Behavioral Assays for Determining Olfactory Performance in Mice

8-4-2011

General Hypothesis

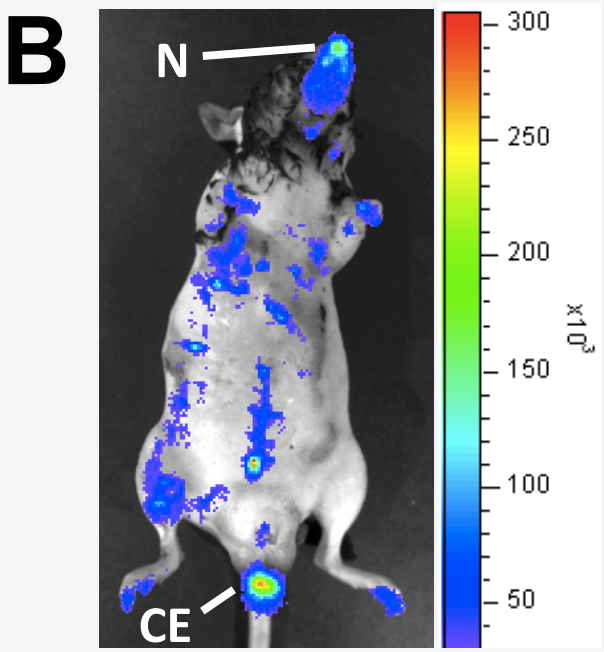
- The presence of ATXN2 in the olfactory system suggests that knocking out the protein, or introducing the mutant protein, will have detrimental effects on basic olfactory function.

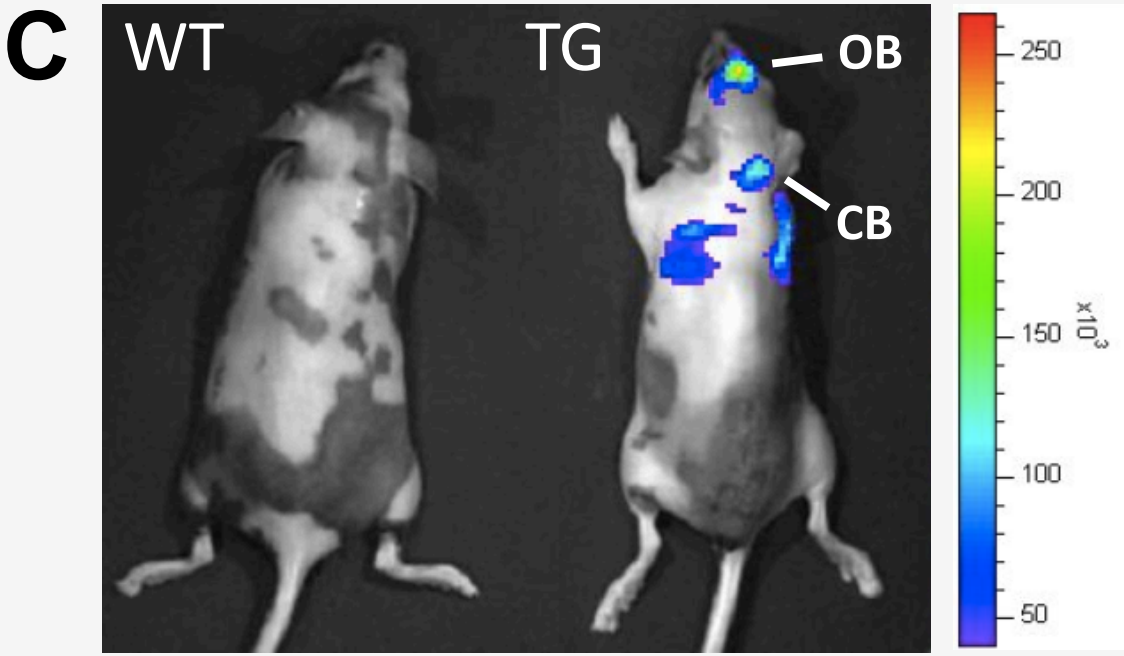
Rationale

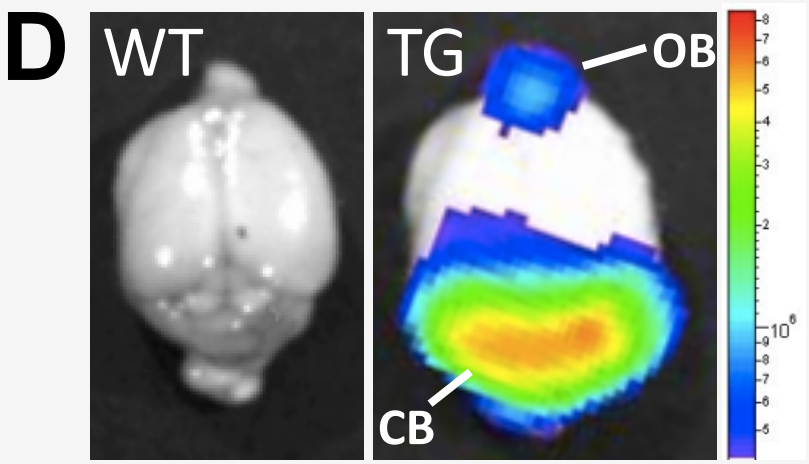
- Dan and Steve have observed high expression of mATXN2 in the olfactory bulb (mitral cells – IHC staining by Steve) in the luciferase mice.

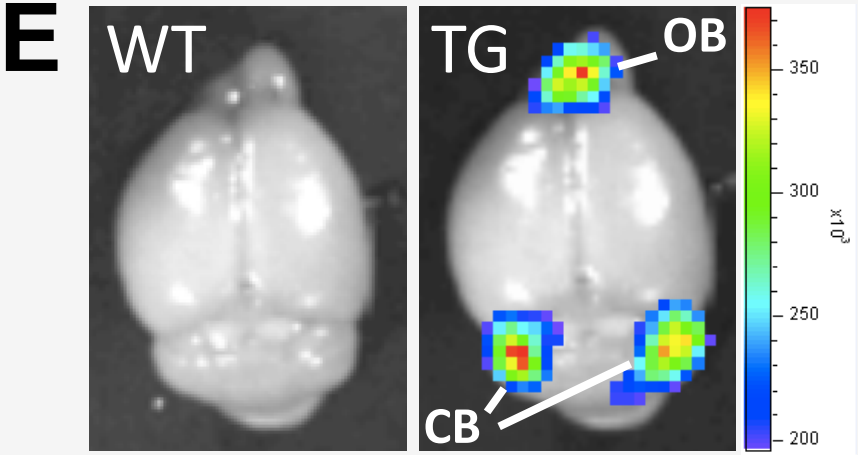
- Clinical trials have shown deficits in olfactory performance in SCA (2,3, and 7) patients (Velazquez-Perez et al., 2011; Connelly et al., 2003).

- SCA2 KO mice show a propensity for weight gain. Maybe related to olfaction?

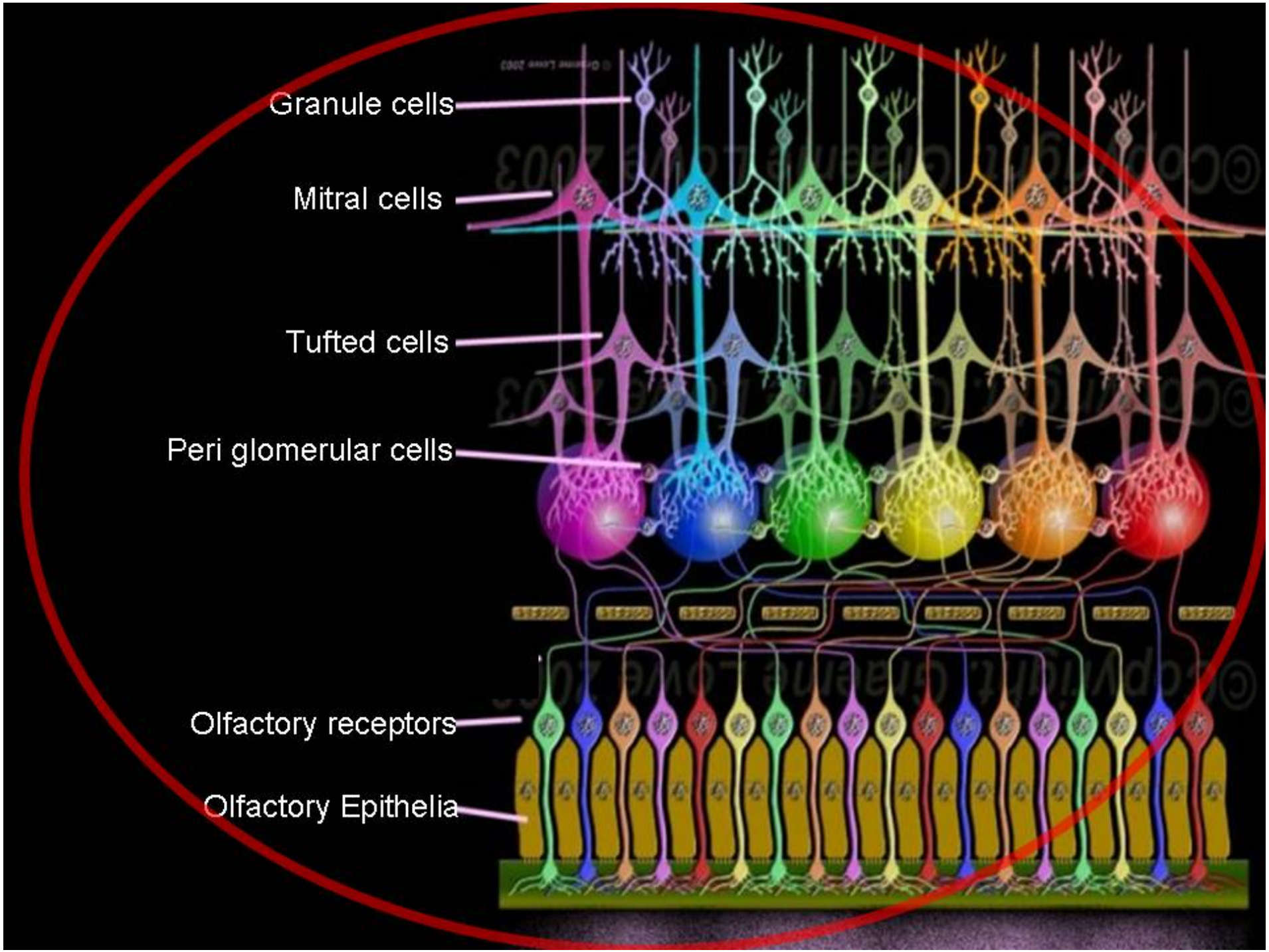




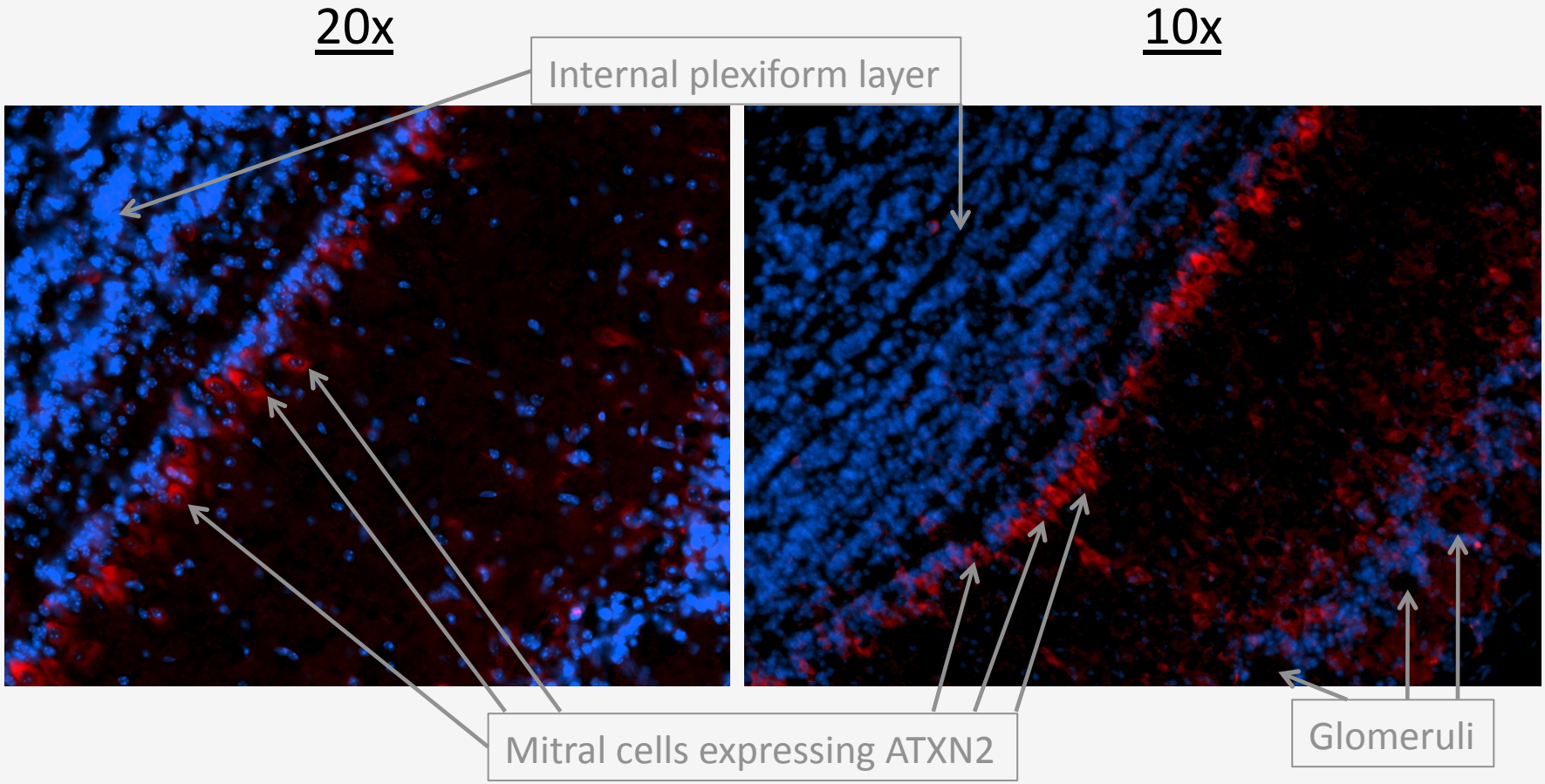




- Scoles et al., 2012ish


Granule cells
Mitral cells
Tufted cells
Peri glomerular cells
Olfactory receptors
Olfactory Epithelia

IHC - mouse olfactory bulb



- Data provided by Dr. Steve (commercial Ab)

Rationale: Clinical Trials

- SCA2, SCA3, and SCA7 patients show deficits in odor recognition and discrimination.
- (***SCA2 - UPSIT***, Velazquez-Perez et al., 2006; ***SCA2,3, & 7 - UPSIT***, Connelly et al., 2011)
- ***UPSIT*** – University of Pennsylvania smell identification test

SCA2 patients have difficulty detecting, identifying, and discriminating odors

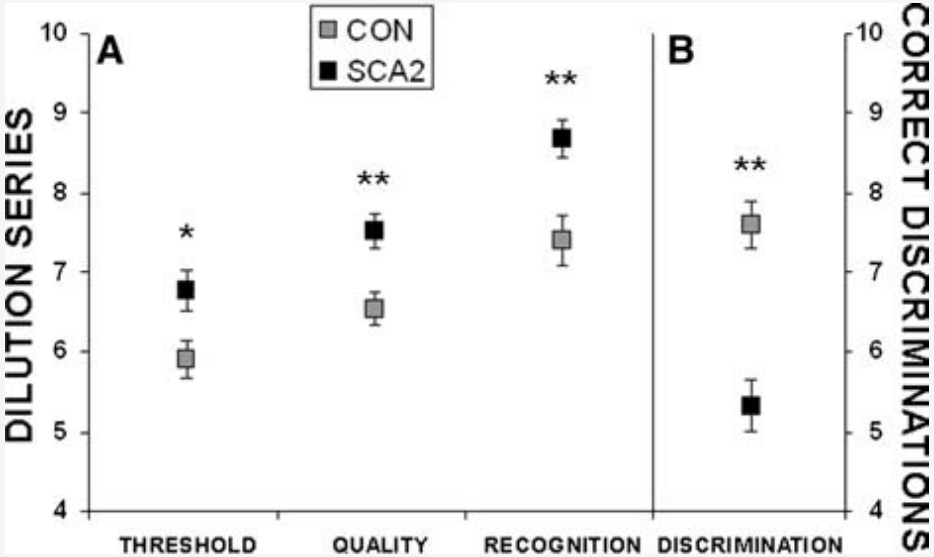


Fig. 2 Comparison of olfactory threshold, quality and recognition (left), and discrimination (right) between control subjects and SCA2 patients. Symbols are means and bars are SEM. Mann-Whitney test, * = $p < 0.05$, ** = $p < 0.01$

- *From Velazquez-Perez et al., 2006*

What are the best behavioral assays for testing olfactory performance?

I initially chose two assays (1-preference & 2-sensitivity) based on a method described by Kobayakawa et al., 2007 and modified by Witt et al., 2009.

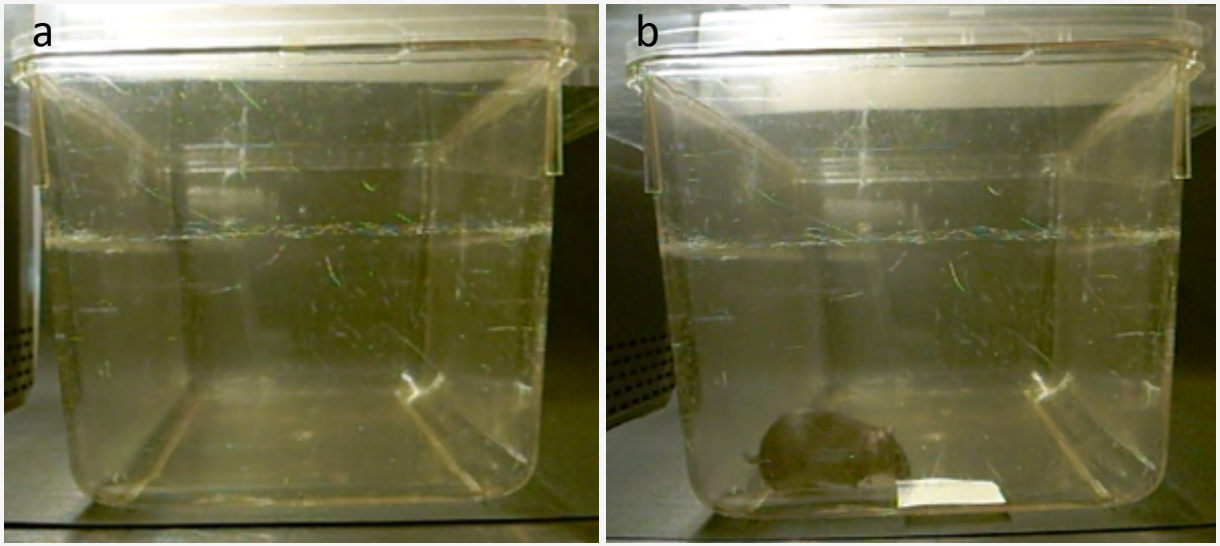
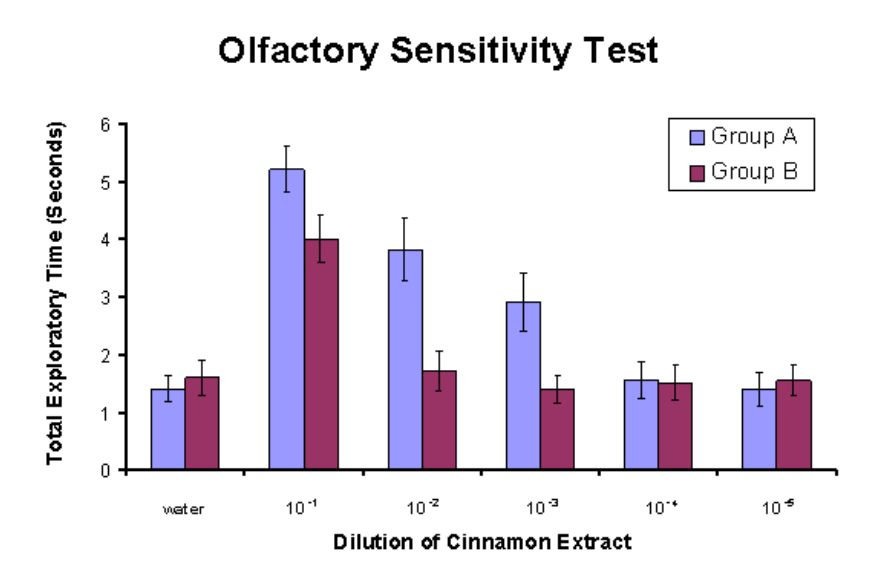
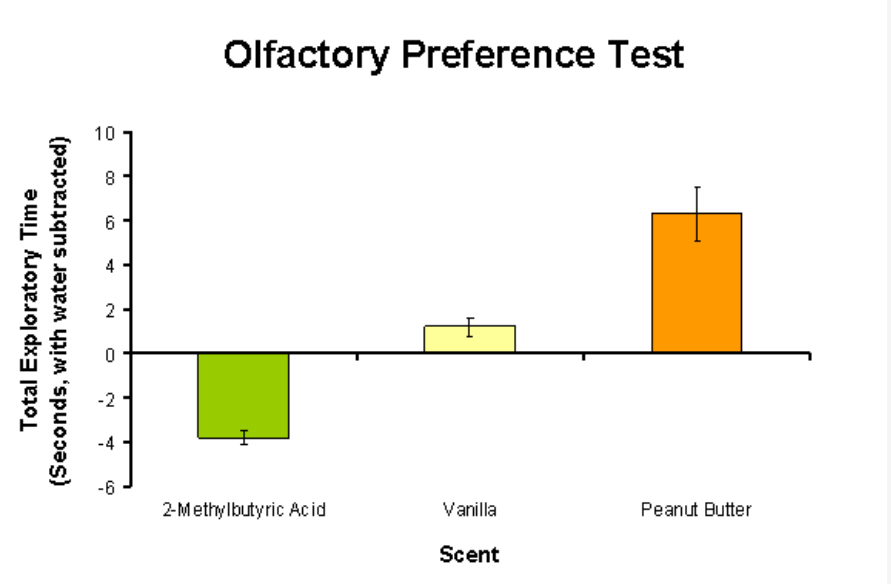


Figure 1. Arena Set-Up. (a) Properly draped, empty arena (b) Arena with subject and scented filter paper.

Begin habituating the subjects. Place the first subject in the first cage for habituation. After 15 minutes, move the first subject to the second
cage. Clean the first cage with Quatricide TB. Next, place the second subject in the first, cleaned cage. Continue this process with
subsequent subjects. Ultimately, each subject should be habituated in all three cages before being placed in the arena (fourth and final cage)
for another 15 minutes of habituation prior to testing.

Representative results





Drawbacks

After speaking with the experts…

- Mary Lucero, Ph.D. – Dept. of Physiology
  - Suggested our assays were fine, but she uses the following:
    - 1) Bury the Malt Ball
    - 2) Bait and Switch
    - 3) Was That You?
    - 4) Guess What I Just Ate

    *She shoots for a sample size of 10 but will settle for 6.

After speaking with the experts…

- Matt Wachowiak, Ph.D. – Dept. of Physiology
  - Studies sniffing behavior in rats. Specifically, how the mechanical behavior of sniffing primes the neural substrate and influences coding properties.
  - Mentioned that there is "loose" evidence showing cerebellar control of sniffing. Was very interested in this line of questioning – how potential mechanical degeneration in SCA2 patients might alter olfactory performance.
  - Suggested similar assays as Lucero.

Decided on

Olfactory deficits in mice overexpressing human wildtype α-synuclein

Sheila M. Fleming[1], **Nicole A. Tetreault**[1], **Caitlin K. Mulligan**[1], **Ché B. Hutson**[1], **Eliezer Masliah**[2], and **Marie-Françoise Chesselet**[1]

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Buried Pellet Test

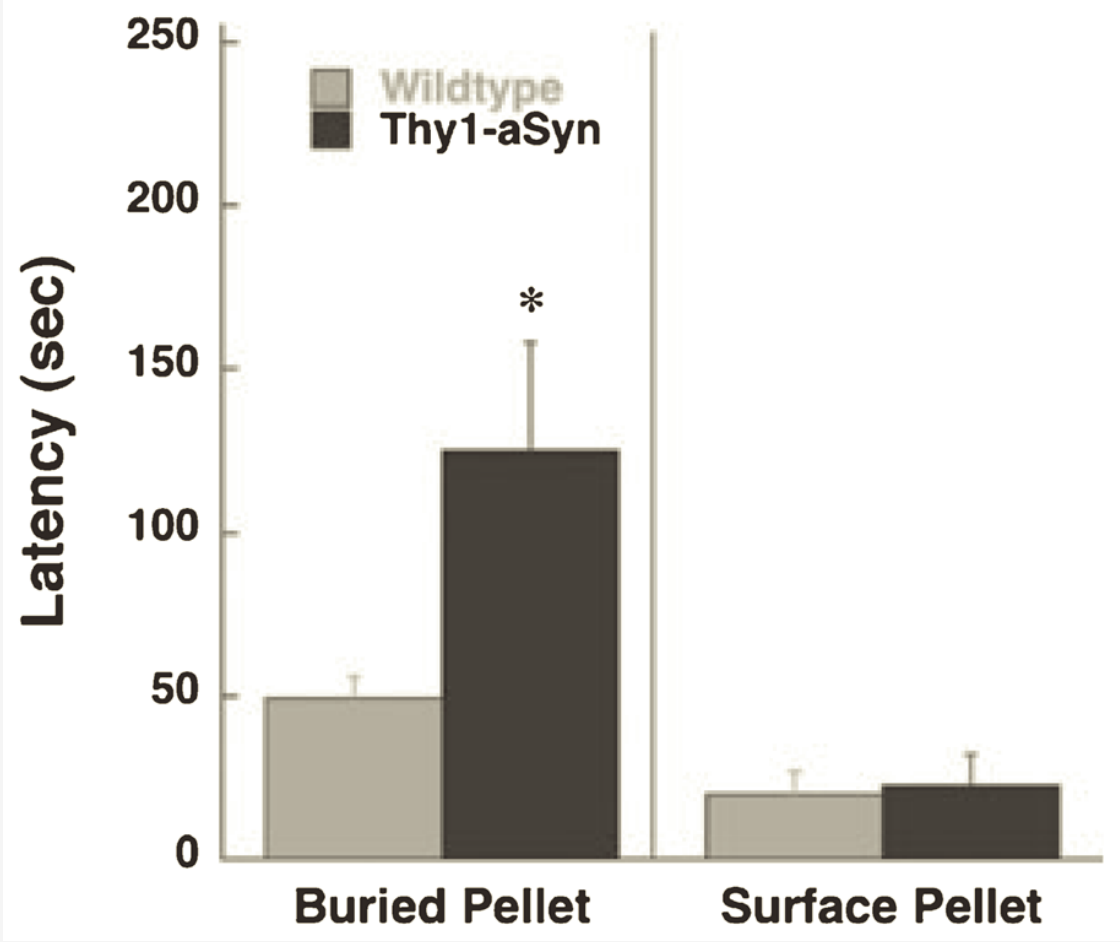

Fig. 1.

Latency to uncover the pellet in 5–6-month-old Thy1-aSyn (*n = 7) and WT (n = 16) mice.*

**P < 0.05 compared with WT (Mann–Whitney U-test).*

Block Test

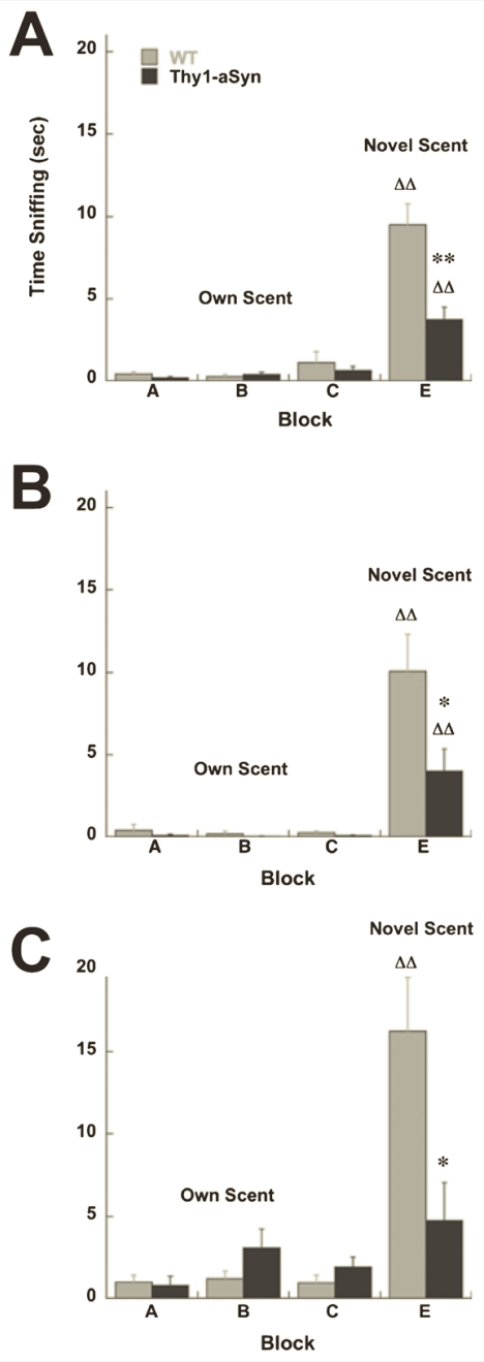

Fig. 2.

(A) Time spent sniffing in the block test in 3–4-month-old Thy1-aSyn (*n = 35) and WT (n =* 36) mice. (B) Time spent sniffing in the block test in Thy1-aSyn (*n = 10) and WT (n = 7)* mice at 9 months. Blocks were kept in the homecage for 7 days prior to testing at 3–4 and 9

months. (C) Time spent sniffing in the block test in Thy1-aSyn (*n = 5) and WT (n = 7) mice* at 11 months. Blocks were kept in the cage for 24 h prior to testing. *, ***P < 0.05 and 0.01,* respectively, compared with WT. Δ*Δ*P < 0.05 and 0.01, respectively, compared with blocks* A–C (Mann–Whitney *U-test and Wilcoxon Signed Rank test).*

Habituation/Dishabituation

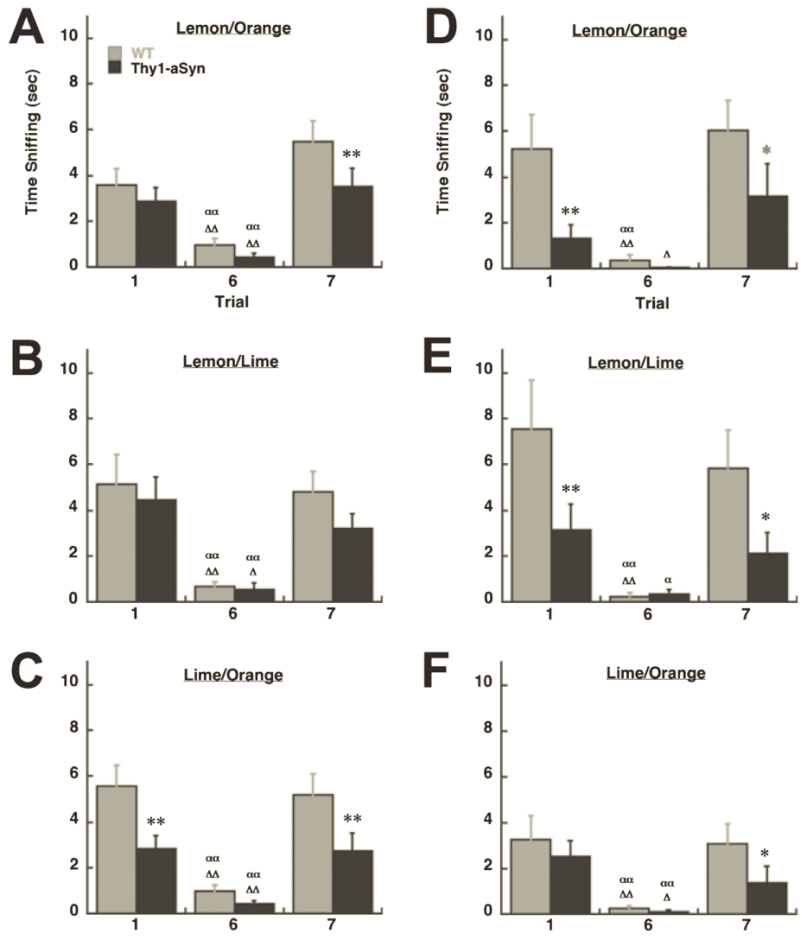

Fig. 4.
(A–C) Time spent sniffing in the habituation/ dishabituation test in 3–4-month-old Thy1-aSyn (*n = 22) and WT (n = 24) mice. (D–F) Time spent sniffing in the habituation/* dishabituation test in 9-month-old Thy1-aSyn (*n = 10) and WT (n = 7) mice. *, **P < 0.05* and 0.01 compared with WT within the same trial; α,αα*P < 0.05 and 0.01, respectively,* compared with Trial 1 within the same genotype; Δ, ΔΔ*P < 0.05 and 0.01, respectively,* compared with Trial 7 within the same genotype (2 × 3 ANOVA followed by Fisher's LSD).

Benefits

- The assays used by Fleming et al. (2008) are similar to, or incorporate similar principles as, those techniques recommended by Lucero.

- Little conditioning, fairly quick tests, and relatively minimal sample sizes

References