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Animal behaviour

Modulation of social space by dopamine in *Drosophila melanogaster*, but no effect on the avoidance of the *Drosophila* stress odorant

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Appropriate response to others is necessary for social interactions. Yet little is known about how neurotransmitters regulate attractive and repulsive social cues. Using genetic and pharmacological manipulations in *Drosophila melanogaster*, we show that dopamine is contributing the response to others in a social group, specifically, social spacing, but not the avoidance of odours released by stressed flies (dSO). Interestingly, this dopamine-mediated behaviour is prominent only in the day-time, and its effect varies depending on tissue, sex and type of manipulation. Furthermore, alteration of dopamine levels has no effect on dSO avoidance regardless of sex, which suggests that a different neurotransmitter regulates this response.

1. Introduction

Social space is a measurable characteristic of individuals in groups, and is based on a balance of attractive and repulsive social cues [1]. Abnormal social spacing is observed in individuals with disorders such as autism spectrum or Williams syndrome [2]. However, few studies have investigated the neural mechanisms underlying this basic social response to others.

We used the genetically tractable *Drosophila melanogaster* model and compared two types of response to another individual: social spacing [3–9], and avoidance of the marking left by flies that have been stressed, i.e. *Drosophila* stress odorant (dSO), composed partially of CO_2 [10,11].

Previous social experience affects social spacing in *Drosophila*; and vision but not classical odorant perception is necessary to maintain this distancing [3,7]. Expression of genes involved in synaptic function have been reported to be necessary for proper social spacing [6–8], and the neural circuitry underlying CO_2 perception has been identified [10]. However, the neurotransmitters involved in these social behaviours have yet to be determined.

The dopamine system is proposed to underlie a conserved network for social decision-making from eusocial insects such as bees [12], through birds and rodents [13], to humans [14]. Therefore, dopaminergic signalling is a strong candidate for the modulation of social interactions. We manipulated tyrosine hydroxylase (TH), the rate-limiting enzyme for dopamine synthesis [15], present in both neuronal and hypodermal dopamine cells [16]. However, intracellular monoamine homeostasis is controlled by the vesicular monoamine

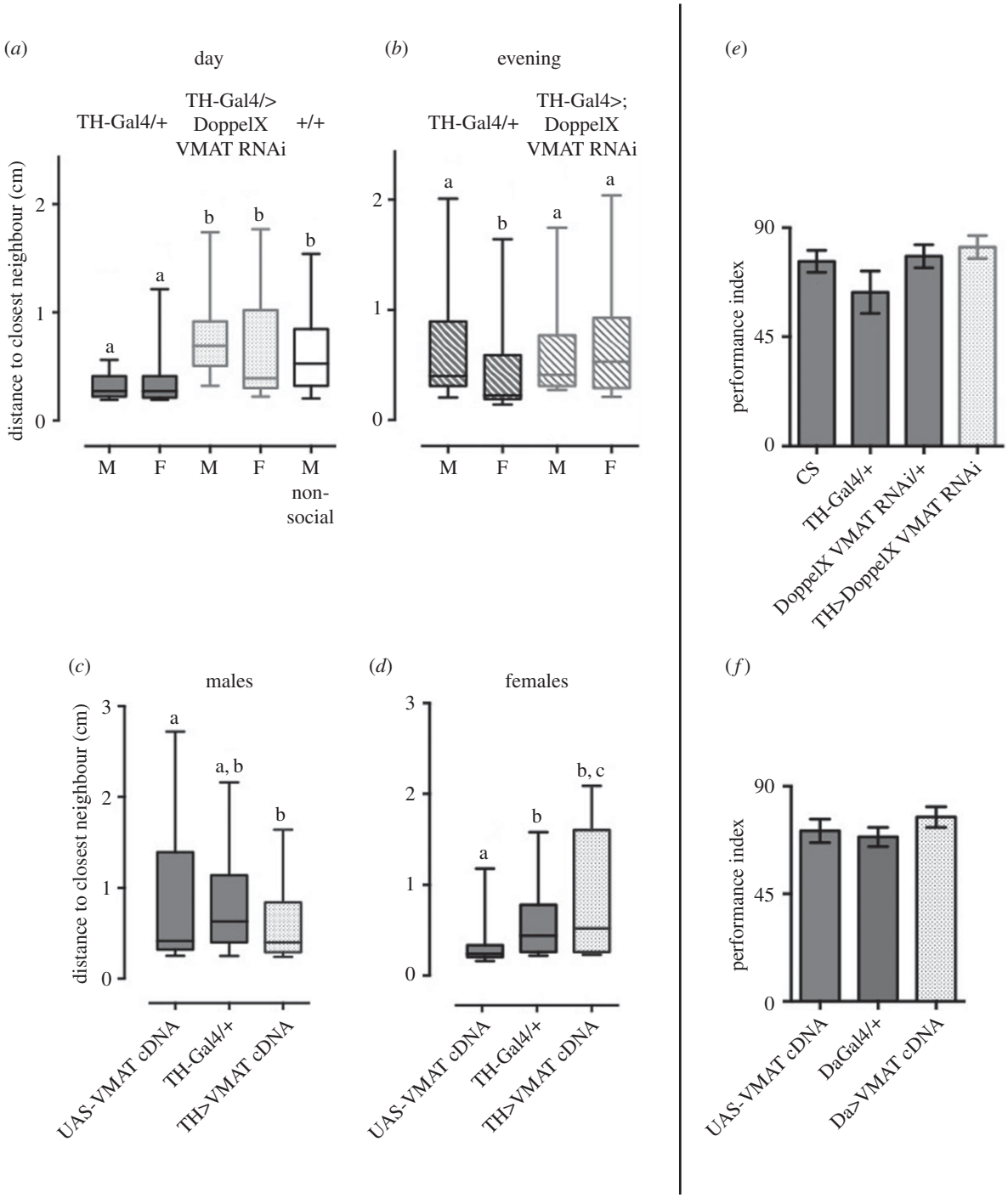

Figure 1. (*a*,*b*) Social spacing of males (M) and females (F) overexpressing VMAT RNAi (DoppelX) with a TH-Gal4 driver during (*a*) the day (number of hours since light turned on (ZT) = 5–7) and (*b*) the evening (ZT = 11–13). (*c*,*d*) Social spacing of (*c*) males and (*d*) females overexpressing UAS-VMAT with a TH-Gal4 driver. (*e*,*f*) dSO avoidance of mixed sex Canon-S (CS) emitter flies by flies of the indicated genotypes. Letters (a, b, c) indicate groups that are statistically different in multiple comparisons (table 1). The data are represented as typical box and whiskers (box: 50% of the data distribution, middle line: median, whiskers 10%–90%).

transporter (VMAT) [17], and is thus also an excellent candidate for studies aiming to identify the role of dopamine modulation on behaviour.

2. Material and methods

All lines of *Drosophila* used are described in the electronic supplementary material. Locomotion was used as an indicator of activity level [18], and is known to be altered in the mutants and conditions tested (in dopamine synthesis [19] and in alteration of VMAT expression [20]). Social space and dSO avoidance were performed as described in [4] and [11]. We fed drugs altering dopamine biosynthesis [15]—30 mM 3-iodotyrosine (a pathway inhibiter) or 1 mM L-DOPA (converted to dopamine)—to 3–4 day old male flies for 24 h. Statistical analyses were performed using GraphPad Prism 7. Detailed descriptions can be found online, in the electronic supplementary material.

3. Results

VMAT loss-of-function mutants—which have less available intracellular dopamine [20]—and flies with a reduced expression of *VMAT* in dopaminergic cells (*TH>VMAT RNAi*) display an increase in social spacing, similar to non-social flies, regardless of their sex (figure 1*a* and table 1, and electronic supplementary material, figure S1*a–c*). However, in

Table 1. Experimental conditions and statistical tests performed.

experiment	replicates and conditions	statistical test performed
figure 1*a*	$n = 2$ independent repeats of 40 flies in large chambers for each genotype	comparison of medians, Kruskal–Wallis test $p < 0.0001$; Dunnet post-test multiple comparison a#b $p < 0.01$
figure 1*b*	$n = 2$ independent repeats of 40 flies in large chambers for each genotype	comparison of medians, Kruskal–Wallis test $p < 0.0001$; Dunnet post-test multiple comparison a#b $p < 0.01$
figure 1*c*	$n = 6$ for VMAT cDNA/+ and TH-Gal4/+, $n = 10$ for TH>VMAT cDNA independent repeats of 15 individuals in small chambers	Kruskal–Wallis test $p < 0.02$; Dunnet post-test multiple comparison a#b $p < 0.02$
figure 1*d*	$n = 6$ for UAS-VMAT/+ and TH-Gal4/+, $n = 10$ for TH>VMAT cDNA independent repeats of 15 individuals in small chambers	Kruskal–Wallis test $p < 0.0001$; Dunn' post-test multiple comparison a#b $p < 0.0002$
figure 1*e*	Canton-S, $n = 6$, TH-Gal4 $n = 8$, DoppelX VMAT RNAi/+, TH>DoppelX VMAT RNAi $n = 9$; 3 independent trials of approx. 30 flies for 60s, with 2–4 internal repeats	one-way ANOVA $p = 0.1113$; *t*-test $p > 0.21$ for each comparison with Canton-S
figure 1*f*	UAS-VMAT cDNA/+ $n = 6$; DaGal4/+, $n = 10$; Da>VMAT cDNA $n = 8$ independent repeats of 30 individuals	one-way ANOVA $p = 0.3751$; *t*-test $p > 0.197$ for each comparison with UAS-VMAT/+

experiment	replicates	control used for normalization
figure 2*a*, male VMAT	TH>VMAT cDNA $n = 12$; control $n = 18$; P VMAT $n = 5$; TH>VMAT RNAi (DoppelX) $n = 12$	Canton-S
figure 2*b*, female VMAT	TH>VMAT cDNA $n = 10$; control $n = 0$; TH>VMAT RNAi (DoppelX) $n = 2$ (in this case, s.e.m. obtained from approx. 80 flies)	Canton-S
figure 2*c*, male biosynthesis	TH>Catsup RNAi $n = 5$, cat^{26}/+ $n = 3$, control $n = 9$, ple^2/+ $n = 3$	for TH>Catsup RNAi control was TH-Gal4/+; for cat^{26}/+ and ple^2 control was Canton-S
figure 2*d*, female biosynthesis	TH>Catsup RNAi $n = 3$, cat^{26}/+ $n = 3$, control $n = 14$, ple^2/+ $n = 3$, pale brain mutant $n = 6$	for TH>Catsup RNAi control was TH-Gal4/+; for cat^{26}/+ and ple^2 control was Canton-S
figure 2*e*, male acute	3 independent repeats with 2–4 internal replicates of 40, such that control $n = 11$, 3-IT $n = 8$ and L-DOPA $n = 9$	Canton-S fed vehicle

the evening, the *TH>VMAT RNAi* lines are not different from the controls (figure 1*b*). In contrast, overexpression of the nerve cells variant, *VMAT-A*, using either *TH-Gal4* driver (expressed in most dopaminergic cells [16]) or *Da-Gal4* driver (not expressed in serotonin neurons [20]) led to sex-specific altered social spacing. Males are closer (figure 1*c* and electronic supplementary material, figure S1*d*), and females are further apart (figure 1*d*). In both sexes, there is no change in dSO avoidance in any manipulation of *VMAT* expression performed (figure 1*e* and electronic supplementary material, figure S2).

Females lacking *tyrosine hydroxylase* in the nervous system [16] have increased social spacing (electronic supplementary material, figure S3*a*). The effect is also strongly diminished in the evening (electronic supplementary material, figure S3*b*). As expected [21], their locomotion is reduced (electronic supplementary material, figure S3*c*). However, male but not female flies that expressed an RNAi against an inhibitor of tyrosine hydroxylase, *catsup*, display an increased social spacing (electronic supplementary material, figure S3*d*). Additional milder mutants, of either *catsup* (*Cat26/+*) or *tyrosine hydroxylase* (*ple2/+*) lead to no effect in males, whereas females appear closer (electronic supplementary material, figure S3*e*).

Finally, we fed male Canton-S flies two drugs, L-DOPA and 3-iodotyrosine (3-IT), respectively increasing and decreasing dopamine synthesis in an acute manner [22]. In contrast to the chronic modifications of dopamine synthesis, both treatments led to a similar increase in social spacing, and no effect on dSO avoidance (electronic supplementary material, figure S3*f*,*g*), but the previously reported effect on their locomotion [19] (electronic supplementary material, figure S3*h*).

In order to compare these different treatments, we normalized the data to their respective controls (figure 2—see electronic supplementary material for details). Dopamine affects social spacing in a sex-specific manner that is dependent on whether only the TH-neurons or all tissues are affected and whether the treatment was chronic or acute.

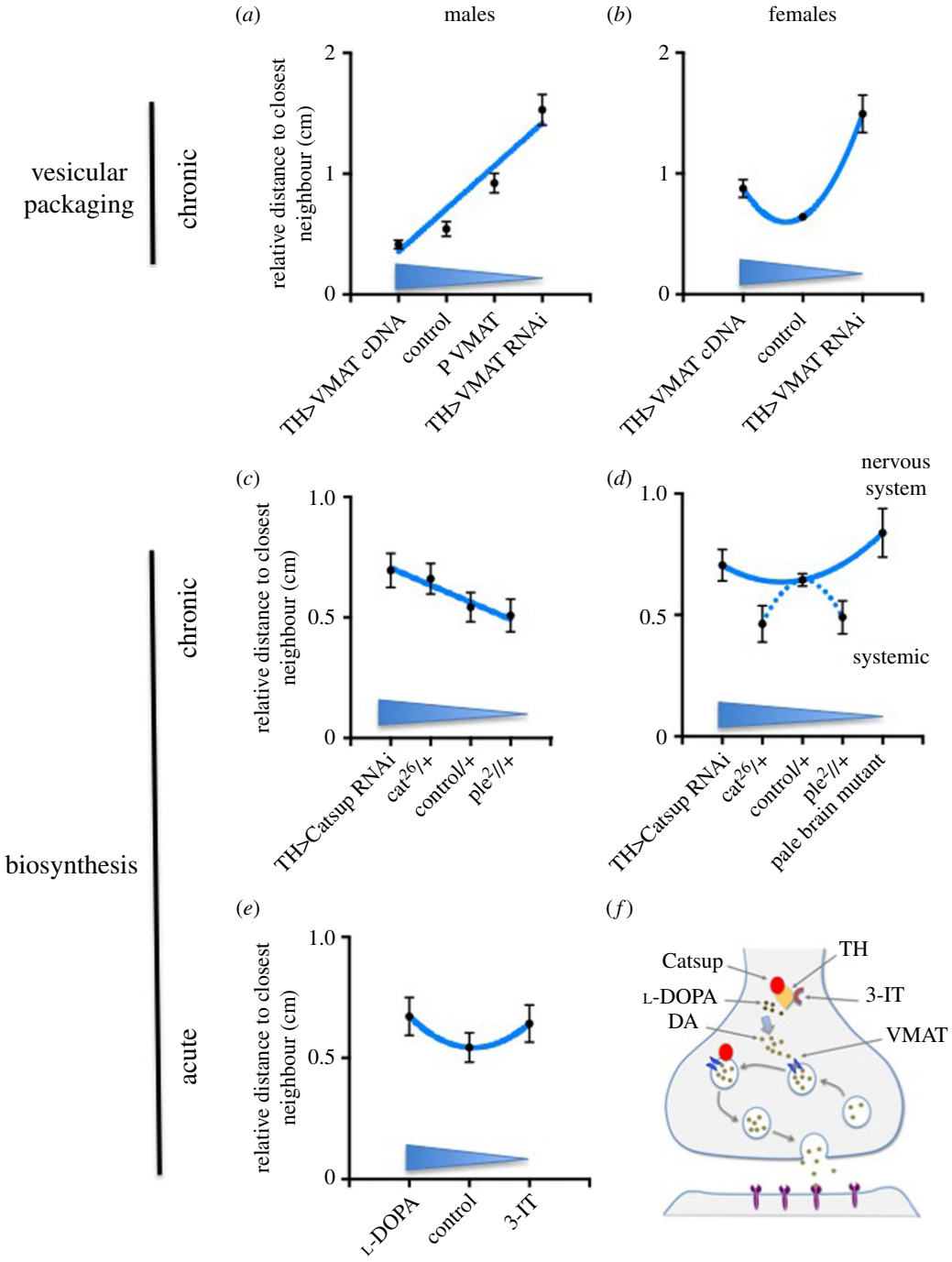

Figure 2. Linear (*a*,*c*) and curvilinear (U-shaped, *b*,*d*,*e*) distribution of social spacing in response to presynaptic modification in dopamine levels, depending on type of alteration (chronic or acute, systemic or nervous system, biosynthesis or vesicular packaging), and sex. The genotypes or treatments are ordered in expected decrease in dopamine (from high to low—blue arrows). Data are represented as relative means (± s.e.m.) of the social space distributions. (*f*) Diagram of a synapse, indicating the proteins altered by the pharmacological and genetic manipulations in this study. DA, dopamine.

4. Discussion

Abnormal expression of *VMAT* in dopaminergic neurons alters social spacing, with no effect on dSO avoidance. Furthermore, both chronic and acute modifications of dopamine synthesis also affect social spacing. Although dopamine and *VMAT* alterations affect locomotion, including in the mutants tested here [3,18,19], we observed no correlation between the social spacing and the locomotion, as reported before [4].

In males, increasing dopamine in the TH-neurons leads to an increase in social space, a result suggested previously, in the context of courtship behaviour [23,24]. However, increasing dopamine in all tissues leads to the opposite effect. In females any manipulation in the TH-neurons leads to increased social space, while manipulation affecting all tissues leads to reduced social space. Although the underlying mechanisms are yet to be understood, these linear (in males) or U-shaped (in females) dose-dependent effects have been proposed by others [25]. It is not surprising to observe different functional consequences for abnormal dopamine concentration in the hypodermal cells versus abnormal dopamine tone at the synapse. Dopamine might affect emission of social cues in hypodermic cells, while affecting the decision process in response to those cues in neuronal cells. Similarly, the sex-specific variations probably reflect differences in how the genetic manipulations performed alter dopamine concentrations in the two sexes. Indeed, adult

females have higher dopamine content than males [26]. Furthermore, flies might rely on sexually dimorphic dopaminergic neurons to generate proper social spacing, as shown previously for stress response [27].

Finally, we found no effect on social spacing of loss of function of *VMAT* in dopaminergic cells in the evening, which supports previous reports of the role of dopamine and *VMAT* in sleep and arousal, although no change in activity patterns themselves has been reported [28–32].

In summary, we show for the first time to our knowledge that dopamine is a key component of the regulation of social space in *Drosophila melanogaster*, probably at the level of both emitting and perceiving social signals. Better understanding of the sex- and cell-specificity of dopamine requirements in these social responses might reveal conserved neural correlates.

Data accessibility. The datasets are available at Dryad: (http://dx.doi.org/10.5061/dryad.dn5tk) [33]. Supplementary material (results, figures, table, material and methods, and references) is provided in an electronic form online (https://dx.doi.org/10.6084/m9.figshare.c.3838141).

Authors' contributions. Data acquisition: R.W.F., A.A.A., M.N., O.F., M.L. and R.R.A., and with A.F.S. data analysis; writing: R.W.F., M.L. and A.F.S.; editing and critiquing: A.A.A., M.N., O.F., R.R.A. and J.M.O.; supervision: A.F.S. and J.M.O.; conception: A.F.S. All authors approved the final version of the manuscript and agree to be held accountable for the content therein.

Competing interests. We declare no conflict of interest.

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