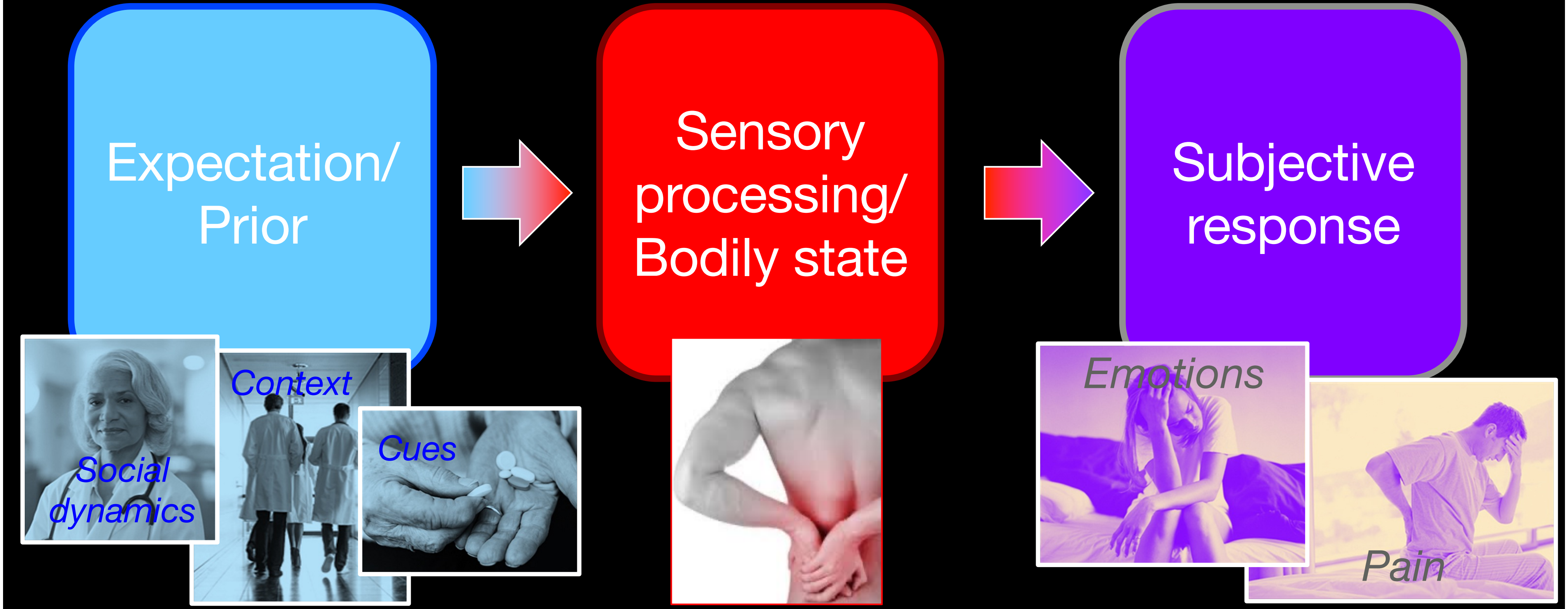
Study 2



Which brain pathways mediate expectancy effects on pain?

Atlas, Bolger, Lindquist, and Wager, 2010, JNeurosci

Working model



Expectation/ Prior

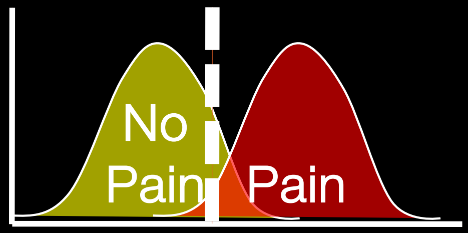
Sensory processing/ Bodily state

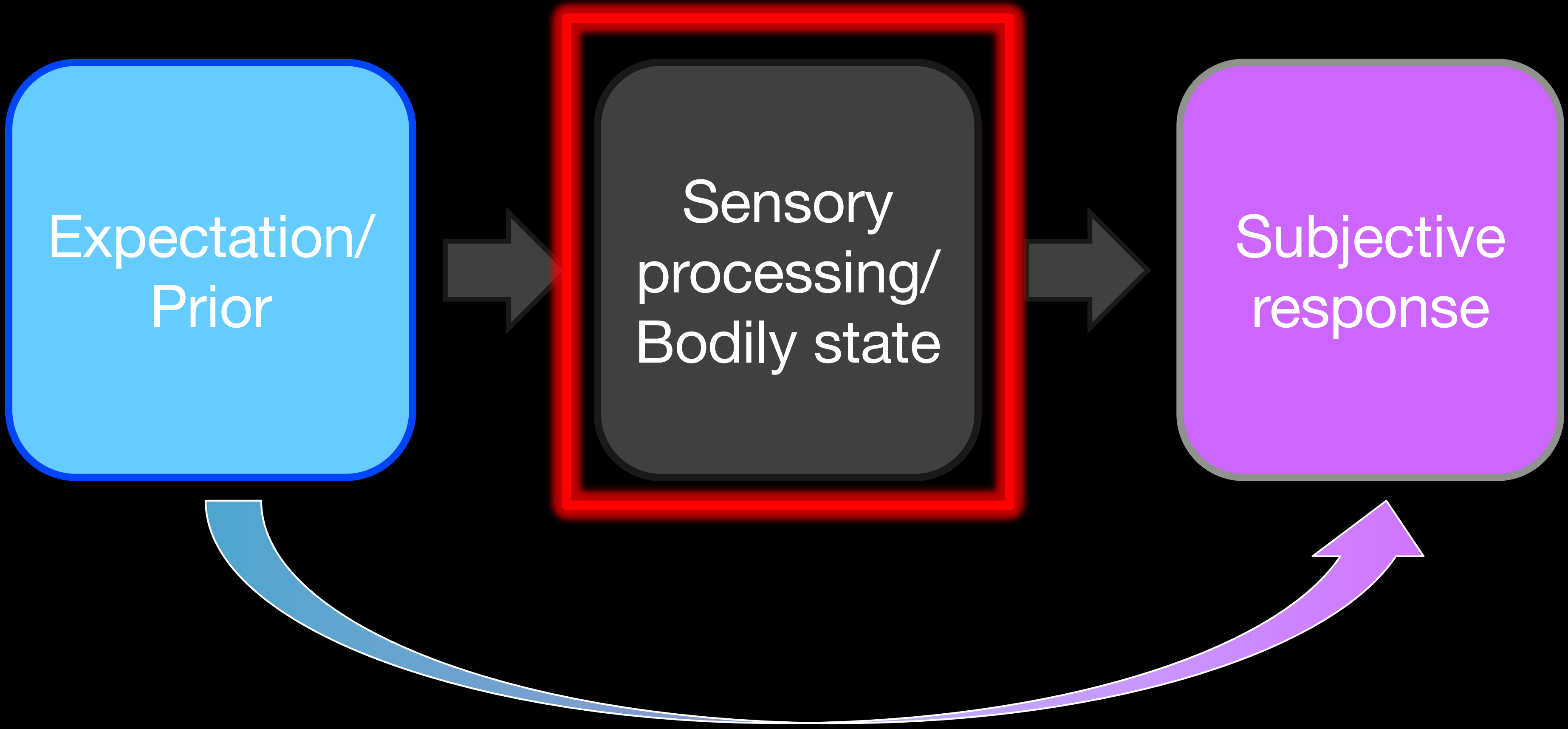
Subjective response

Social dynamics
Context
Cues
Emotions
Pain

Report biases/ Changes in decision-making?

(Hrobjartsson & Gotzsche 2001/04; Allan & Siegel 2002; Clark 2003)

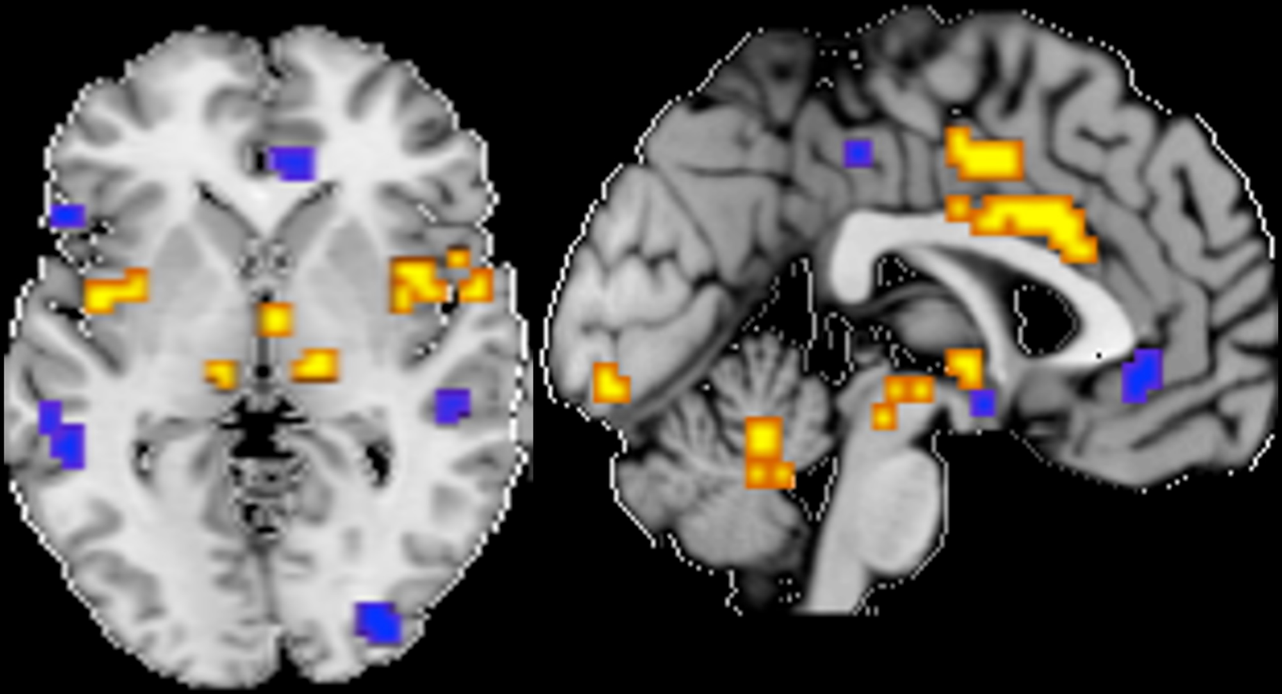



Expectation/ Prior

Sensory processing/ Bodily state

Subjective response

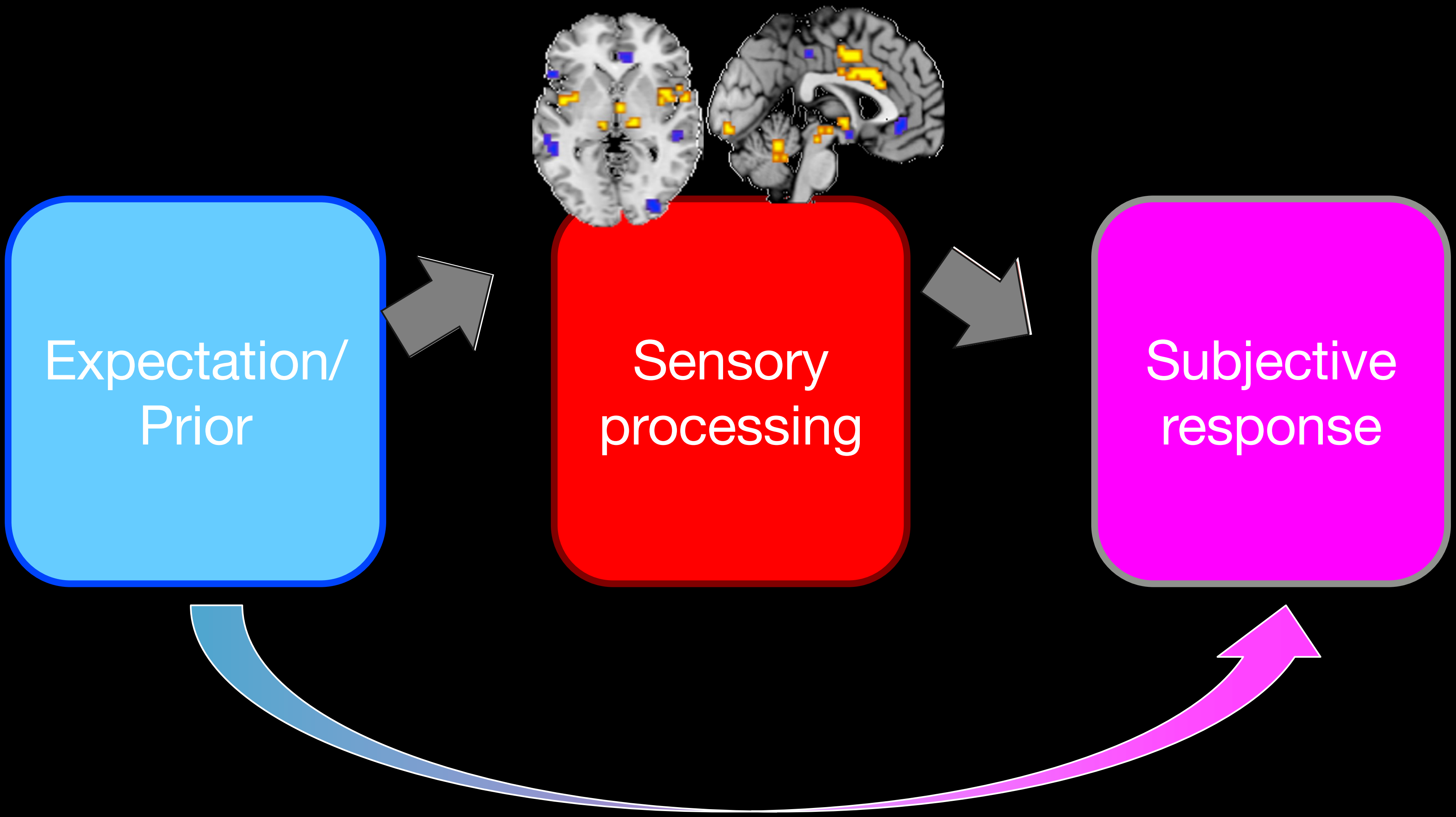
“Neurologic pain signature”



- *Predicts pain in new subjects, in new scanners, in new studies*
- *Highly specific to pain*

Wager, Atlas, et al. (2013), *NEJM*

Do expectations cause changes in pain processing network, and does that give rise to changes in subjective pain?

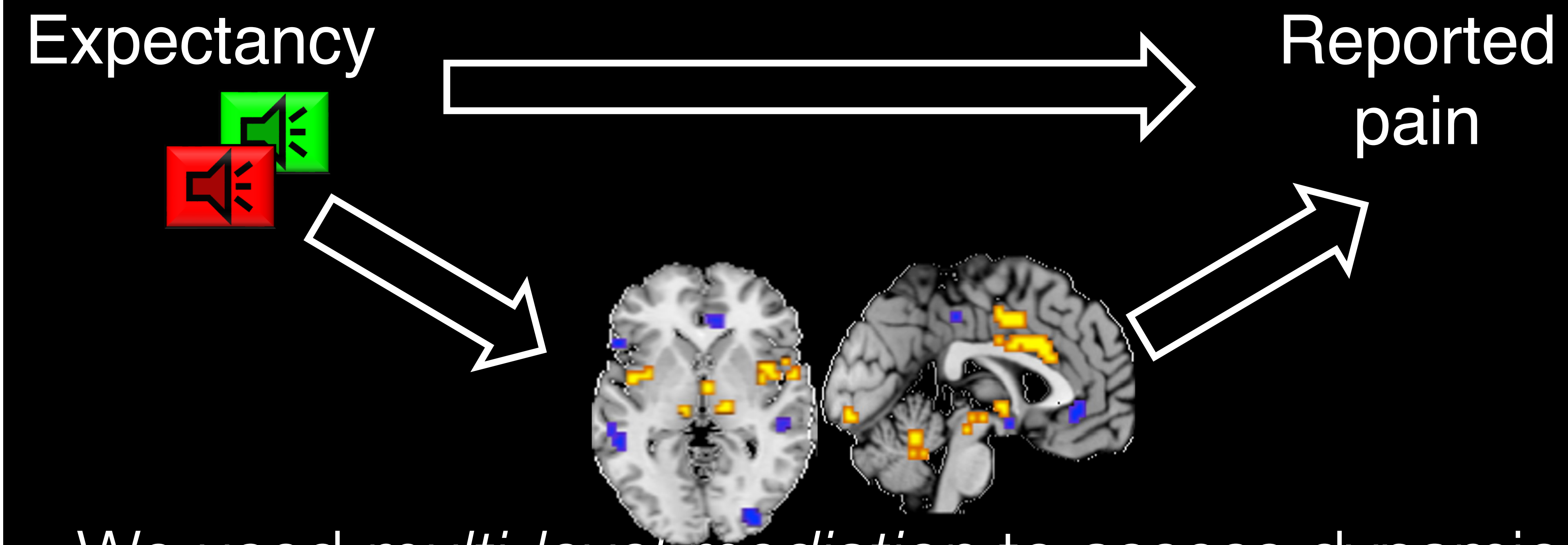


Expectation/ Prior

Sensory processing

Subjective response

Our approach



Expectancy

Reported pain

We used *multi-level mediation* to assess dynamic relationship between trial-by-trial cue-based expectancy, brain activity, and reported pain

Atlas, Bolger, Lindquist, and Wager, 2010, JNeurosci

Expectancy effects on subjective pain

Pain calibration

Level 2
(Slight pain)

Level 5
(Medium pain)

Level 8
(Max pain)

°C
°F
50
40
30
20
10
0
10
20
30
120
100
80
60
40
32
20
0
20

Verbal instructions

🔊 "Low Pain"

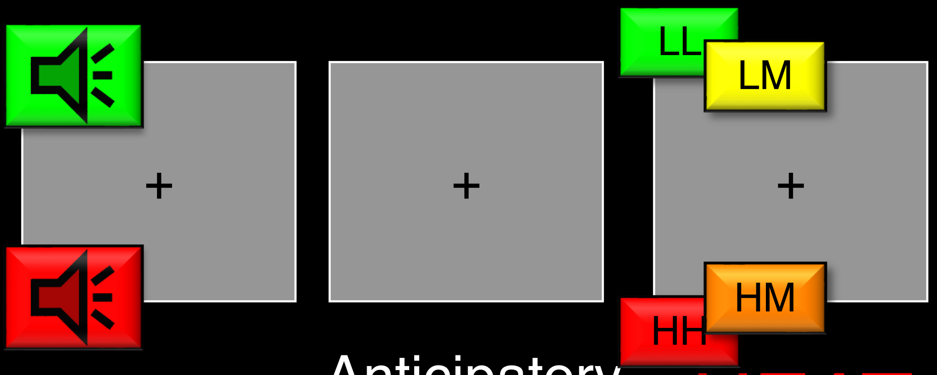
(Counterbalanced)

🔊 "High Pain"

Atlas, Bolger, Lindquist, and Wager, 2010, JNeurosci

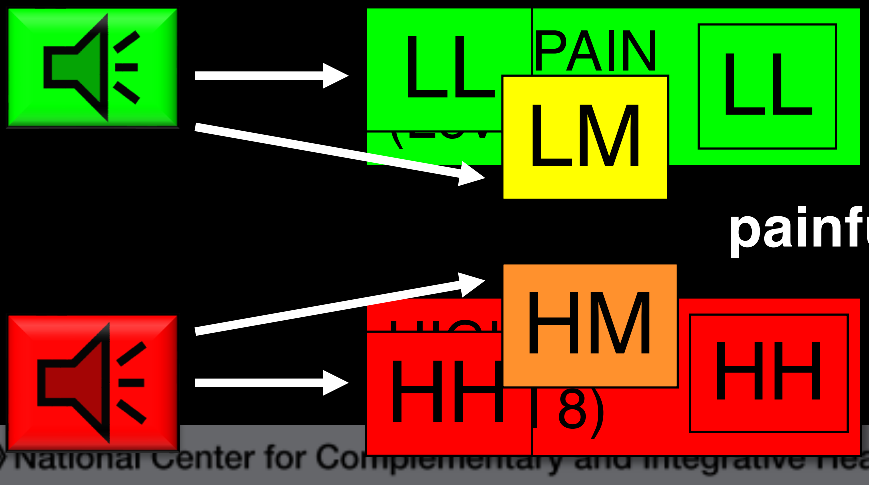
Expectancy paradigm

EXPERIMENTAL PHASE



Anticipatory delay

HEAT

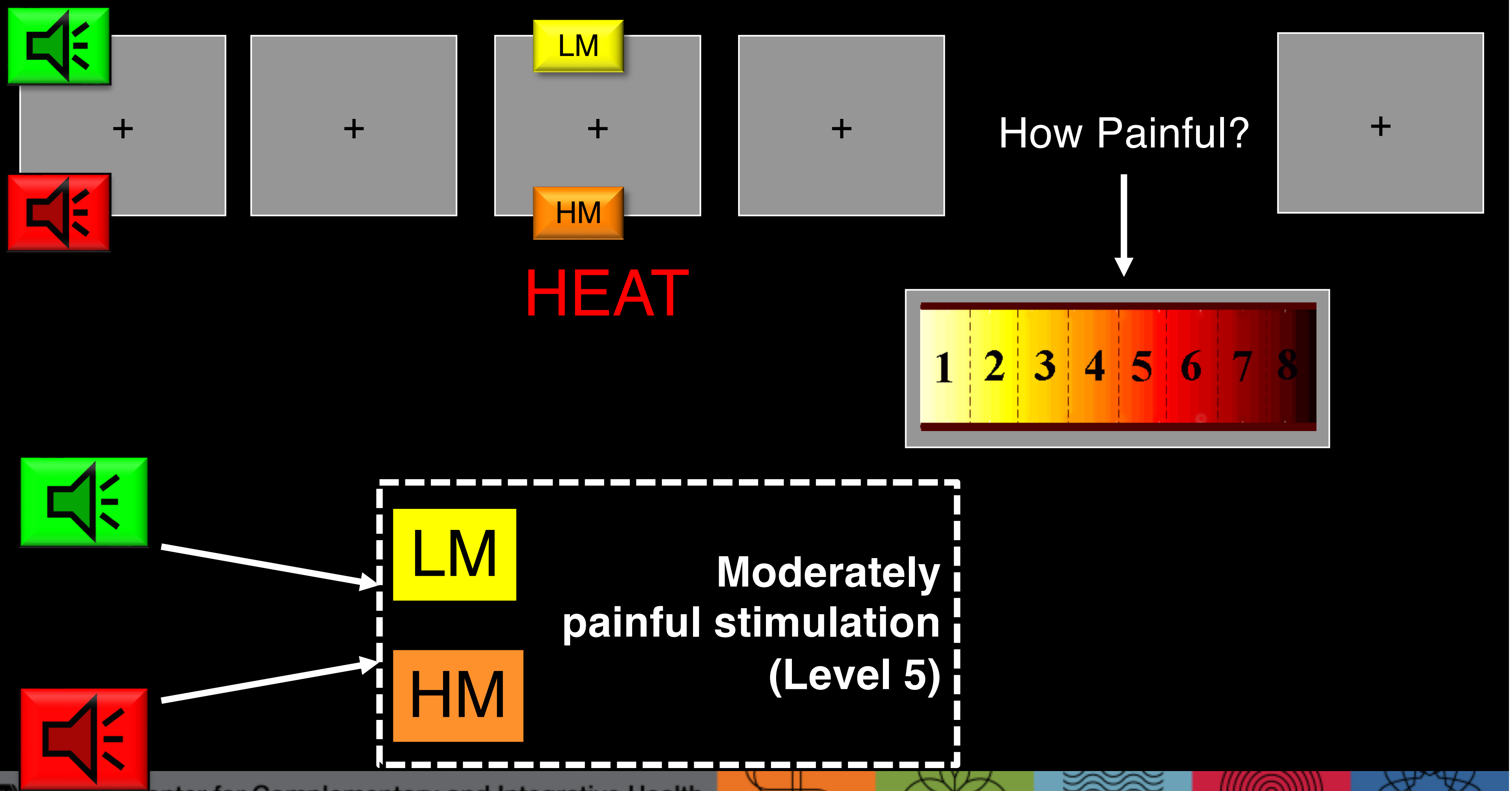

LL
PAIN
LL
LM
HM
HH
8)
HH

Moderately painful stimulation (Level 5)

Six Experimental Runs

Expectancy paradigm

EXPERIMENTAL PHASE



LM

HM

HEAT

How Painful?

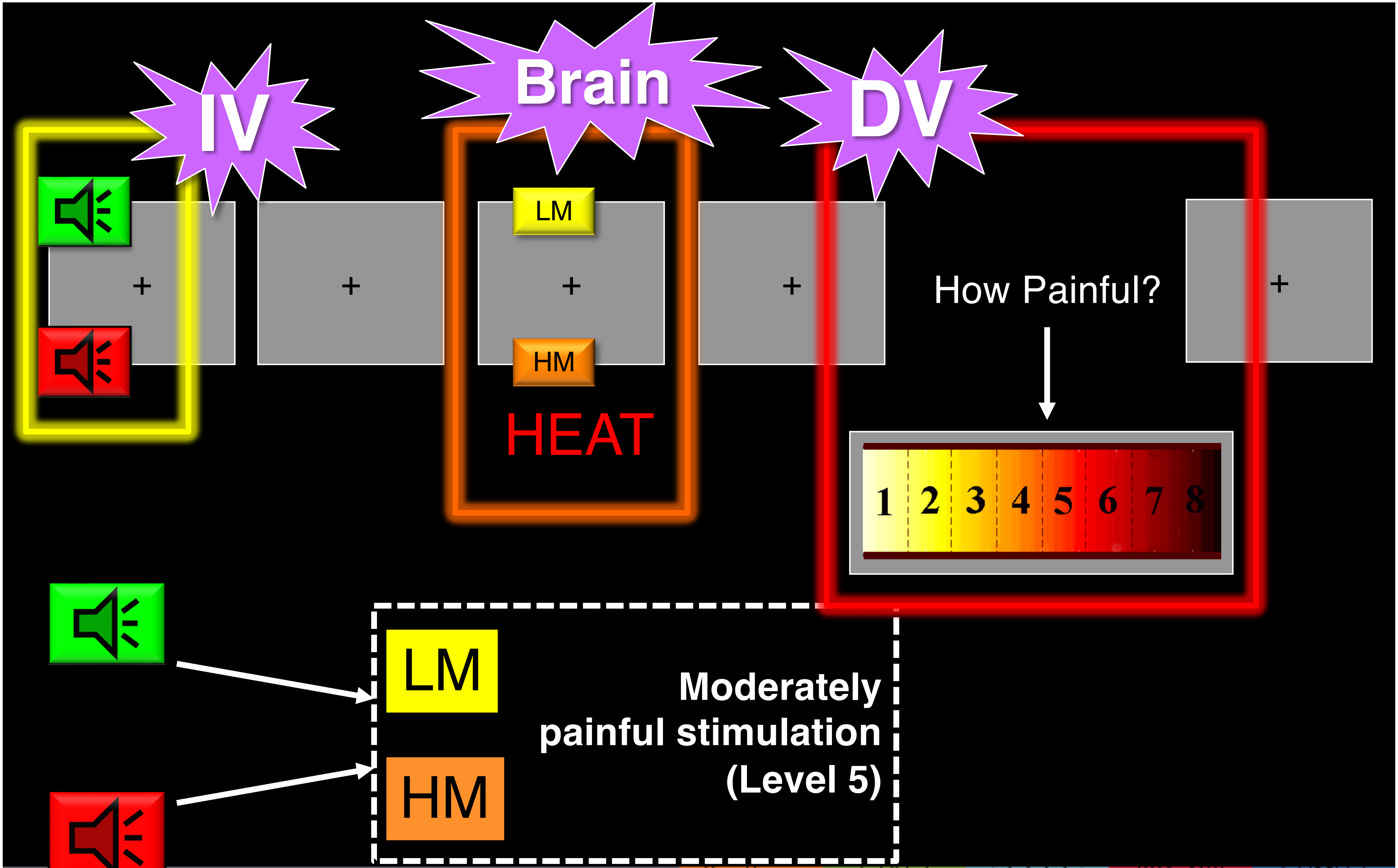
1 2 3 4 5 6 7 8

LM

HM

Moderately painful stimulation (Level 5)

Expectancy paradigm


IV
Brain
DV
+
+
LM
+
HM
+
1
2
3
4
5
6
7
8
+

HEAT

How Painful?

LM

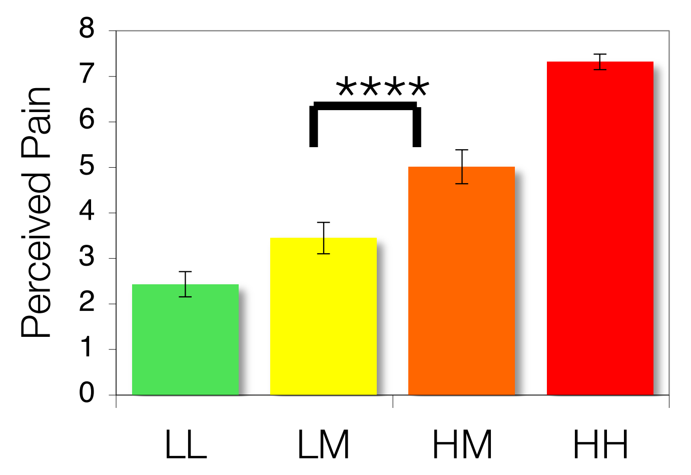
HM

Moderately painful stimulation (Level 5)

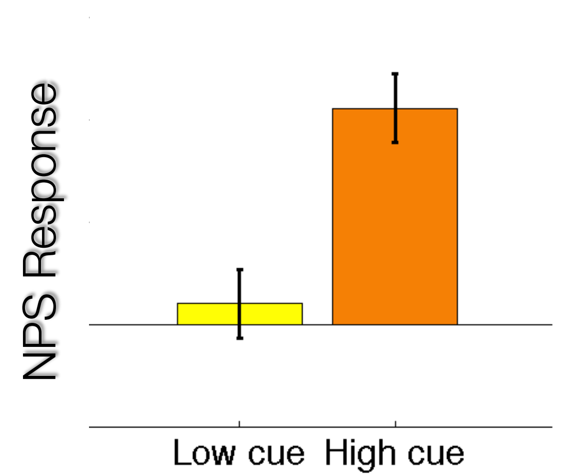
RESULTS

Atlas et al., 2010, *JNeurosci*

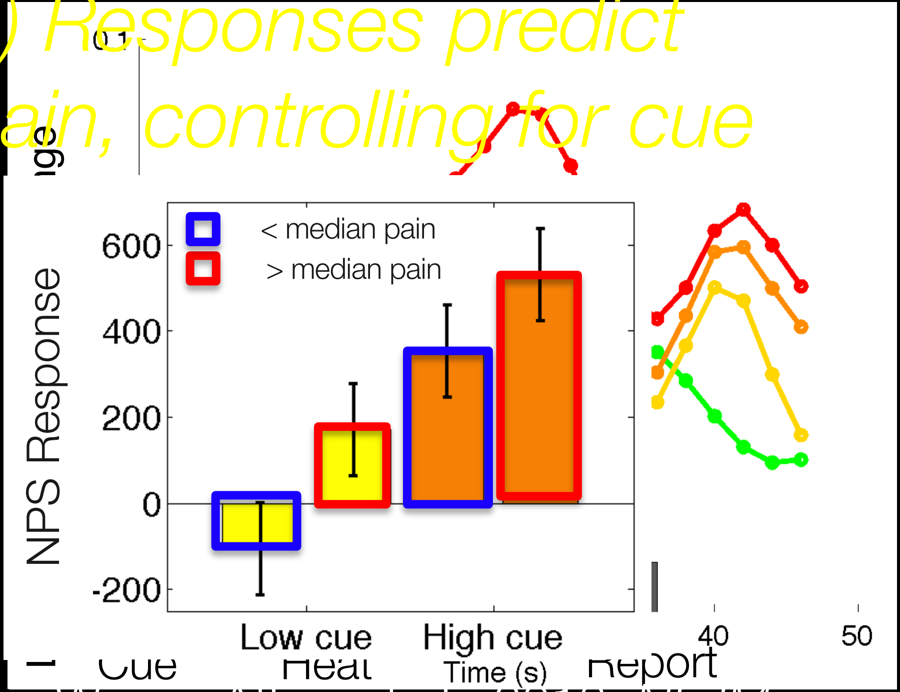
c) Cue-based expectations shape pain



a) Expectations shape responses to heat (HM > LM)

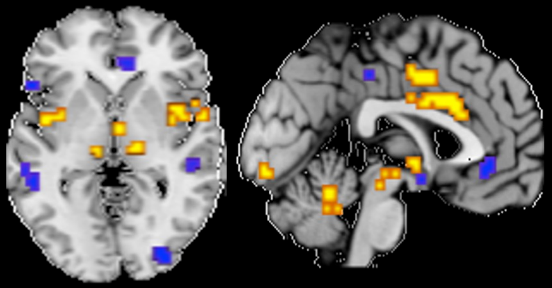


b) Responses predict pain, controlling for cue



Wager, Atlas, et al., 2013, *NEJM*

c-c') Pain network responses mediate cue effects on pain.



Neurologic Pain Signature (NPS)

Atlas et al., 2010, *JNeurosci*

Study 2 summary

Cue-based expectancy effects on pain are mediated by pain-related regions

- Voxelwise analyses (Atlas et al., 2010) reveal other mediating networks as well

Mediation analysis can identify mediators of IV effects on DV

Mediation for effective connectivity

- Cues -> VS and OFC -> Pain-related mediators

Summary

Why use multilevel mediation:

1. Relate independent and dependent variables
2. Test hypothesized pathways
3. Relate individual differences to within-subjects pathway strength

Summary

Consider inferences:

Stronger inferences about directionality if variables are separated in time

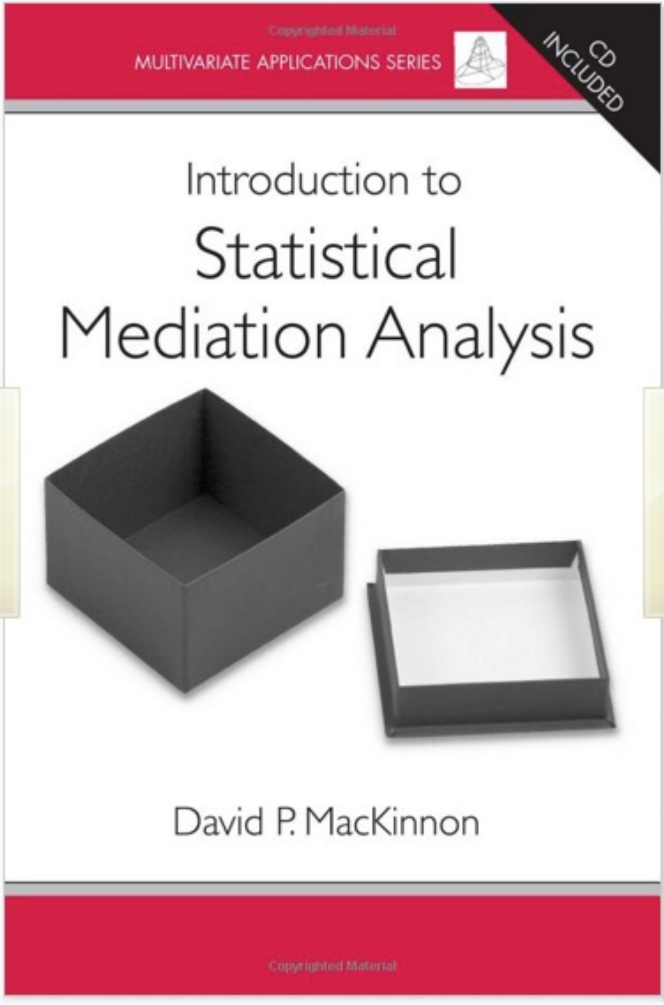
- E.g. Cue -> Anticipation -> Pain -> Report

Strongest inference when you can experimentally manipulate X ***AND*** *M (e.g. using TMS)*

- see Holland, Rubin

Resources

http://wagerlab.colorado.edu/tools


Copyrighted Material

MULTIVARIATE APPLICATIONS SERIES

CD INCLUDED

Introduction to

Statistical Mediation Analysis

David P. MacKinnon

Copyrighted Material

Mediation analysis:

Baron & Kenny, 1986, JPSP

Shrout & Bolger, 2002,
Psychological Methods

Kenny, Korchmaros & Bolger 2003,
Psychological Methods

MEPM:

Wager et al., 2008, Neuron

Wager et al., 2009, Neuroimage

Atlas et al., 2010, JNeuro

Thank you.

Tor Wager

Univ of Colorado, Boulder

Psychology & Neuroscience

Niall Bolger

Columbia

Psychology

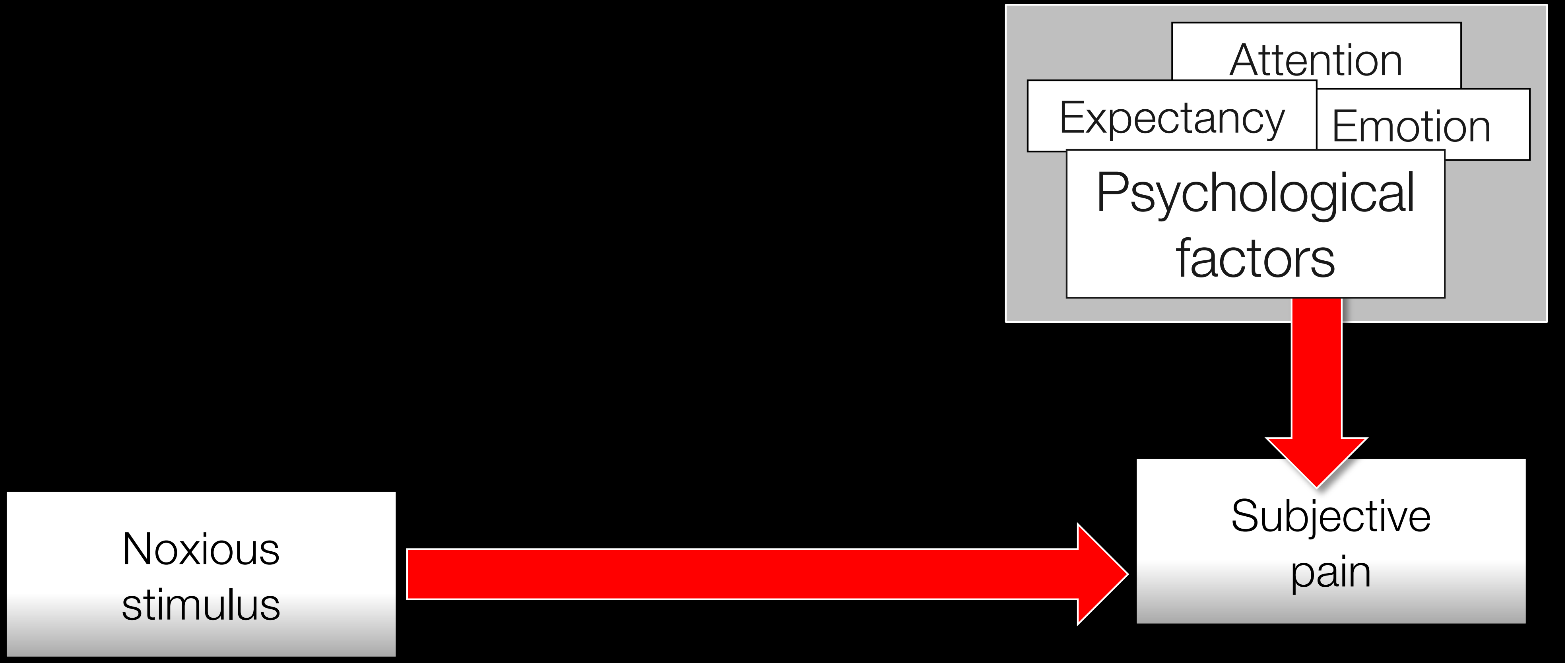
Martin Lindquist

Johns Hopkins

Biostatistics

Affective
Neuroscience &
Pain Lab

NIH National Center for Complementary and Integrative Health



Attention

Expectancy

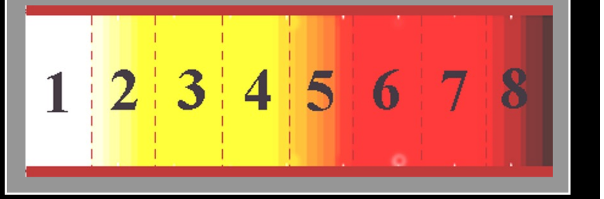
Emotion

Psychological factors

Noxious stimulus

Subjective pain

How Painful?



Attention

Expectancy

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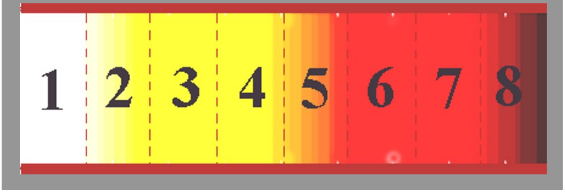
Psychological factors

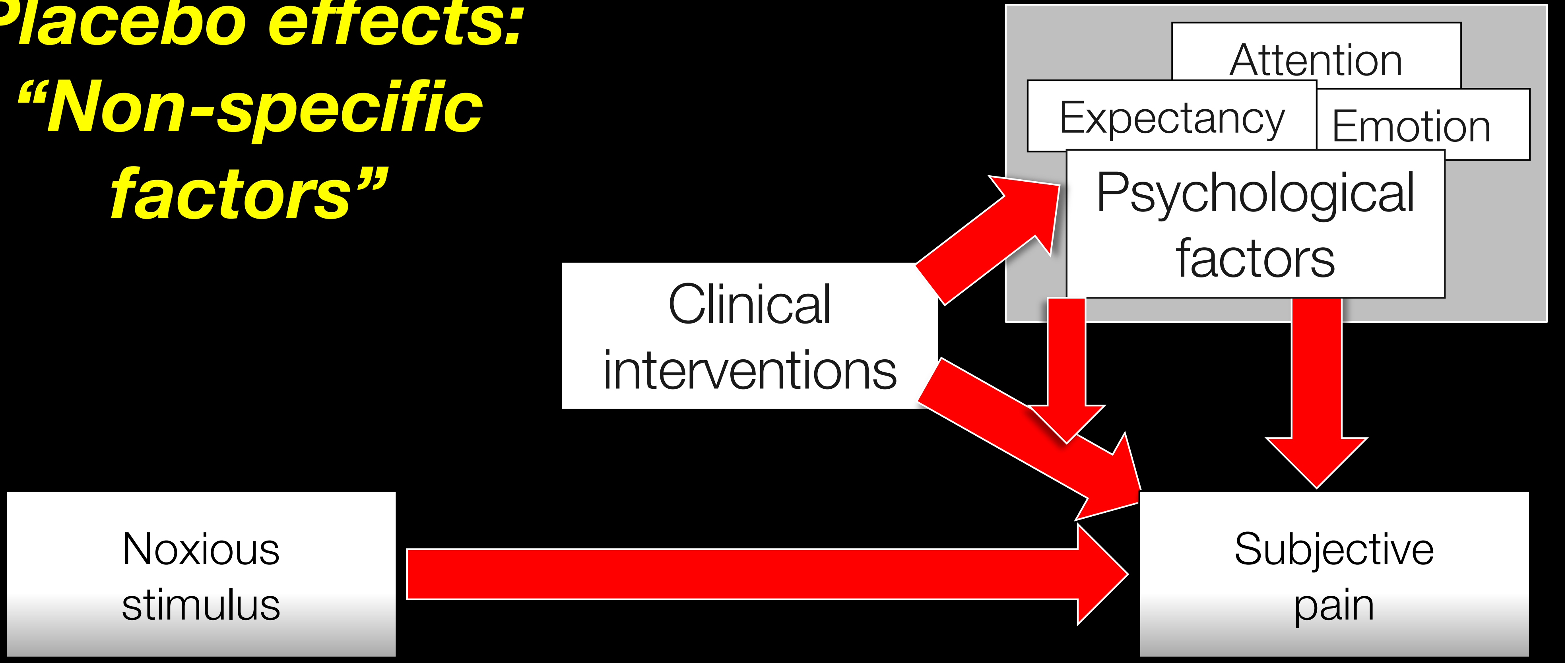
Noxious stimulus

Subjective pain

How Painful?



Placebo effects: "Non-specific factors"



Attention

Expectancy

Emotion

Psychological factors

Clinical interventions

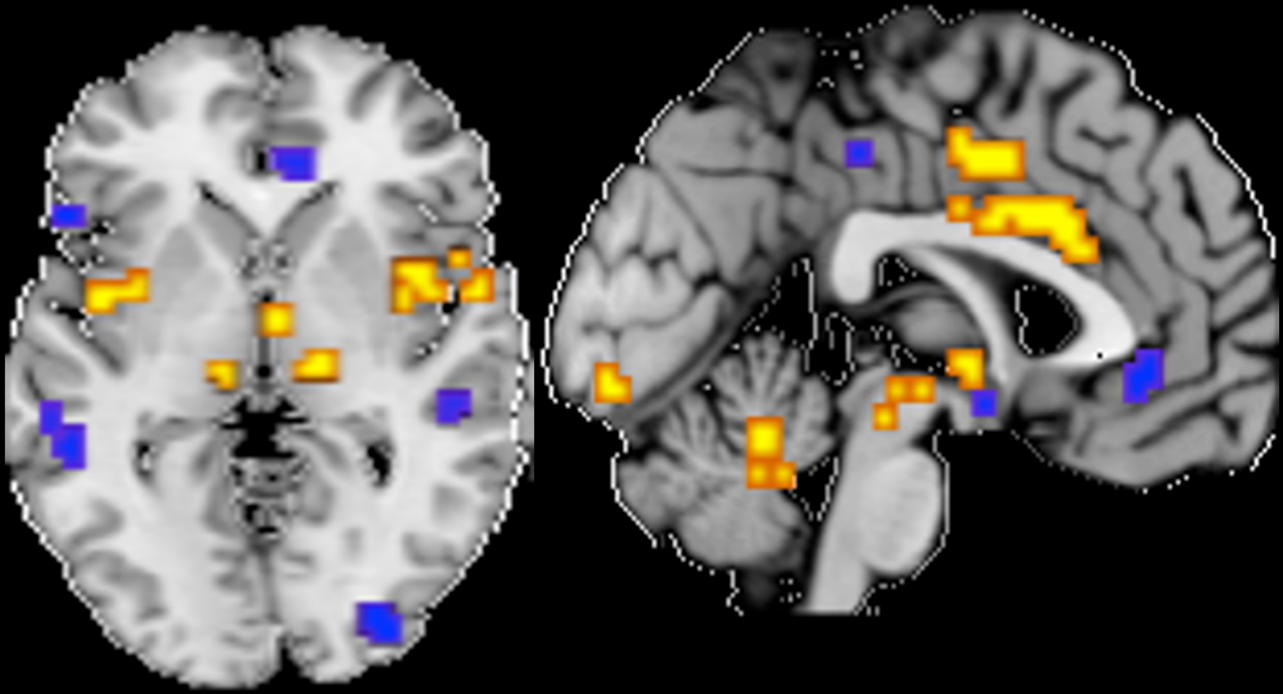
Noxious stimulus

Subjective pain

~116 million American adults affected by chronic pain (Institute of medicine of the National Academies, 2011)

Estimated cost of medical treatment + lost work due to pain
= $635 billion/yr

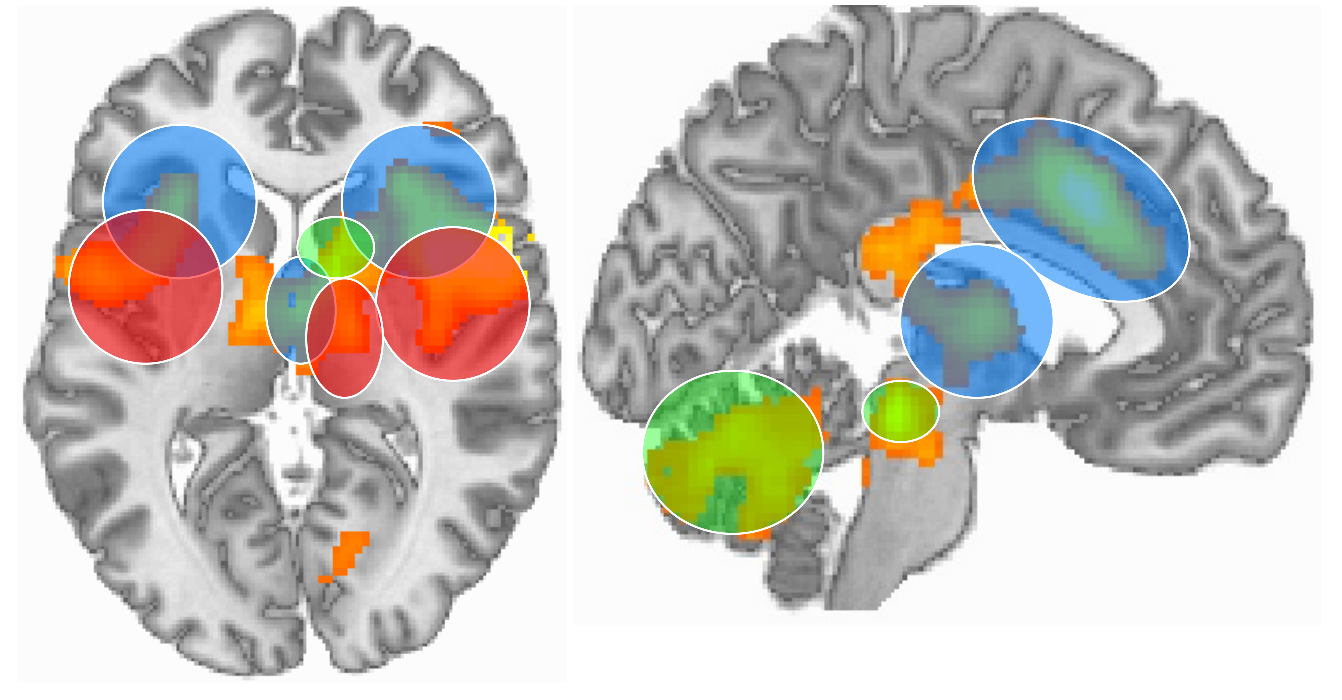
“Neurologic pain signature”



- *Predicts pain in new subjects, in new scanners, in new studies*
- *Highly specific to pain*

Wager, Atlas, et al. (2013), *NEJM*

“The pain matrix”



Sensation

Affect

Lateral thalamus
SI
SII
Posterior Insula

Medial thalamus
Anterior cingulate
Anterior insula

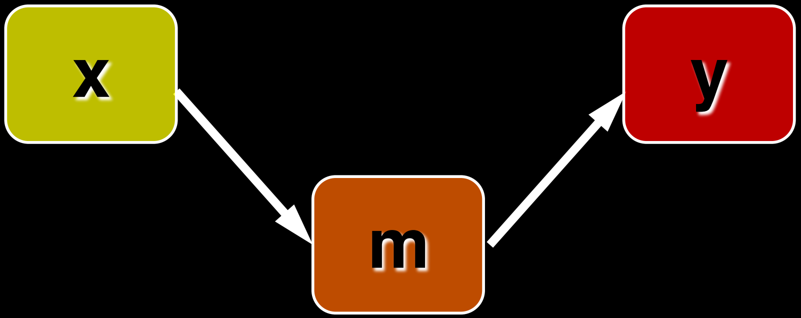
PAG
Cerebellum
Striatum

High vs Low intensity stimulation
Five studies, N = 114
FWE, p<.05

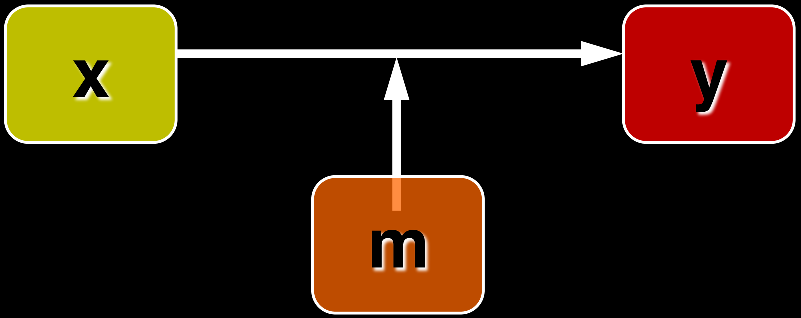
Atlas et al. (2010), *JNeurosci*

Moderation

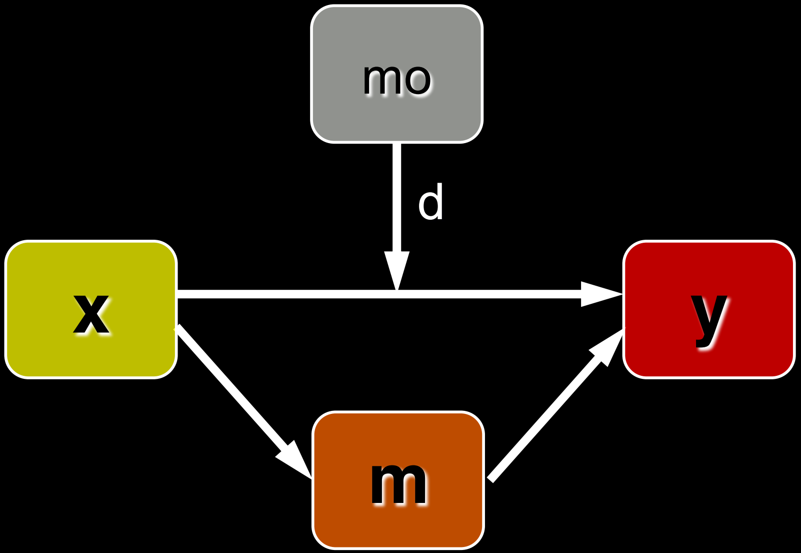
Mediation: does [M] explain some or all of the relationship btwn [X] and [Y]?



Moderation: does the level of [M] influence the relationship btwn [X] and [Y]?



Full model, with mediator and moderator



$$m = i_m + ax + e_m$$

$$y = i_y + bm + c'x + d(mo^*x) + e'_y$$

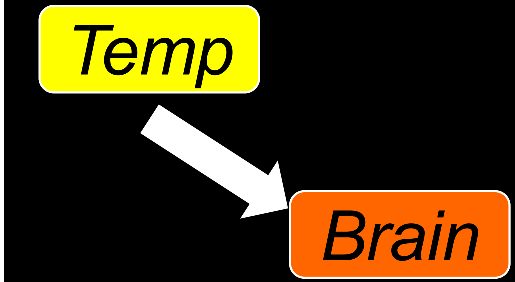
Moderator: Level of mo predicts x-y covariance

Mo*x interaction

Should center *x* and *y* to reduce correlation between moderation and *x* effects

Slide from Tor Wager

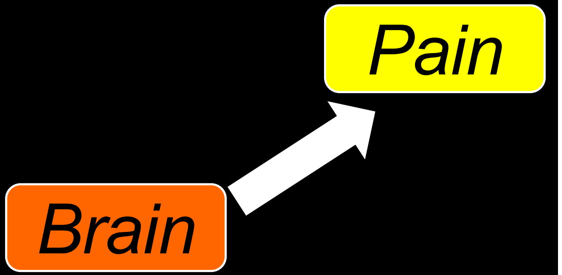
Path a



Temp

Brain

Path b



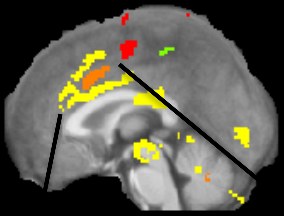
Brain

Pain

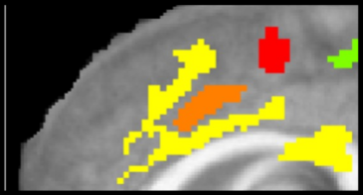
🟨 🟩 Path *a* (temperature), not *b*

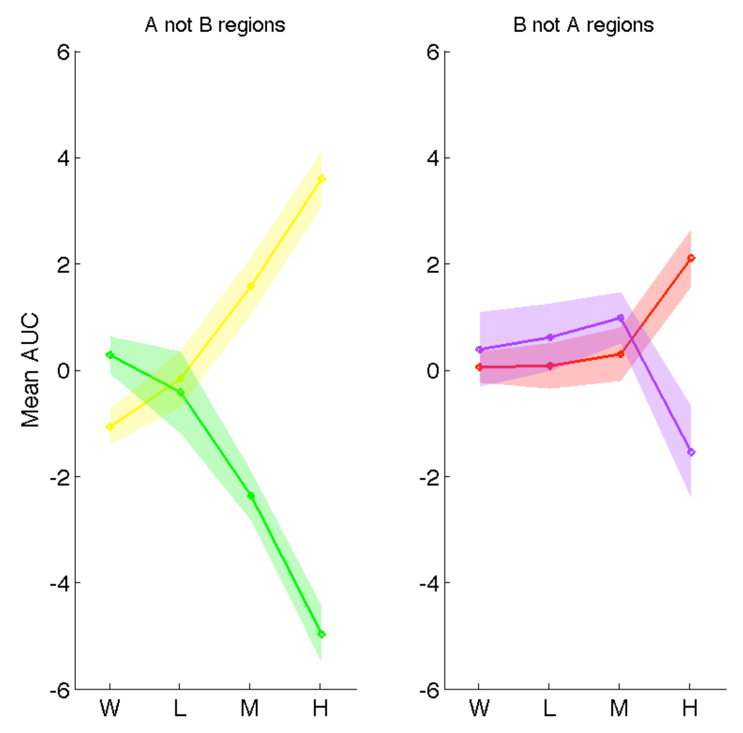
🟧 Mediator, *a* and *b*

🟥 🟪 Path *b* (pain report), not *a*

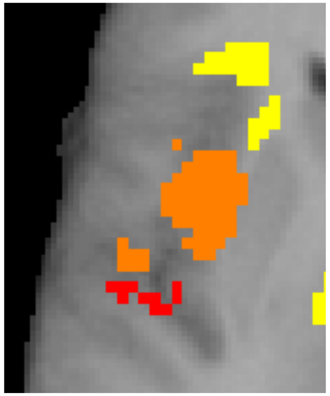


ACC



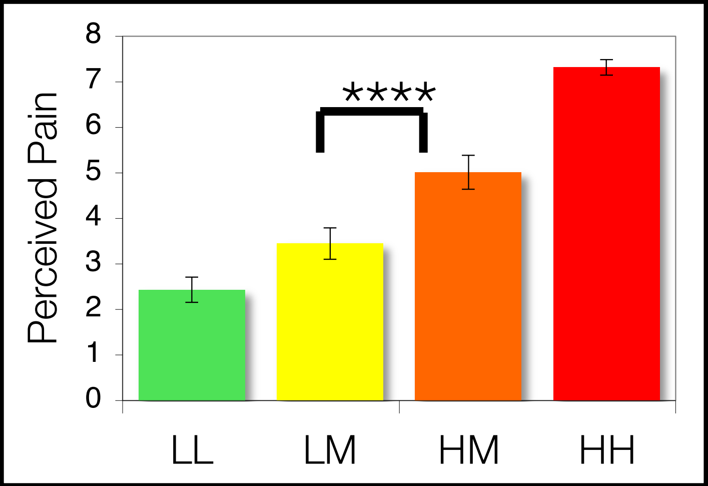


L. Insula

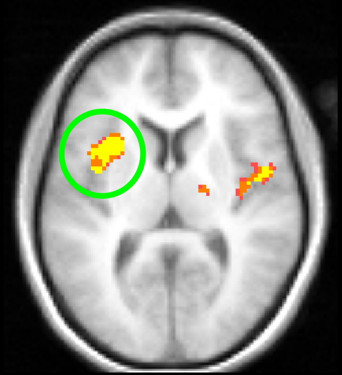


	+	-
p < .001	🟨	🟦
p < .005	🟧	🟦
p < .01	🟥	🟦

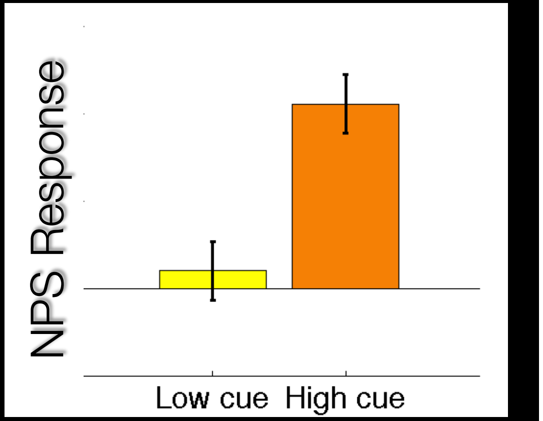
c) Cue-based expectations shape pain



a) Expectations shape responses to heat (HM > LM)

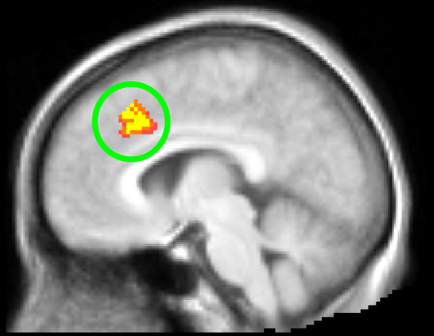


SII, insula, thalamus, dACC, Pre-SMA

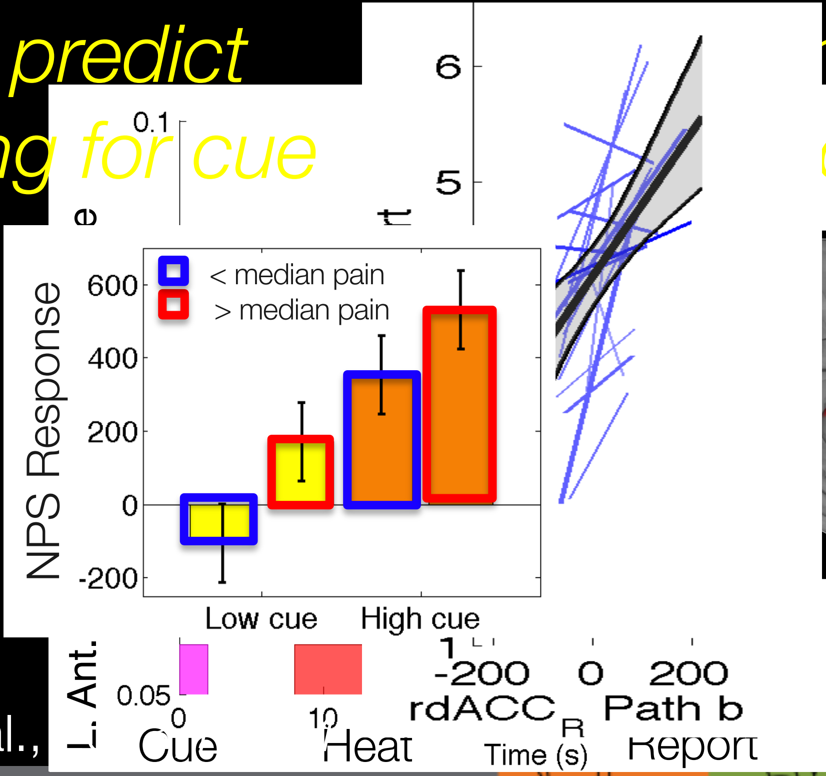


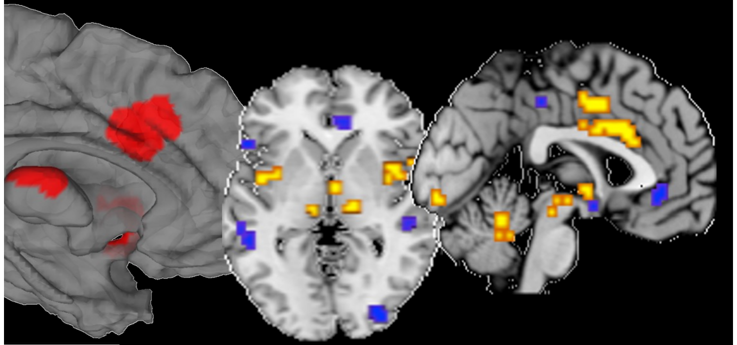
b) Responses predict pain, controlling for cue

n network responses cue effects on pain.



rdACC, insula, SMA, cerebellum



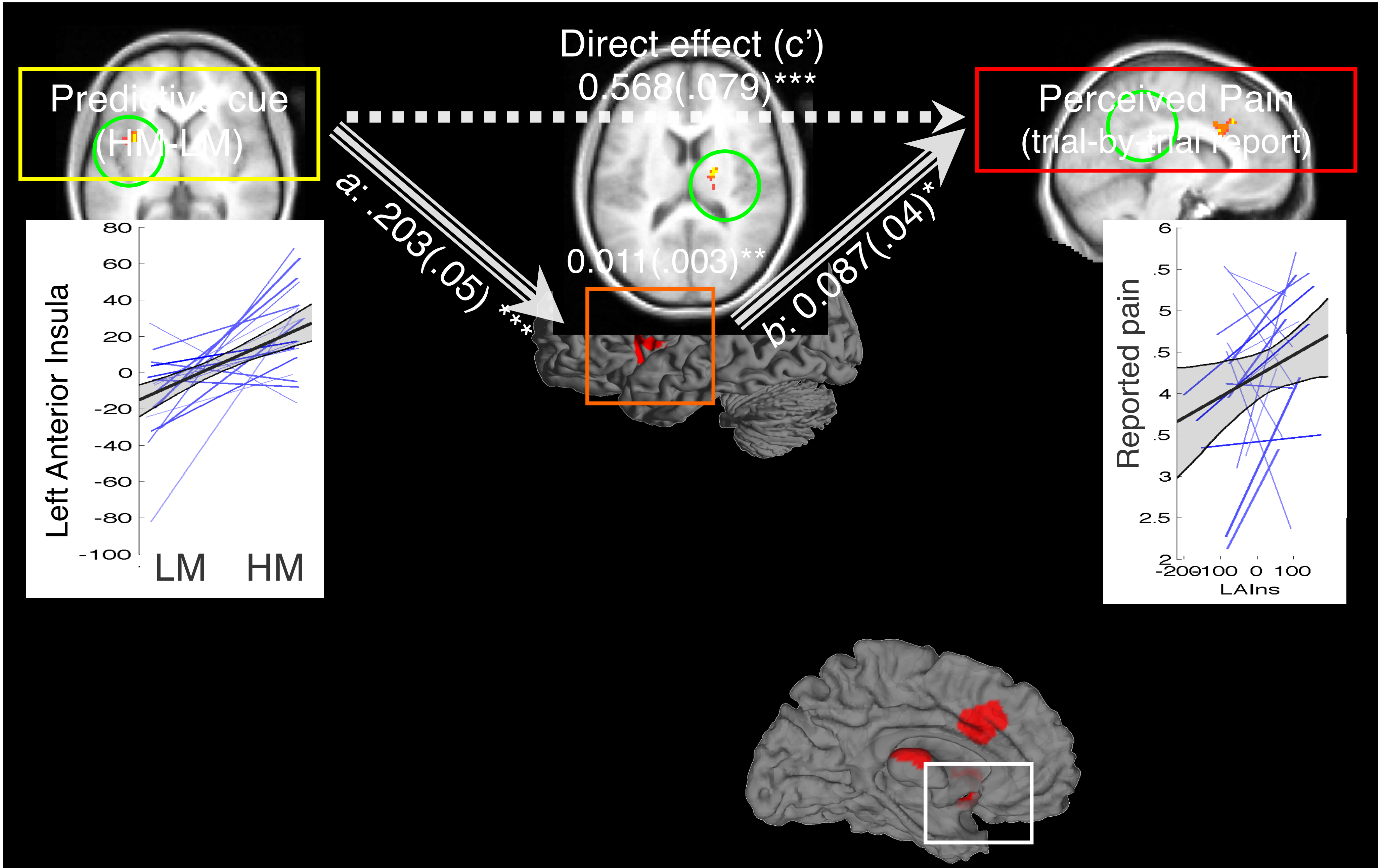


s,

ogic Pain Signature (NPS)

Wager, Atlas, et al.,

tlas et al., 2010, *JNeurosci*



Predictive cue (HM-LM)

Direct effect (c')
0.568(.079)***

Perceived Pain (trial-by-trial report)

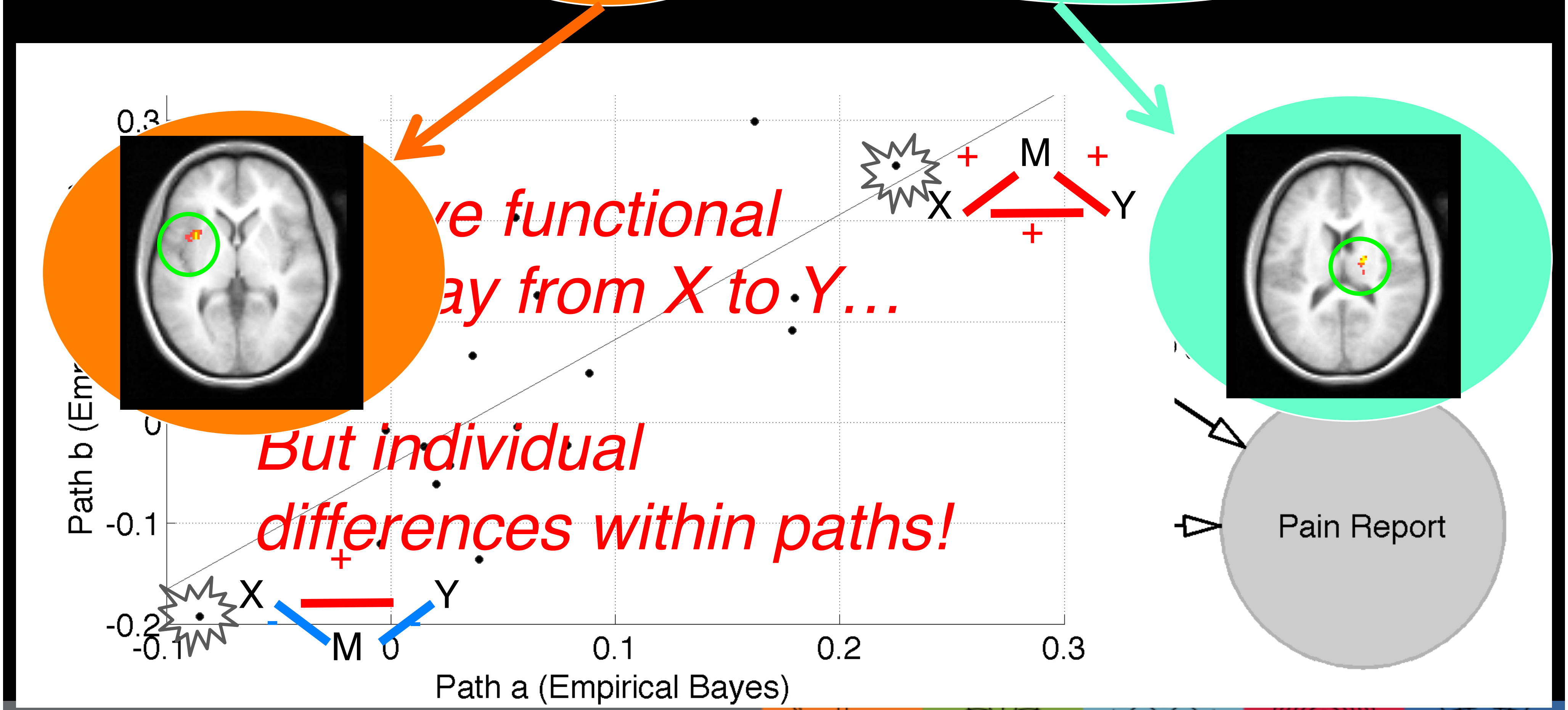
a: .203(.05)***

0.011(.003)**

b: 0.087(.04)*

Covariance and multi-level mediation

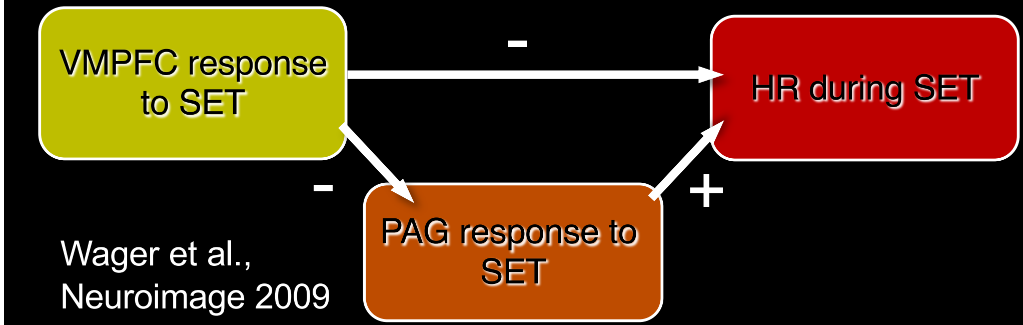
$$c - c' = a*b + \mathrm{cov}(a,b)$$



ve functional
ay from X to Y…

But individual
differences within paths!

Multilevel mediation and functional connectivity


VMPFC response to SET

-

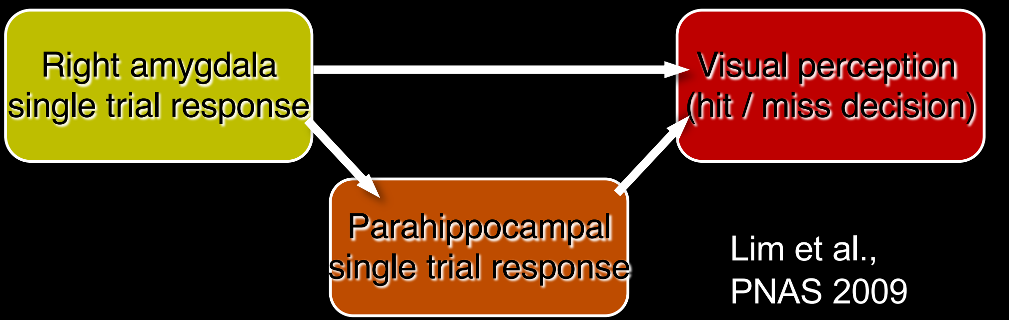
HR during SET

-

PAG response to SET

+

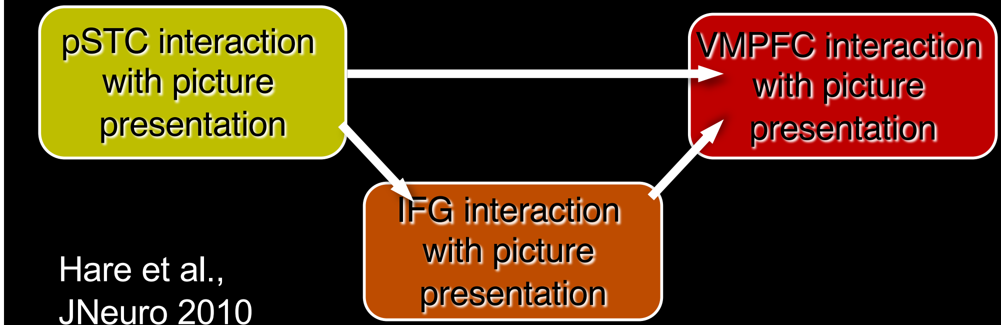
Wager et al., Neuroimage 2009


Right amygdala single trial response

Visual perception (hit / miss decision)

Parahippocampal single trial response

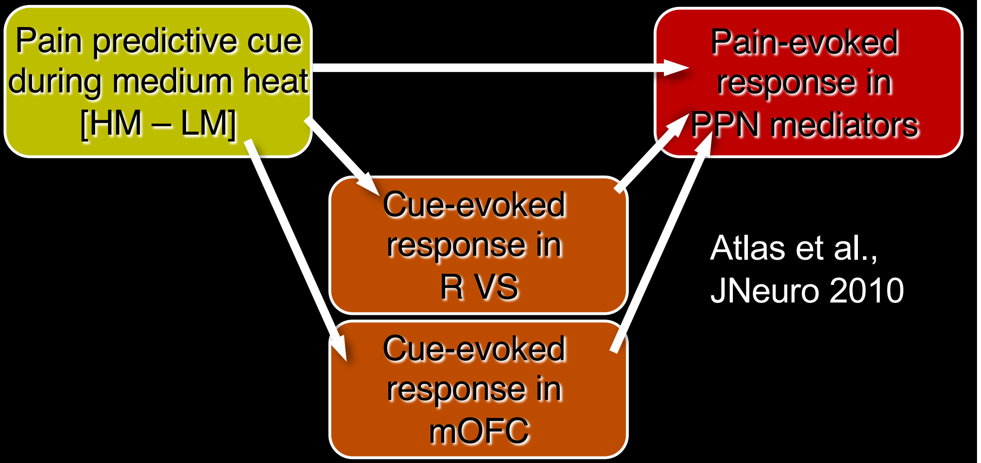
Lim et al., PNAS 2009


pSTC interaction with picture presentation

VMPFC interaction with picture presentation

IFG interaction with picture presentation

Hare et al., JNeuro 2010


Pain predictive cue during medium heat [HM – LM]

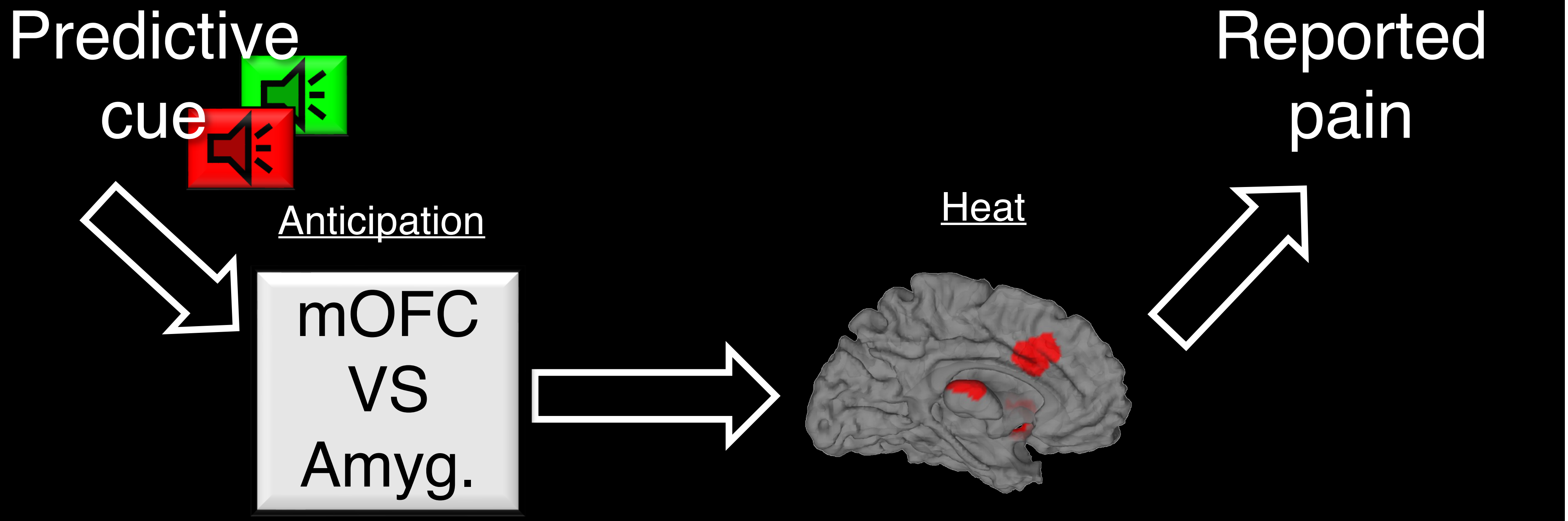
Pain-evoked response in PPN mediators

Cue-evoked response in R VS

Cue-evoked response in mOFC

Atlas et al., JNeuro 2010

How do expectancy effects on the PPN emerge?


Predictive cue
Anticipation
mOFC
VS
Amyg.
Heat
Reported pain

1. Expectations change pain value

(Affective value model)

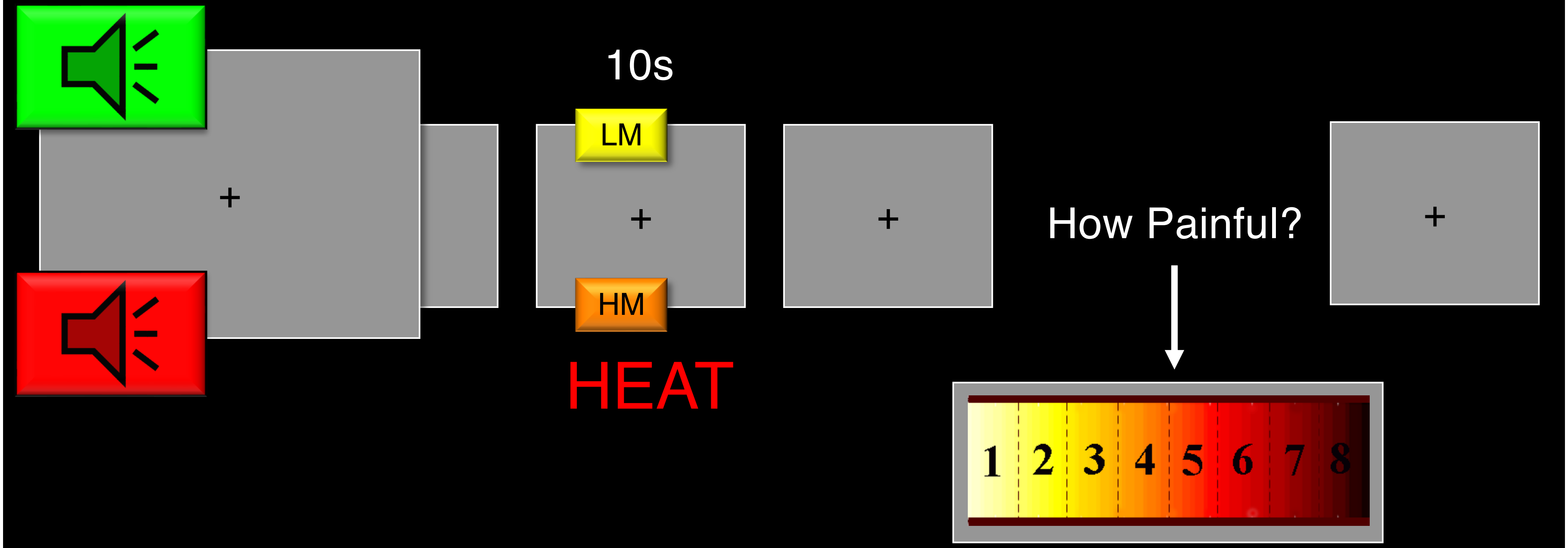
Fields 2007,
Leknes & Tracey 2008

2. Expectations inhibit responses

(Regulation model)

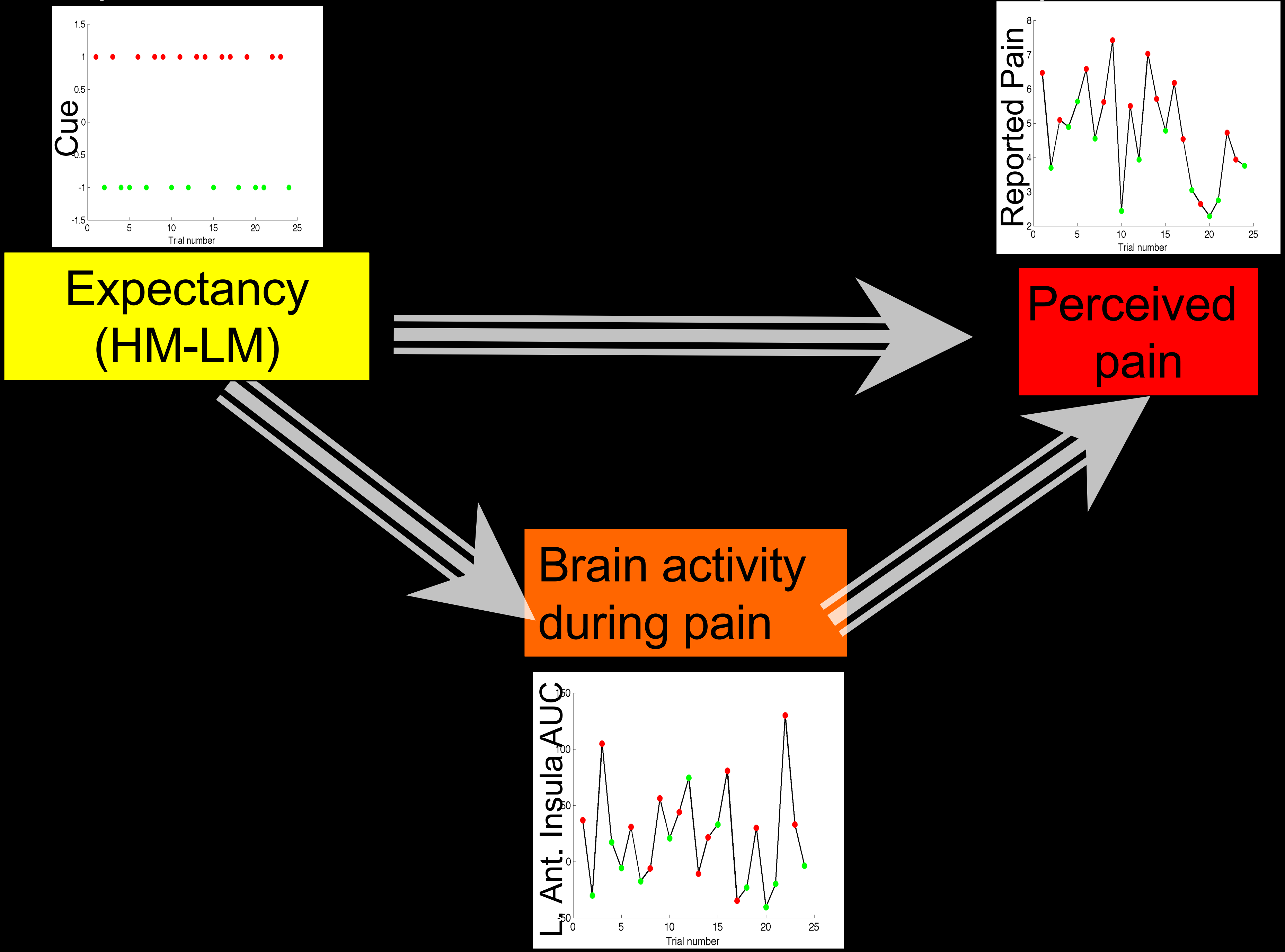
Lieberman 2004,
Wager 2005,
Wiech Ploner & Tracey 2008

Analysis 2: Cue-evoked responses


+
10s
LM
+
HM
HEAT
+
How Painful?
1 2 3 4 5 6 7 8
+

Independent variable

Dependent variable

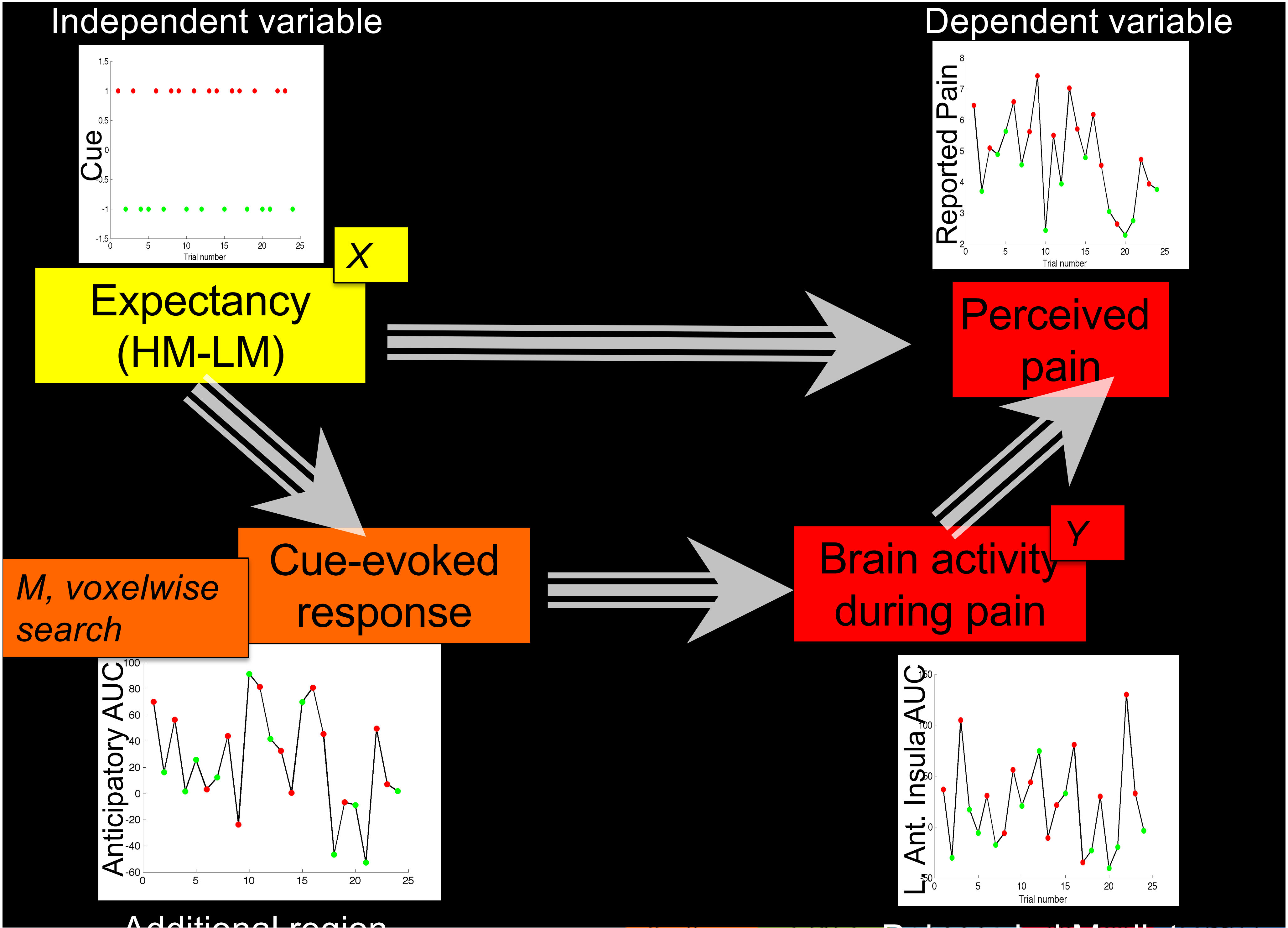

Cue
1.5
1
0.5
0
-0.5
-1
-1.5
0
5
10
15
20
25
Trial number
Reported Pain
8
7
6
5
4
3
2
0
5
10
15
20
25
Trial number
L Ant. Insula AUC
150
100
50
0
-50
0
5
10
15
20
25
Trial number

Expectancy (HM-LM)

Perceived pain

Brain activity during pain

Pain period Mediator


Cue
1.5
1
0.5
0
-0.5
-1
-1.5
0 5 10 15 20 25
Trial number
Reported Pain
8 7 6 5 4 3 2
0 5 10 15 20 25
Trial number
Anticipatory AUC
100 80 60 40 20 0 -20 -40 -60
0 5 10 15 20 25
L. Ant. Insula AUC
150 100 50 0 -50
0 5 10 15 20 25
Trial number

Independent variable

Dependent variable

X

Expectancy (HM-LM)

Perceived pain

Cue-evoked response

M, voxelwise search

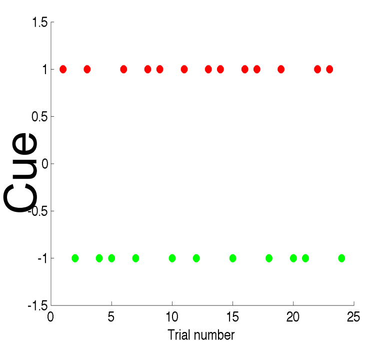
Brain activity during pain

Y

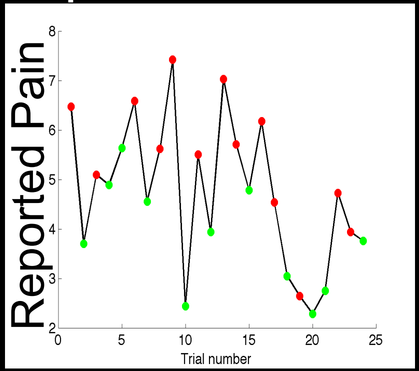
Additional region

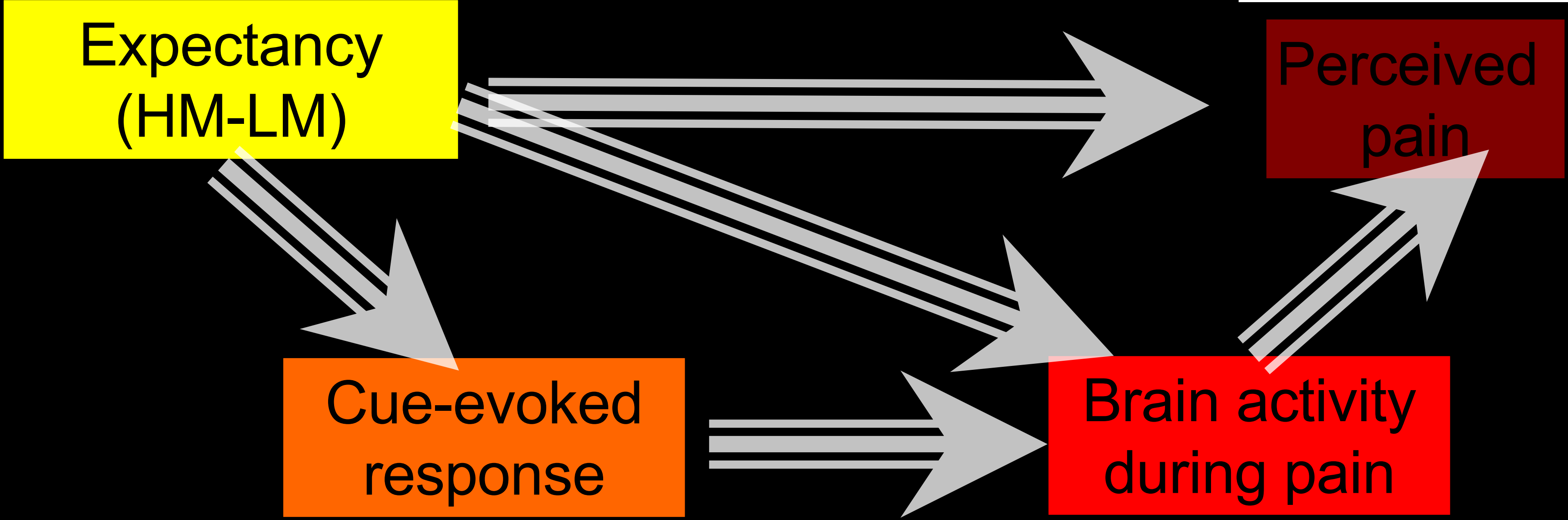
Pain period Mediator

Independent variable



Dependent variable



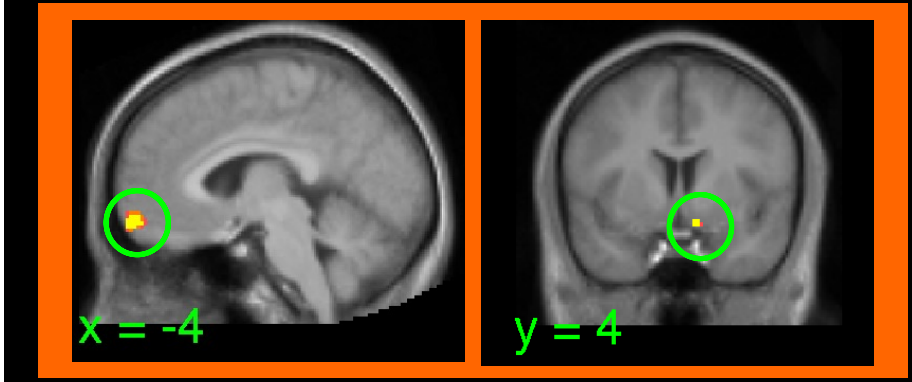


Expectancy (HM-LM)

Perceived pain

Cue-evoked response

Brain activity during pain

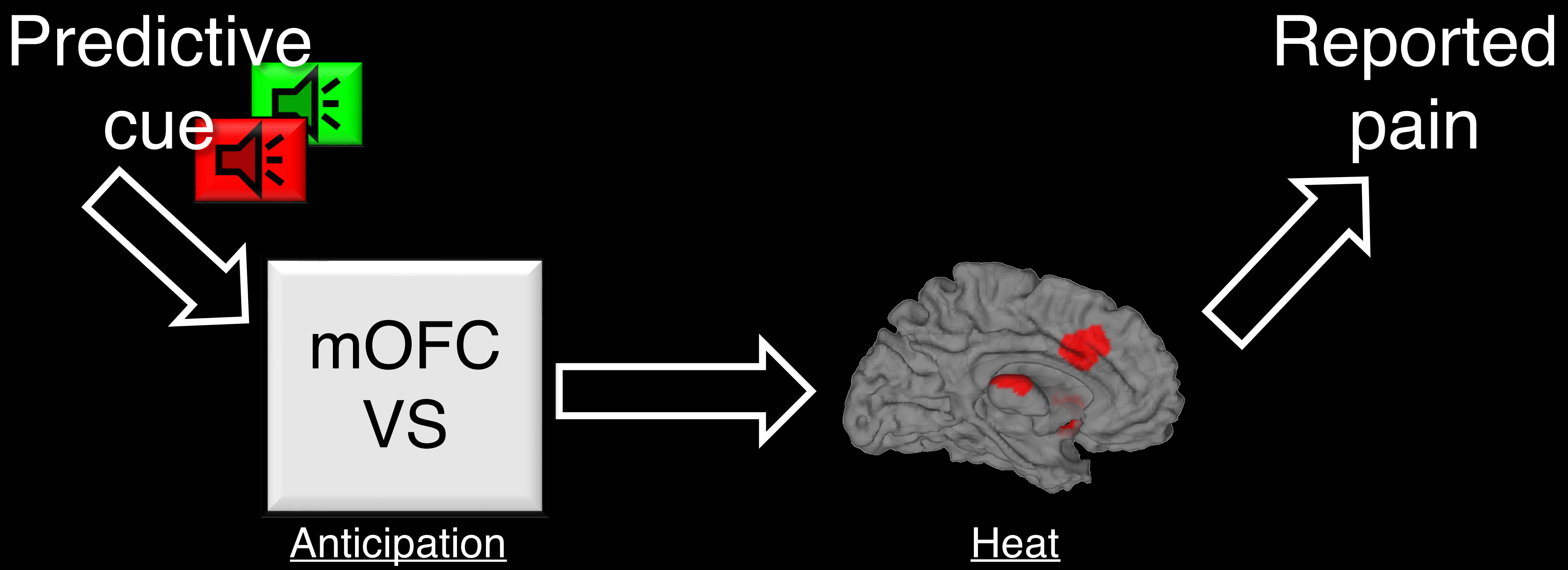


rdACC

L. Ant. Ins

R. Thalamus

Summary


Predictive cue
mOFC
VS
Anticipation
Heat
Reported pain

This suggests that regions involved in evaluating predictive cues and generating value shape PPN responses, which in turn shape subjective experience.

Overview

- Introduction, Single level mediation
- Multi-level mediation:
  - Identifying brain regions that link independent and dependent variables
  - Connectivity analysis
  - Level 2 moderators
- Practical aspects
  - Matlab code, M3 toolbox