Olfactory exposure to males, including men, causes stress and related analgesia in rodents

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We found that exposure of mice and rats to male but not female experimenters produces pain inhibition. Male-related stimuli induced a robust physiological stress response that results in stress-induced analgesia. This effect could be replicated with T-shirts worn by men, bedding material from gonadally intact and unfamiliar male mammals, and presentation of compounds secreted from the human axilla. Experimenter sex can thus affect apparent baseline responses in behavioral testing.

Rodents can discriminate human experimenters by smell, and their behavior can be affected by such perception[1,2], but it has not been shown that human presence can affect the results of laboratory experiments. Our laboratory personnel have reported anecdotally that pain behavior appears to be blunted while experimenters are present (for example, after administering pain-inducing algogens). The recent development of a highly sensitive and totally blinded pain measure, the mouse grimace scale[3], allowed us to evaluate this hypothesis.

Pain was produced by bilateral ankle injections of sterile zymosan A, an inflammatory agent (Online Methods). We placed experimentally naive mice into clear Plexiglas cubicles and recorded facial expressions of pain. We compared facial grimacing in mice tested in the presence of an experimenter (seated quietly at a distance of ~0.5 m) to that of mice tested in an empty room. Four different adult male experimenters produced robust (mean: 36%) and significant decreases in facial grimacing relative to no observer, whereas none of four adult female experimenters produced significant changes (main effect of observer sex: $F_{2,90} = 24.2$, $P < 0.001$; effect of within-sex experimenters: $F_{9,90} = 1.3$, not significant (n.s.)) (**Fig. 1a**). A *t*-test performed on mean data from individual male and female experimenters was also highly significant: $t_6 = 4.3$, $P < 0.01$. Similar effects were seen in rats (**Supplementary Fig. 1**). Both male and female mice displayed this 'male observer' effect, but female mice did so to a greater degree (**Supplementary Fig. 2**). Changing the sex of the technician providing animal husbandry did not alter results (**Supplementary Fig. 3**), nor did changing the sex of the person administering zymosan (**Supplementary Fig. 4**).

The phenomenon appeared to be olfactory in nature, as identical effects were obtained by placing cotton T-shirts, worn the previous night by male or female experimenters, ~0.5 m away from the mice (main effect of T-shirt wearer sex: $F_{5,75} = 6.3$, $P < 0.001$; effect of within-sex T-shirts: $F_{9,75} = 1.2$, n.s.; **Fig. 1b**). A single male-worn T-shirt produced a maximal effect; one that was abolished by the simultaneous presence of a female-worn T-shirt. The analgesic effect of the male T-shirt was found to abate fully by 30–60 min (**Supplementary Fig. 5**). However, the effect did not appear to abate from one day to the next, as mice exposed to a male experimenter for 1 h on a single day or 30 min/d for 5 d displayed equivalent inhibition of facial grimacing on the subsequent day in the presence of the same or a new male experimenter (**Supplementary Fig. 6**).

We next tested whether the phenomenon could be observed on the formalin test, in which, in addition to grimacing, nocifensive behavior (licking and biting directed at the injected hind paw) is observed. Using a low concentration of formalin (1%), we observed a similar inhibitory effect of male-worn T-shirts (**Supplementary Fig. 7a**), statistically significant in only the early phase of the formalin test (0–10 min post injection), likely owing to habituation, as the effect could be reinstated in the late phase by the delayed appearance of a male experimenter (**Supplementary Fig. 7b**). When we used a higher concentration of formalin (5%), olfactory stimuli alone were not sufficient to produce the effect in the formalin test; concurrent visual stimuli were required (**Supplementary Fig. 7c**).

Axillary secretions were likely responsible for the effect, and so we tested a number of volatile acidic and steroidal compounds with secreted concentrations that are known or suggested to be greater in male than in female mammals[4,5]. We observed a concentration-dependent reduction of facial grimacing with gauze saturated with 300 pM–300 µM (*E*)-3-methyl-2-hexenoic acid (3M2H), 0.75–3 mM androstenone and 0.75–3 mM androstadienone

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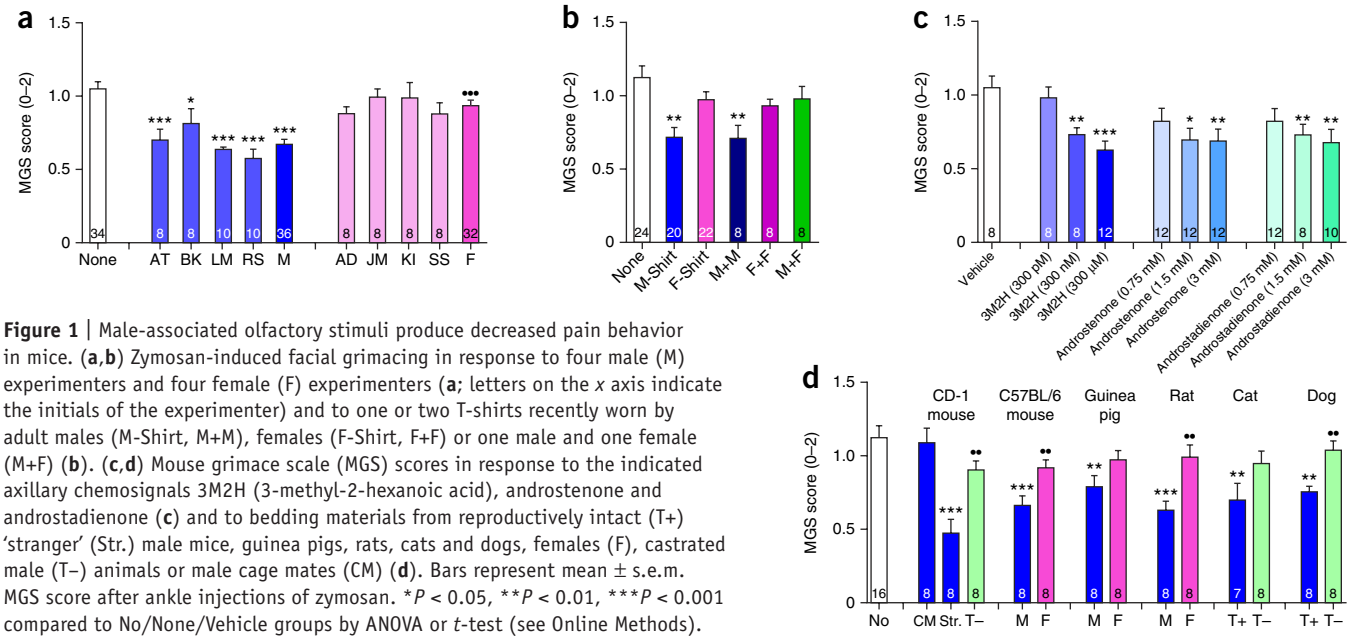

Figure 1 | Male-associated olfactory stimuli produce decreased pain behavior in mice. (**a**,**b**) Zymosan-induced facial grimacing in response to four male (M) experimenters and four female (F) experimenters (**a**; letters on the *x* axis indicate the initials of the experimenter) and to one or two T-shirts recently worn by adult males (M-Shirt, M+M), females (F-Shirt, F+F) or one male and one female (M+F) (**b**). (**c**,**d**) Mouse grimace scale (MGS) scores in response to the indicated axillary chemosignals 3M2H (3-methyl-2-hexanoic acid), androstenone and androstadienone (**c**) and to bedding materials from reproductively intact (T+) 'stranger' (Str.) male mice, guinea pigs, rats, cats and dogs, females (F), castrated male (T–) animals or male cage mates (CM) (**d**). Bars represent mean ± s.e.m. MGS score after ankle injections of zymosan. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ compared to No/None/Vehicle groups by ANOVA or *t*-test (see Online Methods). $••P < 0.01$, $•••P < 0.001$ compared to analogous male-related stimulus group (T– mice compared to T+ or Str. mice) by ANOVA or *t*-test in graphs **a** and **d**. Group sample sizes are provided in each bar.

(4,16-androstadien-3-one) placed ~0.5 m away from the mice ($F_{3,32} = 9.2$, $P < 0.001$; $F_{3,40} = 3.4$, $P < 0.05$; $F_{3,32} = 5.3$, $P < 0.01$, respectively) (**Fig. 1c**). Other putative human pheromones tested were ineffective even when tested at extremely high (30 mM) concentrations (**Supplementary Fig. 8a**). Analgesic effects were not due to olfactory novelty because neither food-related nor aversive odorants affected grimacing (**Supplementary Fig. 8b**). As chemosignaling molecules are structurally invariant in all mammalian species, we tested whether used bedding material from other strains and species might replicate the phenomenon. Bedding material from gonadally intact unfamiliar male mice, nonpredator male guinea pigs and gonadally intact male sympatric predator species (rats, cats and dogs) all produced decreases in facial grimacing ($F_{13,104} = 6.3$, $P < 0.001$; **Fig. 1d**), whereas bedding from the subjects' own cages (including contributions from three cage mates) did not produce changes. The fact that beds from castrated male mice, cats and dogs did not produce the effect directly suggests that androgens are necessary.

There are two possible explanations for the decreases in pain behaviors observed. Mice might simply be consciously inhibiting outward signs of pain, or the stimuli might be producing stress-induced analgesia (SIA), an innate response whereby pain processing is suppressed in the spinal cord by descending modulatory pathways[6]. Both predator odors[7] and the odor of isolated conspecific males (but not from mixed-sex pairs)[8] have been shown to produce SIA. We confirmed that exposure (in the absence of pain) to male experimenters and male-worn T-shirts increased plasma corticosterone levels in mice ($F_{5,34} = 8.8$, $P < 0.001$), an effect abating 30 min after the stimulus was removed. Individual chemosignals produced similar increases compared to vehicle ($F_{3,34} = 3.1$, $P < 0.05$). The corticosterone increases were equivalent to those induced by a 15-min restraint or a 3-min forced swim (**Fig. 2a**). Supporting the assertion that exposure to male odor is stressful is the significant increase in fecal boli deposited by mice in the 30-min testing period in which they were exposed to male- but not female-worn T-shirts ($F_{2,17} = 8.3$, $P < 0.005$; **Fig. 2b**). A final index of stress was the repeated rectal measurement of core body temperature by male or female experimenters, which revealed both highly significant experimenter sex–based differences at all time points (all *P* values < 0.05) and a more rapid progression of hyperthermia when male experimenters were the testers (experimenter sex × repeated measures: $F_{2,92} = 3.7$, $P < 0.05$; **Fig. 2c**).

True SIA should be accompanied by decreased immediate-early gene expression in the pain-processing neurons of the spinal cord dorsal horn; we observed a dramatic (≥50%) decrease in Fos protein–positive neurons in mice injected with zymosan and exposed to male but not female experimenters or shirts (Kruskal-Wallis test statistic: 12.0, degrees of freedom = 4, $P < 0.05$; **Fig. 2d**). Much is known about the neurochemistry of SIA, which exists in opioid (naloxone-reversible[9]), non-opioid (cannabinoid-1 (CB_1) receptor–mediated[10]) and mixed opioid/non-opioid[11] forms. The male experimenter SIA seen here was found to be the latter, as it was blocked (observer × drug interaction: $F_{3,56} = 2.8$, $P < 0.05$) by the broad-spectrum opioid receptor antagonist naloxone (1 mg per kg body weight (mg/kg); $P < 0.05$), by the inverse CB_1 receptor agonist AM-251 (10 mg/kg; $P < 0.05$) and by a combination of the two drugs ($P < 0.01$), at doses not affecting pain sensitivity *per se* (**Fig. 2e**).

Most preclinical pain testing requires an ever-present and looming experimenter who delivers thermal or mechanical stimuli directed at precise body sites. Our data predict that experimenter-subject interactions might alter apparent baseline sensitivity on these tests. We tested this hypothesis by reanalyzing large archival data sets ($n = 226$–610 mice per experimenter sex per assay) from our laboratory. We found that baseline latencies or thresholds were significantly higher when testing was performed by male rather than female experimenters in three different acute pain assays ($n = 4$–6 experimenters per sex; all *P* values < 0.001 for main effect of tester sex; all *P* values < 0.001 for within-sex tester effects; **Fig. 3a**–**c**), indicating a possible analgesic effect.

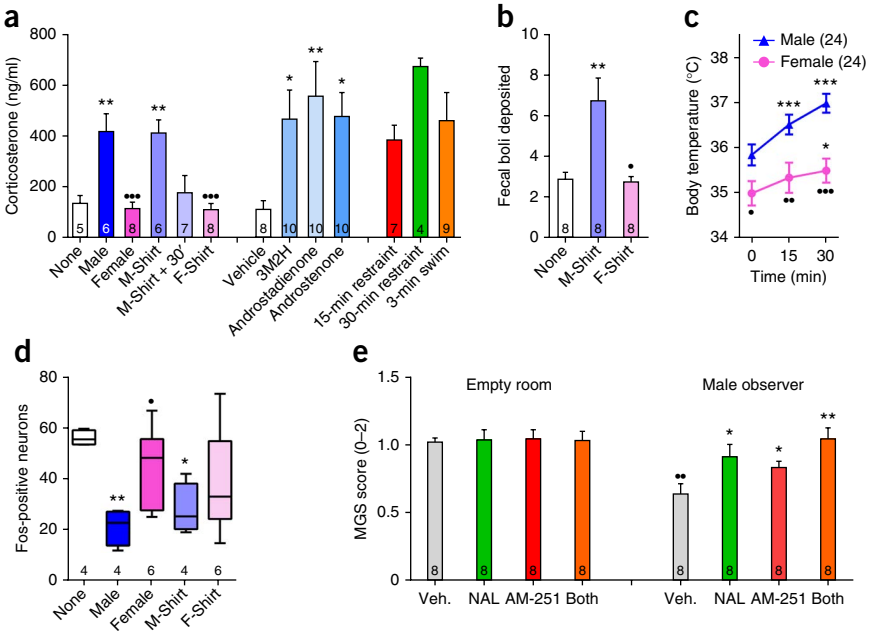

Figure 2 | Observed decreases in pain behavior are due to stress-induced analgesia. (**a**) Stress hormone levels in response to exposure of mice to male experimenters, male-worn T-shirts (M-Shirt; M-Shirt 30 min later (M-Shirt + 30')) or axillary chemosignals (at lowest effective dose from **Fig. 1c**), in the absence of any noxious stimulus. Bars represent mean ± s.e.m. (**b**) Fecal boli deposition after exposure of mice to male-worn T-shirts (mean ± s.e.m.) over a 30-min period. (**c**) Body temperature measurement (mean ± s.e.m.; $n = 24$ mice per condition) in mice handled by male versus female experimenters. (**d**) Decreased expression of the immediate-early gene *Fos* in spinal cord neurons after zymosan injection and exposure to male or female experimenters or shirts. Box-and-whisker plots show median, quartile and extreme Fos-positive neurons. A mean of 12 tissue sections were analyzed per mouse. (**e**) Mouse grimace scale (MGS) scores after blockade of opioid receptors using naloxone (NAL; 1 mg/kg), blockade of CB_1 receptors using AM-251 (10 mg/kg), or their combination (both). Bars represent mean ± s.e.m. Vehicles saline and DMSO were combined (Veh.). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared to None/Vehicle/0 min/Veh. groups (by ANOVA followed by Dunnett's case-comparison test in **a**–**c** and **e**; by Kruskal-Wallis test in **d**). $^{\bullet}P < 0.05$, $^{\bullet\bullet}P < 0.01$, $^{\bullet\bullet\bullet}P < 0.001$ compared to an analogous male stimulus (in **a**–**d**) or empty room (in **e**) group. Group sample sizes are provided in each bar.

Furthermore, in mice tested by male experimenters only, a significant repeated-measures effect was observed such that thresholds were decreased at the second baseline compared to the first, suggestive of abatement of the analgesia over time (**Supplementary Fig. 9**). We independently confirmed robust analgesic effects of the presence of male experimenters and/or T-shirts on von Frey fiber withdrawal thresholds in another laboratory using a within-subject design (main effect of tester sex: $F_{1,114} = 170.6$, $P < 0.001$; effect of within-sex tester: $F_{4,114} = 49.0$, $P < 0.001$; tester sex × repeated-measures (T-shirt effect) interaction: $F_{2,228} = 23.6$, $P < 0.001$; **Fig. 3d**).

Finally, we wished to examine whether male olfactory stimuli would affect another common behavioral assay. We chose the open-field test, in which anxiety is associated with thigmotaxis, or wall-hugging behavior. The simple presence in the room of male- but not female-worn T-shirts significantly exacerbated thigmotaxis in mice (main effect of T-shirt wearer sex: $F_{2,15} = 18.5$, $P < 0.001$; effect of within-sex T-shirts: $F_{5,15} = 0.9$, n.s.; **Fig. 3e**).

The concentrations of androstenone and androstadienone used in this study are several orders of magnitude above those observed in human sweat[12]. However, presentation of androstadienone in such concentrations increased cortisol levels in women[13], via activation of the hypothalamus[14]. Hypothalamic activation, however, is not specific to androstadienone, as a chemically and biologically unrelated but perceptually similar molecule (Polysantol) has the same effect[15], pointing toward perceptual (i.e., olfactory) quality as the key underlying factor. In fact, we observed analgesic effects upon the presentation of three different odorants, with one (3M2H) being chemically unrelated to the others. It is therefore impossible that we observed a substance-specific 'pheromone'-like effect. Our data suggest instead that an odor evoked by a cocktail of chemicals within the body secretions of isolated males (except cage mates) produce stress in rodents, leading to SIA. The effects produced by human males are simply due to the invariant structure of these chemicals across mammalian species. Although it is short lasting, stress caused by male experimenters

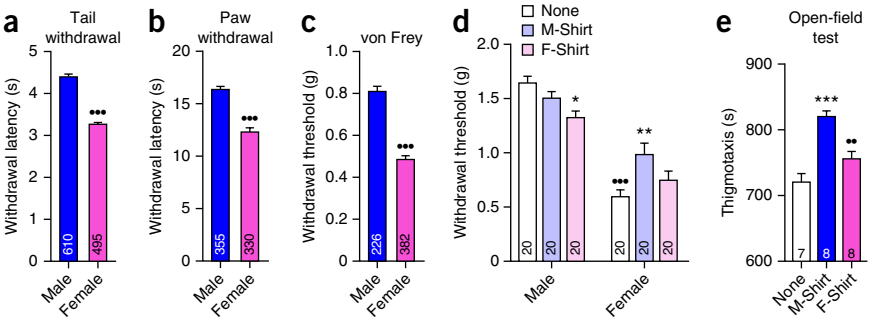

Figure 3 | Mice tested by male experimenters display lower apparent baseline pain sensitivity than those tested by female experimenters. (**a**–**c**) Mean baseline latencies or thresholds ± s.e.m. to withdraw the tail from hot water (**a**), the hind paw from radiant heat (**b**) or the hind paw from von Frey filaments (**c**); mice of both sexes from multiple studies over a 10-year period were tested by male or female experimenters ($n = 4$–6 testers per experimenter sex; mouse sample sizes are shown in bars). (**d**) von Frey withdrawal thresholds in an experiment performed in a different laboratory. C57BL/6 mice ($n = 20$) were tested by six experimenters ($n = 3$ per sex), without or with a male- (M-Shirt) or female-worn (F-Shirt) T-shirt also in the room, in counterbalanced order. Bars represent mean ± s.e.m. (**e**) Thigmotaxis (wall-hugging) behavior in the open-field test. Bars represent mean ± s.e.m. time spent within 3 cm of a wall (out of 900 s total). In **a**–**c**, $^{\bullet\bullet\bullet}P < 0.001$ compared to other sex, by *t*-test. In **d**,**e**, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to the no-shirt condition; $^{\bullet\bullet}P < 0.01$, $^{\bullet\bullet\bullet}P < 0.001$ compared to other sex, by ANOVA followed by Tukey's *post hoc* test.

may represent a confound of much existing animal research, extending even to nonbehavioral studies in which tissues were obtained from live rodents euthanized by either male or female personnel. Our findings strongly suggest that standard laboratory practice should account for experimenter sex when investigating any phenomenon possibly affected by stress.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

R.E.S., S.G.S. and J.S.M. conceived of the study. R.E.S., L.J.M. and J.S.M. designed most experiments. R.E.S., L.J.M., K.A.I., S.G.S., S.R., A.H.T., J.S.W., E.L.A., A. Delaney, B.K., P.L., J.C.S.M., M.M., A. Dokova, G.W., A.P.S., T.Q. and W.F.S. collected and analyzed data. J.F. and C.I.S. designed certain experiments. R.E.S., L.J.M. and J.S.M. wrote the paper.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

All protocols were consistent with national guidelines and approved by local animal care and use committees at McGill University, University of Alabama at Birmingham and the Karolinska Institutet.

Subjects and stimuli. Experimentally naive, adult (6–12 weeks) male and female CD-1 mice (ICR:Crl, Charles River) were used for most experiments; male and female subjects were tested in separate runs. Mice were housed 3–6 per cage in standard shoebox cages with wood-chip bedding, with *ad libitum* access to food (Harlan Teklad 8604) and tap water, in a light-(14:10 h, lights on at 07:00 h) and temperature-controlled (21 ± 2 °C) environment. One experiment used naive, adult (12–14 weeks) female C57BL/6J mice (Charles River, **Fig. 3d**), and one used naive, adult (225–250 g) Wistar rats (Harlan; **Supplementary Fig. 1**). Most mice were bred in-house; others (in **Figs. 2d** and **3d**) were purchased and acclimated to the vivarium for at least 7 d before testing. Husbandry was provided by male staff, except for the study in **Supplementary Figure 3**, in which it was provided by female staff. Animals were used only once and were exposed to only one presentation of one of various stimuli described below, with n = 3–6 animals per stimulus per run. Sample sizes were thus at least n = 8–12 in every stimulus condition in most experiments, representing at least 2 or 3 'runs' in which the same stimulus (or same type of stimulus—for example, a male-worn T-shirt; see below) was presented to 3 or 4 mice. Sample sizes in no-stimulus control conditions were considerably higher, as these runs were interspersed with runs featuring stimuli in order to control for potential day-to-day variability. Human experimenter 'observers' were all adult males and females ($n \geq 3$ per sex); ages and morphometric information are provided in **Supplementary Table 1**. Injectors or observers were unknown to the animals except in the study shown in **Supplementary Figure 6**. In some studies, experimenters (n = 2 or 3 per sex) wore laundered 100% cotton T-shirts for ~8 h while sleeping, sealed them in plastic bags in the morning, and delivered them to the laboratory for presentation. Bedding material from CD-1 mice, C57BL/6J mice, rats and guinea pigs consisted of soiled cage bedding (n = 2 or 3 samples per sex per species; ~50 g) obtained from our laboratory or the Animal Resources Centre at McGill University. For cats and dogs, upholstered beds of male gonadally intact or castrated domesticated pets (n = 4 animals per species) were used. In one experiment, life-size cardboard cutouts (n = 2) were used as humanoid visual display stimuli. All testing occurred at 12:00 p.m. ± 4 h; mice were habituated to the quiet (~55 dB), brightly lit (~550 lx) testing room (21 ± 2 °C) for 30 min before testing commenced. Mice were randomized to stimulus condition within-cage.

Chemicals. Zymosan A (from *Saccharomyces cerevisiae*) and naloxone hydrochloride were obtained from Sigma and dissolved in physiological saline. Formalin was obtained from ACP Chemicals and dissolved in saline. AM-251 (1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide) was purchased from Ascent Scientific and dissolved in 10% dimethyl sulfoxide (DMSO). AM-251 and naloxone doses were chosen on the basis of pilot experiments; lower doses were without effect. Drugs were administered 15 min before introduction of stimuli.

(*E*)-3-methyl-2-hexenoic acid (3M2H), androstenone (5α-androst-16-en-3-one), androstadienone (4,16-androstadien-3-one), epiandrosterone (3β-hydroxy-5α-androstan-17-one), 5α-androstan-3β-ol-16-one (5A3B16) and estratetraenol (estra-1,3,5(10),16-tetraen-3-ol) were purchased from Steraloids and dissolved in propylene glycol. The food-related odors 2-acetylthiazole (nut/popcorn), isoamyl acetate (banana/pear) and vanillin (vanilla), as well as the aversive compound 2-methylbutyric acid[16], were obtained from Sigma and dissolved in propylene glycol. All chemicals were volatilized before use and presented via impregnated sterile gauze (300 µl).

Zymosan-induced facial grimacing. Mice were briefly anesthetized under isoflurane-oxygen, given bilateral intra-articular injections of zymosan (100 µg/20 µl) into the ankle, and returned to their home cages. After 3.5 h, animals were placed individually into Plexiglas cubicles (9 cm long × 5 cm wide × 5 cm high) with clear walls, a wire mesh floor and lids with air holes for a 15-min habituation period. Cubicles were too small to allow forward locomotion; levels of grooming, which have been shown to affect pain[17], were not altered by experimenter presence (data not shown). Following habituation, digital video cameras directed at the front and back of the cubicle were turned on for 30 min. Stimuli were present during the entire recording period, except in no-stimulus conditions. Human observers, T-shirts, bedding material or chemical-impregnated gauze were placed ~0.5 m from the cubicles. For each 30-min recording, one clear facial image was taken for each 3-min interval. We have found this interval to yield the best balance between accuracy and scoring workload. The images were randomized and scored using the mouse grimace scale, as previously described[3], by two raters fully blinded to stimulus, sex and pain status, whose scores were averaged (inter-rater correlation: $r = 0.64$, $P < 0.001$). Group data obtained from one rater compared to the other were almost identical in every case. In the rat study, the Rat Grimace Scale[18] was used instead.

Formalin test. Mice were placed in Plexiglas cylinders (15-cm diameter; 22.5 cm high) atop a glass floor. After a 15-min habituation, mice were removed, 1% or 5% formalin was injected subcutaneously (20 µl) into the plantar surface of the left hind paw, and mice were placed back in the cylinder. Stimuli were presented immediately following formalin injection. Human observers, T-shirts, and/or the cardboard cutouts were placed ~0.5 m from the cylinders. Mice were digitally videotaped from below for 1 h, and the video was later scored for left hind paw licking and biting behavior by blinded observers using 10-s/min sampling. Data are presented as percentages of samples in which licking/biting behavior was observed. The early phase was defined as 0–10 min post injection, and the late phase was conservatively defined as 10–60 min post injection.

Plasma corticosterone. Mice were placed in Plexiglas cubicles as described above and allowed to habituate for 15 min. After this period, mice were exposed to human experimenters, T-shirts or chemical compounds (vehicle, 300 µM 3M2H, 3 mM androstenone, 3 mM androstadienone) as described above. Mice were euthanized immediately after the 30-min presentation of the stimuli, except for one group euthanized 30 min after the cessation of the stimulus presentation. Some mice were instead removed from

the cubicles and tightly restrained in a 50-ml conical tube with air holes for either 15 or 30 min or were forced to swim in room-temperature water for 3 min. No noxious stimulus was injected in any condition. Immediately following this exposure, mice were sacrificed, and trunk blood was collected. Blood samples were kept on ice and centrifuged at 4 °C and 15,000 r.p.m. for 15 min. Plasma was extracted from the samples and frozen at –70 °C until processing. Corticosterone levels were computed using enzyme immunoassay (Cayman Chemical Company, Kit 500655). Samples and standards were assayed in duplicate at a 1:800 dilution according to the manufacturer's protocol. Single absorbance readings for standards and samples were obtained at 405 nm (Tecan Plate Reader), and these values were used for calculation of plasma corticosterone levels (ng/ml) on the basis of linear regression of the standard curve using a log-logit transformation.

Fecal boli. Fecal pellets deposited on the glass floor of the Plexiglas cylinders (15-cm diameter; 22.5 cm high) were counted at the 30-min time point after stimulus presentation by a blinded observer.

Body temperature. Mouse core body temperature was measured at three time points (0, 15 and 30 min) per mouse by either male or female experimenters, using the identical thermistor probe (Fisher Scientific) inserted 2 cm into the anus of a mouse lightly restrained by the experimenter and held for at least 20 s until the reading stabilized.

Fos immunohistochemistry. A separate group of mice were injected with zymosan and exposed 3.5 h later to male or female experimenters or T-shirts as described above. 4.25 h following administration of zymosan, mice were transcardially perfused with 4% paraformaldehyde, and spinal cords were extracted. Tissue was subject to postfixation for 24 h in paraformaldehyde and then cryoprotection by successive 24-h incubations in 10%, 20% and 30% glucose. Specimens were frozen at –70 °C until sectioned (30 μm) on a cryostat (Leica CM1850) through the lumbar enlargement of the spinal cord.

Fos immunohistochemistry was performed on free-floating spinal cord sections using the avidin-biotin-peroxidase method according to standard procedures. Slices were incubated overnight with 1:10,000 polyclonal anti-Fos (Cat. #SC-52; Santa Cruz Biotechnology), followed by secondary antibody (1:400) and avidin-biotin complex (Elite Kit; Vector Laboratories). Sections were incubated with 3,3′-diaminobenzidine (DAB; 5 mg/ml in acetate-imidazole buffer) with nickel sulfate (25 mg/ml) for 4 min. Fos-like immunoreactivity was determined by the presence of black reaction product and quantified by examination of tissue specimens under a microscope equipped with a digital imaging system. The coder responsible for quantifying Fos reactivity was blind to subject sex and experimental condition.

Acute nociceptive assays. The hot-water tail-withdrawal, radiant-heat paw-withdrawal, and von Frey tests were performed as previously described[19]. In the experiment shown in **Figure 3d**, filaments were presented eight times each in ascending order of stiffness; paw-withdrawal threshold was defined by the force exerted by the filament producing at least four withdrawal responses out of eight stimulations.

Open-field test. After a 15-min habituation to the testing room (in their home cages), mice were placed individually into empty, rectangular opaque enclosures (38 cm long × 25.5 cm wide × 12 cm high) on a table top, under ambient illumination. T-shirt stimuli were placed ~0.5 m from the enclosures, and mouse behavior within the open field was recorded with digital video for the next 15 min. Thigmotaxis (wall-hugging) behavior[20] was defined as the time wherein any part of the mouse was within 3 cm of a wall.

Statistics. After being confirmed as normally distributed (Shapiro-Wilk statistic) and featuring homogeneity of variance (Bartlett's test) among groups, data were analyzed (SYSTAT v.13) by Student's *t*-test (**Fig. 3a–c** and **Supplementary Fig. 6**), one-way ANOVA (least-squares method; **Figs. 1a–d**, **2a**,**b** and **3e** and **Supplementary Figs. 1**,**3**,**7** and **8**), one-between, one-within repeated measures ANOVA (**Fig. 2c** and **Supplementary Figs. 5** and **9**), or two-way ANOVA (**Figs. 2e** and **3d** and **Supplementary Figs. 2a**,**c** and **4**), followed where appropriate by Tukey's honestly significant difference *post hoc* test or Dunnett's case-comparison *post hoc* test (one-sided owing to a preexisting hypothesis of analgesia reversal by the antagonists, comparing to None/No/Vehicle/Veh. group). The experiment reported in **Figure 2d**, in which heteroscedasticity was observed in the data set, was analyzed instead using the nonparametric Kruskal-Wallis test. For the experiments reported in **Figures 1a–c** and **3a–c**,**e** and **Supplementary Figure 1**, ANOVAs were performed in which individual experimenters or individual T-shirts were nested within experimenter sex as random effect factors. To achieve a balanced design for this ANOVA, we randomly divided "no stimulus" condition data into as many nested levels as the corresponding "male" and "female" conditions. The ANOVA table from the experiment shown in **Figure 1a** is provided for reference as **Supplementary Table 2**. Note that in all cases, the individual experimenters or T-shirts did not differ significantly from one another within-sex. Data in **Figure 1c**,**d** were analyzed separately by compound or species, compared in every case to a shared vehicle or no-stimulus group. Chemosignal data presented in **Figure 2a** were analyzed separately from experimenter and T-shirt data; they are presented together for purposes of visual comparison. Restraint and swim stressors in **Figure 2a** were not part of the statistical analysis; they are presented solely for purposes of visual comparison. A criterion α level of 0.05 was adopted in all cases, and no violations from normality were detected. Eight data points were excluded from all experiments combined because of their identification as statistical outliers (Studentized residuals >3). In only one case, however, does their inclusion affect the statistical significance of any data set: the experiment reported in **Figure 3e** has a *P* value of 0.052 if the outlier is included. Three formalin test data points were excluded owing to insufficient hind-paw inflammation from the injection (Studentized residuals >3).

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