METHODS & DESIGNS

An olfactometer for small rodents

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An olfactometer that captures and delivers biological odors at experimenter-controlled times, without dilution, is described. The apparatus is quiet, efficient, versatile, animal-safe, and fairly inexpensive. Tests indicate that the device is successful in isolating transitory odors.

One major problem in pheromone research is the devising of a versatile olfactometer that can capture odors surrounding a "donor" animal and deliver them at appropriate and precisely controlled times to a "receiver" animal. The principal difficulties involve the nature of the odor sources. Most olfactometers have nonliving sources, usually liquids or solids, that are highly purified and hence very odorous. These substances are vaporized into a carrier gas, usually filtered air, and often diluted with more air. Air flows through the system continuously, and the odor content of the air remains constant over time (for a review, see Dravnieks, 1975). From a living odor source, the odorous substances are much weaker, much less volatile, and much less plentiful; as a result, effective concentration of odors may be difficult to achieve. Thus, one goal of the present design is to dilute odors minimally.

Another peculiarity of a live odor source is that the odors it produces may vary considerably, both quantitatively and qualitatively, over time. This is especially the case for many pheromones, which may be released during very specific behaviors and which may persist for very short durations. Thus, another unique use of the present olfactometer is to capture odors at precise times and to deliver them at times independent of their times of production. This requires a storage mechanism.

Other prerequisites for a versatile apparatus are as follows: The apparatus should require a minimum of accessory equipment (e.g., pumps). It should carry out air transfers fairly rapidly and quietly and should be easy to calibrate. It should subject the animals neither to pressure fluctuations nor to any danger. It should allow unusual patterns of odor transference. And last, it should be fairly inexpensive to construct. The following is (1) a description of the olfactometer that was designed and built

to meet these needs, and (2) an illustration of the operation of the apparatus through an examination of the response of *Meriones unguiculatus* to Harderian gland odors that are released at the nares of the nose during an autogroom (Thiessen, 1983).

The apparatus, illustrated in Figure 1, was constructed from Plexiglas, Lexan, Tygon, and glass, so that (1) air-borne chemicals would be minimally absorbed by the apparatus, (2) all fluid transfers could be observed directly, and (3) the gerbils could be observed and videotaped. The apparatus functions through entrapment of air pockets above water and movement of the pockets from chamber to chamber through displacement of air with water and vice versa. Gas-pocket entrapment is used in order that scented air be minimally diluted during transfer, an improvement over systems using air to displace air. Water is used for the gas entrapment because the pheromones being studied in this laboratory are all lipid-based, and hence are not easily absorbed in an aqueous medium; however, a thin, clean oil may be recirculated through this apparatus, when water-soluble pheromones are being studied.

None of the stopcocks used in this apparatus are constructed of ground glass, because lubrication with stopcock grease, which might absorb some of the pheromones, would be necessary. The stopcocks controlling the pressure and siphon columns (explained later) have Teflon cores with 6-mm bores. These stopcocks need to work freely and pass water easily, because they are central to each operational step. The remaining stopcocks (Nalgene 6460-0004 and 6470-0004) are of polypropylene and Teflon construction and have 4-mm bores. These stopcocks are inexpensive, compact, tight, maintenance-free, and ideal for panel mounting.

Two types of animal chambers are used in this apparatus: the donor chambers, D1 and D2, and the receiver chamber, R. The donor chambers are constructed from 4.5-in. (i.d.) Plexiglas tubes with screen floors 6 in. from their tops. All lines leading to and from the chambers are located below the floors, so that air pockets are always preserved for the animals (preventing them from drowning, in the event of a procedural error). The top of each

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Figure 1. The olfactometer's air and water tube connections are diagrammed. The pressure and siphon columns (PC and SC) are shown on the left. The remainder of the apparatus is approximately at the same level as the intersection of the SC and PC. Labels on stopcocks, tubes, flasks, and chambers are for use in Table 1. Insets depict the construction of donor and receiver chambers.

tube is sealed airtight by a chamber head. The head consists of a Lexan plate that is sealed to a lip at the top of the chamber tube with cork gasket material and silicone vacuum grease. The plate is clamped to the lip with five bolts and butterfly nuts (for easy removal). Permanently affixed to the top of the plate is a Sprite® 4-in. induction ventilation fan, and attached to the top-side of the fan is a Lexan plate. The fan housing and upper plate are sealed airtight with silicone caulk. The fan is open to the interior of the chamber through a hole in the lower plate, and a plastic screen prevents contact between the gerbil and the fan blades. The fan is not intended to force air downward; rather, it is intended to swirl air gently around the gerbil, so that any pheromones are well evaporated. The fan is normally very quiet, but care must be taken that irregularities in the surfaces of the silicone caulking do not result in ultrasonic "whistling." The receiver chamber is a simple Lexan box, with three air tubes leading to and from it. In the top is a hole that fits a solid

Lexan head similar to those used for the donor chambers. Here, the head contains no fan. The sides of the box are welded together with dichloroethane.

The driving forces behind this apparatus are a water-pressure column (PC) and a siphon column (SC). The pressure column is about 2 m tall and contains a large aspirator bottle at its head (as a reservoir). The column is constantly replenished via a slow-running tap-water supply, and excess water is drained off the top of the column through the drainage tube. The drainage tube is large (.5-in. diameter), in order to prevent gurgling noises that result from a siphoning action. The siphon column consists of a stiff .5-in.-diameter Tygon tube that runs from the apparatus, down below the water level of the sewage pipes leading from the floor drain. A vacuum source rigged with a large water trap will also work.

The remainder of the apparatus (refer to Figure 1) contains four subsystems, and the design of the apparatus may be simplified by elimination of any of these systems. Two

of the subsystems are used for collection of odors from "donor" animals, one is used for collection of odors from crude extracts, tissues, fractions, etc., and one serves as a fresh-air source (for control measures). The first of the former two subsystems (identical to the second) operates as follows: (1) The water level in D1 is low, and that in storage flask 1 (S1) is high; (2) at a time when odors are to be collected from the gerbil in D1, water from the PC is forced into D1, displacing air from D1 into S1; (3) the slightly elevated air pressure in S1 forces water from S1 into the drain; (4) at an appropriate time for delivery of the odors to the receiver chamber, R, water from the PC is forced into S1, displacing the air from S1 into R; (5) the system is reset by drawing water out of D1 via the SC, thereby drawing in fresh air. Either storage or delivery of 1 liter of air requires about 30 sec. Air may also be passed directly from D1 to R using water pressure from PC or air pressure applied to the fresh-air inlet, given the proper stopcock positions.

Both the extract and fresh-air systems work as follows: (1) the water level in S3 (or S4) is high; (2) the water is drawn from S3 (or S4), and air fills the flask (in the case of the extract system, the air first passes through a flask containing the extracts); (3) at an appropriate time, water is forced into S3 (or S4) from the PC, forcing the stored air into R. These systems need no resetting. Either storage or delivery of 1 liter of air requires about 30 sec.

In order to supply the animals with sufficient oxygen and keep them cool while they are not being tested, and in order to flush the system of stray odors between trials, a ventilation system was built into the apparatus. The system ventilates D1 and/or D2 (as required), R, and all ducts leading from the storage flasks to R. During operation, a vacuum applied to the ducts leading to R draws air through R, through D1 and/or D2, and through the remaining receiver ducts. A detailed description of operation steps for all of the above procedures is listed in Table 1. It should be noted that virtually any chamber

Table 1
Operating Procedures

Step	Water Levels	Stopcock Positions	Execution
Preparatory			
1. Flush PC and SC (recharge SC)	SC full of air	A, F, K, and L all off	S,P
2. Lower water level in D1	D1 too high	A13, D13	S
3. Raise water level in D1	D1 too low	A13 (vent system ON)	P
4. Raise water level in S1	S1 too low	A12 (vent system ON)	P
5. Raise water level in S3	S3 too low	K13 (vent system ON)	P
6. Raise water level in S4	S4 too low	L13 (vent system ON)	p
Donor Air Deliver with Store			
1. Capture air from D1 in S1	D1 low, S1 high	A13, B, D23	P
deliver air from S1 to R	S1 low	A12, E, N	P
Donor Air Delivery Without Store			
1. Deliver from D1 to R directly	D1 low	A13, D23, E, N	P
2. Continuous flow from D1 to R	na (not applicable)	C, D23, E, N, vacuum to outflow or pressure to filtered air supply	na
Delivery of Chemical Abstracts			
1. Store extract-scented air in S3	S3 high	K12	S
deliver air to r	S3 low	K13, N	P
Control Procedures			
1. Store fresh air in S4	S4 high	L12	S
deliver air to R	S4 low	L13,N	P
2. Store fresh air in S1	S1 high	A12, D12	S
deliver air to R	S1 low	A12, E, N	P
Ventilation Procedures			
1. Ventilate R	na	M	V
2. Ventilate R and D1	na	M, C, D23, E	V
3. Ventilate R, D1, and D2	na	M, C, D23, E, H, I23, J	V

Note—Commonly used procedures are summarized. Each procedure, indicated by a numeral, may be executed independently of any other procedure. And each procedural step is indicated by a separate line under "Step." The codes representing chambers, tubes, and stopcocks all refer to the labels in Figure 1. Before execution of each step, the water levels (or other conditions) are as indicated under "Water Levels." In order to "switch" the olfactometer to the appropriate functional mode for execution of the step, stopcocks are positioned as indicated under "Stopcock Positions." Here, the following system is used: If a letter is listed, the corresponding stopcock is opened. For three-way stopcocks, only two pathways are connected, indicated by the two numerals following the letter. For instance, "A13" means that stopcock A is opened so that it connects tubes 1 and 3. Finally, each step is executed by opening (and terminated by closing) the stopcock(s) indicated under "Execution." The first and second donor systems operate identically, so procedures are summarized only for the first system (corresponding to D1). And for convenience, stopcock N may be left open for all procedures except ventilation.

connections are possible with this apparatus. As a result, the design has accommodated many procedures not anticipated before the construction. Therefore, Table 1 should not be considered an exhaustive list of possible procedures.

All air entering the system first passes through a charcoal filter, which removes all extraneous odors. The air may also be freed of CO₂ (with Ascarite) or of moisture (with Drierite), as needed. The animal chambers may be temperature controlled by numerous means, including heating with electric heating tapes or heat lamps. Perhaps the most accurate method of temperature control (not yet tested) would involve the placement of heating/cooling coils in the chamber heads. Water would be recirculated through these coils from a thermostatically regulated reservoir. Heat exchange would readily occur because of the rapid circulation of air within the head.

The olfactometer appears complicated to operate, but in fact is easy to use. Proficiency can be acquired in a short time, and gerbils readily habituate to the chambers. The maximum amount of time that a receiver should remain unventilated (without any air transfers), seems to be about 20 min, and the maximum period for a donor depends on the water level (hence air volume) in its chamber. As long as air transfers are carried out between the donor and receiver, approximately every 7 or 8 min, gerbils require no ventilation periods.

DEMONSTRATION RESULTS

A simple experiment was performed in order to determine the efficiency of the device in capturing, storing, and transferring volumes of air. In this experiment, odors were captured from a donor gerbil immediately after the gerbil's release of Harderian substance during autogrooming. Harderian substance is believed to contain a pheromone with attractant qualities for the gerbil, so it was expected that during delivery of the Harderian-scented air ("groom air") to the receiver, the receiver would turn toward the air-delivery port and sniff the incoming air. This orientation response was measured by means of a protractor placed beneath the receiver's (transparent) floor.

Specifically, the air captured during the donor's autogroom was stored until the receiver spontaneously oriented 180° away from the delivery port. Then the air was delivered to the receiver for approximately 30 sec, during which time the receiver's maximum angle of orientation (turning and sniffing) from the 180° mark was recorded. Similarly, either fresh air or air from around the donor gerbil between its autogrooms ("nongroom air") was captured and delivered to the receiver. Lastly, in order to control for spontaneous exploratory activity, the receiver's orientation toward the delivery port was recorded, during a 30-sec period immediately following the gerbil's spontaneous orientation to the 180° mark ("nonpresentation trial"). During both groom and nongroom trials, the storage time of the air (time elapsing

between the beginning of capture and the beginning of delivery) was recorded, in an attempt to account for the absorption and dissipation of odors and/or their spontaneous "deactivation." This parameter, of course, is not applicable for fresh-air or nonpresentation trials. The above four types of trials were alternated randomly. Also random were the storage intervals used during groom and nongroom trials. The results below represent only four or five donors and receivers, so the statistics presented are intended to be illustrative rather than representative of independent samples. All statistical tests are one-tailed Student's *t* tests.

The results of this experiment are plotted in Figure 2. For both the groom and nongroom trials, the data are collapsed into bins, according to air-storage time. The first time bins represent storage times from 1.00 to 1.49 min, the second bins 1.50 to 1.99 min, the third bins 2.00 to 2.49 min, and the fourth bins represent times greater than or equal to 2.50 min. Only two raw data points were determined for storage times of less than 1.00 min, but since the times were extremely close to 1.00 min, these data were included with the 1.00-1.49-min data. Although it is theoretically possible to store air, switch stopcocks, and commence air delivery within a time period of about 35 sec, for this particular experiment, the gerbil must spontaneously orient properly prior to air delivery. This process never required less than 23 sec.

The mean spontaneous orientation (during nonpresentation trials) was $99.7 \pm 61.71^\circ$ ($n=38$ trials), and orientation during fresh air trials was slightly, but insignificantly, higher at $117 \pm 46.18^\circ$ ($n=40$). On the other hand, mean orientation to groom air was significantly greater than that to fresh air, for storage times of both 1.00-1.49 and 1.50-1.99 min, at $180.0 \pm 0.00^\circ$ ($n=6$, $t=8.53$, $p < .001$) and $180.0 \pm 0.00^\circ$ ($n=6$, $t=8.53$, $p < .001$), respectively. For times between 2.00 and 2.49 min and exceeding 2.50 min, mean orientation was insignificantly

Figure 2. Degree of body orientation during presentations of groom versus nongroom body odors, or fresh air. Spontaneous turning to nonpresentation trials is indicated on the left.

higher, at $134.4 \pm 32.70^\circ$ ($n=9$, $t=1.27$) and $134.4 \pm 31.30^\circ$ ($n=9$, $t=1.31$), respectively. Orientation to nongroom air was significantly greater than that to fresh air only for 1.00-1.49-min storage ($177.5 \pm 6.61^\circ$, $n=8$, $t=8.52$, $p < .001$). Mean orientations for 1.50-1.99-, 2.00-2.49-, and ≥ 2.50 -min storage were $142.5 \pm 37.67^\circ$ ($n=4$), $111.43 \pm 33.99^\circ$ ($n=7$), and $117.1 \pm 47.72^\circ$ ($n=7$), respectively. Thus, nongroom air seems to fall to a "baseline effectiveness" (typified by fresh air) faster than groom air, during storage. This differential rate of loss of effectiveness is further evidenced by the fact that mean orientation to groom air for 1.50-1.99-min storage is significantly higher than that to nongroom air, for the same storage time ($t=1.99$, $p < .05$), whereas differences are not significant for greater storage times.

The lack of any difference between orientation responses during fresh-air and nonpresentation trials suggests that the olfactometer successfully delivers air to the receiver in a manner that does not distract or disturb it, at least with respect to the measurement used. It was found, however, that the gerbil tended to poke its nose in the delivery port during the delivery of air in some trials. Some preliminary data suggest that this nose-poking behavior is independent of the presence of any odors and is dependent possibly on air delivery rate and probably on distance from the port (using a single flow rate). At a flow rate of 2 liters/minute, gerbils seemed to be sensitive to air flow within about a 4-cm radius of the air-delivery port (measured to the nose of the gerbil).

The robust orientation responses to groom and non-

groom air, for short storage times, clearly demonstrate the olfactometer's ability to capture and transfer odors from one chamber to another. The reduction in response as storage time increases suggests that the odors are absorbed into the apparatus or are spontaneously deactivated during storage. The former possibility seems unlikely, however, because the duration of effectiveness of the groom air (about 2 min) is very similar to the period of time, following a groom, that a gerbil retains its attractive properties to other gerbils (Thiessen, 1977). Lastly, the receiver gerbils show a different pattern of response to groom air than to nongroom air. Thus, it would seem that the present design of olfactometer can be used to isolate odors that occur during brief, episodic behaviors.

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