How do we assign motivational significance to sensory stimuli?
How do we identify something as good or bad?

Introduction

Intensity / Arousal

Neutral

Valence / Hedonic Value

Negative

Positive

“Two-Dimensional Theory of Emotion”
Adapted from: Lang (1995)
How do we identify something as good or bad?

Stimulus

Is it important? (saliency/arousal) |n|

NO
neutral

Yes

Is it bad or good? (valence)

-n +n

avoid approach

“Two-Factor Theory of Emotion”
Adapted from: Schachter and Singer (1962)

“Two-Dimensional Theory of Emotion”
Adapted from: Lang (1995)
Perturbations of motivational valence

Intensity / Arousal

Valence

Negative Neutral Positive
Perturbations of motivational valence

Interoception

Intense / Arousal

Negative Neutral Positive

Valence

Anxiety
Perturbations of motivational valence

Introduction
Neural Circuits of Emotional Valence: 
Amygdala circuitry

Amygdala important for emotional processing of environmental stimuli
(Brown & Schafer 1888; Kluver & Bucy, 1937; Weiskrantz, 1956)

Figure 3: A monkey with Klüver-Bucy syndrome has lost his natural fear of snakes

Adapted from: Amaral et al., 2003
Neural Circuits of Emotional Valence: Amygdala circuitry

Patient S.M. following bilateral amygdala damage lost fear to snakes and spiders, ability to recognize emotion in faces — but showed autonomic responses related to fear upon suffocation. (Tranel and Hyman, 1990; Adolphs et al., 1994; Feinstein et al., 2013)
BLA = Basolateral amygdala
CeA = Central nucleus of the amygdala

1. Where do circuits encoding positive and negative valence diverge?

2. How do positive and negative circuits interact?

3. When do valence-coding circuits engage in bottom-up v. top-down?

4. Overview & Outlook
The Amygdala: a primitive analog of the cortico-striatal circuit

Basolateral Amygdala (BLA) is “cortical-like”
- 90% glutamatergic pyramidal neurons

Central Amygdala (CeA) is “striatal-like”
- 95% GABAergic medium spiny neurons

Carlsen and Heimer (1988)
Swanson and Petrovich (1998)
Support for the BLA as a candidate divergence site

1. Neurons encode positive and negative valence

- Fuster and Uyeda (1971)
- Schoenbaum et al., (1999)
- Tye et al. (2007)
- Shabel and Janak (2009)
- Redondo et al. (2014)
- Gore et al., (2015)

BLA: Basolateral amygdala
Amygdala encoding of positive and negative valence

1. Neurons encode positive and negative valence

2. Sensory info converges

Romanski et al (1993)
Bordi and LeDoux (1992)
Fontanini et al (2009)

US: Unconditioned stimulus
CS: Conditioning stimulus
BLA: Basolateral amygdala
Amygdala encoding of positive and negative valence

1. Neurons encode positive and negative valence

2. Sensory info converges

3. Learning induces plasticity

- Quirk et al. (1995)
- Rogan et al. (1997)
- McKernan et al. (1997)
- Rumpel et al. (2005)
- Tye et al. (2008)
- Clem and Huganir (2010)

US: Unconditioned stimulus
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Amygdala encoding of positive and negative valence

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US: Unconditioned stimulus
CS: Conditioning stimulus
BLA: Basolateral amygdala
AMP/NMDA ratio: a proxy for glutamatergic synaptic strength

**Background**

**Long-Term Potentiation (LTP):**
AMPA receptor phosphorylation and delivery

**Long-Term Depression (LTD):**
AMPA receptor dephosphorylation and endocytosis

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*Adapted from:* Mark Bear, Rob Malenka and others
Fear conditioning increases AMPA:NMDA ratio in thalamo-BLA synapses

Background

Rumpel et al., *Science* (2005)

US: Unconditioned stimulus
CS: Conditioning stimulus
BLA: Basolateral amygdala
Reward conditioning also increases AMPA:NMDA ratio in thalamo-BLA synapses


US: Unconditioned stimulus
CS: Conditioning stimulus
BLA: Basolateral amygdala
Amygdala encoding of positive and negative valence

How can the same mechanism underlie fear and reward conditioning?

US: Unconditioned stimulus
CS: Conditioning stimulus
BLA: Basolateral amygdala
How can the same mechanism underlie fear and reward conditioning?

1) Maybe the amygdala just encodes salience

2) Maybe the amygdala is the site of valence assignment via distinct projections

Stimulus

Is it important? (salience/arousal)

NO YES

Is it good or bad? (valence)

approach avoid
CeM is critical for the expression of fear

Disconnecting BLA and CeM abolishes fear expression
Jimenez and Maren, 2009

Optogenetically stimulating CeM neurons evokes freezing responses
Ciocchi et al. 2010
Haubensak et al., 2010

But see…
Holland & Gallagher, de Araujo, Tonegawa, Palmiter, Bruchas and Klein!

BLA: Basolateral amygdala
CeM: Centromedial amygdala
NAc: Nucleus accumbens
Divergent pathways for expression of behavior

NAc is important for reward-related processes
Cador et al., 1989
Schultz et al., 1992

Optogenetically stimulating BLA terminals in NAc supports self-stimulation and place preference
Stuber et al., 2011
Britt et al., 2012

BLA: Basolateral amygdala
CeM: Centromedial amygdala
NAc: Nucleus accumbens
What is the circuit mechanism for assigning positive or negative valence?

Praneeth Namburi
Anna Beyeler
Hypothesis: BLA neuron projection target predicts learning-induced synaptic plasticity
Hypothesis: BLA neuron projection target predicts learning-induced synaptic plasticity
Examining Valence-Specific Potentiation in Projection-Identified BLA neurons

Results

Synapses onto BLA-CeM undergo LTP after fear conditioning and LTD after reward learning.

Synapses onto BLA-NAc undergo LTD after fear conditioning and LTP after reward learning.
Opposite changes in synaptic strength after fear and reward conditioning

Learning-induced synaptic strength

<table>
<thead>
<tr>
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But is there a causal relationship?
BLA-NAc Supports Positive Reinforcement, BLA-CeM Supports Punishment

Intracranial self-stimulation

In Collaboration with Ian Wickersham

Results

**BLA-NAc** Supports Positive Reinforcement, **BLA-CeM** Supports Punishment

RV-ChR2-Venus or RV-Venus

Real-Time Place Avoidance

In Collaboration with Ian Wickersham

If this was an NMDAR-dependent mechanism...

...Then hyperpolarizing postsynaptic neuron would prevent learning

*Adapted from: Collingridge (1986); Bliss, Collingridge, Morris, and many others*
Photoinhibition of BLA-CeM Impairs Fear Learning and Enhances Reward Learning

Photoinhibition of BLA-CeM Impairs Fear Learning and Enhances Reward Learning

BLA is a site of valence assignment

1. Opposite synaptic changes map onto projection

2. Activation of projections causes either approach or avoidance

3. Inhibition of CeM projectors impairs fear, but enhances reward learning
Opposite synaptic changes map onto projection

Activation of projections causes either approach or avoidance

Inhibition of CeM projectors impairs fear, but enhances reward learning

*But is it really that simple?*
From circuits to behaviour in the amygdala

Patricia H. Janak\textsuperscript{1,2} & Kay M. Tye\textsuperscript{3}

Although we champion the ability to manipulate circuit components that can be isolated with genetic or anatomical features, the existing tools still have limitations that prevent a comprehensive understanding of these circuits. Genetically encodable tools for neural manipulation allow far greater targeting specificity than before, but it is unlikely that all of the neurons that project from one region to another, or that share a genetic marker, have identical functions. Thus, we may still be observing a ‘majority vote’ for a given behavioural readout when manipulating any circuit component, and only more specific targeting strategies will reveal the functional minority populations. Along these lines, the synchrony and timing of most photostimulation experiments are not physiological and could disturb important rhythmic interactions across distal networks in ways we do not understand. To solve these issues, we need a greater library of tools that can be more selectively and spatially targeted.

Thus, we may still be observing a ‘majority vote’ for a given behavioural readout when manipulating any circuit component, and only more specific targeting strategies will reveal the functional minority populations. Along these lines, the synchrony and timing of most photostimulation experiments are not physiological and could disturb important rhythmic interactions across distal networks in ways we do not understand. To solve these issues, we need a greater library to manipulate circuit compositional or anatomical features, the existing tools still have limitations that prevent a comprehensive understanding of these circuits. Genetically encodable tools for neural manipulation allow far greater targeting specificity than before, but it is unlikely that all of the neurons that project from one region to another, or that share a genetic marker, have identical functions.

“Optogenetic tools tell us what neurons can do, not what neurons do do.” —Eve Marder

Although we champion the ability to manipulate circuit components that can be isolated with genetic or anatomical features, the existing tools still have limitations that prevent a comprehensive understanding of these circuits. Genetically encodable tools for neural manipulation allow far greater targeting specificity than before, but it is unlikely that all of the neurons that project from one region to another, or that share a genetic marker, have identical functions. Thus, we may still be observing a ‘majority vote’ for a given behavioural readout when manipulating any circuit component, and only more specific targeting strategies will reveal the functional minority populations. Along these lines, the synchrony and timing of most photostimulation experiments are not physiological and could disturb important rhythmic interactions across distal networks in ways we do not understand. To solve these issues, we need to develop new tools to reveal the fundamental properties of neural circuits.

What is each projection-defined neuron encoding?

1. Where do circuits encoding positive and negative valence diverge?
Is it really that simple?
How heterogeneous are these populations?
(Minimal) Criteria for valence encoding in single cells

1. Task responsive
2. Differential responding
3. Independent of stimulus features

Namburi et al., *NPP* (2015)
Investigating valence processing *in vivo*

Thanks to Jeremiah Cohen and Nao Uchida

Beyeler*, Namburi* et al., *Neuron* (2016)

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**Methods**

**Recording and Photo-identification**

- **day 7**
  - 2h

- **day 8**
  - 2h

**Learning and Recall**

- Sucrose
- Licks
- Quinine

---

**Performance**

- n=10 mice

**Training Session**

- 0.2
- 0.4
- 0.6
- 0.8
- 1.0

**Recall**

- 1
- 2
- 3
- 4
- 5
- 6
- 7
- 8
Results

Beyeler*, Namburi* et al., Neuron (2016)
Investigating valence processing \textit{in vivo}: Recordings from 1000+ neurons
Photostimulation-assisted Identification of Neuronal Populations: 
A strategy to overlay structure and function

1. Single-unit activity of neurons recorded during behavior

Technique: Lima et al. (2009)
Slide Courtesy of Fergil Mills
1. Single-unit activity of neurons recorded during behavior
2. After behavior, “phototagging” with light pulse delivery allows identification of ChR2+ neurons (Neuron B)

Methods

Photostimulation-assisted Identification of Neuronal Populations: A strategy to overlay structure and function

Technique: Lima et al. (2009)
Slide Courtesy of Fergil Mills
Determining appropriate phototagging criteria

Caveat: Recurrent Excitation

- ChR2-expressing
- Non-expressing neighbor not receiving input
- Non-expressing neighbor receiving input
Determining photoresponse latency thresholds

*Caveat: Recurrent Excitation*

- **ChR2-expressing**
- **Non-expressing neighbor receiving input**
Divergent routing of positive and negative information from the amygdala during memory retrieval

- **BLA-NAc** predominantly encodes positive valence
- **BLA-CeA** predominantly encodes negative valence
- **BLA-vHPC** does not have a significant bias for either CS

Beyeler*, Namburi* et al., *Neuron* (2016)
1. Where do circuits encoding positive and negative valence diverge?

2. How do positive and negative circuits interact?

3. When do valence-coding circuits engage in bottom-up v. top-down?

4. Overview & Outlook
1. Where do circuits encoding positive and negative valence diverge? BLA is a site of divergence for positive and negative valence.

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3. How do these circuits orchestrate competing motivational signals?

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4. Overview & Outlook
Clues that local interactions exist between BLA-CeM and BLA-NAc in vivo

Results

BLA-CeM cells have greater “influence” over neighbors

Results

Beyeler, Chang, et al., Cell Reports (2018)
Intermingled gradients of projection-defined BLA neurons

Results

CS (Auditory inputs) US (negative) US (positive) BLA CeM vHPC NAc

Beyeler, Chang, et al., Cell Reports (2018)
Whole brain imaging of BLA populations - CLARITY

BLA-NAc  BLA-CeM

Thanks to Kwanghun Chung
Advantage of intermingling: Local interactions to aid action selection

Dual Valence Model

Do BLA neurons encoding fear and reward interact? How?
Possible connectivity

- Unspecified Principal Neuron
- Reward-encoding Principal Neuron
- Fear-encoding Principal Neuron

Adapted from Janak and Tye, Nature (2015)
How does homeostatic need influence emotion? (and decision-making?)

Source: PNAS
How do these functionally-distinct projection-defined BLA neurons interact?

Gwendolyn Calhoon  Amy Sutton

Chia-Jung Chang
Local interactions of BLA-NAc and BLA-CeM cells: Net Effect (Naive Condition)

Local interactions of BLA-NAc and BLA-CeM cells: Asymmetric/Unidirectional relationship

How does this fit with everything else we know?

Asymmetric/Unidirectional relationship

Learning-induced synaptic strength

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Asymmetric/Unidirectional relationship

Discussion and Speculation

Learning-induced synaptic strength

Fear  Reward

BLA-NAc

BLA-CeM
Why might the brain work this way?

Asymmetric/Unidirectional relationship

Speculation:
reward-seeking is inherently risky,
priming escape is a good insurance policy
How do animals change their responses to stimuli depending on homeostatic need?
Local interactions of BLA-NAc and BLA-CeM cells: Net Effect (Food Deprived Condition)

Tracking Activity of BLA-NAc Neurons Across States:

*In vivo 2-photon deep brain imaging*

How do individual cells change across homeostatic states?

Summary of ex vivo ephys data

Firing Rate Difference (Hz)

- Sated
- Food
- Dep

Chia-Jung Chang
Tracking Activity of BLA-NAc Neurons Across States: *In vivo 2-photon deep brain imaging*

Food Deprivation Increases Activity of BLA-NAc neurons:  
*In vivo 2-photon deep brain imaging of calcium transients*

Results

Food Deprivation Decreases Activity of BLA-CeM neurons:

*In vivo 2-photon deep brain imaging of calcium transients*

**Results**

Sated (before) Food Deprived Sated (after)

2P Imaging of BLA-CeM in Awake Headfixed Mice

CeM: CAV2-Cre
BLA: CAG-FLEX-GCaMP6m

Food Deprived
Food Deprivation Induces Opposite Changes in BLA-NAc and BLA-CeM neurons:

*In vivo 2-photon deep brain imaging of calcium transients*

**Results**

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<td><strong>Food Deprived</strong></td>
<td><img src="Image" alt="Graph" /></td>
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**Bar charts:***

- **BLA-NAc**
  - Session 1: 5
  - Session 2: 10
  - Session 3: 15

- **BLA-CeM**
  - Session 1: 3
  - Session 2: 6
  - Session 3: 9

**Significance:**

- **BLA-NAc**
  - Session 1: **p < 0.01**
  - Session 2: **p < 0.01**

- **BLA-CeM**
  - Session 1: *p < 0.05*
  - Session 2: **p < 0.01**
Microcircuit interactions change with 24 hrs food deprivation

1. Relationship between competing BLA-NAc and BLA-CeM circuits is state-dependent

2. Activity of BLA-NAc increases, activity of BLA-CeM decreases, in vivo after food deprivation

Calhoon, Sutton, Chang et al., BioRxiv (2018)
Microcircuit interactions change with 24 hrs food deprivation

Relationship between competing circuits is state-dependent

As theorized
Microcircuit interactions change with 24 hrs food deprivation

Relationship between competing circuits is state-dependent
Microcircuit interactions change with 24 hrs food deprivation

Relationship between competing circuits is state-dependent

Discussion and Speculation
1. Where do circuits encoding positive and negative valence diverge? BLA is a site of divergence for positive and negative valence.

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   Asymmetrically, and dynamically (depending on state)

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   Asymmetrically, and dynamically (depending on state)

3. When do valence-coding circuits engage in bottom-up v. top-down?
   *Examples*: Bottom-up in rapid responses, Top-down in social contexts

4. Overview & Outlook
Amygdala circuits conserved across evolution

Collaborators: Mark Ungless, Li-Huei Tsai, Nick Gilpin, Jesse Gray, Emery Brown, Alcino Silva, Peyman Golshani, Denise Cai, James Curley, Liam Paninski, Alice Ting, Feng Zhang, Ila Fiete, Kwanghun Chung, Kerry Ressler

Reagents: Eric Kremer (CAV-Cre), Ian Wickersham (RV), Rachael Neve (HSV), GENIE, Ed Boyden, Silvia Arber, Byungkook Lim, Inscopix, Karl Deisseroth, Alon Chen

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Questions?