The Habenula and Nicotine Withdrawal: From Mouse to Human

Ramiro Salas, Ph.D.

Department of Psychiatry
Baylor College of Medicine
Introduction

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Why do you smoke?

“I just can’t quit”  Withdrawal
“If I am anxious I’ll smoke”  Anxiety
“It makes me feel sharper”  Cognitive

Why did you start smoking?

“All my friends were smoking”  Social component
“It was cool”
Elevated Plus Maze to Study Anxiety-Related Behavior in Rodents

Validation: drugs like benzodiazepines that are tranquilizers in humans increase open arm exploration in rodents

Animal 8 (Test 8)  
Animal 9 (Test 9)  

Less anxious-like mouse  
More anxious-like mouse
β4 -/- mice behave as less anxious in the elevated plus maze

If I’m anxious, I’ll smoke
β4 -/- and α5 -/- mice behave as less anxious in the elevated plus maze.

(A) Time in Open Zone (s) for α5

(B) Time in Open Zone (s) for β4

(C) Time in Open Zone (s) for α7

(D) Entry Ratio for α5

(E) Entry Ratio for α7

(F) Entry Ratio for β4

* denotes significant difference.
β4 -/- mice show abnormal social behavior: Intruder test

Cognitive and social component

2 min interaction of adult resident with smaller intruder

- Day 1: intruder A
- Day 2: intruder A
- Day 3: intruder B

Non-social olfactory memory was normal
β4 -/- mice show abnormal social behavior.
Normal non-social memory

Cognitive and social component

Mice were trained to find chocolate under sand mixed with different scents. For each of 3 pairs of scents, one predicted chocolate and one did not.
β4 -/- mice are resistant to the hypolocomotive effects of nicotine

The effect of the first cigarette predicts later risk for tobacco addiction

Nic 0.5 mg/Kg (ip)

![Graph showing the effect of nicotine on total distance traveled by β4 -/- mice. The graph compares the total distance (cm) traveled by +/+ PBS, +/+ Nic, -/- PBS, and -/- Nic groups. There is a significant difference (*) between the +/+ Nic and +/+ PBS groups.]
The effect of the first cigarette predicts later risk for tobacco addiction.

$\beta_4$ -/- mice are less sensitive to nicotine-induced seizures.

Nicotine (ip)

Observe 5’
Nicotine withdrawal in the mouse

The osmotic minipump

Delivers drug sc continually for 2 weeks
Nicotine withdrawal in the mouse

I just can’t quit

Withdrawal signs
- Increased shaking
- Increased grooming
- Increased scratching
The $\beta 4$ subunit is necessary for nicotine withdrawal

I just can’t quit...
The $\beta_4$ (but not the $\beta_2$) subunit is necessary for nicotine withdrawal.

I just can’t quit.
The $\alpha_2$ and $\alpha_5$ subunit are also necessary for nicotine withdrawal.

- **Chronic:**
  - Saline
  - Nicotine

- **Acute:**
  - Mecamylamine ip

- **Withdrawal signs:**
  - $+/+$ Saline
  - $+/+$ Nicotine
  - $\alpha_2$ -/-$+$ Saline
  - $\alpha_5$ -/-$+$ Nicotine

* indicates a significant difference.
Where in the brain does all this happen?
β4 mRNA in the CNS
β4 and β2 mRNA in the CNS
α5 and α2 nAChR subunit expression pattern

From the Allen atlas
www.brain-map.org
Microinjection of mecamylamine in the habenula is sufficient to precipitate nicotine withdrawal.

I just can’t
Microinjection of mecamylamine in the habenula or IPN, but not cortex, is sufficient to precipitate nicotine withdrawal.
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If you ask a mouse “How do you feel?”

Happy  Confused  Upset  Sad
Excited  Appalled  Anxious  Confident
Horrified  Joyful  Worried  Bored
Is any of this relevant to human health?
Can we see the habenula on fMRI?

Structural

10 minutes in the MRI voxel is 1 x 1 x 1 mm

functional

2 sec per slice in the MRI voxel is 3 x 3 x 4 mm
All humans are different

It is easy to identify the habenula in structural MRI, but a brain-by-brain analysis may be needed to see functional activity.
Passive learning with juice reward

A

Normal events

light → 6s → juice

Catch events

light → 6s → nothing → 4s → juice

B

Restricted slab

collected during functional scans

C

run 1
23 normal events
rest in scanner

run 2
22 normal events
rest in scanner

run 3
12 normal events
6 catch events
rest in scanner

run 4
12 normal events
6 catch events
rest in scanner

Use anti-correlation method to identify habenula voxels

Analyze catch event time series from habenula voxels identified in run 1
Manual co-registration

For each of 50 subjects, we manually co-registered structural and functional images, and manually defined coordinates for the right and left habenulae.
Anticorrelation approach to identify Hb voxels

1. Manually place ROIs on unregistered images

2. Compute correlation with striatal time series in C

3. Identify habenula as negatively correlated voxels

Normal event in run 1 - predicted

run 1

6s

striatum
time (sec)
habenula

% signal change (Str)

% signal change (Hb)
Negative prediction errors activate the habenula.
Summary

β4 subunit-containing nAChRs in the medial habenula might be critical mediators of several effects of nicotine and related behaviors.

β4 -/-, α5 -/-, and α2 -/- mice:

- Behave as less anxious than wild type littermates (β4 and α5 -/-)

- Display social amnesia (β4 -/-)

- Are insensitive to acute nicotine (β4 -/-, α5 -/-, and α3)

- Do not show nicotine withdrawal symptoms (β4 -/-, α2 -/-, α5 -/-)
Summary 2

- Blocking nAChRs in the habenula or ipn precipitates nicotine withdrawal in “addicted” mice

- The human habenula activates during “disappointment”

GWAS showed that SNPs in $\alpha_5$ (and $\alpha_3$, $\beta_4$) account for smoking risk in humans.

Speculation: Is drug withdrawal a state of continuous disappointment due to habenula hyperactivation?
Current work

• Human habenular activation in non-smokers, sated smokers and smokers in withdrawal.
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