**Animal models of postoperative delirium research**

Zhongcong Xie, M.D., Ph.D.
Geriatric Anesthesia Research Unit
Department of Anesthesia, Critical Care and Pain Medicine
Massachusetts General Hospital
Harvard Medical School
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**Why do we need the animal model to study postoperative delirium?**

- Mechanistic hypothesis testing:
  - Interaction of Aβ/Tau and neuroinflammation.
- Vulnerable window assessment:
  - Age dependent?
- Are there less provocative anesthetic:
  - Isoflurane versus desflurane.
- Potential treatment and prevention:
  - Anti-Aβ, anti-Tau and anti-inflammation.

**Animal studies of delirium**

- There are few animal models available to study delirium at the present time.
- It is important to establish animal models of delirium.
- We then can determine whether the perioperative factors (e.g., anesthesia, surgery, pain, and sleep deperivation, which could contribute to POCD) can also contribute to the postoperative delirium.

**T-maze alternation: working memory**

Dr. Colm Cunningham

“Since the nature of these deficits are acute and transient, with impairments in attention, recall, and short-term/working memory, this model displays key core features of delirium as defined by DSM-IV (American Psychiatric Association, 1994) and ICD-10 (World Health Organization, 1992) and will be useful in delineating mechanisms.”

(Murray et al., Neurobiology of Aging, 2012)

**Neurobiology of Disease**

Cyclooxygenase-1-Dependent Prostaglandins Mediate Susceptibility to Systemic Inflammation-Induced Acute Cognitive Dysfunction

Éadaoin W. Griffin,* Donal T. Skelly,* Carel L. Murray, and Colm Cunningham

Trinity College Dublin, Ireland.
**T-maze alternation: working memory**  
Dr. Colm Cunningham

- Mice will escape from shallow water.
- It needs training period (10 trails).
- It assesses working memory in rodents.

*(Murray et al., Neurobiology of Aging, 2012)*

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**Animal studies of delirium**

- We have set out to observe several animal nature behaviors following the treatment of scopolamine and following the abdominal surgery under isoflurane anesthesia.
- The ultimate goal is to develop a method of "CAM in mice" or even "3D-CAM in mice".

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**“CAM in mice”**

- Acute onset and fluctuating course  
  - Timecourse studies.
- Inattention  
  - Attention deficit assessment, buried food.
- Disorganized thinking  
  - Freezing behavior, Y-maze
- Altered level of consciousness  
  - Open field test and others.

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**Nature behavioral observation**

- Attention level *(Millecamp et al., 2004)*.
- Freezing episodes.
- Open field tests.
- Timecourse investigation.

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**Attention level**

\[
\text{Attention level} = \frac{\text{Duration of the new object exploration}}{\text{Total duration of all cumulated objects exploration (i.e. 3 familiar + the new one)}} \times 100
\]

*(Millecamps et al., 2004)*

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**Freezing episodes**

- Definition: No movement except respiration.
- Detected and analyzed by Any-Maze (Stoelting, Wood Dale, IL).
Open field test

- Definition: The time spent in the zone near the wall during the open field test.
- Detected and analyzed by Any-Maze (Stoelting, Wood Dale, IL).

Scopolamine in mice

A

![Graph showing time spent exploring new object (100%)](image)

B

![Graph showing number of freezing episodes](image)

C

![Graph showing time spent near the wall (100%)](image)
Anesthesia and surgery in mice

(Ren et al., 2015)

Effects of Anesthesia and Surgery on mice “CAM”

<table>
<thead>
<tr>
<th>Scopolamine</th>
<th>Anesthesia &amp; Surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-8-month mice</td>
<td>2-8-month mice</td>
</tr>
<tr>
<td>30 min</td>
<td>12h</td>
</tr>
<tr>
<td>Near the wall</td>
<td>↑</td>
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<tr>
<td>Attention level</td>
<td>↓</td>
</tr>
<tr>
<td>Freezing episode</td>
<td>↓</td>
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</tbody>
</table>

*↑ and ↓ indicate significant increase (P < 0.05) and decrease (P < 0.05) compared with saline or sham group at the same time point, respectively.

On going studies

Methods

CAM-in mice battery

Control group (n=14)

C57BL/6J mice (4 months old)

Surgery+anesthesia group (n=14)

Baseline 6h 9h 24h

Surgery+anesthesia

Buried food test

Open field test

Two trial recognition Y-maze test
**Buried food test**

![Graph showing latency to eat pellets for control and surgery + anesthesia groups at baseline, 6h, 9h, and 24h.](image)

- Control
- Surgery + anesthesia

**Two trial recognition Y-maze test**

![Graphs showing number of arm visits for control and surgery + anesthesia groups at baseline, 6h, 9h, and 24h.](image)

- Control
- Surgery + anesthesia

**Open field test**

![Graphs showing time spent in the center and immobile time for control and surgery + anesthesia groups at baseline, 6h, 9h, and 24h.](image)

- Control
- Surgery + anesthesia

**Effects of Surgery and Anesthesia on mice “CAM”**

<table>
<thead>
<tr>
<th></th>
<th>6h</th>
<th>9h</th>
<th>24h</th>
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<tbody>
<tr>
<td><strong>Buried food test</strong></td>
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<td></td>
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<tr>
<td>Latency to eat pellets</td>
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<tr>
<td>Levels of attention and executive function</td>
<td>-</td>
<td>↑</td>
<td>-</td>
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<tr>
<td><strong>Open field test</strong></td>
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<td></td>
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<tr>
<td>Total distance</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Locomotor activity</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Time spent in the center</td>
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<td></td>
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<tr>
<td>Anxiety-like behavior</td>
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<tr>
<td>Immobile time</td>
<td></td>
<td></td>
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<tr>
<td>Hypoactivity/hyperactivity</td>
<td>-</td>
<td>↑</td>
<td>-</td>
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<tr>
<td><strong>Two trial recognition Y-maze test</strong></td>
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<tr>
<td>Number of arm visits</td>
<td></td>
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<tr>
<td>Locomotor activity</td>
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<tr>
<td>Duration of novel arm visits</td>
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<tr>
<td>Short-term spatial recognition memory</td>
<td>↓</td>
<td>-</td>
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<tr>
<td>Number of novel arm visits</td>
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</tbody>
</table>

↑ and ↓ indicate significant increase (P < 0.05) and decrease (P < 0.05) compared with control group at the same time point, respectively.

**Limitations of the animal models in studying postoperative delirium**

- Mouse and human – alike, yet different
  - Genomes: only 85% same
  - Mouse brain is 70% neurons and 3% glia; human brain is opposite
- Human delirium ≠ mouse behavior
  - Mouse does not show disorganized thinking
  - We cannot measure level of consciousness in mice
  - Inattention: Maybe

**Animal models of perioperative factors**

- Acute onset and fluctuating course: Yes
**General anesthesia**

Animal: Rats.

Anesthesia: 1.2% isoflurane, 70% nitrous oxide, and 30% oxygen for two hours.

Cognitive function determination: 12-arm radial maze.

*(Culley et al., Anesthesiology, 2004)*

Animal: Mice.

Anesthesia: Halothane (0.8–1%) or isoflurane (0.9–1%) in 30% oxygen, balanced by N2 for two hours.

Cognitive function determination: Morris Water Maze.

*(Bianchi et al., Neurobiology of Aging, 2008)*
Animal: Mice.
Anesthesia: 1.4% isoflurane for two hours.
Cognitive function determination: Fear conditioning system.

Animal: Mice.
Surgery: Hepatectomy.
Anesthesia: Chloral hydrate (i.p.).
Cognitive function determination: Morris Water Maze.

Animal: Rats.
Surgery: Splenectomy.
Anesthesia: Fentanyl and droperidol (i.p.).
Cognitive function determination: Y-Maze.

Animal: Mice.
Surgery: Splenectomy.
Anesthesia: 1.4% isoflurane for two hours.
Cognitive function determination: Fear conditioning system.

Animal: Mice.
Surgery: Hepatectomy.
Anesthesia: Chloral hydrate (i.p.).
Cognitive function determination: Morris Water Maze.

(Bianchi et al., Neurobiology of Aging, 2008)
(Zhang et al., Annals of Neurology, 2012)
(Wan et al., Anesthesiology, 2007)
(Wan et al., Critical Care Medicine, 2010)

Fig. 1. Schematic time-line of the experimental paradigm. RMT = reference memory testing.
Animal: Mice.

Surgery: Open tibial fracture.

Anesthesia: 2.1% isoflurane briefly.

Analgesia: Buprenorphine (Buprenex, 0.1 mg/kg s.c).

Cognitive function determination: Fear conditioning system.

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Surgery plus local anesthesia

Animal: Mice.

Surgery: Opening and closing abdomen.

Anesthesia: local injection of bupivacaine.

Analgesia: EMLA (local anesthetics).

Cognitive function determination: Fear conditioning system and Morris Water Maze.

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Pain

Animal: Mice.

Surgery: Incisional or chemical pain.

Cognitive function determination: Fear conditioning system.

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Sleep deprivation

Animal: Mice.

No sleep for 24 hours.

Cognitive function determination: Fear conditioning system.

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Summary and Conclusion

- The employment of several animal models has suggested that perioperative factors, e.g., anesthesia, surgery, pain and sleep deprivation, may contribute to the cognitive impairment in rodents.

- Whether these perioperative factors can contribute to postoperative delirium remains largely to be investigated.

- Thus, it is important to establish animal models of postoperative delirium.
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