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Version 2

Iodine-starch sweating assay V.2

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Fanglin Lu¹

¹MD, MS



Fanglin Lu

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Protocol status: Working

We use this protocol and it's working

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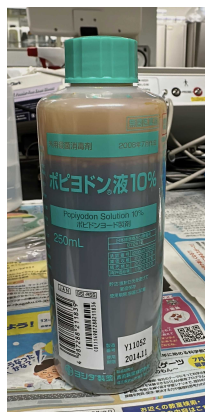
Abstract

This protocol outlines the procedure for assessing the sudomotor function in mice under restraint stress and under systemic pilocarpine stimulation. Both two kinds of sweating assays were based on the iodine-starch reaction.

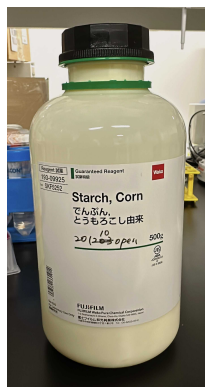
Guidelines

Please follow the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guideline.
It is favorable to not perform these two different kinds of sweating assays on the same day.

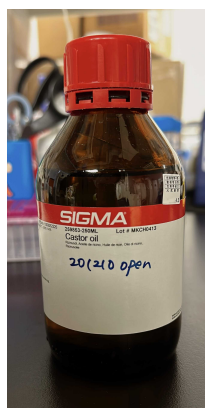
Materials



10% povidone–iodine solution (Yoshida Pharmaceutical Co., Ltd.)



Starch (FUJIFILM Wako Pure Chemical Corp., Osaka, Japan; catalog number: 193-09925)



Castor oil (Sigma-Aldrich, St. Louis, MO; catalog number: 259853)



Mini makeup brushes (we use them instead of cotton buds)



Microscope (Olympus SZ61) with digital camera Moticam 1080



Self-made restrainer (The nose piece is **BROKEN. USE A NEW ONE.**)



Note

The restrainer is made of a nose piece of commercially available mouse handling tube and a 50mL syringe. Mouse will bite the nosepiece under restraint stress, and get injured by the broken nosepiece. Change the nose piece once it is not intact. Or use round nylon nose piece, which is safer.

Pilocarpine hydrochloride (Sigma-Aldrich; catalog number: PHR1493)

Before start

Check if the restraint tube is intact.

Prepare 100% starch–castor oil (1 g starch per 1 mL castor oil)

Prepare pilocarpine (diluted in 150 μ L 0.9% saline).

Restraint stress-induced sweating

6m

- 1 The mouse is immobilized in a self-made restraint tube.

2m



self-made restraint tube

- 2 Apply 10% povidone-iodine solution to the plantar surface of bilateral hind paws with a cotton bud. Once dry, coat the skin surface with a suspension of 100% starch-castor oil.

1m

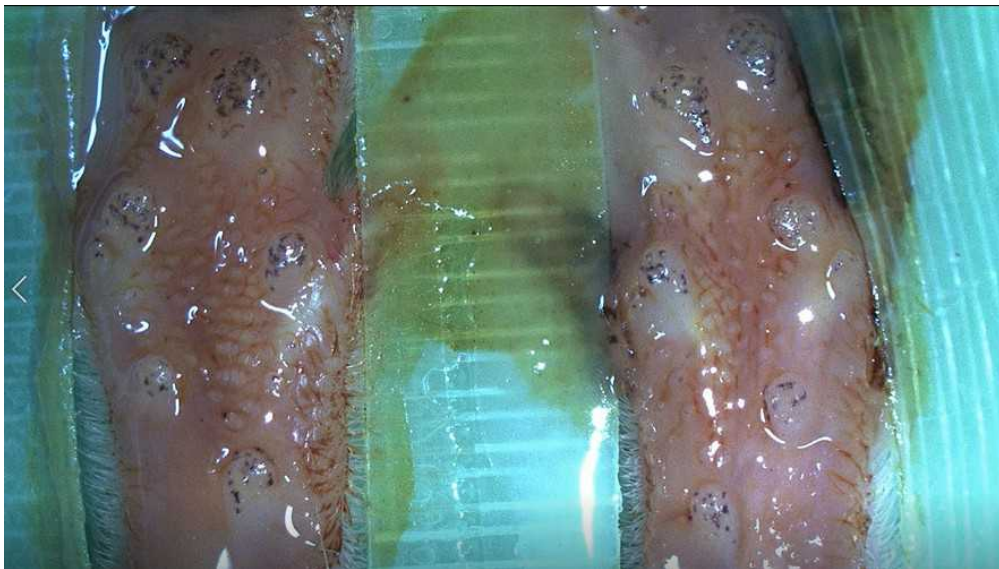


- 3 Changes on footpads are video-recorded for 3 min. The number of dark spots was counted for each paw.

3m

Expected result

Sweat droplets are visualized as dark-colored spots because of iodine–starch reactions.



Pilocarpine-induced sweating

7m 10s

- 4 The mouse is placed in the supine position under sevoflurane anesthesia (4 volume% in 1 L/min oxygen).

1m

Note

Sevoflurane inhalation was used, as it is safe, non-invasive, rapid, and easy to control. Adequate anesthetic depth was ascertained by loss of response to tail clamp.

- 5 Apply 10% povidone–iodine solution to the plantar surface of bilateral hind paws with a cotton bud. Once dry, coat the skin surface with a suspension of 100% starch–castor oil.

1m



- 6 Inject 100 μ g pilocarpine hydrochloride intraperitoneally.

10s

 150 μ L diluted in 0.9% saline

- 7 Changes on footpads are video-recorded for 5 min. The number of dark spots was counted for each paw.

5m

Expected result

