Not So Fast: Searching for Behavioral Effects of Putrescine in Direct and Conceptual Replications of Wisman and Shrira (2015).

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Author Note:

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Abstract

Understanding olfactory signal perception in humans is important for advancing basic scientific questions about the role of odor in cognitive and social processes. Here we review animal research on behavioral consequences of exposure to putrescine, a trace amine found in bodily tissues and which is produced by decay processes. Wisman and Shrira (2015) exposed human participants to putrescine and other aversive substance odors, gathered hedonic ratings, and reported heightened vigilance and increased threat and escape-related cognitions and behavior in putrescine conditions. In Wisman & Shrira and the present experiments, participants and experimenters were blind to substance condition. We conducted a direct replication of Wisman and Shrira's supraliminal exposure ratings and walking speed studies (Experiments 2 and 3) and a conceptual replication of a subliminal presentation defensive threat effect found in their Experiment 4. In our direct replication, putrescine and ammonia were rated similarly on intensity and repugnance, matching results obtained by Wisman and Shrira. Putrescine exposure was not associated with increased walking speed. In our conceptual replication, low-level putrescine exposure was not associated with ratings of potential aggressiveness of white and Black facial targets. Whether putrescine exposure reliably elicits threat-related cognition and behavior deserves further investigation.

Keywords: Putrescine, olfactory cognition, threat signals, face processing

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Diamines such as putrescine and cadaverine are produced in decaying flesh by decarboxylation of amino acids and are strongly malodorous. Putrescine is one of many trace amines found in the body and may be useful diagnostically as a tumor marker (Soda, 2011) and as a risk factor for post-surgery confusion in the elderly (Pan et al., 2019). Putrescine is found in saliva upon awakening and after eating (Cooke, Leeves, & White; 2003) illustrating the highly interactive presence of these amines in food and in our bodies. Trace amines exist throughout the microbiome and may be modifiable by microbiotic means as a method to understand food-caused inflammation (Berry, Gainetdinov, Hoener, & Shahid, 2017)

Volatile aromatic components of these compounds act on the nasal epithelium, which contains odorant receptors and trace amine-associated receptors (TAARs), a class of g-protein-coupled receptors (Izquierdo, Gómez-Tamayo, Nebel, Pardo, & Gonzalez; 2018). Research in zebrafish (Hussain et al., 2013) and rat models (Dewan, Pacifico, Zahn, Rinberg & Bozza, 2013) has been aimed at understanding the precise genetic mechanisms of TAAR receptivity that modulate avoidance behaviors across species. The extent to which conservation in TAAR receptors is found in humans, rodents and bony fishes (Izquierdo et al., 2018) is under active investigation and new research by Fei et al. (2020) found coordination of two TAAR enhancers to be highly evolutionarily conserved across jawed vertebrates. A recent genetic survey examined a large sample of Iceland's population (Gisladottir et al., 2020), finding a relationship between TAAR5 expression and sensitivity to the fishy smell of trimethylamine. A TAAR5 variant in 2% of the population rendered them less sensitive to the odor of aging or fermented fish as observed in qualitative verbal reports and pleasantness ratings. The authors remark it may be unsurprising fermented fish is used in Icelandic dishes more than in many other European cuisines.

Exposure to these substances has been found to trigger avoidance behavior in zebrafish (Hussain et al., 2013) but approach in omnivorous goldfish (Rolen, Sorensen, Mattson, & Caprio, 2003). Species-specific behavior is also observed in rodents; mice avoid cadaver mice in a maze (Prounis & Shields, 2013), rats showed greatly enhanced burial time for dead conspecifics and

putrescine and cadaverine-sprinkled dowel substitutes compared to control rats, anosmic rats, and to other odiferous compounds in a series of experiments by Pinel, Gorzalka, and Ladak (1981), and rats have a taste preference for cadaverine-scented food pellets (Heale, Petersen, & Vanderwolf, 1996). Hussain et al. (2013) comment on the social nature of some of the findings above and in other contexts such as sensitivity to urine components in feline marking (Dewan et al., 2013).

Given evidence for functional effects of TAAR receptors in governing odor perception, their extensive conservation across species, and a number of behavioral effects seen in lower animals to substances detected by these receptors, a reasonable next step would be to look for behavioral effects in humans after exposure to these odors. There appears to be only one investigation in humans of the behavioral correlates of putrescine exposure (Wisman and Shrira, 2015). In a series of laboratory and field experiments, the authors exposed participants to putrescine or other negatively-valenced odors and observed escape and threat-related behaviors. In Experiment 1, participants smelled putrescine, ammonia or water, provided ratings of intensity, repugnance and familiarity, then performed a speeded vigilance task. Dot detection was faster after putrescine relative to the other odors. In Experiments 2 and 3 participants walked away from the experiment site faster after providing smell ratings for putrescine compared to the other odors (over 80 meters and 60 meters, respectively) and completed word stems with more escape-related words (Experiment 3). Putrescine and ammonia were generally rated equal in repugnance and intensity (with intensity higher in the ammonia condition than for putrescine in one experiment). In Experiment 4, participants were exposed to 5% putrescine solution or water or ammonia at a subliminal level (and participants did not report odor presence). After putrescine exposure (compared to the other two conditions) affective reactions were more strongly negative to a scenario in which a minority (outgroup) student criticized the university. Wisman and Shrira saw their results as consistent with human evolutionary threat-management mechanisms incorporating olfactory cues –consciously or not – in ways that facilitate social functioning, citing other domains of odor cognition research such as behavioral consequences of exposure to human sweat.

Visual information from faces conveys social cues to which perceivers are highly attuned. One major model defines face space along two key dimensions; emotional valence / trustworthiness and power / dominance (Oosterhof & Todorov, 2008). An abundance of recent research has been

aimed at understanding the potential influence of face shape characteristics on judgments of perceived aggression. For example, researchers have closely examined the relationship between a specific measurement of facial structure, the facial width-to-height ratio (fWHR), and aggressiveness judgments and behavior. The fWHR is defined as the bizygomatic distance divided by the distance between the brow and the upper lip. Carré and McCormick (2008) found higher fWHR in men predicted lab-produced aggression and was positively related to penalty minutes awarded to varsity and professional hockey players. Loehr and O'Hara (2013) show a positive relationship between increased fWHR and lifetime reproductive success and a negative relationship with achieved rank in a sample of 795 photographs of Finnish soldiers from World War II. The relationship between high fWHR and increased perceived aggressiveness in neutral emotion faces is maintained when the face is blurred, preserving facial configuration information but eliminating specific visual cues, even though participants cannot state explicitly that configural information guided their judgments (McCormick, Mondloch, Carré, & Short, 2010). Taken together, these results suggest fWHR is an evolved cue to threat that provides a basis for approach or avoidance behavior. The metaanalytically derived effect size has been characterized as small (Haselhuhn, Ornison, & Wong, 2015) and consistent (Geniole, Denson, Dixon, Carré & McCormick, 2015).

Although static, neutral-expression faces may stably convey information about behavioral propensity toward aggression, face perception unfolds in variable affective, cognitive and social contexts. Downstream of immediate perceptual effects face memory is modulated by competitive interaction, as Balas and Thomas (2015) found; post-interaction face reconstructions in a face puzzle task exhibited higher fWHR after competition compared to cooperation.

Given the extensive consideration of fWHR as a potential marker of aggression, perhaps it is surprising there is only a developing body of research in which face race is manipulated. Wade and Renninger (2016) showed single category exemplars of different black male face shapes (e.g. trapezoidal, oval) to participants and found significant relationships between trait impressions and the fWHR computed from the exemplar. These relationships may be examined descriptively in trait judgment norms provided as part of the Chicago Face Database (CFD; Ma, Correll, & Wittenbrink, 2015).

Li, Moallem, Paller and Gottfried (2007) found subliminal scents guide likeability judgments of neutral faces in an odor-valence consistent way. At consciously-perceived levels, however, odor-valence consistent liking was not observed. Subliminal odor presentation can guide oculomotor search for congruent emotional faces (Dollion, Baudouin, Durand & Schaal, 2014) and odor valence and morphed face emotion information combine congruently; a lowered amount of information is required to judge ambiguous faces in the presence of butyric acid compared to strawberry aroma (Leleu et al., 2015). Syrjänen et al., 2018 found odor presence increased arousal to faces generally and that odor valence and facial emotion incongruence effects may be emotion and measure-specific. Happy faces showed very low arousal ratings in the presence of an unpleasant odor and the N170 marker of face detection was enhanced for disgust face displays in the presence of a pleasant odor. Findings such as these informed the development of our Experiment 2 conceptual replication.

In Experiment 1 we report a direct replication of the double-blind hedonic ratings and walking speed experiments reported in Wisman & Shrira (2015). We collected ratings of intensity, repugnance and familiarity for water, ammonia and putrescine as in the original study, and surreptitiously recorded walking times over a fixed distance as participants left the location. We expected to replicate the Wisman & Shrira relative ratings results with putrescine being generally equal in intensity ratings compared to ammonia, and for the two substances to be equal in repugnance. Crucially, a replication of the supraliminal walking speed studies would show clear speed increases after putrescine exposure but not after ammonia or water exposure.

In Experiment 2 participants were exposed to putrescine at the subliminal level used in Wisman and Shrira's (2015) Experiment 4 (5%), at half that level (2.5%) and water. Participants and experimenters were blind to odor condition. Participants made aggressiveness judgments to high and low fWHR black and white faces. We developed a standardized deployment method to test the effect of subliminal putrescine exposure on face aggressiveness ratings, hypothesizing that if putrescine reliably elicited threat or escape-related cognitions and behaviors, overall facial aggressiveness ratings in the presence of putrescine would be higher than in a control condition. In Wisman & Shrira Experiment 4, the rating of an outgroup member who expressed hostility toward an institution in a presented scenario is ecologically valid for a student participation pool, and judging faces of peer-aged

men is also highly likely to be a relevant social task where combinatory effects of an olfactory threat cue – particularly at subliminal levels as in Li et al. (2007) – could influence judgments.

Experiment 1

General Description of Sampling Method

The bulk of the data from Experiment 1 was obtained in a study conducted outdoors from September – October 2020. The procedure was completed in a self-service manner at staffed stations to maintain COVID-19 safety. The Wittenberg University IRB approved the procedure (#s 013-201819 and 002-202021). Our sample of ratings and walking times was collected by convenience. We asked pedestrians to participate if they were alone and walking between one of two stations, arranged 53 meters apart, at one of two possible campus locations. The two-station, two-experimenter arrangement allowed us to sample bidirectional pedestrian traffic walking times by surreptitious timing after the ratings task, but also resulted in lost participant walking times if the walker changed direction between stations. We emailed instructions to an additional group of students that we would be testing at an outdoor running track and set up one station and demarcated an ending point (for surreptitious timing) 60 meters from the rating location. Finally, we collected odor ratings from a previous experiment (Fall 2019) using the same measure and method (individually) but in a Chemistry laboratory. In Experiment 1 we evaluate the hedonic ratings of intensity, repugnance and familiarity with the full data set and analyze walking times only for those participants for whom we also have ratings.

Sample Size Considerations

Our goal was to exceed the N of participants with rating and walking speed measurements in the larger of the two Wisman & Shrira (2015) walking speed experiments (N = 60, Experiment 3).

Method

Participants

Double-blind ratings (N=120) were provided for three substances (water; N=37, ammonia; N=43, putrescine; N=40). Walking speed was collected for 72 of these participants (water; N=25, ammonia; N=24, putrescine; N=23). No personally identifying information was collected and no demographic information was collected.

Materials and Procedure:

For the majority of the ratings (N=89) and associated walking times (N=72) participants were asked if they wished to participate if walking alone. If answering affirmatively, experimenters pointed to and read consent and instruction sheets which contained a photograph of how the pad should look after substance delivery and included the three scale items. All materials were placed on a portable 4-ft diameter table. Verbal consent was obtained and participants chose a coded 2-oz plastic bottles from a set of 6 such dispensers (2 containing each substance). After rating, dispensers were placed in a used area (thus there was selection without replacement for successive participants). The remainder of the ratings (N=31) were obtained in an indoor chemistry laboratory by self-selection of a coded 4-oz jar which contained a pad with the odor substance. Ratings of intensity, repugnance and familiarity were obtained verbally and recorded by experimenters. Outdoor participants were debriefed by telling them we hoped to replicate a ratings pattern for aversive substances seen previously. The substances under investigation were identified as water, ammonia (“a cleaning chemical”) and putrescine (“a natural substance that occurs in the body and is associated with decay”). Participants were thanked and the experimenter started a timer when they left the table. When participants reached the opposing station, a second experimenter stood to initiate a second debriefing of participants and the first experimenter stopped the present timing. Participants were then told about our walking speed measurement and that we were trying to replicate a pattern of people walking faster after exposure to aversive substances. We asked participants in the second debriefing not to discuss the aims of our work with others.

Putrescine and ammonia solution preparation

A 25% (w/v) solution of Putrescine (1,4-Diaminobutane, Sigma-Aldrich D13208) was created by dissolving 5g of Putrescine in 20mL of deionized water and stirred until dissolved. A 5% (v/v) solution of Ammonium Hydroxide (Fisher A669-212) was prepared by diluting 5mL of Ammonium Hydroxide to 100mL of deionized water and stirred. Equal sample sizes were aliquoted into coded plastic 2-oz containers with screw-on felt-tip tops. Codes were created and maintained by Chemistry staff.

Results

Our analysis method was modeled closely on that of Wisman and Shrira (2015) for the hedonic ratings and walking times. In the original work, ratings of each type (intensity, repugnance and

familiarity) were submitted to separate one-way ANOVAs with the two aversive substances compared (ammonia and putrescine). We conducted independent-samples t-tests on the means for ammonia and putrescine. In the original work, walking times (in seconds) for all three conditions (including water) were analyzed in a one-way ANOVA and we do the same (with times transformed to meters per second).

Intensity ratings for putrescine ($M=4.53$, $SD=2.32$) were equal to those for ammonia ($M=3.51$, $SD=2.51$), $t(81)=1.91$, $p=.06$. Similarly, repugnance ratings for putrescine ($M=4.75$, $SD=2.66$) were equal to those for ammonia ($M=3.72$, $SD=2.35$), $t(81)=1.87$, $p=.07$. Finally, familiarity ratings for putrescine ($M=5.30$, $SD=2.58$) were equal to those for ammonia ($M=4.47$, $SD=2.66$), $t(81)=1.45$, $p=.15$.

Figure 1: Hedonic ratings distribution, and descriptive and inferential statistics for comparisons between Intensity, Repugnance and Familiarity ratings as a function of aversive substance, Experiment 1.

Condition means are represented by the thin vertical line.

A one-way between-participants ANOVA with substance as the factor and walking time as the DV revealed no effect of odor condition, $F(2, 46)=0.17$, $p=.85$. In Figure 2, horizontal dashed lines indicate the fastest and slowest walking speeds, in meters/sec, among the odor conditions in the two experiments reported by Wisman and Shrira (2015).

Figure 2: Walking speed as a function of substance exposure, Experiment 1. Demarcated with dashed lines are the slowest (Left: the water exposure condition of Experiment 2) and fastest walking speeds (Right: the putrescine exposure condition from Experiment 3) from Wisman and Shrira (2015).

Finally, we examined each odor condition separately to see if walking times showed a regular relationship to rating strength by computing Pearson correlations and examining scatterplots. In the water condition there was no relationship between rated intensity, rated repugnance or rated familiarity and walking speed in M/S, all r s < $|.14|$ and all p s > $.63$. In the ammonia condition there was no relationship between rated intensity or rated familiarity and walking speed in M/S, all r s < $|.12|$ and all p s > $.58$. For participants exposed to ammonia, however, there was a significant positive relationship between the repugnance rating and speed, $r=0.48$, $N=24$, $p=.02$. Lastly, in the putrescine condition there was no relationship between rated intensity, rated repugnance or rated familiarity and walking speed in M/S, all r s < $|.14|$ and all p s > $.51$. Please see Figure 3.

Figure 3: Relationship between Intensity and Repugnance ratings and walking times (in M/S) for the two aversive substance used in Experiment 1.

Discussion

In Wisman and Shrira (2015), hedonic ratings of the two aversive substances were generally the same across experiments. This pattern informed the conclusion that mechanisms specific to the odor of putrescine elicited activations of threat and escape behavior rather than a reaction to any aversive scent. With a larger pool of ratings, we replicate these results, noting in three Wisman and Shrira studies (Experiments 1, 2 and 3) the relative degree of repugnance for the scent of ammonia and putrescine changed polarities (although with no significant differences between the conditions). Such instability of the relative amplitudes of these measurements may reflect enhanced variability due to repeated use of small samples sizes. Inspecting the N of the relevant Wisman and Shrira (2015) studies reveals 45 participants in Experiment 2 (with no indication of division among odor subgroups, which if equal would be 15 per group) and the N was 60 for Experiment 3 (again, with no indication of division into odor conditions, which if equal would be 20 per group).

We failed to replicate Wisman and Shrira's Experiment 2 and 3 findings that walking speed increased after putrescine exposure. Walking speeds were identical across odor conditions despite including a total sample 20% larger than the larger of the two original studies. The range of our walking speeds in the middle of – and less variable than – those reported by Wisman and Shrira (2015). Finally,

our results show that for ammonia-exposed participants, increased repugnance ratings were related to faster walking speed, results that should be replicated as they directly contradict the original findings.

Experiment 2

Wisman and Shrira's (2015) Experiment 4 involved presenting written scenarios to participants, who made judgments about the hostility of a target person. The hostility metric was composed of four items. We note again the small sample size of Experiment 4 ($N=69$, with no indication of subsample sizes for the three odor conditions) and also that the time required to fill out the experimental questionnaire was recorded as a proxy for escape but the authors do not report those data.

Here, participants made aggressiveness judgements of 50 Black and 50 white faces, sets which were selected to be substantively different in fWHR within each face race. These judgments were made in the presence of water or putrescine placed on a small filter pad directly in front of the participant. Participants and experimenters were blind to odor condition. If odors can be a cue within an evolved threat management mechanism – and in particular if putrescine constitutes a reliable threat cue – aggressiveness ratings may show main effects of putrescine or interactive effects of putrescine in combination with the face stimulus factors that we do not find in the presence of water or ammonia.

Sample Size Considerations

We calculated a d effect size from the means and standard deviations presented by Wisman and Shrira (2015) in their probe of the main effect in the one-way ANOVA reported for Experiment 4, contrasting putrescine, ammonia and water exposure conditions. The d was 0.82, equivalent to a Cohen's f of 0.41. We used this effect size and G*Power version 3.1 to calculate a-priori sample size for ANOVA repeated measures, between factors using an alpha level of 0.05, power of 0.95, 3 groups, 100 measurements, and a correlation among the measures estimated at .80. The required number of participants was 78 by these calculations.

Method

Participants

Students at Wittenberg University ($N=123$, 87 women; $M_{Age}=20.94$ years, $SD_{Age}=4.66$ years) participated for partial course credit or were volunteers. Participant racial self-identification was white ($N=108$) more often than other racial categories ($N=12$ Black, $N=3$ other or more than one race).

Assignment to odor condition was blinded for participants and experimenters by virtue of vial selection from a randomized and coded set of vials, rotated regularly by Chemistry department staff. There were 44 participants in the water condition (30 women), 36 participants in the 2.5% putrescine condition (25 women), and 43 participants in the 5% putrescine condition (31 women).

Face stimuli

A set of 100 neutral expression color face photographs was constructed by selecting the 25 highest fWHR Black and white male faces and the 3rd - 27th lowest fWHR Black male faces and the 25 lowest fWHR white male faces from the Chicago Face Database, version 2.0 (Ma et al., 2015). We did not include two very low fWHR Black male exemplars, enabling low fWHR faces to match in fWHR across race ($M_{\text{LowfWHRBlack}} = 1.70$, $SD_{\text{LowfWHRBlack}} = .06$ and $M_{\text{LowfWHRWhite}} = 1.72$, $SD_{\text{LowfWHRWhite}} = 0.06$), $t(48) = 1.32$, $p = .19$. High fWHR faces also matched in fWHR across race ($M_{\text{HighfWHRBlack}} = 2.03$, $SD_{\text{HighfWHRBlack}} = .09$ and $M_{\text{HighfWHRWhite}} = 2.00$, $SD_{\text{HighfWHRWhite}} = .06$, $t(48) = 1.48$, $p = .15$). Figure 4 presents example face stimuli, CFD identification number, fWHR and fWHR ranges.

Figure 4: Example Face Stimuli. Two examples of each of the four groups of 25 Chicago Face Database photographs presented to participants with CFD ID number, fWHR of the stimulus, and the range for the group.

Putrescine solution preparation and delivery

Putrescine (1,4-Diaminobutane) was obtained from Sigma-Aldrich (Item number D13208). Putrescine melts at 25-28 deg C, so is solid in the jar. Gentle warming in a beaker of warm water enabled use of an eyedropper. To 95.03 g deionized water was added dropwise 4.99 g of putrescine to make a 5% solution. 30 mL of this was measured in a graduated cylinder and diluted to 60 mL with deionized water to make 2.5% solution. This stock was used to fill vials for the experimental stations.

Drop count was considered for delivery of the solutions and plastic pipettes are convenient for this purpose. Testing plastic pipettes with water and putrescine solutions showed that for pure water 1 mL in pipette required 20 drops to empty. For the 5% putrescine solution, 1 mL in pipette required 23-24 drops to empty. Drop size with putrescine was thus significantly smaller and likely depends linearly on concentration. Because the amount of solution delivered by a drop varied with concentration in plastic pipettes, digital pipets were used for drop delivery of 0.05 mL each.

Delivery of the dosing drop to standard copy paper was tested. A single drop of water wet the paper only very slowly, soaking in after about 45 minutes. Instead of standard copy paper, experimenters delivered a single drop per participant under a fume hood to 5.5 cm filter paper disks (Fisher P-8: stock number 09-795A), each stapled to a 5.5 cm x 21.5 cm piece of standard copy paper. The long strip of paper was then placed into position on the tabletop in front of the participant, directly under the CRT, as they adjusted their seating prior to beginning the practice trials.

Apparatus

Faces were displayed on a Sony 21-inch monitor placed on a table in front of the participant. Participant position relative to the monitor was not standardized but participants were encouraged to sit as close to the desk and monitor as possible. The height of the chair was adjusted to position the participant's head level with the center of the monitor. Seating distance to the center of the monitor and to the position of the filter paper was approximately 55 cm (Figure 5).

Faces were presented using SuperLab (Cedrus Corporation), version 4.5. Each face subtended approximately 9 x 5 degrees of visual angle at the average seating distance. Responses were collected using the 1-7 keys on a MacBook Pro laptop. The laptop screen was covered with a folder and participants were instructed to look straight ahead, viewing the faces on the large monitor.

Figure 5: Seating of participant relative to monitor and filter paper with water, 2.5% or 5% putrescine.

Procedure

Participants were tested in a Chemistry laboratory. Labs had two or more large ventilation hoods opened to trigger air recirculation at least 30 minutes prior to the first participant of the day and for at least 30 minutes between participant sessions. Participants read the consent form and were told they would or would not be presented with an odor while they viewed faces and made face judgments. They were told the odor, if present, was meant to be subliminal, described as being below the level of conscious awareness. Participants were informed there was no danger from exposure to the odor substance as determined by the University's Chemical Hygiene Officer and the Institutional Review Board approved our protocol for use with human participants. Prior to the experiment participants provided demographic information and completed a 4-item handedness measure.

Participants saw four practice trials, one face at a time, to familiarize them with the procedure. They were informed that the practice trials contained two Black and two white faces presented in single alternation, and that in the full set of 100 trials they would see either 50 Black faces or 50 white faces first, with the first face race randomly determined. Two different random orders of the differing race face lists were created for presentation to participants in a random order.

On each trial, participants saw a fixation plus sign for 1000, 1200, or 1400 ms, randomly determined by the computer program. The fixation marker was removed and a face was centrally presented for 2000 ms. The face was then replaced with a Likert-type item, as used in Carre, McCormick, and Mondloch (2009); "How aggressive do you believe this person would be if provoked?"

with a scale containing the numbers 1 through 7. The scale was labeled on each trial with number 1 as “not at all aggressive” and 7 being “most aggressive.” Participants proceeded through the trials in a self-paced manner, pressing the space bar for successive trials when ready.

After the trials participants were presented a sheet of paper with the prompt “During this experiment, did you smell anything unusual?” written at the top accompanied by the answer options “yes” and “no”. If participants answered yes, they were asked to describe what they smelled in a space below the prompt. Participants were given a debriefing form which explained they would not be informed of their odor condition immediately but that they could inquire more about the experiment at a later date. They were thanked for their participation and exited the laboratory. The duration of the experiment was approximately 15 minutes.

Results

Two participants were removed from further analysis. In one case (a white male participant) all responses were identical for the 50 Black faces; the participant provided all 1 (not at all aggressive) responses, and volunteered after the experiment that they did so deliberately. In the other case, a Black male participant was somewhat visibly disturbed during the experiment. Later inspection of his reaction times to make the judgments (RTs were collected by the computer program with the aggressiveness ratings) in the within-participant conditions (race x fWHR) showed RTs greater than 5 SDs above the mean for each condition and so his data were not considered.

Mean aggressiveness ratings were submitted to a 2 (Face list order) X 2 (Face race order) X 3 (Odor condition) X 2 (fWHR; Low or High) X 2 (Race; Black or White) mixed-factor ANOVA. There were no significant main effects of the List and Race order conditions, $F_s < 1$, *ns*, nor was there a significant main effect of the Odor condition on judgments, $F(2, 109) = .03$, $p = .97$. Mean aggressiveness ratings for participants in the water condition ($M = 3.71$, $SD = .86$), the 2.5% putrescine condition ($M = 3.67$, $SD = .86$) and the 5% putrescine condition ($M = 3.67$, $SD = .88$) were essentially identical. None of the between-participants experimental factors were found to interact, all $F_s < 1.24$, *ns*.

Few participants explicitly claimed to smell anything in the post-experiment questionnaire. Four of 44 participants (9.1%) showed a false alarm, claiming to smell something in the water condition. Six of 36 (16.7%) claimed to smell something in the 2.5% putrescine condition and 7/41

(17.1%) claimed to smell something in the 5% putrescine condition. There was little commonality in the open-ended smell description responses, which ranged from flowers or sweetness in a few cases to idiosyncratic reports of sourness, cologne, urine or vinegar. One male participant in the 5% condition claimed to smell the odor of tree flowers or semen, scents the majority of experimenters thought were most similar to the putrescine solution odor when brought directly adjacent to the nostrils (particularly Pear tree blossoms). We asked the participant if he touched the filter or brought it to his nose during the experiment and the response was negative.

We did not analyze the results quantitatively as a function of explicit smell reports because there were so few reports overall. The degree to which we were successful in maintaining supraliminal exposure across the three odor-exposed groups of participants might be roughly computed as follows. If the false alarm rate in the water condition is more widely applicable across conditions and thus might be subtracted from the smell hit rate, the claim of real “smelling” might only be about 8% in our two putrescine groups.

Discussion

Wisman and Shrira (2015, Experiment 4) reported putrescine-selective increased hostility (presumed to reflect defensive threat and measured by an index of four questions) in student participants after reading a description of a foreign student criticizing the university. In Experiment 2, our method allowed viewing of racial ingroup and outgroup stimulus faces by our primarily white student participants, faces that also varied maximally on a well-studied marker of potential aggressiveness. We think it important from a validity standpoint to have used a large and variable set of face stimuli. The facial identity variety inherent in our full set of stimuli should provide more reliable and potentially more externally valid estimates of judged aggression than in some earlier work. These seemed like prime conditions under which to search for putrescine effects. We specifically manipulated putrescine exposure to be the same (5%) as the replicated study and also lower, in case we might capture subliminal-to-supraliminal boundary performance changes as seen in Li et al. (2007).

We found no effects of putrescine exposure on aggressiveness ratings of face stimuli and no interactions of odor condition with fWHR or face race in predicting aggressiveness ratings. These null findings were observed despite using an effect size calculation based on the earlier Wisman and Shrira

(2015) study, planning sample sizes via a priori power calculations and exceeding those sample size targets substantively. We exerted careful control over every aspect of putrescine exposure, including creating and dispensing the solution and blinding of participants and experimenters to odor condition. In particular we think our method, involving placement of the putrescine solution on absorbent filter paper disks, probably resulted in a less variable distribution of the odor substances to participants, as there would be no chance for liquid to run off the surface of the standard copy paper; we observed very slow absorption in our pre-experiment tests. Wisman and Shrira's delivery of a drop to the top of a questionnaire may have enabled participants to be closer to the odor source than in our setup but we cannot be sure of this; in any case, participant seating relative to the odor source may have been more variable in the original research compared to our method.

General Discussion

Our central findings stand in opposition to Wisman and Shrira's (2015) single published set of experiments on human behavior in reaction to the trace amine putrescine. We find null behavioral effects of putrescine at low exposure levels and at conscious levels. However, we replicated the overall similarity of rated intensity and repugnance for ammonia and putrescine using the same instrument.

There are plausible explanations to resolve these conflicting findings. First, although the *t* distribution was developed specifically to be sensitive for the use of small samples and is generally robust even at very small independent group sample sizes of $N=5$ or fewer, there is an inflation of Type I error rates when sample sizes are low, unequal, and when the smaller N group has lower variance than the larger N comparison group (de Winter, 2013). Repeated sampling and testing over several experiments in Wisman and Shrira (2015) with N s ranging from 15-23 leaves open the possibility of compounded Type I error rates, an explanation for Wisman and Shrira's results that should not be discounted. The experiments reported here increased sample sizes over the original research by at least 20% to try to combat this statistical issue.

Despite our best efforts to find a conceptual replication environment that would be well-suited to revealing effects of putrescine, it is possible that judgments of likely aggression from briefly viewing face stimuli are not as engaging of defensive threat responses compared to outgroup criticism of a highly personally-relevant ingroup institution for college students. That is, perhaps the effects of putrescine are

only likely to be seen in certain highly engaging and more deep social interactions and not in those required for a passing face judgment, a proposition that is empirically testable. With continued research and further understanding of olfactory cognition, we may encounter a day soon when the behavioral and cognitive consequences of exposure to trace amines, and to putrescine in particular, are more reliably demarcated and consequently understood.

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