Female urine sniffing test

1. Estrus urine determination

In the morning (8:00–9:00 am) of the experiment, the vaginal estrus condition of the female donors was assessed by inserting the tip of a plastic pipette filled with 10 μL of saline (NaCl 0.9%) into the mouse vagina and vaginal secretions was collected. Then the vaginal fluid was transferred to a glass slide. By examining the proportion and the morphology of leukocytes and epithelial cells present in the smear, the four stages (proestrus, estrus, metaestrus, and diestrus) of the estrus cycle was determined.

1.1. Estrus urine collection

Female mice in estrus were being held on by their skin on the nape and were placed on a flat surface covered with clean aluminum foil. Handling is usually sufficient to induce urination; otherwise, the ventral area should be rubbed in an anterior-to-posterior direction. The urine on the foil was collected immediately, and ∼60 μL is pipetted directly from the aluminum surface onto a clean cotton swab.

2. Test

2.1. Setup

Animals were singly housed 1 week prior to the FUST in a conventional vivarium (temperature 22  ±  1c; 12 h light-dark cycle; food and water ad libitum).

2.2. Procedure

One hour prior to the test, rodents were habituated to a sterile ­cotton-tipped applicator inserted into their home cage. After 1 h, the sterile cotton-tipped applicator was removed and each rodent, separately, was transferred to a dimly lit room (∼3 lux lighting). The FUST had three phases: (1) Water – one exposure (3 min) to a cotton tip dipped in sterile water, during which sniffing duration is measured; (2) Habituation – the rodent cage was transferred from the experiment room to the habituation area for an interval of 45 min during which no cotton tip was presented; and (3) Urine – the animal was transferred back to the experiment room for one exposure (3 min) to a cotton-tipped applicator infused with fresh urine collected from females in estrus (of the same strain), during which sniffing duration was measured (Malkesman et al., 2010).