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Bead expulsion

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Protocol status: Working

We use this protocol and it's working

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Abstract

This assay measures mouse rectal function using a rectally-inserted glass bead. Animals are timed for how quickly they are able to expel the bead.

Materials

Heating pad

Glass beads - 1.5-2mm size (autoclaved)

Metal straight gavage tubes

- Marked at 2cm with sharpie

Vaseline

Small weigh boats for holding beads

Clean empty standard mouse cages

Sticky notes

Optional - second researcher to watch for bead expulsion while the other performs insertions

Set up

- 1 Habituate animals to behavior testing room for 1hr prior to start of assay.
- 2 Clean and lay out heating pad - set to low. Clean isoflurane box and nose cone and set up on top of the heating pad.
- 3 Set up beads in weigh boats, vaseline, gavage tubes. Mark 2cm on gavage tube.
- 4 Set up isoflurane machine according to institution guidelines.
- 5 Label empty, bedding-free mouse cages with animal numbers.

Procedure

- 6 Place mouse into isoflurane chamber
- 7 Wait for mouse to be anesthetized, looking for about 1 breath/second and no movement (can tilt box sideways to make mouse roll)
- 8 Once fully asleep, transfer animal to nose cone facing belly-down. Check for response using a firm paw press.
- 9 Optional - Put eye ointment on mouse eyes to prevent drying (if learning/working slowly and mouse will be unconscious for more than ~2 min)
- 10 Dip gavage tube in vaseline
- 11 Lift up mouse tail and move away fur from anus while rubbing in vaseline
- 12 Clean out any feces in rectum: Gently insert gavage tube, moving very slowly, up to 2cm mark.



- Angle of rectum goes down at first then levels out - try to follow curve with tube
- 12.1 Slowly take out tube, repeat if there are any feces
Once tube goes in easily and there are no more feces, proceed to inserting bead.
- 13 Pick up a bead with fingers and gently press it onto anal opening, holding it there until feel it has gone in.
- 14 Use gavage tube to push in the bead further, up to the 2cm mark, and gently remove.
- 14.1 Before taking the tube out, check that can feel the bead in there by making small movement up and down/side to side with tube to try to feel glass scratching on metal tube.
Also check that the bead is in far enough by squeezing sides of mouse rectum (on the rump) to see if you can feel the bead (should not be able to if it is placed correctly)
- 15 Take animal off nose cone and let wake up for ~1 min on heating pad
- 16 Wipe down and disinfect all equipment in between animals.
- 17 Check for responsiveness by turning onto side - if the animal is able to right itself, then transfer to individual clean cage. Record the start time.
- 17.1 Move cage to a well-light table top to observe for bead expulsion. Record time (to the minute) of when the animal has expelled the bead, with a maximum time of 60 minutes.
- 18 After the bead has been expelled, animal can be returned to the home cage.

Protocol references

1. Camilleri, Michael, and David R. Linden. "Measurement of gastrointestinal and colonic motor functions in humans and animals." *Cellular and molecular gastroenterology and hepatology* 2.4 (2016): 412-428.