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Mating increases *Drosophila melanogaster* females' choosiness by reducing olfactory sensitivity to a male pheromone

Philip Kohlmeier¹, Ye Zhang², Jenke A. Gorter¹, Chih-Ying Su² and Jean-Christophe Billeter¹✉

Females that are highly selective when choosing a mate run the risk of remaining unmated or delaying commencing reproduction. Therefore, low female choosiness would be beneficial when males are rare but it would be maladaptive if males become more frequent. How can females resolve this issue? Polyandry would allow mating-status-dependent choosiness, with virgin females selecting their first mate with little selectivity and becoming choosier thereafter. This plasticity in choosiness would ensure timely acquisition of sperm and enable females to increase offspring quality during later mating. Here, we show that *Drosophila melanogaster* females display such mating-status-dependent choosiness by becoming more selective once mated and identify the underlying neurohormonal mechanism. Mating releases juvenile hormone, which desensitizes Or47b olfactory neurons to a pheromone produced by males, resulting in increased preference for pheromone-rich males. Besides providing a mechanism to a long-standing evolutionary prediction, these data suggest that intersexual selection in *D. melanogaster*, and possibly in all polyandrous, sperm-storing species, is mainly the domain of mated females since virgin females are less selective. Juvenile hormone influences behaviour by changing cue responsiveness across insects; the neurohormonal modulation of olfactory neurons uncovered in *D. melanogaster* provides an explicit mechanism for how this hormone modulates behavioural plasticity.

In most species, the costs associated with reproduction, such as energy investment into gamete production and non-reproductive postcopulatory periods, are higher in females than in males. A consequence of female-biased reproductive investment is that the selective pressure to choose high-quality mating partners is higher in females than in males^{1,2}. However, females might have difficulties finding a male that meets their criteria when male density or quality is low. Such male limitations can occur during seasonal changes, when migrating into a new habitat or as a result of habitat destruction. Under these conditions, high choosiness can become costly for females because it engenders the risk of delayed or reduced reproductive output³, which can contribute to population extinction⁴.

Theory suggests that these risks drive the evolution of mating-status-dependent choosiness: virgin females mate unselectively and become choosier regarding male quality or attractiveness when re-mating^{5,6}. Mating-status-dependent choosiness can be beneficial for females since it allows them to reduce the risk of dying as a virgin without lowering offspring quality⁶. In contrast, a stable, non-plastic choosiness either increases the risk of virgin death (if choosiness is constantly high) or reduces offspring quality (if choosiness is constantly low). When potential mating partners are scarce, low virgin female choosiness relaxes sexual selection on males and increases the likelihood of fertilization. If an already mated female encounters males of higher quality or attractiveness later in life, increased postmating choosiness enables her to re-mate specifically with those males⁶. Elevated choosiness during re-mating is particularly beneficial in species with last male sperm precedence, a phenomenon where most eggs are fertilized by the sperm of the last male the female mated with, because it results in the production of offspring mostly from the higher-quality male⁶.

Despite strong theoretical support for its benefits, empirical evidence for the existence of mating-status-dependent choosiness is scarce and has mainly been produced using indirect measurements of female choosiness, such as receptivity^{7–10}. Moreover, knowledge about the underlying mechanisms that regulate transitions in choosiness is lacking. This gap in research impairs our understanding of the dynamics that underlie sexual selection and how environmental conditions impact the strength of sexual selection faced by males. The fruit fly *Drosophila melanogaster* is an ideal model for closing this gap because it fulfils the conditions for the evolution of plasticity in mate choice: females naturally mate with multiple males¹¹, most eggs are fertilized by the last male they mated with¹² and population density undergoes seasonal fluctuations leading to periods of reduced mate availability¹³. In this study, we reveal that *D. melanogaster* female choosiness is higher in mated than in virgin females. We deploy neurogenetic tools available in *D. melanogaster* to dissect the molecular and neuronal mechanisms that underlie this plasticity. Our data demonstrate that this increase in choosiness is mediated by a neurohormonal pathway that specifically leads to a postcopulatory desensitization of Or47b Odorant receptor neurons, a class of olfactory sensory neurons responding to a pheromone produced by males. Thus, these data demonstrate the existence of mating-status-dependent choosiness and provide a mechanism for this plasticity.

Results

Mating status modulates female mate choice through juvenile hormone. To test whether *D. melanogaster* females display mating-status-dependent choosiness, we offered wild-type (WT) *Canton-S* females the choice to mate and re-mate with males of two established WT strains, *Netherlands* (NL) and *Tai*. We found that

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Fig. 1 | Increased choosiness between virgin and mated state in *D. melanogaster* females. Choosiness of virgin and/or mated females of the indicated genotypes that mated with *Tai* males when exposed to one *NL* and one *Tai* male. The blue bars indicate virgin females and the magenta bars mated females. **a**, *Canton-S* females mate choice indicated in percentages (left) and translated to change in choosiness (Δ virgin $\rightarrow$ mated female choosiness) (right) measured as the proportion of females that selected *Tai* males for mating after transitioning from virgin to mated state (mean $\pm$ s.e.m.). The asterisks indicate a statistically significant increase in choosiness compared to 0 (dashed red line). **b**, Percentage of *Canton-S* mated females that mated with *Tai* males after having mated as virgins with males of the indicated genotypes. What is referred to as SP^{-} in the text is $SP^0/\Delta 130$. **c**, Difference in choosiness between virgin and mated females of the indicated genotypes (mean $\pm$ s.e.m.). **d**, Virginal mate choice of females either fed the juvenile hormone analogue methoprene or the ethanol vehicle. The numbers in the histograms and between brackets indicate the sample sizes. Histograms labelled with the same letter are not statistically different. ** $P < 0.01$, *** $P < 0.001$. Data were analysed using a binomial generalized linear mixed model (**a**, left; **b**, **d**), one-sided t-test (**a**, right) and ordinal logistic regression (**c**). For detailed statistical analysis, see Supplementary Table 2.

female mate choice was indeed mating-status-dependent since no preference for one of the two males was observed in virgins whereas already mated females preferred *Tai* over *NL* males for re-mating (Fig. 1a, left). This finding additionally establishes *Tai* as the more attractive strain than *NL* due to their higher mating success with mated females. In support of this we found that, when exposed to only a single male, mating latency of *Canton-S* female virgins is independent of male genotype but already mated females re-mate faster with a *Tai* than with a *NL* male (Extended data Fig. 1). We then transformed the mating preference data into a Δ virgin $\rightarrow$ mated female choosiness score based on which we tested whether females displayed the predicted increase in female choosiness⁶. This score equals -1 if the female selected the *Tai* male as a virgin and the *NL* male as a mated female (that is, a decrease in choosiness because the male selected as virgin was more attractive than the male selected as a mated female). It equals 0 if the female selected the same male genotype as virgin and mated female and $+1$ if the female selected the *NL* male as a virgin and the *Tai* male as a mated female (that is, an increase in choosiness because the male selected as virgin was less attractive than the male selected as a mated female). If mating-status-dependent choosiness exists, this Δ virgin $\rightarrow$ mated female choosiness score would be close to 0.5 with virgin females mating unselectively (that is, equally choosing *NL* or *Tai* males) and 100% of these females re-mating with *Tai* males. If mating-status-dependent choosiness does not exist, then both virgin and mated females would select the attractive *Tai* male for mating; hence, the Δ virgin $\rightarrow$ mated female choosiness score would equal 0 . In our experiment, the Δ virgin $\rightarrow$ mated female choosiness score was 0.52 , which demonstrates an increased choosiness in females after mating (Fig. 1a, right).

Postmating increases in female choosiness have previously been reported in a few species^{7,14,15}. However, it is unclear whether such shifts in choosiness resulted from the acquisition of experience by mated females, who might have had limited information on male quality as virgins, or are mediated by a dedicated mechanism that increases selectivity postmating irrespective of experience^{6,14}. To distinguish between these two potential explanations, we investigated the molecular mechanisms that trigger this postmating shift

in choosiness. *D. melanogaster* females undergo well-documented physiological and behavioural changes in response to the first mating¹⁶. Most of these female postmating responses are independent of experience since they are triggered by the receipt of sex peptide (SP), a peptide in the male ejaculate that binds to SP receptor (SPR)-expressing neurons in the female uterus^{17–20}. We found that this SP–SPR pathway also regulates mating-status-dependent choosiness: females that were mated as virgins with mutant males lacking SP were less selective during re-mating than females that were mated as virgins with control males transferring SP (Fig. 1b). Similarly, mutant females lacking SPR showed increased choosiness after the first mating less strongly than control females (Fig. 1c). Together, these results demonstrate that mating-status-dependent choosiness is an SP-dependent postmating response in *D. melanogaster*.

One of the downstream effects triggered by mating, and potentially by SP²¹, is the increased biosynthesis of juvenile hormone (JH)^{22,23}, a pleiotropic hormone that, among others, stimulates oogenesis²⁴. We tested the hypothesis that a postmating increase in choosiness is mediated by elevated JH titres by treating virgin females with the JH analogue methoprene. In support of this hypothesis, methoprene-treated virgin females displayed a stronger preference for *Tai* males than virgin females treated with the ethanol vehicle alone (Fig. 1d). Taken together, these data demonstrate that both the SP–SPR pathway and an increase in JH titres are necessary to increase female choosiness. This increase in choosiness is likely controlled by a dedicated, experience-independent mechanism because, first, the experience of mating did not increase choosiness in females where the SP–SPR pathway was disrupted and second, an application of methoprene was sufficient to increase choosiness in virgins without the experience of mating. Thus, we provide empirical evidence for the existence of mating-status-dependent choosiness in *D. melanogaster*.

Or47b olfactory neuron is the neuronal substrate for state-dependent mate choice. The observed preference for *Tai* over *NL* males in mated females raises the question of how females distinguish between these males. We focused on pheromones since

Fig. 2 | Or47b ORN is necessary for plastic female choosiness and decreases in sensitivity to palmitoleic acid after mating. **a**, Difference in choosiness between virgin and mated females of the indicated genotypes (mean $\pm$ s.e.m.). **b**, Difference in choosiness between virgin and mated females of the indicated genotypes (mean $\pm$ s.e.m.). **c**, Left: representative traces (top), raster plots (middle) and peri-stimulus time histograms (bottom) of single-unit recordings of at4 sensillum housing Or47b ORN in virgin and mated *Canton-S* females in response to a palmitoleic acid puff (shaded grey). Line width, s.e.m. Grey bar, stimulus duration. Right: dose-response curves comparing Or47b ORN spike responses to palmitoleic acid in mated and virgin females. **d**, Dose-response curves comparing Or67d ORN spike responses to *cis*-vaccenyl acetate in mated and virgin females. **e**, Dose-response curves comparing Or67d ORN spike responses to methyl palmitate in mated and virgin females. The error bars indicate the s.e.m. NS, non-significant; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Data were analysed using ordinal logistic regression (**a,b**) and two-tailed *t*-tests (**c-e**). The number of replicates per group is indicated in the histograms or between brackets. For detailed statistical analysis, see Supplementary Table 2.

they influence female mate choice and different *D. melanogaster* strains display stable quantitative differences in pheromone profiles^{25–27}. Since pheromones are often sensed by olfactory receptor neurons (ORNs)^{28–33}, we hypothesized that the olfactory system is required for females to increase choosiness after mating. In support of this hypothesis, we found that the choosiness of *Orco* mutant females, whose ORNs are non-responsive to odour stimulation³⁴, increased less strongly after mating compared to females where *Orco*

expression was rescued (Fig. 2a), showing that responsive ORNs are necessary for a postmating increase in female choosiness.

To identify the specific ORNs that mediate mating-status-dependent choosiness, we tested the mate choices of females mutant for either of the three odorant receptors documented to respond to male pheromones in *D. melanogaster*: *Or47b*, *Or67d* and *Or88a*^{28,29,31–33}. While *Or88a* and *Or67d* mutant females displayed increased choosiness after mating, *Or47b* mutant females did not

increase choosiness between virgin and mated state (Fig. 2b). These data show that mating-status-dependent choosiness is modulated through olfaction and specifically requires a functioning *Or47b* odorant receptor.

How can *Or47b* ORNs mediate changes in female choosiness? Previous studies demonstrated that changes in the sensitivity of a chemosensory neuron to its ligand or changes in firing rate can modulate responsiveness to specific cues and by that behaviour^{32,35–38}. Thus, we hypothesized that mating-status-dependent choosiness is based on a postmating change in the physiology of the ORNs expressing *Or47b*. To test this, we performed single-sillum recordings in virgin and mated females on the *Or47b*, *Or67d* and *Or88a* ORNs. The spike response of *Or47b* ORNs in response to their ligand palmitoleic acid³² was lower in mated than virgin females, with an approximately 50% difference at the highest palmitoleic acid dose (Fig. 2c, left and right). This postmating neuronal desensitization is specific to *Or47b* ORNs since neither the *Or67d* nor *Or88a* ORNs responses to their pheromone ligands changed after mating (Fig. 2d,e). These data suggest that mating-status-dependent choosiness is specifically mediated by the mating-status-dependent sensitivity of *Or47b* ORNs.

JH specifically acts on *Or47b* ORNs and desensitizes their response to palmitoleic acid. We then asked whether the reduced postmating sensitivity of *Or47b* ORNs is caused by elevated JH titres, which we found to be sufficient to increase choosiness in virgin females (Fig. 1d). To answer this, we treated virgin females with methoprene and measured the spiking response of *Or47b* ORNs to palmitoleic acid. Methoprene decreased *Or47b* ORN sensitivity in virgin females suggesting that elevated JH titres, which are characteristic of mated females^{22,23}, are sufficient to lower *Or47b* ORN sensitivity (Fig. 3a, left and right). To assess whether JH reduces *Or47b* ORN sensitivity to palmitoleic acid by directly acting on these neurons, we knocked down the JH receptor *methoprene-tolerant* specifically in *Or47b* ORNs. In these females, *Or47b* ORN sensitivity to palmitoleic acid remained at virgin level after mating, which shows that the binding of JH to these neurons is necessary for their postmating desensitization (Fig. 3b, left and right). We then tested whether knocking down *methoprene-tolerant* in *Or47b* ORNs does not only inhibit the postmating decrease in neuronal sensitivity but also the increase in choosiness. Indeed, females in which *methoprene-tolerant* was knocked down specifically in *Or47b* ORNs displayed a weaker increase in choosiness after mating compared to females with an unmanipulated *methoprene-tolerant* expression (Fig. 3c).

Our data suggest a model where a postmating increase in JH titres desensitizes *Or47b* ORNs, which results in a reduced sensitivity to the aphrodisiac pheromone palmitoleic acid in mated compared to virgin females. Therefore, males that produce large quantities of palmitoleic acid will be more likely to be accepted by mated females for re-mating than males who produce low quantities. In support of this, we found that *Tai* males produce larger quantities of palmitoleic acid than *NL* males (Fig. 3d), as well as most other known pheromones (Supplementary Table 1). This model predicts that elevated JH titres in females and differences in palmitoleic acid quantities between males are sufficient to explain mating-status-dependent choosiness. To test this, we applied additional palmitoleic acid to *NL* males and pitted these palmitoleic acid⁺ *NL* males against vehicle-only-treated *NL* males for mating. We exposed these males to two types of virgin females: methoprene-treated virgins, which display the higher choosiness of mated females; and ethanol-treated virgins, which display the lower choosiness of virgin females (Fig. 1d). Methoprene-treated virgin females selected palmitoleic acid⁺ *NL* males more frequently for mating than vehicle-treated virgin females (Fig. 3e). This demonstrates that high JH titres increase female choosiness for males with larger amounts of palmitoleic acid, while virgin JH levels lead

to females that lack a preference for males with larger amounts of palmitoleic acid. Taken together, these data show that a specific postmating decrease in *Or47b* ORN sensitivity mediated by JH increases mate choosiness towards pheromone-rich males.

Genetic mechanisms regulating *Or47b* ORN sensitivity. Via which mechanisms does JH reduce *Or47b* sensitivity to palmitoleic acid in mated females? The JH receptor methoprene-tolerant functions as a JH-dependent transcription factor³⁹ that influences gene expression^{40–42}. In *D. melanogaster* males, binding of JH to methoprene-tolerant increases the *Or47b* ORN firing rate in response to palmitoleic acid stimulation by upregulating the expression of the male-specific transcription factor *Fruitless^M* (*Fru^M*)⁴³. Increased expression of *Fru^M* results in increased expression of *pickpocket25* (*ppk25*), an ion channel subunit that amplifies electrical signals generated after ligand binding^{42,43}. This pathway is responsible for mediating increased sensitivity of *Or47b* ORNs to palmitoleic acid as males age or when they are part of a social group, resulting in increased courtship intensity^{32,42,43}.

Thus, the *Or47b*–JH neurohormonal pathway we identified in females has a male counterpart. However, JH has opposite effects in the two sexes since JH binding increases *Or47b* ORN sensitivity in males but decreases *Or47b* ORN sensitivity in females. This predicts that the mechanism via which JH influences *Or47b* ORN sensitivity differs between males and females in line with their different functions, that is, increasing courtship intensity in males and increasing choosiness in females. To test this, we used RNA sequencing (RNA-seq) and compared antennal gene expression between virgin and mated females. We identified a total of 340 differentially expressed genes with 188 of them being more strongly expressed in virgin females and 152 of them being more strongly expressed in mated females (Fig. 4a,b). In line with what has been described in males⁴³, we found that *fruitless* female-specific transcripts (*fru^F*) derived from the same promoter as *fru^M*⁴⁴ are more abundant in mated females, which are characterized by elevated JH titres, than in virgin females (false discovery rate (FDR) < 0.0001; Fig. 4a). However, *ppk25* (or other members of that gene family) did not change in expression between virgin and mated females suggesting that in females, a change in *fruitless* expression does not translate into a change in *ppk25* expression and that a decrease in *Or47b* ORN sensitivity is not mediated through modulation of *ppk* genes (Fig. 4c and Supplementary Fig. 2). Thus, the *Or47b*–JH neurohormonal pathway has different downstream elements in males and females.

Besides changes in *ppk* genes, the ORN firing rate can also be decreased by upregulating genes encoding odorant degrading enzymes that inactivate pheromones before they can bind to their respective receptor^{45,46}. The observed decrease in *Or47b* ORN sensitivity is unlikely to be caused by an increased degradation of palmitoleic acid since none of the candidate odorant degrading enzymes were upregulated after mating (Extended data Fig. 2). Moreover, we found no olfactory or ionotropic receptor genes including *Or47b*, (Fig. 4c and Supplementary Fig. 2) to be differentially expressed in the antennae of virgin and mated females. This indicates that changes in receptor expression are not mediating *Or47b* ORN sensitivity. The mechanisms through which JH affects *Or47b* ORN sensitivity in females are thus different than in males and await further investigation.

Discussion

Theory has predicted the existence of mating-status-dependent choosiness as a solution to the risk of females delaying mating due to high choosiness⁶. In this study, we provide experimental evidence for this long-standing prediction and reveal a neurohormonal mechanism through which *D. melanogaster* females adjust choosiness. In particular, increased JH titres, which are characteristic of

Fig. 3 | JH reduces Or47b ORN sensitivity. **a**, Left: sample traces of single-unit recordings of at4 sensillum housing Or47b ORN in vehicle- and methoprene-treated virgin females in response to a palmitoleic acid puff (shaded grey). Right: dose-response curves of Or47b ORN spike responses to dilutions of palmitoleic acid from control and methoprene-treated virgin females (mean $\pm$ s.e.m.; $n=9$ from 4–5 flies). **b**, Left: same as in **a** but in virgin and mated females of the indicated genotypes. Right: comparison of Or47b ORN responses to palmitoleic acid (10^{-1}) in virgin and mated females of the indicated genotypes. **c**, Difference in choosiness between virgin and mated females of the indicated genotypes (mean $\pm$ s.e.m.). **d**, Quantification of palmitoleic acid in individual *NL* and *Tai* males (mean $\pm$ s.e.m.). **e**, Mate choice of virgin females treated with either methoprene or vehicle towards *NL* males treated with palmitoleic acid. The numbers between brackets indicate the sample sizes. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Data were analysed using two-tailed *t*-tests (**a**, **b**), ordinal logistic regression (**c**), unpaired two-tailed *t*-tests (**d**) and a binomial generalized linear mixed model (**e**). For detailed statistical analysis, see Supplementary Table 2.

mated females^{22,23}, cause a decrease in sensitivity of the Or47b ORN and by that render mated females more selective for males with higher palmitoleic acid quantities.

Mating-status-dependent choosiness influences the dynamics of sexual selection. Our demonstration of the existence of mating-status-dependent choosiness combined with the

Fig. 4 | Transcriptomic differences between the antennae of virgin and mated females. **a**, Heatmap of genes differentially expressed between virgin and mated females and dendrogram of replicate mRNA antennal libraries based on differentially expressed genes showing that they cluster by female mating status (virgin or mated). **b**, Venn diagram displaying the number of differentially expressed transcripts and of those transcripts with no change in expression. **c**, Normalized read counts of *fruitless* female-specific transcript (*fru^f*), *ppk25* and *Or47b* in the antennae of virgin (blue) and mated (pink) females (mean $\pm$ s.e.m.). ***FDR $P < 0.001$. The FDR P value was obtained from the gene expression analysis. For detailed statistical analysis, see Supplementary Table 2.

well-documented high last male sperm precedence in *Drosophila* suggests that the strength of sexual selection is influenced by an interaction between female mating status and the availability of attractive males in this species. These mechanisms predict that if population density is low or if the environment limits the production of attractive males, mating-status-dependent choosiness can relax the strength of sexual selection on male traits and makes it more likely that a virgin female will mate with the first male it encounters⁶. This ability to reproduce even when population density is low can, for instance, be beneficial when migrating into new environments. Conversely, if population density is high or if the environment allows the production of attractive males, sexual selection on male traits is largely determined by mated females because genes will be passed on predominantly from males attractive enough to be selected for mating by non-virgin, that is, selective females⁶. Thus, the conventional practice of using virgin females for studies on sexual selection is likely to have masked the strength and dynamics of selective pressure imposed by *D. melanogaster* females and potentially of polyandrous species in general. Our study thus stresses the fundamental importance of factoring female mating status and availability of attractive males when studying sexual selection.

JH as a widespread modulator of behaviour. JH-dependent modulation of sensory sensitivity is found across insects where it governs state-dependent changes in behaviour. For instance, an increase in JH titres with age increases ORN sensitivity to a stimulatory pheromone in *D. melanogaster* males, which results in elevated courtship behaviour in older males and resembles one factor that contributes to the higher mating success of older compared to younger males³². JH-dependent pheromone responsiveness in the moth *Agrotis ipsilon*

modulates male responsiveness to sex pheromones and by that the timing of reproduction⁴⁷. In this case, JH does not influence the pheromone responsiveness of ORNs but instead of olfactory antennal lobe interneurons showing that JH can impact behaviour through the modulation of both cue sensing and the processing of sensory input. In the migratory locust *Locusta migratoria*, JH influences expression of olfactory-related genes, such as olfactory receptors, potentially influencing the attraction to or repulsion of volatiles from conspecifics and thus gregarious versus solitary behaviour⁴⁸. Non-reproductive division of labour in the eusocial honeybee *Apis mellifera* is influenced by JH-dependent responsiveness to task-related cues. Foragers have both a higher JH titre and sucrose responsiveness than nurses^{49,50}; treating nurses with JH analogues increases sucrose responsiveness⁵¹ and induces foraging⁵². Our study adds an adaptive change in mate choice to the list of behaviours modulated by JH. JH and its modulation of the sensing or processing of cues is thus emerging as a widespread and versatile neurohormonal mechanism modulating state-dependent beneficial behaviours that has been recycled in different species with different levels of social organization. To what extent such neurohormonal mechanisms interact with pathways that perceive the experience of mating is to be studied.

The evolution of sex-specific ORN functioning. In this study, we showed that JH is responsible for a mating-status-dependent change in olfactory sensitivity allowing for adaptation by females to the risk of delaying reproduction due to high choosiness. The net result of increased JH postmating is a decrease in *Or47b* ORN sensitivity in mated females. This decrease is beneficial since it increases choosiness in females who have already secured sperm for fertilization from their first mate. However, this female

Table 1 | List of flies

Full genotype	Short name	Source	Identifier
<i>w¹¹¹⁸</i>	<i>w¹¹¹⁸</i>	Vienna <i>Drosophila</i> Resource Centre	VDRC: 60000
<i>Canton-S</i>	CS	Billeter lab	
<i>Oregon-R</i>	OR	Billeter lab	
<i>Wildtype: Netherlands</i>	NL	Global Diversity Lines, Andrew Clarke	
<i>Wildtype: Tai</i>	<i>Tai</i>	Jean-François Ferveur	
<i>+,+; SP⁰/TM3, Sb;+</i>	<i>Sp⁰</i>	Ref. ¹⁷	BDSC: 77892
<i>+,+;Delta¹³⁰/Tm3, Sb;+</i>	<i>Δ130</i>	Ref. ¹⁷	
<i>w;Df(1R)SPR/FM7;+;+;+</i>	<i>SPR</i>	Ref. ⁶³	BDSC: 7708
<i>w;Orco-gal4;Orco²;+</i>	<i>Orco-gal4</i>	Ref. ⁶⁴	
<i>w;UAS-Orco;Orco²;+</i>	<i>UAS-Orco</i>	Ref. ³⁴	
<i>+,+;Orco²;+</i>	<i>Orco²</i>	Ref. ³⁴	BDSC: 23130
<i>+,Or47b³;+;+</i>	<i>Or47b³</i>	Refs. ^{31,32}	BDSC: 51307
<i>w;p{Or47b-gal4,w⁺};+;+</i>	<i>OR47b-gal4</i>	Ref. ⁶⁵	BDSC: 9983
<i>y,w;UAS-Methoprene-tolerant RNAi;+;+</i>	<i>UAS-Met-RNAi</i>		VDRC:100638
<i>w;+; OR88a⁺;+</i>	<i>OR88a⁺</i>	Ref. ³³	
<i>+,+;Or67d⁺gal4;+</i>	<i>OR67d⁺</i>	Ref. ²⁹	

response should create a conflict with Or47b ORN functioning in males, in which elevated JH titres increase—not decrease—neuron sensitivity. If JH had similar effects on Or47b ORNs in both sexes, it would be disadvantageous for one of the two sexes because either male courtship intensity would decrease with age or females would not be able to increase choosiness after the first mating. Such opposite olfactory responses of the sexes to the same stimulus (palmitoleic acid) and modulator (JH) bears the hallmark of the resolution of intralocus sexual conflict. In the course of evolution, a gene may acquire a function that is beneficial to one sex but costly to the other resulting in opposite reproductive values when in a male than when in a female genome. In such a situation, evolutionary modelling predicts strong selection for the evolution of mechanisms restricting expression of the gene to the sex where it is beneficial⁵³. Such sex-specific expression can be achieved by placing the gene's transcription under the control of transcription factors of the sex determination pathway. One such transcription factor in *Drosophila* is *Fru^M*, which is produced in the nervous system of males only⁵⁴. The promoter regulating *fru^M* transcription in Or47b neurons has been previously shown to increase activity in response to elevated JH in males⁴³. The resulting *Fru^M* protein then initiates the transcription of *ppk25* ion channels⁴², which results in an enhanced amplification of the electrical signals in Or47b ORNs generated after palmitoleic acid binding⁴². Our RNA-seq data suggest that, in females, JH has a similar effect on the expression of *fru* but not on the expression of any *pickpocket* genes downstream of *fru*. This observation is consistent with intralocus sexual conflict resolution because of the sex-specific expression of *fru*⁵³. The *fru^F* transcripts made in females are not translatable into a transcription factor due to insertion of an early stop codon^{44,55}. This can explain why, unlike in males, in females the upregulation of *fru* does not result in transcriptional changes of any *pickpocket* genes. How JH exactly decreases Or47b ORN in females is to be determined but our study firmly establishes JH and Or47b ORN as the mechanism regulating changes in female choosiness. Such mechanisms can include posttranslational modifications of proteins involved in modulating neuronal sensitivity, such as odorant receptor coreceptor dephosphorylation⁵⁶, or yet undescribed mechanisms since 40.2% of genes differentially expressed between the antennae of virgin and mated females were uncharacterized or non-annotated proteins.

Methods

Rearing conditions. Flies (Table 1) were reared at 25°C in a 12:12h light–dark cycle (LD 12:12). Rearing was done on food medium containing agar (10 g l⁻¹), glucose (167 mM), sucrose (44 mM), yeast (35 g l⁻¹), cornmeal (15 g l⁻¹), wheatgerm (10 g l⁻¹), soya flour (10 g l⁻¹), molasses (30 g l⁻¹), propionic acid (5 ml of 1 M) and tegosept (2 g in 10 ml of ethanol). Virgin adults were collected using CO₂ anaesthesia and aged in same-sex groups of 15 in food vials (2.3 × 9.3 cm) for 5–7 d until testing. Flies were aged in the same light and temperature conditions as rearing. To ensure consistency in male quality, *NL* and *Tai* males were collected only for 2 d after hatching started from their culture bottles.

Mate choice assays. Disposable mating arenas were produced by piercing a hole (diameter approximately 3 mm) into the side wall of a 35 × 10 mm Petri dish (Greiner Bio-One) and by covering the bottom of the dish with 3 ml of fly food. Fifteen age-matched *NL* and *Tai* males were transferred to a colouring vial and the vial was rotated for 3 s. Colouring vials were produced by giving 0.5 g of green or pink fluorescent powder (Slice of the moon) into a fresh vial and rotating it until the inner surface of the vial was entirely covered. Residual powder was removed by tapping vials upside down. Colours were assigned randomly on every experimental day. One *NL* and one *Tai* male were transferred into a mating arena using a mouth pipettor and allowed to acclimatize for 30 min. A single female was added to the arena and was inspected for mating every 3–5 min for mating. In the experiment testing whether elevated JH levels delay mating latency, flies were checked once per minute. The male selected by the female was inspected for colouration using an ultraviolet flashlight (EFI 4; Perel). Virgin females that did not mate within 120 min were discarded. After mating ended, males were removed and single females were kept individually in the mating arena for 24 h. The next day, new mating arenas including one *NL* and one *Tai* male were prepared as described above. Mated females were transferred individually to the new mating arena and re-mating choice was recorded. Females that did not re-mate within 10 h were discarded. Since *Or47b* mutant females displayed low receptivity, re-mating was observed for 30 h. All experiments were performed at 25°C in an LD 12:12. To suppress potential leaky expression of *UAS-Met-RNAi*, all females in this experiment were raised at 18°C and kept at 18°C during the isolation phase between first mating and re-mating. To compensate for daily fluctuations, data collection for each experiment was done over several days. The laboratory bench was cleaned with acetone before and after every experiment to remove potential pheromonal residues. The tip of the mouth pipettor was renewed after each genotype to prevent pheromone transfer between groups.

Methoprene treatment. *Canton-S* virgin females were starved for 7 h by keeping them in groups of 15 in a vial without food but with a moist cotton ball to prevent desiccation. Then, 15 μl 0.25% (v/v) of methoprene (Sigma-Aldrich) dissolved in 100% ethanol were spread over the surface of food in a vial, a validated method for delivering methoprene⁵⁷. To ensure equal spreading, we used vials with an even food surface and no bubbles. To control for vehicle, 15 μl of 100% ethanol were used. Residual ethanol was evaporated by keeping the vials unplugged for 2 h. Starved *Canton-S* female virgins were transferred to the methoprene or ethanol feeding vials and flies were tested after 18 h.

Single-sensillum recordings. A fly was wedged into the narrow end of a truncated plastic 200- μ l pipette tip to expose the antenna, which was then stabilized between a tapered glass microcapillary tube and a coverslip covered with double-sided tape. Single-unit recordings were performed essentially as described by Ng et al.⁵⁸. Briefly, the electrical activity of the ORNs was recorded extracellularly by placing a sharp electrode filled with artificial haemolymph solution⁵⁹ in the at4 sensillum (Or47b ORN and Or88a recordings). For the at1 (Or67d ORN) sensillum recordings, 0.6 $\times$ sensillum lymph Ringer solution⁶⁰ was used. The reference electrode filled with the same respective solution was placed in the eye. No more than three sensilla from the same antenna were recorded per fly.

Odour stimuli. Both palmitoleic acid (4.5 μ l per filter disc) and *cis*-vacenyl acetate (10 μ l per filter disc) were diluted in ethanol and delivered via a 500-ms air pulse at 250 ml min⁻¹ directly to the antenna from a close range, as described previously⁵⁸. Ethanol was allowed to evaporate for 1 h in a vacuum desiccator before the experiments. Methyl palmitate was diluted in paraffin oil, applied as 100- μ l aliquots on filter discs and delivered to the antenna via a 500-ms air pulse at 200 ml min⁻¹ through the main airstream (2,000 ml min⁻¹).

Cuticular hydrocarbon and fatty acid quantification and treatment of flies with synthetic pheromones. Cuticular hydrocarbon (CHC) and fatty acid quantification were performed as in Billeter et al.²⁶ and Lin et al.³², respectively. Ten *Tai* or *NL* male flies were anaesthetized and placed in a 2-ml glass vial (Sigma-Aldrich) containing 200 μ l of an internal standard made of hexane spiked with 10 μ g ml⁻¹ of octadecane (C18) and 10 ng ml⁻¹ of hexacosane (C26). Vials were vortexed at minimum speed for 2 min and the flies were removed using a clean metal pin. The resulting extract was split in half (2 $\times$ 100 μ l) and evaporated under a nitrogen flow. One half was used for fatty acid quantification; it was resuspended in 80 μ l of methanolic acid (Sigma-Aldrich) and incubated at 60 °C for 1 h for transesterification, after which methanolic acid was evaporated under a nitrogen flow and the sample resuspended in 100 μ l of hexane. The other half for CHC analysis was directly resuspended in 100 μ l of hexane.

Two microlitres of the resulting CHC or fatty acid extracts were analysed using an Agilent 7890 gas chromatograph with a flame ionization detector, an Agilent DB-1 column (diameter: 0.180 mm; film: 0.18 μ m) and a split/splitless injector set at 250 °C with 40 ml min⁻¹ splitless flow. The injector valve opened 1.5 min after injection in splitless mode with helium as the carrier gas (flow: 37.2 cm s⁻¹). The oven temperature programme started at 50 °C for 1.5 min, ramping at 10 °C min⁻¹ to 150 °C, then ramping at 4 °C min⁻¹ to 280 °C and holding for 5 min. The ChemStation software (Agilent Technologies; Rev. C.01.03) was used to quantify compounds based on peak areas relative to the internal standards C18 and C26. Palmitoleic acid was only detectable in the transesterified fatty acid samples and was identified based on both retention time³² and by spiking extracts with synthetic palmitoleic acid.

To apply palmitoleic acid on *NL* flies, 0.5 μ l of a 333 ng μ l⁻¹ solution of palmitoleic acid in acetone (Sigma-Aldrich) was pipetted directly onto the dorsal abdomen of male flies anesthetized on ice, using a positive displacement micropipette with capillary pistons (MICROMAN; Gilson). Control males received the same treatment but only with acetone. After palmitoleic acid/acetone application, both palmitoleic acid-treated and control-treated males were placed back in food vials for 30 min to recover before undergoing the mate choice assays with ethanol- or methoprene-treated virgin females as described above.

RNA-seq of virgin and mated female antennae. Mated females were produced by mating 10 *Canton-S* females (5–6 d old) with 15 *NL* males in a vial for 2 h. After 2 h, males were removed and females were kept in the vial for 24 h. Virgin females were kept in vials in groups of 10 for 24 h. The antennae of 200 mated or virgin females were pooled in 150 μ l of Trizol (Ambion), homogenized with RNase-free micro pestles and stored at -80 °C. Whole messenger RNA extraction and low-input library construction were performed by Novogene. A total of 6 libraries (3 virgin and 3 mated female samples) were indexed, pooled and sequenced on a single lane of an Illumina Novogene 6000 resulting in 68.3 $\pm$ 4.8 million (mean $\pm$ s.e.m.) 150-base-pair-long paired-end reads per sample. Quality-filtered and adaptor-trimmed raw reads were mapped to the *D. melanogaster* genome (release 6 plus ISO1 MT; August 2014) using STAR v.2.7 (ref. ⁶¹). Differentially expressed genes were identified by running a pairwise comparison between virgin and mated female samples in edgeR v.3.30.3 (ref. ⁶²). Read counts were normalized for sequencing depth by dividing the read counts by the total number of million reads in the library.

Statistical analysis. For the experiments where we compared choosiness among females of the same mating status (for example, Figure 1b), we ran a binomial generalized linear mixed model including selected male (*Tai* versus *NL*) as a response variable, genotype/treatment as an explanatory factor and date of the experiment as a random factor. For the experiments where we tested whether females display an increase in choosiness, we used ordinal logistic regression to test whether the ordinal Δ virgin $\rightarrow$ mated female choosiness score (see Results for how this score was calculated) differed between females of different genotypes. To additionally test whether the Δ virgin $\rightarrow$ mated female choosiness score was

different from 0, we used a one-sample *t*-test. All analyses were run in R v.4.0.0 loaded with the packages car, MASS, lme4 and lmerTest.

For the dosage curve of the odour concentration, two-tailed *t*-tests comparing the experimental and control groups were performed for each concentration; the *P* value is indicated in the figure by asterisks (**P* < 0.05; ***P* < 0.01; ****P* < 0.001). These data are presented as the mean $\pm$ s.e.m. and plotted in Igor Pro v.6.32A (<https://www.wavemetrics.com/products/>). Dose-response curves were fitted with the Hill equation for illustration purposes.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Raw sequencing reads are available at the National Center for Biotechnology Information Short Read Archive (<https://www.ncbi.nlm.nih.gov/sra/SRX9940561>); accession no. PRJNA694952. All other raw data are available at Dataverse-NL (<https://doi.org/10.34894/454UBX>).

Code availability

R scripts and the gene expression analysis code are available on Dataverse-NL.

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Author contributions

P.K., C.-Y.S. and J.-C.B. designed the study. P.K. and J.-C.B. performed the behavioural experiments, analytical chemistry, RNA-seq and data analysis. Y.Z. performed the single-sensillum recordings. J.A.G. provided the reagents. P.K., Y.Z., C.-Y.S. and J.-C.B. wrote the manuscript with feedback from all authors.

Competing interests

The authors declare no competing interests.

Additional information

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Extended Data Fig. 1 | Mating latency is influenced by an interaction between female mating status and male genotype. *Canton-S* virgin females were offered a single male (*NI* or *Tai*) for mating. After mating, females were isolated for 24h and offered a new single male (*NI* or *Tai*) for remating. Virgin and mated female mating latency was recorded. Plots display (Mean $\pm$ s.e.m.). A generalized linear mixed model including mating latency as a response variable, male genotype (*NI* vs. *Tai*), female mating status (virgin, mated) and their interaction as explanatory factors and female ID as a random factor revealed that mating latency is influenced by an interaction between male genotype and mating status (GLMM: $p = 0.038$). The interaction was then explored using posthoc tests.

Extended Data Fig. 2 | Expression of transcripts potentially modulating neuronal sensitivity. Normalized and log transformed read counts of antennal transcriptomes separated by mating status. Isoforms marked with (*) were differentially expressed between virgin and mated females (Mean $\pm$ s.e.m). *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

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Software and code

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Data collection All data were collected in Excel

Data analysis Data were analyzed in R v 4.0.0. RNA sequencing data were processed using STAR 2.7 and edgeR 3.30.3. CHC analysis was run in ChemStation

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All data and script were uploaded to Dataverse-NL (Link provided in the manuscript). Raw sequencing reads were uploaded to NCBI Short Read Archive PRJNA694952.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample sizes are high in all cases because many of our measures involve binomial tests that can easily be swayed in small replicate conditions. We thus ensured that we did no less than 30 replicates (and often more than 60) per group. The manuscript has high amounts of replicates in general.
Data exclusions	Virgin females that did not mate within 2 hours and mated females that did not remate within 30 hours were excluded. This is indicated in the Methods. For virgins, Justification is that such long mating latencies are unnatural and thus the sign of a damaged test animal. For the mated females, it was simply not possible for an experimentalist to stay awake for more than 30 hours in a row, hence why we introduce this cutoff.
Replication	All experiments were replicated at least twice, but most were replicated on three different occasions spread over one year.
Randomization	Colours used to mark males were randomized. Otherwise controls and experimentals were always run side by side.
Blinding	All genotypes were kept blind to the experimentalist. Vials of flies were coded with random letters each experimental day.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☐ Antibodies
☐	☐ Eukaryotic cell lines
☐	☐ Palaeontology and archaeology
☐	☐ Animals and other organisms
☐	☐ Human research participants
☐	☐ Clinical data
☐	☐ Dual use research of concern

Methods

n/a	Involved in the study
☐	☐ ChIP-seq
☐	☐ Flow cytometry
☐	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Describe all antibodies used in the study; as applicable, provide supplier name, catalog number, clone name, and lot number.
Validation	Describe the validation of each primary antibody for the species and application, noting any validation statements on the manufacturer's website, relevant citations, antibody profiles in online databases, or data provided in the manuscript.

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	State the source of each cell line used.
Authentication	Describe the authentication procedures for each cell line used OR declare that none of the cell lines used were authenticated.
Mycoplasma contamination	Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	Name any commonly misidentified cell lines used in the study and provide a rationale for their use.

Palaeontology and Archaeology

Specimen provenance	<i>Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information).</i>
Specimen deposition	<i>Indicate where the specimens have been deposited to permit free access by other researchers.</i>
Dating methods	<i>If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.</i>
☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.	
Ethics oversight	<i>Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.</i>

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	<i>Drosophila melanogaster. Details (e.g. genotype, sex) given in manuscript</i>
Wild animals	<i>Provide details on animals observed in or captured in the field; report species, sex and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.</i>
Field-collected samples	<i>For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.</i>
Ethics oversight	<i>Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.</i>

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about [studies involving human research participants](#)

Population characteristics	<i>Describe the covariate-relevant population characteristics of the human research participants (e.g. age, gender, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."</i>
Recruitment	<i>Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.</i>
Ethics oversight	<i>Identify the organization(s) that approved the study protocol.</i>

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration	<i>Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.</i>
Study protocol	<i>Note where the full trial protocol can be accessed OR if not available, explain why.</i>
Data collection	<i>Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.</i>
Outcomes	<i>Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.</i>

Dual use research of concern

Policy information about [dual use research of concern](#)

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes
☐	☐ Public health
☐	☐ National security
☐	☐ Crops and/or livestock
☐	☐ Ecosystems
☐	☐ Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes
☐	☐ Demonstrate how to render a vaccine ineffective
☐	☐ Confer resistance to therapeutically useful antibiotics or antiviral agents
☐	☐ Enhance the virulence of a pathogen or render a nonpathogen virulent
☐	☐ Increase transmissibility of a pathogen
☐	☐ Alter the host range of a pathogen
☐	☐ Enable evasion of diagnostic/detection modalities
☐	☐ Enable the weaponization of a biological agent or toxin
☐	☐ Any other potentially harmful combination of experiments and agents

ChIP-seq

Data deposition

- ☐ Confirm that both raw and final processed data have been deposited in a public database such as [GEO](#).
- ☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links

May remain private before publication.

For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.

Files in database submission

Provide a list of all files available in the database submission.

Genome browser session

(e.g. [UCSC](#))

Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.

Methodology

Replicates

Describe the experimental replicates, specifying number, type and replicate agreement.

Sequencing depth

Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.

Antibodies

Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.

Peak calling parameters

Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.

Data quality

Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.

Software

Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.

Flow Cytometry

Plots

Confirm that:

- ☐ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☐ All plots are contour plots with outliers or pseudocolor plots.
- ☐ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.

Instrument

Identify the instrument used for data collection, specifying make and model number.

Software

Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.

Cell population abundance

Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.

Gating strategy

Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.

- ☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

Design type

Indicate task or resting state; event-related or block design.

Design specifications

Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.

Behavioral performance measures

State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).

Acquisition

Imaging type(s)

Specify: functional, structural, diffusion, perfusion.

Field strength

Specify in Tesla

Sequence & imaging parameters

Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.

Area of acquisition

State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.

Diffusion MRI

☐ Used

☐ Not used

Preprocessing

Preprocessing software

Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).

Normalization

If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.

Normalization template

Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.

Noise and artifact removal

Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).

Volume censoring

Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.

Statistical modeling & inference

Model type and settings

Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).

Effect(s) tested

Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ BothStatistic type for inference
(See [Eklund et al. 2016](#))

Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.

Correction

Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).

Models & analysis

n/a | Involved in the study

- ☐ ☐ Functional and/or effective connectivity
- ☐ ☐ Graph analysis
- ☐ ☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity

Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).

Graph analysis

Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).

Multivariate modeling and predictive analysis

Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.