Asymmetric Overlap in Neuronal Sensation Constraints Rational Choice in *C. elegans*

Dror Cohen[1,2], Meshi Volovich[1,2], Yoav Zeevi[2,3], Kenway Louie[4], Dino J Levy[2,5,$], Oded Rechavi[1,2,$]

[1]Department of Neurobiology, Wise Faculty of Life Sciences, Tel Aviv University, Israel.

[2]Sagol School of Neuroscience, Tel Aviv University, Israel.

[3]Statistics and Operation Research, Tel Aviv University, Israel.

[4]Center for Neural Science, New York University, 4 Washington Place, Room 809, New York, New York, 10003, USA.

[5]Coller School of Management, Tel Aviv University, Israel.

$Equal contribution.

#Corresponding authors: dinolevy@post.tau.ac.il&odedrechavi@gmail.com

Abstract

Rational choice theory in economics assumes optimality indecision-making. One of the basic axioms of economic rationality is “Independence of Irrelevant Alternatives” (IIA), according to which a preference ratio between two options should be unaffected by introducing additional alternatives to the choice set. Violations of IIA have been demonstrated in both humans and in various animals, and could therefore stem from common neuronal constraints. We

used the nematode *Caenorhabditis elegans*, an animal with only 302 neurons and a fully mapped connectome, to examine when and why economic rationality and violations of rationality occur. We developed tests for IIA violations by characterizing the choices that *C. elegans* make in olfactory chemotaxis assays. In each assay, we exposed the worm to different odors that activate only specific neurons, thus involving in the choice process only defined neuronal networks, and tested whether particular neuronal architectures are prone to producing irrational choices. We found that *C. elegans* are capable of maintaining robust binary olfactory preferences irrespectively of the presence of a third attractive odor. However, in very specific olfactory contexts, which we term *asymmetric overlaps*, the preference ratio between the two odors was altered due to the addition of a third inferior odor, in a manner that violates IIA, and in certain cases can be considered "irrational" based on the economic definition of rationality. Our results suggest that different network configurations vary in their propensity to give rise to inconsistent decision making. Thus, non-optimal choices, assumed to be an outcome of high-order cognitive and mental processes, could result from much more basic attributes of neuronal activity and constrained computational mechanisms.

Introduction

Decision-making is a crucial process that enables organisms to flexibly respond to environmental demands in changing conditions. The choice process has been extensively studied in humans, but it is a general

phenomenon extending to the simplest of organisms. Normative models of decision-making, such as rational choice theory in economics and foraging theory in ecology, assume optimality in the behavior of individual choosers. Their core principle is *utility maximization*, which assumes that choosers act to maximize an internal measure of satisfaction. Economists originally proposed that decisions rely only on outcome probability and magnitude, or *expected value*. However, this simple model fails to describe how human choosers actually behave, leading to the idea that choosers instead transform expected value into an internal *subjective value*, incorporating a subjective aversion to risk(Bernoulli, 1738).

More recently, Samuelsson and others developed the axiomatic approach to choice analysis, revolutionizing economics by defining rationality as the choices consistent with maximizing a subjective, internal representation of value or *utility* (Houthakker, 1950; Samuelson, 1937; Von Neumann & Morgenstern, 1944). Choice optimality is a general principle, extending to the ecological study of animal behavior (Stephens & Krebs, 1988). Rather than decisions about money or goods, optimal foraging theory posits that animals choose by maximizing their net rate of energy intake, considered to be a proxy for reproductive fitness (Charnov, 1976; Emlen, 1966; Macarthur & Pianka, 1966). Optimality-based models accurately describe choice behavior in diverse species and ecological niches, from insects to birds to primates.

The economic and ecological frameworks share the notion that choosers act to efficiently maximize the specific currency of interest. A central biological question is whether and how the internal value signals posited by these normative choice theories are represented by the underlying neural

systems (Paul W. Glimcher, Michael C. Dorris, 2005; Zhang, Lu, & Bargmann, 2005). The economic definition of rationality only requires that decision makers choose "as if" they are maximizing a theoretical utility quantity. However, recent electrophysiological (Kenway Louie & Glimcher, 2012; Schultz, 2004; Webb, Glimcher, & Louie, 2014) and neuroimaging data (Bartra, McGuire, & Kable, 2013; Levy & Glimcher, 2012; Rangel & Hare, 2010) show that a number of brain areas represent subjective value information. Such findings suggest that rationality-based models are approximated, at the level of information coding, in biological decision circuits.

While rationality remains central to economic thought, empirical choice behavior often violates normative theory (Kahneman & Tversky, 1979, 1992). However, why such irrational behaviors occur is still unknown. Failures of "rationality", this inconsistency in preferences, may reflect the implementation of decision-making in biological nervous systems facing intrinsic physical and metabolic constraints (H. A. Simon, 1955, 1956). A better understanding of the conditions in which economic rationality and violations of rationality occur may offer insight into the biological basis of decision making.

Psychological studies (Kahneman & Tversky, 1979, 1992) have documented a wide range of suboptimal choice patterns. Contradicting the rational notion of stable value functions and choice consistency, preferences are often constructed at the time of elicitation and are remarkably dependent on the decision context. Significant context-dependence and irrational behavior occurs in monkeys (K. Louie, Khaw, & Glimcher, 2013; Yamada, Tymula, Louie, & Glimcher, 2013), birds (Bateson, Healy, & Hurly, 2003; Hurly & Oseen, 1999), fish (Royle, Lindström, & Metcalfe, 2008), and insects

(Shafir, 1994; Shafir, Waite, & Smith, 2002), paralleling human irrationalities such as intransitivity, attribute based preference reversals (Huber, Payne, & Puto, 1982; Simonson, 1989; Tversky & Simonson, 1993), and the paradox of choice (Iyengar & Lepper, 2000; Schwartz, 2003). These similarities occur despite varying nervous system architectures, suggesting that rationality and deviations from rationality arise from general computational principles rather than specific biological implementations. According to the idea of *bounded rationality*, the computational or informational load required to make truly optimal decisions exceeds the capacity of our nervous systems.

While cognitive capacity limits are well documented in behavior (Gigerenzer & Gaissmaier, 2011), little is known about how they are instantiated at the neural level. The amount of sensory information in the environment far exceeds the brain's coding capacity, which is limited by physical and metabolic constraints. Given considerable regularity in the statistical structure of the world, sensory systems must reduce the informational redundancies in sensory representations. Thus, true optimality (a complete representation of the world) is impossible but the nervous system has adopted computational strategies to approach a constrained optimality (Laughlin, 1981; Simoncelli, 2003; Simoncelli & Olshausen, 2001). The behavioral limits of biological perception are evident in the form of perceptual illusions, many of which can be traced to the underlying neural computations (Schwartz, Hsu, & Dayan, 2007). In this manuscript, we propose an analogous framework to examine the relationship between suboptimal choice and the biological mechanism of decision-making.

One central requirement of rationality and stable value functions is *independence of irrelevant alternatives*, or IIA (Luce., 1959). According to this axiom, a preference between two options should be unaffected by the number or quality of any additional options, and the relative choice ratio between options A and B (pA/pB) should remain constant regardless of the choice set. However, contextual factors such as choice set size significantly alter animal and human decisions. This rational principle of regularity and the constant-ratio rule can be examined by comparing choices between options in binary (only the two options present) versus trinary trials, in which a new option is added to the choice set. For example, as will be demonstrated in this paper, choice behavior between two odors “A” and “B” could be examined in the presence and absence of a third odor “C”. The critical variable is the relative preference between A and B. Rational choice predicts that this ratio remains unchanged despite the presence of other alternatives.

We established a test for IIA violations using *Caenorhabditis elegans* nematodes. We examined the consistency of the worm’s choices using olfactory chemotaxis assays (relative preferences of two odors in the absence and presence of a third inferior odor).*C. elegans* has only 302 neurons, 32 of which are chemosensory neurons, and chemoreceptor genes comprise a very significant portion of the worm’s gene content (7%-10%), highlighting the centrality of chemosensation for the nematode (Bargmann & Mori, 1997). Using its simple nervous system, *C. elegans* uses chemotaxis to achieve sophisticated behaviors, including simple forms of ethologically-relevant decision making, such as exploration-exploitation foraging behavior (Jarrell et al., 2012) and male mating behavior (Barrios, 2014; Borne, Kasimatis, &

Phillips, 2017; Leighton, Choe, Wu, & Sternberg, 2014; White et al., 2007). However, little is known about economic choice behavior or the extent of rationality in nematodes.

While particular chemoreceptor genes are expressed in specific pairs of chemosensory neurons (Chen et al., 2005; Colosimo et al., 2004; Steven A. McCarroll & Bargmann, 2005; Sun et al., 2001; Troemel, Chou, Dwyer, Colbert, & Bargmann, 1995) the number of different worm chemoreceptor genes greatly exceeds the number of olfactory neurons. Thus, a single olfactory sensory neuron often expresses many different odorant receptor genes (Bargmann & Kaplan, 1998). Just two pairs of amphid sensory neurons, AWC and AWA, are required for chemotaxis toward attractive volatile odors (Bargmann, Hartwieg, & Horvitz, 1993b). Both AWA and AWC respond to the change in attractant concentration over time, and not to the absolute level of attractant (Pierce-Shimomura, Morse, & Lockery, 1999). AWC detects at least six attractive odors: benzaldehyde, 2-butanone, heptanone, isoamyl alcohol, 2,3-pentanedione, and 2,4,5-trimethylthiazole. AWA detects at least three: diacetyl, pyrazine, and 2,4,5-trimethylthiazole. AWA and AWC neurons synapse onto several postsynaptic interneurons: AIA, AIB, AIY and AIZ. These first layer interneurons are also interconnected to each other (White, J. G., Southgate, E., Thomson, J. N., & Brenner, 1986). AIB and AIY interneurons enhance and suppress turning rates, respectively, facilitating gradient climbing (Gray, Hill, & Bargmann, 2005; Larsch et al., 2015; Wakabayashi, Kitagawa, & Shingai, 2004)(see **Supplementary Figure1**).

The two AWC neurons are structurally similar but functionally distinct from each other. One detects 2-butanone (AWC^{ON}), while the other detects 2,3-pentanedione (AWC^{OFF}). Therefore, in the current manuscript, we refer to these neurons as separate individual neurons. Both AWC neurons detect benzaldehyde, isoamyl alcohol and 2,4,5-trimethyltiazole (Wes & Bargmann, 2001). AWC asymmetry is required for odor discrimination between the differentially detected odors benzaldehyde and 2-butanone (Wes & Bargmann, 2001). We examined how the worm chooses between two odorants that activate specifically AWC^{ON}, AWC^{OFF} or the AWA neurons. While in most cases the worms behaved "rationally", and displayed consistent choices, we found that "imbalanced" activation of this minimal neuronal circuit by a less attractive third odorant, can lead to non-optimal decision making (IIA violations), and even choice reversal, namely "irrational" decision making, according to the economic definition of rationality.

Results

To investigate the decision-making process in *C. elegans*, we conducted odor preference tests, as previously described (Bargmann, Hartwieg, & Horvitz, 1993a; Ward, 1973), by placing a population of adult hermaphrodites in the center of an agar plate, equidistant from two or three attractive volatile compounds (the "choice set"). We measured the relative preference between two attractant spots, in which odor "A" (most attractive) and odor "B" (less attractive) were placed, in the presence or in the absence of the least attractive third attractant, odor "C". As controls, we performed also

binary choice assays, in which the third spot contained only a drop of ethanol, the solvent used to dissolve all the attractive volatile compounds (**Figure 1**). The attractiveness of each odor depends on the odor's concentration (Bargmann et al., 1993a; Ward, 1973), thus, by changing the concentrations of the odors that were used in each test, we could decide whether any of the specific odor chemicals would serve as option "A", "B", or "C" (from most attractive to the least attractive) in each particular assay.

Our main interest was the extent to which a relative preference between two attractive odor spots, "A" and "B", would be affected by a third less attractive alternative, odor "C". By testing this preference, we examined if *C. elegans* exhibits non-optimal choice behavior, or *"IIA violations"*. According to the principle of IIA, the ratio of the preference between two options should be independent of other, less preferred options.

An introduction of a third alternative ("C") may change the relative preference between two alternatives (where "A">"B") by increasing the value of the preferred alternative "A", or by reducing it. However, not every change in preference caused by an introduction of a third alternative to a choice set can be considered as an IIA violation. If, for instance, when administered in specific concentrations, the newly added odor which should serve as alternative "C" is more attractive than the less preferred option of the original two ("B"), then the relative preference of "A" over "B" will get stronger, not as a result of the influence that option "C" has on the decision process in hand, but simply because choice "C" actually becomes the new option "B" (the old "B" has been rendered irrelevant). *Bone fide* IIA violations require "C" to be the least preferred alternative of the three. Thus, an IIA violation is demonstrated

if the presence of "C" weakens the relative preference of "A" over "B". An organism affected in this way would be acting inconsistently, since the addition of the new, least preferred alternative, "C", to the choice set weakened the relative preference of the originally preferred odor "A", while increasing the preference of the originally less preferred alternative "B".

Worms make consistent decisions in the presence of a third irrelevant odor

We first examined a minimal decision-making neuronal circuit, by performing choice assays using only odorants that are detected exclusively by the two AWC neurons: 2-butanone is sensed by the AWC^{ON} neuron, and 2,3-pentanedion is sensed by the AWC^{OFF} neuron, while benzaldehyde is sensed by both AWC neurons ("AWC^{BOTH}"). We determined the concentration in which 2-butanone, (AWC^{ON}) odor "A" in this case, was more attractive than odor "B", 2,3-pentanedione (AWC^{OFF}) (~80% of the worms preferred "A" over "B", **Sup.Figure2**). We first examined a *balanced* set of neuronal inputs. Namely, we hypothesized that benzaldehyde, which activates both AWC neurons, could potentially disrupt the sensing of both odor "A" and odor "B", and therefore used this chemical as the third "irrelevant" attractant, odor "C". To make benzaldehyde the least attractive odor on the plate (to utilize it as odor "C") in this experiment, benzaldehyde was administered in low concentrations. We first tested the effect that different concentrations of odor "C", benzaldehyde, would have on the relative preference between 2-butanone ("A") and 2,3-pentanedione ("B").

The chemotaxis index (C.I) of 2-butanone was calculated, as previously described (Bargmann et al., 1993a, 1993b) based on its relative preference. That is, the number of worms at the 2-butanone spot, divided by the number of worms found at the two constant attractant spots (**Figure 2a**):

$$\cdot\frac{\#A}{\#A+\#B}$$

Our results show that odor "C" (benzaldehyde) in this case does not lead to violations of rationality, as the preference ratio between odor "A" (2-butanone) and odor "B" (2,3-pentanedione) did not change in a statistically significant or physiologically relevant way, and when increasing concentrations of odor "C" were applied, we did not observe a trend of a reduction in the preference of odor "A" over "B". At one concentration point (10^{-3}) we observed very weak changes in the preference of "A" over "B" (up to 3%), however these physiologically irrelevant differences were statistically significant only since we performed a very high number of experiments (n=13 triplicates, in both the experiment and control groups). In one case, a high concentration of odor "C" led to a small change in the ratio of "A" over "B" (3.6% change), however in this case "C" was more attractive than "B" (and therefore “C” became the “real” option “B”). Thus, in this setup the worms maintain a stable preference of "A" over "B", despite the addition of odor "C" ($C=10^{-2}$: W=332, p=0.367 ; C=1/500: W=1395, p=0.003 ; $C=10^{-3}$: W=1280, p=0.017 ; C=1/3000: W=82 , p=0.495, **Figure 2a**). We considered the possibility that the relative preference did not change due to the relatively high starting preference of odor “A” over odor “B” (~80/20 ratio). Thus, we repeated the experiment with a higher concentration of odor “B”, to make odor “B” relatively more attractive (2,3-pentandeione, 10^{-3}). In this setup, the relative preference between “A”

and “B” was closer to 50%. Nevertheless, also in this setup, we did not observe any significant differences upon the addition of benzaldehyde, odor “C” (C=1/500: W=175, p=0.167 ; C=10^{-3}: W=141, p=0.642 ; C=1/3000: W=96 , p=0.57; n=4, **Figure 2b**). Despite many attempts to find conditions (by adjusting the concentrations of odors A, B, and C) under which the worm exhibits preference changes, we could not detect any violations of IIA. In such “balanced” setups, where the third option “C” (benzaldehyde) was sensed by the neurons that sense both the preferred odor “A” (2-butanone), and the less preferred odor “B” (2,3-pentandeione), the worms’ behavior was rational (**Figure 2a-c**).

Dopamine is not required for consistent decision-making

In many organisms the dopaminergic system has a strong effect on decision-making by signaling positive and negative rewards (Doya, 2008; Rogers, 2011) and by serving as a reward prediction error (Fee & Goldberg, 2011; Montague, Dayan, & Sejnowski, 1996; Phillips, Walton, & Jhou, 2007; Pool, Sennwald, Delplanque, Brosch, & Sander, 2016; Schultz, 2004). The *cat-2* gene encodes for tyrosine hydroxylase, the rate limiting enzyme in the synthesis of dopamine. To assess the relevance of dopamine pathways for rational decision-making in worms, we subjected *cat-2* mutants to the same choice task described above. First, we verified the mutants’ ability to discriminate between the odors. Wild-type and mutant animals were forced to choose between 2-butanone (10^{-3}) and 2,3-pentanedione (10^{-4}). We did not find significant differences between wild-type (N2) and *cat-2* mutants in the

relative preferences of the odors, or in the ability to sense and discriminate between the odors (2-butanone: W=38, p=0.28, n=8; 2,3-pentanedione: W=18, p=0.28; n=8, **Figure 2d**).

Next, using *cat-2* mutants, we examined the effect of adding benzaldehyde as odor "C" on the relative preference between 2-butanone ("A") and 2,3-pentanedione ("B"). As observed in wild type animals, and perhaps surprisingly, we did not observe any statistically significant differences between wild-type and *cat-2* mutants in the preference between odors "A" and "B" in the present or in the absence of option "C" (C=1/500: W=175, p=0.167; C=10^{-3}: W=141, p=0.642; C=1/3000: W=96, p=0.57; n=4, **Figure2e**). To allow detection of potentially subtler effects of dopamine on the decision-making process, we enhanced the assay's sensitivity by broadening the range of concentrations of the third attractant that was applied. To minimize technical and environmental variability, we performed simultaneously all the experimental conditions in which we tested the effect of different concentrations of odor "C". Still, the introduction of the third attractant did not have any significant effect on the preference between "A" and "B" (C=10^{-7}: W=172, p=0.718; C=10^{-6}: W=148, p=0.314; C=10^{-5}: W=206, p=0.602; C=10^{-4}: W=193, p=0.862; n=4, **Figure 2f**). This suggests that in *C. elegans* the lack of dopamine signaling does not lead to IIA violations.

Even in decisions that involve a more complicated neuronal circuit, worms make consistent decisions

In the experiments described above, all three odorants ("A", "B", and "C") were sensed by just two neurons, AWC^{ON} and AWC^{OFF}. It is possible that the minimal neuronal circuit that we examined so far was "too simple" to give rise to inconsistent behaviors – perhaps irrationality stems from complexity? To increase the complexity of the neuronal circuit underlying the decision process, we examined whether a third attractant odor ("C") that is sensed by a different set of neurons, the two AWA neurons, can affect the relative preference between two odors sensed by the AWC^{ON} and AWC^{OFF} neurons (2-butanone and 2,3-pentanedione, respectively). Similarly to the case in the previous experiments, we first examined a "balanced" third odor "C", a chemical which is not sensed preferentially only by the neurons that sense odor "A" (AWC^{ON}) or only by the neurons that that sense odor "B" (AWC^{OFF}). Two different odors served, in different experiments, as a third AWA-sensed odor"C", diacetyl and pyrazine. The introduction of an AWA-sensed third attractant odor did not significantly affect the relative preference between odor "A", 2-butanone, and odor "B", 2,3-pentanedione (Diacetyl: 10^{-7}: W=33, p=0.645 ; $C=10^{-6}$: W=40, p=0.232; $C=10^{-5}$: W=39, p=0.277; $C=10^{-4}$: W=38, p=0.327, **Figure 3a;** Pyrazine: $C=10^{-7}$: W=127, p=0.368; $C=10^{-6}$: W=113, p=0.735 , $C=10^{-5}$: W=77, p=0.288; $C=10^{-4}$: W=119, p=0.563; $C=10^{-3}$: W=134, p=0.236, **Figure 3b**). In reciprocal experiments (also using a "balanced" distractor), we tested the effect of a third odor sensed by both AWC neurons (benzaldehyde) on the relative preference between "A" and "B" odors which are sensed by both AWA neurons (diacetyl and pyrazine). Also in this setup, no significant effect was observed ($C=10^{-7}$: W=32, p=0.121 ; $C=10^{-6}$: W=42,

p=0.366 , $C=10^{-5}$: W=65, p=0.641; $C=10^{-4}$: W=36, p=0.197 ; $C=10^{-3}$: W=57, p=1 ; $C=10^{-2}$: W=55, p=0.925, **Figure 3c**).

Together, these results suggest that the involvement of an additional pair of neurons in the neuronal circuit that senses the third odor does not lead to inconsistencies in decision-making. This rule applies at least in cases where odor “C” is sensed both by the neurons that sense odor “A”, and the neurons that sense odor “B”. In other words, the disturbance caused by the “irrelevant” alternative is “symmetrical” or “balanced”, since odor “C” is not sensed preferentially only by the neurons that sense odor “A” or only by the neurons that sense odor “B”.

IIA violations may arise in neuronal networks with an imbalanced pattern of olfactory sensation

The results presented above demonstrate that the worm’s decision-making process can be consistent and robust when the irrelevant alternative is sensed symmetrically, in a balanced way, by the neurons that sense odor “A”, and the neurons that sense odor “B”. To increase the complexity of the neuronal circuit underlying the decision-making process, we broke the symmetrical pattern of olfactory inputs, in a more complicated setup that combines odors which are sensed by two different pairs of olfactory sensory neurons.

We examined the effect that AWC^{ON}-sensed odor “C” (2-butanone) has on the relative preference between an AWA-sensed odor (pyrazine, “A”), and

an AWC^{BOTH}-sensed odor (benzaldehyde, "B"). We tested increasing concentrations of the AWC^{ON}-sensed odor "C" (2-butanone). In these experiments we observed, for the first time, that odor "C" affected the relative preference between odors "A" and "B". In the presence of odor "C" (2-butanone), the relative preference of pyrazine ("A") over benzaldehyde ("B") significantly increased from around 60% to 80% ($C=10^{-3}$: W=193, p<0.000; $C=10^{-3}/2$: W=184, p<0.000; C=1/3000: W=169, p<0.000; n=4, **Figure 4a**). However, the change in the relative preferences resulted from the favoring of attractant "C" over the less preferred alternative, "B" (odor "B" is now rendered irrelevant), and therefore this result cannot be considered as a violation of IIA.

Next, we examined if violations of IIA would be observed, in a different setup using the exact same odors, with similar concentrations of odor "C" (2-butanone), but when the preference ordering of odors "A" and "B" would be flipped (by changing the odors' concentrations). Here we used concentrations of benzaldehyde (10^{-3}) and pyrazine (10^{-4}) in which benzaldehyde is more attractive than pyrazine (thus, this time benzaldehyde served as odor "A", and pyrazine served as odor "B"). At these concentrations, the relative preference between benzaldehyde and pyrazineis approximately 80%/20%. Importantly, although the same odors were used, in this setup, for the first time, we witnessed a true IIA violation in *C. elegans*. The presence of the third, least preferred alternative, 2-butanone ("C"), weakened the preference for the originally favored alternative, benzaldehyde ("A"), increasing the value of the originally less favored alternative, pyrazine ("B") ($C=10^{-6}$: W=66, p=0.019; $C=10^{-5}$: W=54, p=0.2 ; $C=10^{-4}$: W=43, p=0.758; $C=10^{-3}$: W=17, p=0.059 ; C=1/500: W=5, p=0.003 ; $C=10^{-2}$: W=0, p<0.000 ; n=6, **Figure 4b**). It is

important to emphasize that the same odor "C", 2-butanone, in the same concentration, did not alter the worm's ability to "rationally" choose between the exact same two odors (pyrazine and benzaldehyde), when high concentrations of pyrazine (serving as "A") and low concentrations of benzaldehyde (serving as "B") were tested. The only difference between these two experiments was which of the two odors, pyrazine and benzaldehyde, served as "A" and which served as "B". This suggests that IIA violations do not occur because specific three odors are used, but rather due to a more complicated interaction which depends not only on the neurons which are activated, but also on the relative preferences of the odors tested. Therefore, we continued to test different hypotheses regarding the source of this perplexing asymmetry that causes IIA violations.

We next tested whether the observed inconsistencies in the worm's decision-making stem from the increase in the task's *complexity* (the involvement of both AWC and AWA neurons), or perhaps solely due to an imbalanced sensation of odor "C" only by the neurons that sense odors "A", but not by the neurons that sense odor "B". Towards this aim, we tested a different "imbalanced" setup, which we considered to be less "complex", since only odors that are sensed by the AWC neurons were involved in the assay. This time, benzaldehyde (10^{-2}), sensed by both AWC^{ON} and AWC^{OFF}, served as odor "A", and 2,3-pentaedione (10^{-3}), sensed only by AWC^{OFF}, served as the less preferred alternative, "B". We tested the effect of 2-butanone, as the third attractant "C", on the relative preference between "A" and "B". Interestingly, similarly to what we found in the previous setup, when also the AWA neurons were involved, the presence of the least preferred odor "C", 2-

butanone, weakened the relative preference for the preferred alternative benzaldehyde (from 60% to 40%), increasing the value of the originally less preferred alternative, 2,3-pentaedione ($C=10^{-6}$: W=15, p=0.166; $C=10^{-5}$: W=16, p=0.38 ; $C=10^{-4}$: W=6, p=0.547; $C=10^{-3}$: W=0, p=0.023 ; C=1/500: W=0, p=0.023 ; $C=10^{-2}$: W=0, p=0.023 ; n=3, **Figure 4c**). This suggests that IIA violations can occur due an *asymmetric overlap* between odors "A" and "C", independently of the complexity of the circuit (in this case, only the AWC neurons were involved). In other words, IIA violations can occur because of an imbalanced sensation of odor "C" by the neurons that sense odor "A" but not odor "B".

IIA violations arise also independently of interactions between 2-butanone and benzaldehyde.

An alternative explanation to the results that we obtained is that the violations are odor-specific, since in both of the setups in which we found IIA violations, odor "A" was benzaldehyde, and odor "C" was 2-butanone **(Figure 5)**. Note, however, that when we flipped the attractiveness of the exact same odors by changing their concentrations, so that 2-butanone served as choice "A", and benzaldehyde served as choice "C", no violations were observed (see **Figure 2a-c**).

We examined whether the IIA violations that we found stem from specific interactions between 2-butanone and benzaldehyde, in AWC^{ON}, the one overlapping neuron that senses both odorants (as specified above, 2-butanone is sensed only by the AWC^{ON} neuron, while benzaldehyde is

sensed by both AWC^{ON} and AWC^{OFF}). It was previously reported that mutants that express the *str-2* gene (an AWC^{ON} marker) in both AWC neurons, which have two pairs of AWC^{ON} neurons at the expense of the AWC^{OFF} neuron pair, lose the ability to identify benzaldehyde on an agar surface containing 2-butanone. A similar phenomenon was observed in AWC^{OFF}-ablated animals. Notably, the same study found that attraction of wild type animals toward benzaldehyde is only slightly reduced in the presence of 2-butanone (Wes & Bargmann, 2001). The authors hypothesized that 2-butanone can attenuate benzaldehyde signaling in the AWC^{ON} neuron, and that benzaldehyde sensing by the AWC^{OFF} neuron is therefore important in order to continue and mediate normal chemotaxis toward benzaldehyde when 2-butanone is present.

We conducted two different experiments to study the interaction between 2-butanone and benzaldehyde, to test if the conclusions that we drew so far, regarding *asymmetric overlap*, are specific to these two odors. First, we tested if the odor combination of 2-butanoneand benzaldehyde is unattractive. When present alone, both benzaldehyde and 2-butanone are each attractive odors (Bargmann et al., 1993b), however, little is known about the ecology of *C.elegans* (Bargmann et al., 1993b; Sagasti, Hobert, Troemel, Ruvkun, & Bargmann, 1999; Schulenburg & Félix, 2017), and it is therefore possible that the combination of the two odors in the wild is associated with an unattractive or even repulsive substance. If this scenario is indeed the case, then the change that we observed in the preference of benzaldehyde (reduced preference of “A” relative to “B”), in the presence of 2-butanone (“C”), might be considered a “feature” of the system, and not a “bug” (which results due to information constraints of the neural network). That is, it would

be considered reasonable and rational to avoid a new unattractive odor, formed upon the combining of benzaldehyde and 2-butanone on the same plate.

Therefore, if 2-butanone masks sensation of benzaldehyde or diminishes its value, a mixed solution of 2-butanone and benzaldehyde should be *less* attractive than the sum of the two odors when they are presented separately. To test this possibility, we examined if worms prefer benzaldehyde over a mixed combination of benzaldehyde and 2-butanone (placed in the same spot on the plate, see methods). We found that the combination of 2-butanone and benzaldehyde was more attractive than benzaldehyde alone ($W=16.5$, $p=0.0000$; $n=6$). No special interaction was observed between the two odors (as would be expected if the combination formed a novel smell with different qualities), as the spot that contained both odors was as attractive as would be expected based on the simple summation of the attractiveness of each of the odors alone. Thus, introducing 2-butanoneon to the plate does not create a new unattractive odor (with benzaldehyde) that can explain the IIA violation that we observed; rather,2-butanone, when serving as odor “C”, changes the choice context, causing benzaldehyde to be less attractive. These results strengthened the hypothesis that the IIA violations that we observed are due to constrains on the neural system – that is, it’s a “bug”, not a “feature” (**Figure 6a**).

We conducted a second experiment to distinguish between the two possible explanations: (1) that the IIA violations we observed stem from a specific effect that sensation of 2-butanone has on sensation of benzaldehyde, (2) that the IIA violations result from a more general limitation

to odor sensation, i.e. *asymmetric overlap*, arising in neuronal networks that employ the same neurons for sensing of multiple molecules (AWC^{ON} in this case). To discriminate between the two possibilities, we tested if 2-butanone, serving as odor "C", influences the attractiveness of the odor isoamyl alcohol (serving as odor "A"). Isoamyl alcoholis sensed, exactly like benzaldehyde, by both AWC^{ON} and AWC^{OFF} neurons. We found that increasing the concentrations of 2-butanone ("C") significantly reduced the relative preference for isoamyl alcohol ("A") over pyrazine ("B") ($C=10^{-5}$: W=16, p=0.78; $C=10^{-4}$: W=18, p=0.527; $C=10^{-3}$: W=22, p=0.163; C=1/500: W=25, p=0.042; $C=10^{-2}$: W=27, p=0.012; n=4,**Figure 6b**). Importantly, this finding suggests that the IIA violations we observed are not the result of unique interactions between 2-butanone and benzaldehyde, but stem from more general network properties such as *asymmetric overlap* in the AWC^{ON} neuron. When 2-butanone, which is detected by the AWC^{ON} neuron, is serving as odor "C", it will significantly reduce the worm's attraction toward the preferred odors, when these odors ("A") are detected by both AWC^{ON} and AWC^{OFF} neurons, while the less preferred odor "B" is sensed by different neurons, that do not sense 2-butanone (either AWC^{OFF} or AWA neurons).

2-butanone as a third attractant does not necessarily induce IIA violations.

Until now, in all the IIA violations that we documented, butanone constituted the third least preferred odorant ("C"). Importantly, when 2-butanone served as odor "A", no IIA violations were observed (**Figure2a-c**).

Hence, it is possible that in any setup in which 2-butanone will serve as odor "C", it would lead to violations. In order to rule out this possibility, we conducted another experiment where 2,3-pentanedione (AWC^{OFF}) served as odor "A", pyrazine (AWA) served as odor "B", and we systematically introduced increasing concentrations of 2-butanone serving as odor "C". We found that the preference ratio between 2,3-pentanedione ("A") and pyrazine ("B") was not influenced by the addition of 2-butanone ("C") ($C=10^{-6}$: W=12, p=0.342; $C=10^{-5}$: W=11, p=0.485 ; $C=10^{-4}$: W=12, p=0.342; $C=10^{-3}$: W=11, p=0.485 ; C=1/500: W=8, p=1 ; $C=10^{-2}$: W=8, p=1 ; n=4, **Figure 6c**). As the introduction of 2-butanone as a third attractant did not change the relative preference between these odorants, the violations we previously identified do not stem from an innate property of 2-butanone. Therefore, 2-butanone cannot be considered as a general "distractor" or "confusant" that drives the IIA violations we observed. A different "confusant" substance was recently described, see (Dennis et al., 2017). Rather, in our experiments, 2-butanone, when serving as odor "C", gives rise to IIA violations only in very specific contexts – when it asymmetrically overlaps the neurons that sense odor "A" but not the neurons that sense odor "B" (**Figure 4b-c**,and **Figure6b**).

Increasing the sensation "bandwidth" of odor "A" does not prevent IIA violations.

In the next experiment, we wanted to expand our understanding of the *asymmetric overlap* rule using a different odor "A", a chemical which has a broader effect, influencing more sensory neurons. We tested if the worm

would be able to behave rationally, to overcome the *asymmetric overlap* constraint, even though odor "C" will still be sensed only by neurons that sense odor "A" and not "B", if sensation of odor "A" would be distributed across more neurons (odor "A" will be sensed by all four neurons, both AWCs and AWAs). That is, will the additional neurons that sense odor "A" reduce the impact of the *asymmetric overlap* between odors "A" and "C" in AWC^{ON}, so that an IIA violation will not occur. Towards this aim, we used 2,4,5-trimethyltiazole which is detected by all four neurons, (AWC^{BOTH} &AWA^{BOTH}), as odor "A", and 2,3-pentanedione (AWC^{OFF}) served as odor "B". We tested the influence of 2-butanone as odor "C" on the relative preference between these two odors "A" and "B". We found that adding 2-butanone ("C") generated a decrease in preference of 2,4,5-trimethyltiazole ("A"), giving rise to an IIA violation($C=10^{-6}$: W=10, p=0.428; $C=10^{-5}$: W=21 p=0.329; $C=10^{-4}$: W=20, p=0.428; $C=10^{-3}$: W=9, p=0.329; C=1/500: W=23, p=0.177; $C=10^{-2}$: W=30, p=0.004; n=6, **Figure 7a**). Hence, expanding the "bandwidth" of the sensation of odor "A" does not protect from IIA violations, as long as there is an asymmetric overlap between odors "A" and "C" in the AWC^{ON} neuron.

IIA violations are not observed when a larger neural circuit is involved, despite an *asymmetric overlap* setup.

Until now, we only observed IIA violations when the circuits displayed an *asymmetric overlap*, namely, the neurons that sensed odor "A", sensed also odor "C", while the neurons that sensed odor "B" did not. We wanted to examine if we could expand the *asymmetric overlap* rule to configurations that

involve not only the AWC^{ON} and AWC^{OFF} neurons. Therefore, we examined whether a different asymmetric configuration (but similar in principle/architecture), that involves more neurons, would produce IIA violations. For this purpose, in the next experiment, the imbalanced choice set included odorants sensed by both the AWA and AWC neurons, but with an expanded *asymmetric overlap* configuration. We hypothesized that the use of multiple neurons to sense particular odors could "buffer" against the distracting effects of the other odors, sensed by the same neurons. Also in this expanded architecture, only the neurons that sense odor "A" sense odor "C", the neurons that sense odor "A" also sense odor "B", and the neurons that sense odor "B" do not sense odor "C". We tested the influence of isoamyl-alcohol ("C", AWC^{BOTH}) as a third attractant on the relative preference between 2,4,5-trimethyltiazole ("A", AWC^{BOTH}&AWA^{BOTH}) and pyrazine (B, AWA^{BOTH}). This experiment did not reveal any IIA violations ($C=10^{-6}$: W=11, p=0.536; $C=10^{-5}$: W=7 p=0.792; $C=10^{-4}$: W=10, p=0.428; $C=10^{-3}$: W=9, p=0.547; C=1/500: W=10, p=0.428; $C=10^{-2}$: W=9, p=0.329; n=6, **Figure 7b**).To further strengthen the notion that setups with expanded asymmetry are not prone to IIA violations, we conducted an additional experiment using a different set of odors, that tests the limitations of a similar asymmetric architecture. We examined the influence of pyrazine ("C", AWA^{BOTH}) on the relative preference for 2,4,5-trimethyltiazole ("A", AWC^{BOTH}&AWA^{BOTH}) over benzaldehyde ("B", AWC^{BOTH}). As in the previous cases which gave rise to IIA violations, also in this experiment odor "C" was sensed by half of the neurons which sense odor "A", and not by the neurons that sense odor "B". Thus, the network architecture in this assay is analogous (but with an expanded

architecture) to the network architectures which revealed IIA violations in the previous experiments. Nevertheless, in this experiment we did not observe IIA violations or significant changes in the relative preference of "A" over "B" ($C=10^{-6}$: W=16, p=0.547; $C=10^{-5}$: W=16, p=0.547; $C=10^{-4}$: W=17, p=0.420; $C=10^{-3}$: W=18, p=0.309; C=1/500: W=16, p=0.547; $C=10^{-2}$: W=12, p=1; n=5, **Figure 7c**). As no violations were observed in both of these experiments, this raises the possibility that indeed even in an "imbalanced" circuit, the involvement of more neurons in the sensation of the odors can "buffer" the distracting effect of the irrelevant odor, and/or that the asymmetric overlap is specific to the interactions of odors "A" and "C" within the AWC^{ON} neuron (regarding the latter option, see more in the Discussion).

Discussion

In this work we demonstrate for the first time that even *C. elegans*, with its extremely minimal nervous system, displays IIA violations in decision making. We found that in most cases worms are capable of preserving the preferred relations between two odorants, even when a third less attractive odorant is added to the choice set. We initiated our research by probing the simplest olfactory decision-making task that we could test, involving just two odor pairs, the AWC^{ON} and AWC^{OFF} neurons. We examined whether the capacity of worms to choose consistently is affected by the number of neurons involved in sensing the odorants used for the task, or by the

complexity and symmetry of the neural circuit underlying the decision process. We found that if the irrelevant odor "C" was sensed, in a *symmetrical* or *balanced* fashion, by both neurons that sense odor "A" and the neurons that sense odor "B", the animals behaved "rationally", and kept their relative preferences between the most attractive odors. Upon breaking of this symmetry, the worm lost its capacity to choose rationally, and displayed IIA violations (**Figure 5**). Our results show that violations of IIA occur due to an *asymmetric overlap* phenomenon. That is, IIA violations occur when a single neuron needs to sense multiple different odors – we observed violations in imbalanced situations when the neurons that sensed odor "A", but not the neurons that sensed odor "B", sensed also odor "C". Importantly, we also showed that the *asymmetric overlap* caused IIA violations even when odor "A" was sensed by more neurons.

However, our attempts to expand the asymmetric overlap rule to a larger neuronal architecture that involves both pairs of AWA and AWC neurons did not result in IIA violations. This could suggest that the *asymmetric overlap* arises due to specific interactions in the AWC^{ON} neuron. Moreover, this could suggest that involvement of more neurons in the sensation of the odors could "buffer" the distracting effect of the irrelevant odor. Thus, basic constrains on the neuronal sensation give rise to IIA violations and a broader neuronal architecture might compensate these distortions.

In addition, we showed that the IIA violations arise not because the addition of the third odor generates a "new odor" that has a negative value to the worm (a "feature"), but rather that in the trinary choice setup there needs to be a specific interaction, *asymmetric overlap*, between the representation of

odor “C” and odor “A”. This might suggest that IIA violations are a result of neural constraints carved by evolution in order to maximize information under limited time and resources. This hypothesis is in line with previous studies showing that IIA violations can be explained by a divisive normalization framework (K. Louie, Grattan, & Glimcher, 2011; K. Louie et al., 2013; Webb et al., 2014), which is a general and robust rule of cortical computation (Heeger, 2016).

The fact that an extremely simple organism with only 302 neurons displays IIA violations, suggests that non-optimal behavior originates in basic neural mechanisms that are common to many species. Moreover, these neural mechanisms directly relate to the high-level descriptions that are commonly used to explain non-optimal behavior in general, and IIA violations in particular, such as “cognitive overload” or “biased attention”.

Expanding the variety of recognizable odors may come at the expense of the consistency among various decisions, which may lead to non-optimal decision processes. Indeed, in *C. elegans*, a single neuron can express many different chemoreceptor genes (Axel, 2005; Bargmann & Kaplan, 1998; Buck & Axel, 1991; Chess et al., 1994). The worm is able to distinguish different odors efficiently, presumably by using sophisticated signal transduction machinery (Hodgkin, 2001). Evolution may therefore have adopted a pattern of increased biochemical complexity in order to compensate for the lack of neural plasticity and the diminished neuroanatomical complexity in the nematode nervous system (Hodgkin, 2001; Nickell, Pun, Bargmann, & Kleene, 2002). These constraints may render such tradeoffs between olfactory repertoire and decision optimality profitable.

The notion of this tradeoff between olfactory repertoire and decision optimality is strengthened by our finding that the *asymmetric overlap* rule that we identified as inducing IIA violations involves the AWC^{ON} neuron. We speculate that this could be related to the *antisymmetry* displayed by the AWC neurons. Neuronal asymmetry can be broadly divided into two main categories (A. R. Palmer, 1996). In *directional asymmetry*, one of the neurons in a pair obtains a certain defined terminal fate, while the second neuron obtains another. In *C. elegans*, the ASE gustatory neurons present a clear case of such directional asymmetry (Cochella et al., 2014; Cochella & Hobert, 2012; Pierce-Shimomura, Faumont, Gaston, Pearson, & Lockery, 2001; Poole & Hobert, 2006; Yu, Avery, Baude, & Garbers, 1997). This may be considered a biased process because the fate of each of the ASE neurons is genetically fixed. In *antisymmetry*, considered as an unbiased process, each of the two neurons randomly adopts one of two possible fates (A. R. Palmer, 1996). Importantly, AWC olfactory neurons display an anti-symmetry (Chang, Johnston, & Hobert, 2003; A. R. Palmer, 1996). A study that examined the neuronal asymmetry throughout evolution suggested that anti-symmetry might represent an evolutionary intermediate step between symmetry and directional asymmetry, and that the transition from anti-symmetry to directional asymmetry is more likely to occur as compared to a direct transition from neuronal symmetry to directional asymmetry (R. Palmer, 2004).

The default state of AWC neurons at the forth larval stage, before antisymmetry develops, is AWC^{OFF} (Chuang, VanHoven, Fetter, Verselis, & Bargmann, 2007; VanHoven, Bauer Huang, Albin, & Bargmann, 2006).

Hence, it is possible that once the AWC anti-symmetry appeared in the worms' evolution, as a change from a previous symmetric state (palmer, 1996, 2004), one of the AWC neurons (the AWC^{ON} to be) gained the ability to sense2-butanone (in addition to benzaldehyde), but lost its ability to sense 2,3-pentanedion (which is sensed only by the AWC^{OFF} neuron). Because of AWC's anti-symmetry, sensation of benzaldehyde by AWC^{ON} is masked by the presence of 2-butanone, when 2-butanone is mixed into the agar (Wes & Bargmann, 2001). Accordingly, our results suggest that IIA violations arise when there is an imbalanced overlap at the AWC^{ON} neuron. In the decision making experiments we conducted, we showed that the relative preference for benzaldehyde declines (and also of other odorants which are sensed by both AWC neurons) in the presence of a third alternative spot containing increasing concentrations of 2-butanone. We showed that this context-dependency of AWC^{BOTH} sensed odors, generated by the anti-symmetry determining event, may lead to suboptimal and in some cases even irrational behavior. While we do not know enough about the worm's ecology, our results may suggest that although the AWC anti-symmetry process expands the repertoire of odors between which *C. elegans* can discriminate, it may come at the cost of consistency in economic decisions. Hence, this demonstrates a simple example of the phenomenon of the "paradox of choice" (Iyengar & Lepper, 2000; Schwartz, 2003), where more options are normatively considered better but in many cases, lead to suboptimal choices.

To conclude, our findings increase our understanding on the neural basis of optimal choice in general, and IIA violations in particular. We propose that understanding the building blocks of choice in an animal with a compact,

deciphered, rigid, and stereotypic connectome, sheds light on the fundamental biological constraints and principles that generate (non)-rational behavior in simple as well as in complex organisms. Exposing the limitations of the nervous system would allow identification of situations, where sub optimal behavior is hardwired in the brain's most basic design principles. When it comes to visual coding and perception, the boundaries are easily exposed by optical illusions. In the domain of decision making, sub-optimality is revealed as non-consistent and non-rational choice (Kahneman, 2007; Kahneman & Tversky, 1979, 1992). Despite general near-optimality, human choosers display a range of characteristic violations of rationality in specific choice scenarios. Crucially, we observed similar choice inconsistencies in a simple organism, suggesting fundamental, conserved constraints inherent in biological decision processes.

Acknowledgments

We thank all the Rechavi lab and Levy lab members for helpful discussions. D.C and M.V wish to thank the Sagol School of Neuroscience. O.R is thankful to the Adelis foundation grant #0604916191 and ERC grant #335624 for funding. D.J.L is thankful to the ISF grant #1104/13 and to the Henry Crown Institute of Business Research for funding.

Materials and methods

Strains and husbandry

All the strains were provided by the CGC, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440). The strains used in this work: Bristol N2 wild type and*cat-2* (n4547). All strains were maintained at 20°C on NGM plates supplemented with the antifungal agent Nystatin and fed with *E. coli* OP50 (Stiernagle, 2006).

Obtaining synchronized worms (“Egg-prep”)

A synchronized population of worms was obtained by employing a standard “egg-prep” procedure, as previously described (Stiernagle, 2006).

Chemotaxis Assays

Chemotaxis assays were based on classical chemotaxis assays, (Bargmann et al., 1993b; Ward, 1973). Assay plates were square 12X12cm dishes containing 30 ml of 1.6% BBL agar (Benton-Dickinson), 5mM potassium phosphate (pH 6.0), 1mM CaCl2 and 1mM MgSO4. Three marks

were made on the back of the plates equidistant from the center of the plate (3cm) and from each other (5.2cm). The diluted attractants (1 µl) was placed on the agar over one marks. In the control plates (binary choice), 1 µl of 100% ethanol was placed over the third mark (all attractants were diluted in ethanol). The tested animals were placed at the center of the plate, equidistant from the three marks. Attractants were obtained from Sigma-Aldrich. Pure pyrazine is a solid, so pyrazine dilutions are weight:volume rather than volume:volume as for other attractants.

Well-fed adult animals were washed three times with wash buffer (0.5% Gelatin, 5mM potassium phosphate (pH 6.0), 1mM CaCl2 and 1mM MgSO4), then placed near the center of a plate equidistant from the attractants (and the control spot when present). Approximately one hour after the assay began, the numbers of animals at the three areas (2 cm radius of each attractant) were determined, as well as the total number of animals in the assay, the number of animals that were not at any attractant area, and the number of animals that stayed in the starting point (did not cross a 1cm diameter circle around the center of the plate). A specific C.I was calculated as:

$$\mathbf{A}\ \text{Chemotaxis Index} = \frac{\text{Number of animals at attractant } \mathbf{A}}{\text{Number of animals at attractants } \mathbf{A} \text{ and } \mathbf{B}}$$

The C.I could vary from 1.0 to -1.0. The animals were anesthetized when they reached the attractant. 1µl of Sodium azide 1M was placed at each one of the three spots, 15 minutes in advanced. Sodium azide anesthetized animals within about a 1 cm radius of the attractant.

"Bug" or "Feature" assays

We measured the relative preference between 2ul of benzaldehyde (10^{-2}) (A) and 2ul of butanone (10^{-2}) (B), and compared it to the relative preference between 2ul of benzaldehyde (10^{-2}) (A), and a mixture of 1ul of butanone (1/50) and 1ul of benzaldehyde (1/50) (A'B'). The butanone spot (B) and the butanone+benzaldehyde (A'B') spot, contain the same amount of butanone molecules, as well as an equal volume of ethanol. The "A'B'" spot contains, in addition to butanone, the same amount of benzaldehyde molecules as presented by “A”.

Statistical analysis

Data are presented as mean +/− SEM. Statistical significance of differences in chemotaxis index between control and test plates were analyzed by **A Wilcoxon Signed-Ranks Test** (*P*<0.05 was regarded as significant). "*" means p< 0.05, "**" means p<0.01, "***" means p< 0.001, and "****" means p<0.0001.)

Literature cited

Axel, R. (2005). Scents and sensibility: A molecular logic of olfactory perception (Nobel Lecture). *Angewandte Chemie - International Edition*, *44*(38), 6111–6127. https://doi.org/10.1002/anie.200501726

Bargmann, C and Mori, I. (1997). Chemotaxis and Thermotaxis. In *SourceC. elegans II.* (2nd ed.).

Bargmann, C. I., Hartwieg, E., & Horvitz, H. R. (1993a). Odorant- selective genes and neurons mediate olfation in C. elegans. *Cell*, *74*, 515–527.

Bargmann, C. I., Hartwieg, E., & Horvitz, H. R. (1993b). Odorant-selective genes and neurons mediate olfaction in C. elegans. *Cell*, *74*(3), 515–527. https://doi.org/10.1016/0092-8674(93)80053-H

Bargmann, C. I., & Kaplan, J. M. (1998). Signal Transduction in the Caenorhabditis Elegans Nervous System the Structure and Analysis of the Caenorhabditis Elegans Nervous System. *Annu. Rev. Neurosci*, *21*, 279–308. https://doi.org/10.1146/annurev.neuro.21.1.279

Barrios, A. (2014). Exploratory decisions of the caenorhabditis elegans male: A conflict of two drives. *Seminars in Cell and Developmental Biology*, *33*, 10–17. https://doi.org/10.1016/j.semcdb.2014.06.003

Bartra, O., McGuire, J. T., & Kable, J. W. (2013). The valuation system: A coordinate-based meta-analysis of BOLD fMRI experiments examining neural correlates of subjective value. *NeuroImage*, *76*, 412–427. https://doi.org/10.1016/j.neuroimage.2013.02.063

Bateson, M., Healy, S. D., & Hurly, T. A. (2003). Context-dependent foraging decisions in rufous hummingbirds. *Proceedings of the Royal Society B: Biological Sciences*, *270*(1521), 1271–1276.

https://doi.org/10.1098/rspb.2003.2365

Bernoulli, D. (1738). Exposition of a New Theory on the Measurement of Risk. *Econometrica*, *22*(1), 23–36.

Borne, F., Kasimatis, K. R., & Phillips, P. C. (2017). Quantifying male and female pheromone-based mate choice in Caenorhabditis nematodes using a novel microfluidic technique, 1–14. https://doi.org/https://doi.org/10.1101/196733

Buck, L., & Axel, R. (1991). A novel multigene family may encode odorant receptors: A molecular basis for odor recognition. *Cell*, *65*(1), 175–187. https://doi.org/10.1016/0092-8674(91)90418-X

Chang, S., Johnston, R. J., & Hobert, O. (2003). A transcriptional regulatory cascade that controls left/right asymmetry in chemosensory neurons of c. elegans. *Genes and Development*, *17*(17), 2123–2137. https://doi.org/10.1101/gad.1117903

Charnov, E. L. (1976). Optimal foraging, the marginal value theorem. *Theoretical Population Biology*, *9*(2), 129–36.

Chen, N., Pai, S., Zhao, Z., Mah, A., Newbury, R., Johnsen, R. C., … Stein, L. D. (2005). Identification of a nematode chemosensory gene family. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(1), 146–51. https://doi.org/10.1073/pnas.0408307102

Chess, A., Simon, I., Cedar, H., Axel, R., Barlow, D. P., Stöger, R., … Nathans, J. (1994). Allelic inactivation regulates olfactory receptor gene expression. *Cell*, *78*(5), 823–34. https://doi.org/10.1016/S0092-8674(94)90562-2

Chuang, C. F., VanHoven, M. K., Fetter, R. D., Verselis, V. K., & Bargmann,

C. I. (2007). An Innexin-Dependent Cell Network Establishes Left-Right Neuronal Asymmetry in C. elegans. *Cell*, *129*(4), 787–799. https://doi.org/10.1016/j.cell.2007.02.052

Cochella, L., & Hobert, O. (2012). Embryonic priming of a miRNA locus predetermines postmitotic neuronal left/right asymmetry in C. elegans. *Cell*, *151*(6), 1229–1242. https://doi.org/10.1016/j.cell.2012.10.049

Cochella, L., Tursun, B., Hsieh, Y. W., Galindo, S., Johnston, R. J., Chuang, C. F., & Hobert, O. (2014). Two distinct types of neuronal asymmetries are controlled by the Caenorhabditis elegans zinc finger transcription factor die-1. *Genes and Development*, *28*(1), 34–43. https://doi.org/10.1101/gad.233643.113

Colosimo, M. E. (2004). Identification of Thermosensory and Olfactory Neuron-Specific Genes via Expression Profiling of Single Neuron Types. *Current Biology*, *14*.

Dennis, E. J., Jin, X., Dobosiewicz, M., Duvall, L. B., Hartman, P. S., Bargmann, C. I., & Leslie, B. (2017). A natural variant and an engineered mutation in a GPCR promote DEET resistance in C . elegans. *bioRxiv*, 1–15. https://doi.org/10.1101/198705

Doya, K. (2008). Modulators of decision making. *Nature Neuroscience*, *11*(4), 410–416. https://doi.org/10.1038/nn2077

Emlen, J. M. (1966). The role fo time and energy in food preference. *American Naturalist*, *100*, 611-.

Fee, M. S., & Goldberg, J. H. (2011). A hypothesis for basal ganglia-dependent reinforcement learning in the songbird. *Neuroscience*, *198*, 152–170. https://doi.org/10.1016/j.neuroscience.2011.09.069

Gigerenzer, G., & Gaissmaier, W. (2011). Heuristic Decision Making. *Annual Review of Psychology*, *62*(1), 451–482. https://doi.org/10.1146/annurev-psych-120709-145346

Gray, J. M., Hill, J. J., & Bargmann, C. I. (2005). A circuit for navigation in Caenorhabditis elegans. *Proceedings of the National Academy of Sciences*, *102*(9), 3184–3191. https://doi.org/10.1073/pnas.0409009101

Heeger, D. J. (2016). Theory of cortical function. *PNAS*. https://doi.org/10.1073/pnas.1619788114

Hodgkin, J. (2001). What does a worm want with 20,000 genes? *Genome Biology*, *2*(11), COMMENT2008. https://doi.org/10.1186/gb-2001-2-11-comment2008

Houthakker, H. S. (1950). Revealed Preference and the Utility Function, *17*(66), 159–174. https://doi.org/10.1111/j.l468-0335.2010.00859.x

Huber, J., Payne, J. W., & Puto, C. (1982). Adding Asymmetrically Dominated Alternatives: Violations of Regularity and the Similarity Hypothesis. *Journal of Consumer Research*, *9*(1), 90. https://doi.org/10.1086/208899

Hurly, T. A., & Oseen, M. D. (1999). Context-dependent, risk-sensitive foraging preferences in wild rufous hummingbirds. *Animal Behaviour*, *58*(1), 59–66. https://doi.org/10.1006/anbe.1999.1130

Iyengar, S. S., & Lepper, M. R. (2000). When choice is demotivating: Can one desire too much of a good thing? *Journal of Personality and Social Psychology*, *79*(6), 995–1006. https://doi.org/10.1037/0022-3514.79.6.995

Jarrell, T. A., Wang, Y. Y., Bloniarz, A. E., Brittin, C. A., Xu, M., Thomson, J. N., … Emmons, S. W. (2012). The Connectome of a Decision Making

Neural Network. *Science*, *in press*(6093), 437–444.

Kahneman, D. (2007). Rational Choice and the Framing of Decisions Rational Choice and the Framing of Decisions, *59*(4).

Kahneman, D., & Tversky, A. (1979). Prospect Theory - Analysis of Decision under Risk, *47*(2), 263–292.

Kahneman, D., & Tversky, A. (1992). Advances in Prospect Theory : Cumulative Representation of Uncertainty, *323*, 297–323.

Larsch, J., Flavell, S. W., Liu, Q., Gordus, A., Albrecht, D. R., & Bargmann, C. I. (2015). A Circuit for Gradient Climbing in C. elegans Chemotaxis. *Cell Reports*, *12*(11), 1748–1760. https://doi.org/10.1016/j.celrep.2015.08.032

Laughlin, S. (1981). A simple coding procedure enhances a neuron???s information capacity. *Zeitschrift Fur Naturforschung - Section C Journal of Biosciences*. https://doi.org/10.1515/znc-1981-9-1040

Leighton, D. H. W., Choe, A., Wu, S. Y., & Sternberg, P. W. (2014). Communication between oocytes and somatic cells regulates volatile pheromone production in *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences*, *111*(50), 17905–17910. https://doi.org/10.1073/pnas.1420439111

Levy, D. J., & Glimcher, P. W. (2012). NIH Public Access, *22*(6), 1027–1038. https://doi.org/10.1016/j.conb.2012.06.001.The

Louie, K., & Glimcher, P. W. (2012). Efficient coding and the neural representation of value. *Annals of the New York Academy of Sciences*, *1251*(1), 13–32. https://doi.org/10.1111/j.1749-6632.2012.06496.x

Louie, K., Grattan, L. E., & Glimcher, P. W. (2011). Reward Value-Based Gain Control: Divisive Normalization in Parietal Cortex. *Journal of*

Neuroscience, *31*(29), 10627–10639. https://doi.org/10.1523/JNEUROSCI.1237-11.2011

Louie, K., Khaw, M. W., & Glimcher, P. W. (2013). Normalization is a general neural mechanism for context-dependent decision making. *Proceedings of the National Academy of Sciences*, *110*(15), 6139–6144. https://doi.org/10.1073/pnas.1217854110

Luce., R. . D. (1959). ndividual choice behavior: A theoretical analysis.

Macarthur, R. H., & Pianka, E. R. (1966). On Optimal Use of a Patchy Environment. *The American Naturalist*, *100*(916), 603–609.

Montague, P. R., Dayan, P., & Sejnowski, T. J. (1996). A framework for mesencephalic dopamine systems based on predictive Hebbian learning. *Journal of Neuroscience*, *16*(5), 1936–1947. https://doi.org/10.1.1.156.635

Nickell, W. T., Pun, R. Y. K., Bargmann, C. I., & Kleene, S. J. (2002). Single ionic channels of two Caenorhabditis elegans chemosensory neurons in native membrane. *Journal of Membrane Biology*, *189*(1), 55–66. https://doi.org/10.1007/s00232-002-1004-x

Palmer, A. R. (1996). From symmetry to asymmetry: phylogenetic patterns of asymmetry variation in animals and their evolutionary significance. *Proceedings of the National Academy of Sciences of the United States of America*, *93*(25), 14279–14286. https://doi.org/10.1073/pnas.93.25.14279

Palmer, R. (2004). Symmetry breaking and the evolution of development. *Science (New York, N.Y.)*, *306*(2004), 828–33. https://doi.org/306/5697/828 [pii]

Paul W. Glimcher*, Michael C. Dorris, and H. M. B. (2005). Physiological

utility theory and the neuroeconomics of choice. *Games Econ Behav.* https://doi.org/10.1016/j.jacc.2007.01.076.White

Phillips, P. E. M., Walton, M. E., & Jhou, T. C. (2007). Calculating utility: Preclinical evidence for cost-benefit analysis by mesolimbic dopamine. *Psychopharmacology*, *191*(3), 483–495. https://doi.org/10.1007/s00213-006-0626-6

Pierce-Shimomura, J. T., Faumont, S., Gaston, M. R., Pearson, B. J., & Lockery, S. R. (2001). The homeobox gene lim-6 is required for distinct chemosensory representations in C. elegans. *Nature*, *410*(6829), 694–698. https://doi.org/10.1038/35070575

Pierce-Shimomura, J. T., Morse, T. M., & Lockery, S. R. (1999). The fundamental role of pirouettes in Caenorhabditis elegans chemotaxis. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *19*(21), 9557–9569.

Pool, E., Sennwald, V., Delplanque, S., Brosch, T., & Sander, D. (2016). Measuring wanting and liking from animals to humans: A systematic review. *Neuroscience and Biobehavioral Reviews*, *63*, 124–142. https://doi.org/10.1016/j.neubiorev.2016.01.006

Poole, R. J., & Hobert, O. (2006). Early Embryonic Programming of Neuronal Left/Right Asymmetry in C. elegans. *Current Biology*, *16*(23), 2279–2292. https://doi.org/10.1016/j.cub.2006.09.041

Rangel, A., & Hare, T. (2010). Neural computations associated with goal-directed choice. *Current Opinion in Neurobiology*, *20*(2), 262–270. https://doi.org/10.1016/j.conb.2010.03.001

Rogers, R. D. (2011). The roles of dopamine and serotonin in decision

making: Evidence from pharmacological experiments in humans. *Neuropsychopharmacology*, *36*(1), 114–132. https://doi.org/10.1038/npp.2010.165

Royle, N. J., Lindström, J., & Metcalfe, N. B. (2008). Context-dependent mate choice in relation to social composition in green swordtails Xiphophorus helleri. *Behavioral Ecology*, *19*(5), 998–1005. https://doi.org/10.1093/beheco/arn059

Sagasti, A., Hobert, O., Troemel, E. R., Ruvkun, G., & Bargmann, C. I. (1999). Alternative olfactory neuron fates are specified by the LIM homeobox gene lim-4. *Genes and Development*, *13*(14), 1794–1806. https://doi.org/10.1101/gad.13.14.1794

Samuelson, P. A. (1937). A Note on Measurement of Utility. *The Review of Economic Studies*, *4*(2), 155. https://doi.org/10.2307/2967612

Schulenburg, H., & Félix, M. A. (2017). The natural biotic environment of Caenorhabditis elegans. *Genetics*, *206*(1), 55–86. https://doi.org/10.1534/genetics.116.195511

Schultz, W. (2004). Neural coding of basic reward terms of animal learning theory, game theory, microeconomics and behavioural ecology. *Current Opinion in Neurobiology*, *14*(2), 139–147. https://doi.org/10.1016/j.conb.2004.03.017

Schwartz. (2003). The Paradox of Choice Chapter 4, 1–2.

Schwartz, O., Hsu, A., & Dayan, P. (2007). Space and time in visual context. *Nature Reviews Neuroscience*, *8*(7), 522–535. https://doi.org/10.1038/nrn2155

Shafir, S. (1994). Intransitivity of preferences in honey bees: Support for

"comparative" evaluation of foraging options. *Animal Behaviour*. https://doi.org/10.1006/anbe.1994.1211

Shafir, S., Waite, T. A., & Smith, B. H. (2002). Context-dependent violations of rational choice in honeybees (Apis mellifera) and gray jays (Perisoreus canadensis). *Behavioral Ecology and Sociobiology*, *51*(2), 180–187. https://doi.org/10.1007/s00265-001-0420-8

Simon, H. A. (1955). A Behavioral Model of Rational Choice. *The Quarterly Journal of Economics*, *69*(1), 99–118. https://doi.org/10.2307/1884852

Simon, H. A. (1956). Rational choice and the structure of the environment. *Psychological Review*, *63*(2), 129–138. https://doi.org/10.1037/h0042769

Simoncelli, E. P. (2003). Vision and the Statistics of the Visual Environment E ffi cient Coding, *13*(April).

Simoncelli, E. P., & Olshausen, B. A. (2001). Natural image statistics and neural representation. *Annu Rev Neurosci*, *24*, 1193–1216. https://doi.org/10.1146/annurev.neuro.24.1.1193

Simonson, I. (1989). Choice Based on Reasons: The Case of Attraction and Compromise Effects. *Journal of Consumer Research*, *16*(2), 158. https://doi.org/10.1086/209205

Stephens, J. and Krebs, D. (1988). FORAGING THEORY. *Journal of Mammalogy*, *69*(4), 877.

Steven A. McCarroll, H. L., & Bargmann, and C. I. (2005). Identification of transcriptional regulatory elements in chemosensory receptor genes by probabilistic segmentation. *Current Biology*, *15*(2005), 347–352.

Stiernagle, T. (2006). Maintenance of C. elegans. *WormBook*, (1999), 1–11. https://doi.org/10.1895/wormbook.1.101.1

Sun, M., Paciga, J. E., Feldman, R. I., Yuan, Z. Q., Coppola, D., You Yong Lu, … Cheng, J. Q. (2001). Phosphatidylinositol-3-OH kinase (PI3K)/AKT2, activated in breast cancer, regulates and is induced by estrogen receptor α (ERα) via interaction between ERα and PI3K. *Cancer Research*, *61*(16), 5985–5991. https://doi.org/10.1038/nature

Troemel, E. R., Chou, J. H., Dwyer, N. D., Colbert, H. A., & Bargmann, C. I. (1995). Divergent seven transmembrane receptors are candidate chemosensory receptors in C. elegans. *Cell*, *83*(2), 207–218. https://doi.org/10.1016/0092-8674(95)90162-0

Tversky, A., & Simonson, I. (1993). Context-Dependent Preferences. *Management Science*, *39*(10), 1179–1189. https://doi.org/10.1287/mnsc.39.10.1179

VanHoven, M. K., Bauer Huang, S. L., Albin, S. D., & Bargmann, C. I. (2006). The Claudin Superfamily Protein NSY-4 Biases Lateral Signaling to Generate Left-Right Asymmetry in C. elegans Olfactory Neurons. *Neuron*, *51*(3), 291–302. https://doi.org/10.1016/j.neuron.2006.06.029

Von Neumann, J., & Morgenstern, O. (1944). *Theory of games and economic behavior*. Princeton University Press.

Wakabayashi, T., Kitagawa, I., & Shingai, R. (2004). Neurons regulating the duration of forward locomotion in Caenorhabditis elegans. *Neuroscience Research*, *50*(1), 103–111. https://doi.org/10.1016/j.neures.2004.06.005

Ward, S. (1973). Chemotaxis by the nematode Caenorhabditis elegans: identification of attractants and analysis of the response by use of mutants. *Proceedings of the National Academy of Sciences of the United States of America*, *70*(3), 817–21. https://doi.org/10.1073/pnas.70.3.817

Webb, B. R., Glimcher, P. W., & Louie, K. (2014). Rationalizing Context-Dependent Preferences : Divisive Normalization and Neurobiological Constraints on Choice. *Choice*, 1–56. https://doi.org/10.2139/ssrn.2462895

Wes, P. D., & Bargmann, C. I. (2001). C. elegans odour discrimination requires asymmetric diversity in olfactory neurons. *Nature*, *410*(6829), 698–701. https://doi.org/10.1038/35070581

White, J. G., Southgate, E., Thomson, J. N., & Brenner, S. (1986). The structure of the nervous system of the nematode Caenorhabditis elegans. *Philosophical Transactions of the Royal Society B.*

White, J. Q., Nicholas, T. J., Gritton, J., Truong, L., Davidson, E. R., & Jorgensen, E. M. (2007). The Sensory Circuitry for Sexual Attraction in C. elegans Males. *Current Biology*, *17*(21), 1847–1857. https://doi.org/10.1016/j.cub.2007.09.011

Yamada, H., Tymula, A., Louie, K., & Glimcher, P. W. (2013). Thirst-dependent risk preferences in monkeys identify a primitive form of wealth. *Proceedings of the National Academy of Sciences*, *110*(39), 15788–15793. https://doi.org/10.1073/pnas.1308718110

Yu, S., Avery, L., Baude, E., & Garbers, D. L. (1997). Guanylyl cyclase expression in specific sensory neurons: A new family of chemosensory receptors. *Proceedings of the National Academy of Sciences*, *94*(7), 3384–3387. https://doi.org/10.1073/pnas.94.7.3384

Zhang, Y., Lu, H., & Bargmann, C. I. (2005). Pathogenic bacteria induce aversive olfactory learning in Caenorhabditis elegans. *Nature*, *438*(7065), 179–184. https://doi.org/10.1038/nature04216

Figure 1

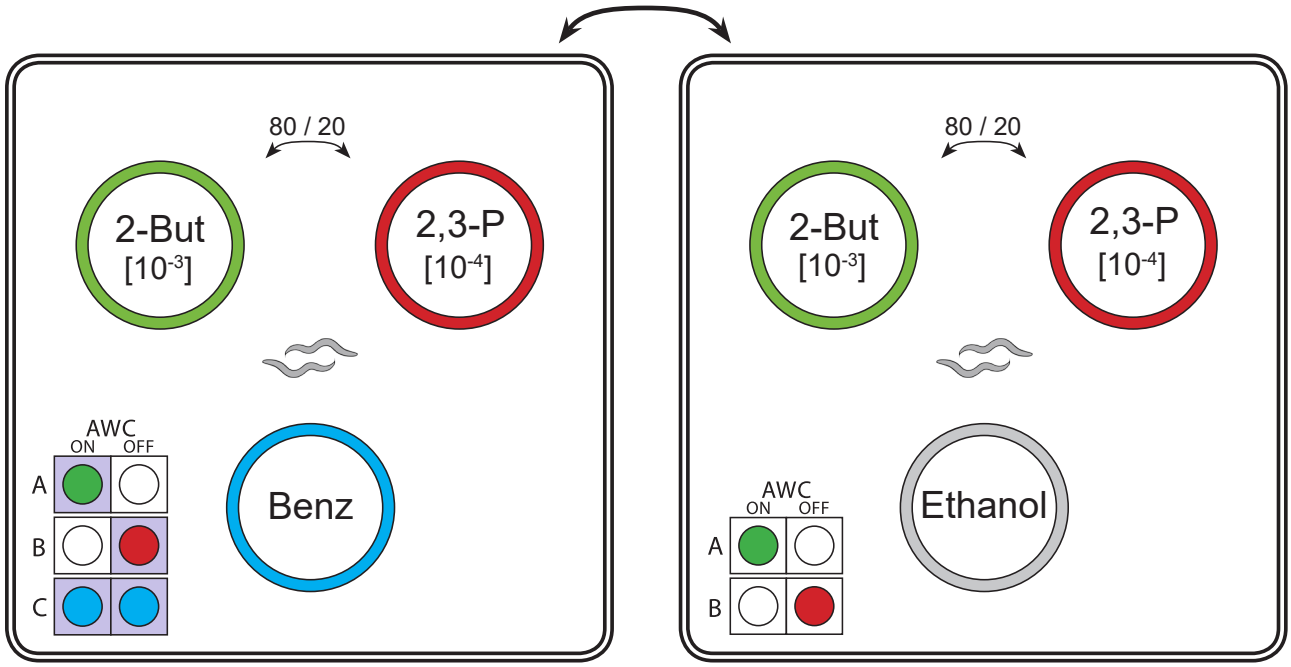
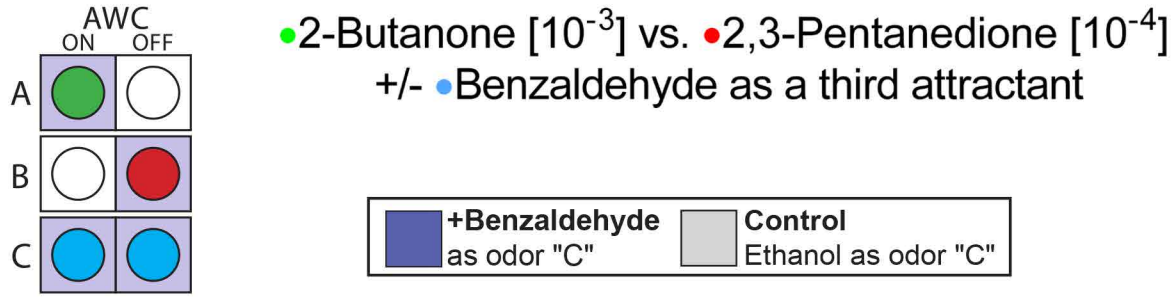


Figure 1. A scheme for the Independence of Irrelevant Alternatives chemotaxis assays

A thin layer of agar in a square 12x12cm Petri dish was used as a substrate for chemotaxis. Between 250 and 350 washed adult hermaphrodite animals were placed at the center of an assay plate equidistant from three spots, 1µl drop of diluted odor at each. In the binary choice assays which served as controls, one of the spots contained only ethanol (the solvent for every odor materials used), with no additional odorant. 1µl of 1M Sodium azide (an anesthetic) was used to capture animals at the attractant and control areas. Once a worm reaches a certain attractant spot it will be paralyzed, ending its choice task. After one hour in 20˚c incubation, the number of animals at each attractant area was counted. A chemotaxis index (C.I) was calculated based on the relative population preference to the favored attractant (number of worms in A, divided by the number of worms in A and B together). The chemotaxis index can vary from +1 (absolute preference for A) to -1 (absolute preference for B). Every test plate (ternary choice) was coupled with a control plate (binary choice with ethanol at the third point as a control). At the bottom left corner, we present a scheme describing the olfactory neurons involved. Colors correlate between an odor and the specific neuron that senses it.

Figure 2



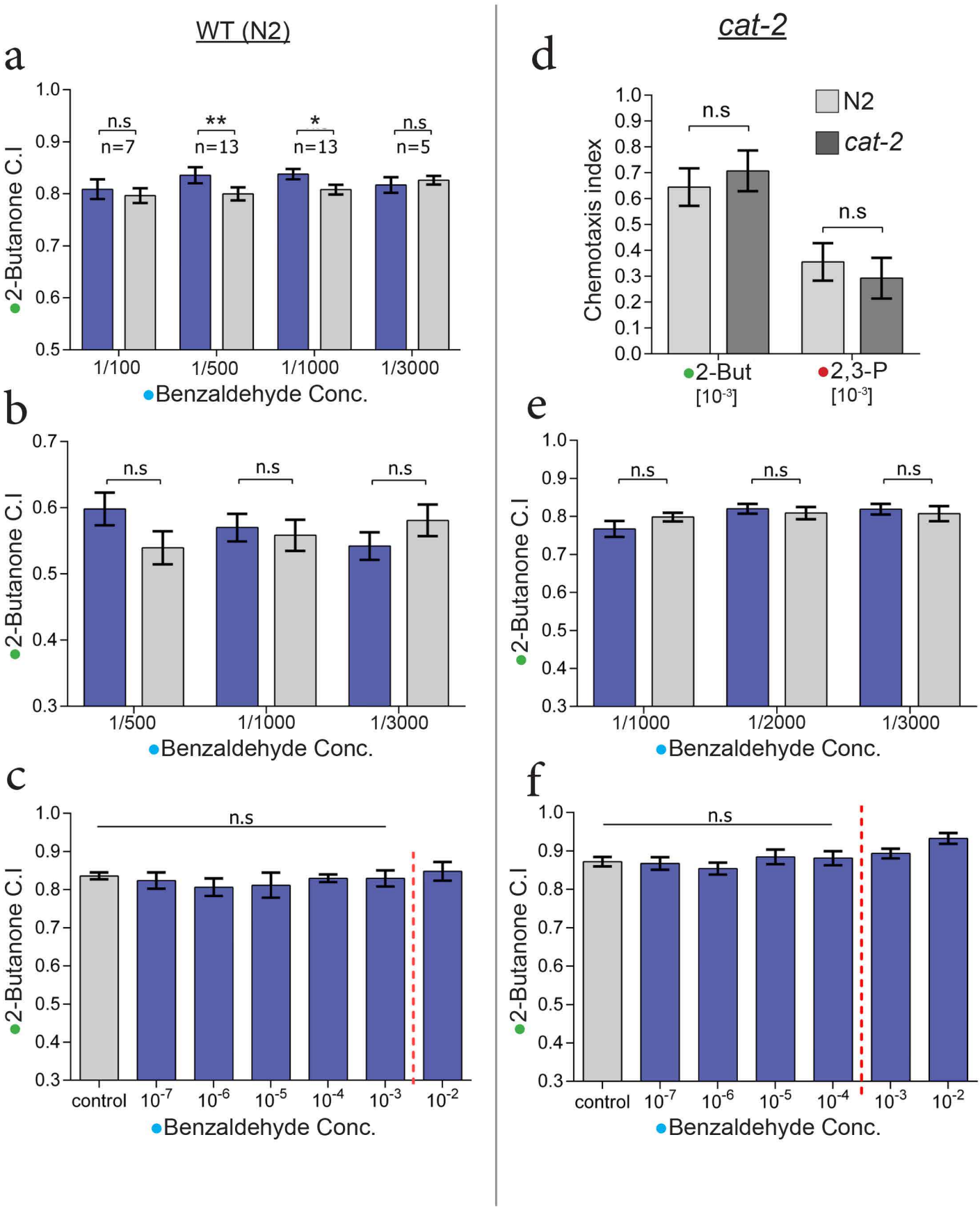


Figure 2. The effect of benzaldehyde (AWC^{BOTH}) as a third attractant on the relative preference between 2-butanone (AWC^{ON}) and 2,3-pentanedione (AWC^{OFF}), in wild type and *cat-2* mutants.

(a,b) Benzaldehyde as a third attractant does not influence the relative preference between **(a)** 2-butanone (10^{-3}) and 2,3-pentanedion (10^{-4}) **(**C=10^{-2}: W=332, p=0.367 ; C=1/500: W=1395, p=0.003 ; C=10^{-3}: W=1280, p=0.017 ; C=1/3000: W=82, p=0.495, n=4, namely 4 biological replications. The very weak differences that were observed here were considered physiologically irrelevant, as detailed in the main text) and **(b)** 2-butanone (10^{-3}) and 2,3-pentanedion (10^{-3}) **(**C=1/500: W=175, p=0.167 ; C=1/1000: W=141, p=0.642 ; C=1/3000: W=96 , p=0.57; n=4). Each replication consisted of 3 experimental plates and 3 control plates. Each plate contained 200-400 worms. **(c)** The relative preference for 2-butanone (10^{-3}) over 2,3-pentanedion (10^{-4}) is unaffected by increasing concentration of benzaldehyde as a third attractant (C=10^{-7}: W=56, p=0.5 ;C=10^{-6}: W=49, p=0.293 ; C=10^{-5}: W=69, p=1 ; C=10^{-4}: W=61, p=0.686; C=10^{-3}: W=59, p=0.608 ; n=6). Error bars represent the standard error of the mean C.I. **(d)** There are no significant differences between wild-type (N2) and *cat-2* mutants in their relative preferences between 2-butanone (10^{-3}) and 2,3-pentanedion (10^{-4}) (2-butanone: W=38 ,p=0.28 ; 2,3-pentanedione: W=18 , p=0.28 ; n=8, namely 8 biological replications). Error bars represent standard deviation. **(e)** The effect of benzaldehyde as a third attractant on the relative preference between 2-butanone (10^{-3}) and 2,3-pentanedion (10^{-4}) in *cat-2* mutants (C=1/500: W=175, p=0.167 ; C=10^{-3}: W=141, p=0.642 ; C=10^{-3}/3: W=96 , p=0.57 ; n=4). **(f)** *cat-2* mutants' relative preference between 2-butanone (10^{-3}) and 2,3-

pentanedion (10^{-4}) is unaffected by the introduction of benzaldehyde as a third attractant. Bars represent the mean C.I of 2-butanone. Error bars represent standard error of the mean C.I ($C=10^{-7}$: W=172, p=0.718 ; $C=10^{-6}$: W=148, p=0.314 ; $C=10^{-5}$: W=206, p=0.602 ; $C=10^{-4}$: W=193, p=0.862; n=4). The dashed red line indicates the point where the benzaldehyde concentration was too attractive for our purposes, i.e. it rendered 2,3-pentanedione irrelevant to the choice task Colors correlate between an odor and the specific neuron recognizing it.

Figure 3

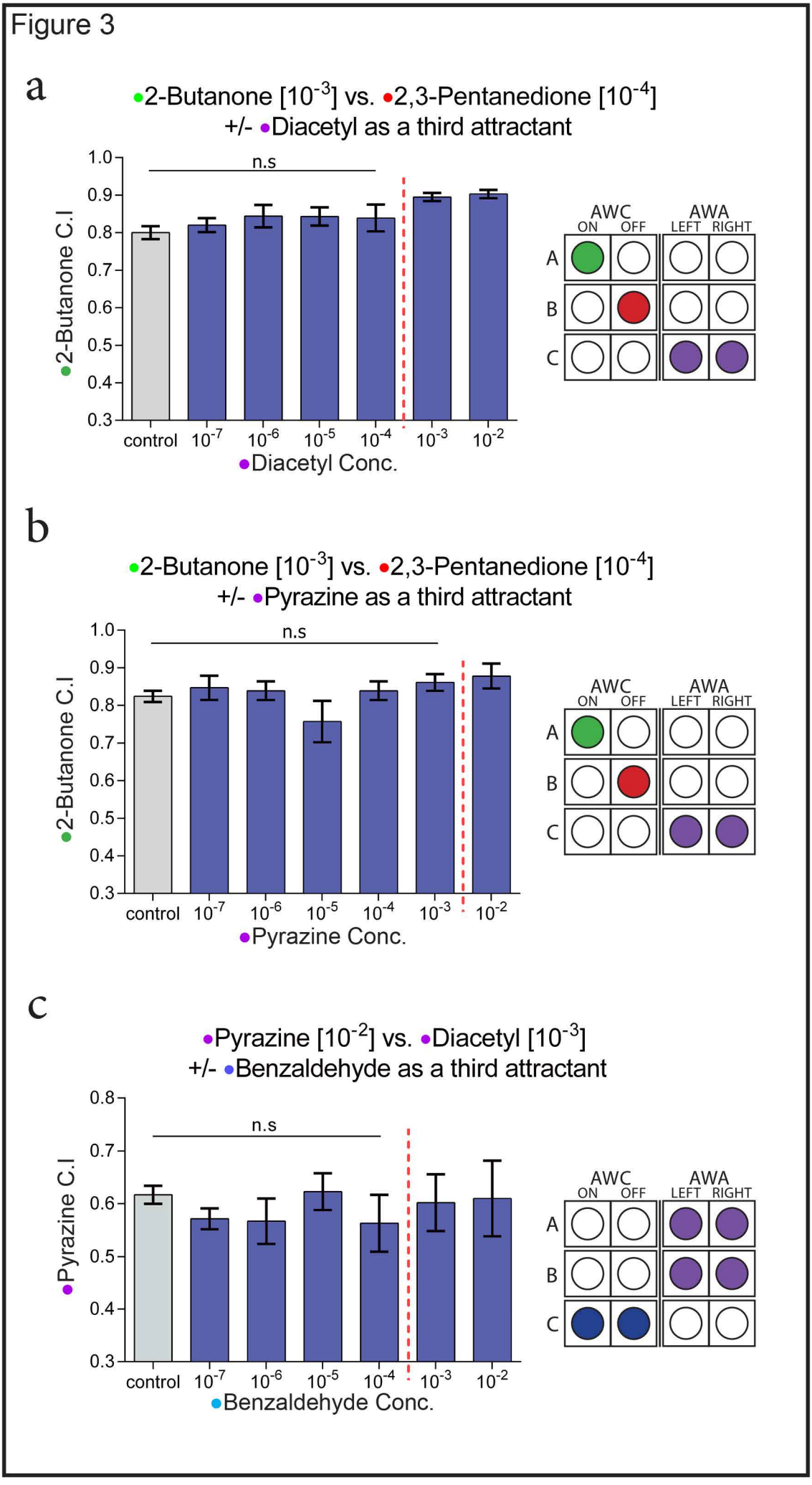


Figure 3. Introducing AWA-sensed odorants to the choice set does not lead to choice inconsistencies in WT animals

(a, b) introducing AWA sensed odorants, diacetyl **(a)** and pyrazine **(b)** as a third attractant, does not influence the relative preference between 2-butanone (AWC^{ON}) (10^{-3}) and 2,3-pentanedione (AWC^{OFF}) (10^{-4}) (**a**: 10^{-7}: W=33, p=0.645 ; $C=10^{-6}$: W=40, p=0.232 ; $C=10^{-5}$: W=39, p=0.277; $C=10^{-4}$: W=38, p=0.327 n=4, Each plate contained 200-400 worms; **b**: $C=10^{-7}$: W=127, p=0.368 ; $C=10^{-6}$: W=113, p=0.735 , $C=10^{-5}$: W=77, p=0.288; $C=10^{-4}$: W=119, p=0.563 ; $C=10^{-3}$: W=134, p=0.236925 n=8, Each plate contained 200-400 worms). Bars represent the mean C.I of 2-butanone) **(c)** The effect of AWC sensed odorant benzaldehyde as a third attractant, does not affect the relative preference between the two AWA sensed odorants pyrazine (10^{-2}) and diacetyl (10^{-3}). Bars represent the C.I of pyrazine. ($C=10^{-7}$: W=32, p=0.121 ;$C=10^{-6}$: W=42, p=0.366 , $C=10^{-5}$: W=65, p=0.641; $C=10^{-4}$: W=36, p=0.197 ; $C=10^{-3}$: W=57, p=1 ; $C=10^{2}$: W=55 p=0.925n=6, Each plate contained 200-400 worms). Error bars represent standard error of the C.I. Colors correlate between an odor and the specific neuron recognizing it. Dashed red lines indicate the point where the third odor point is too attractive for our purposes, i.e. rendering 2,3-pentanedione or diacetyl irrelevant to the choice task.

Figure 4

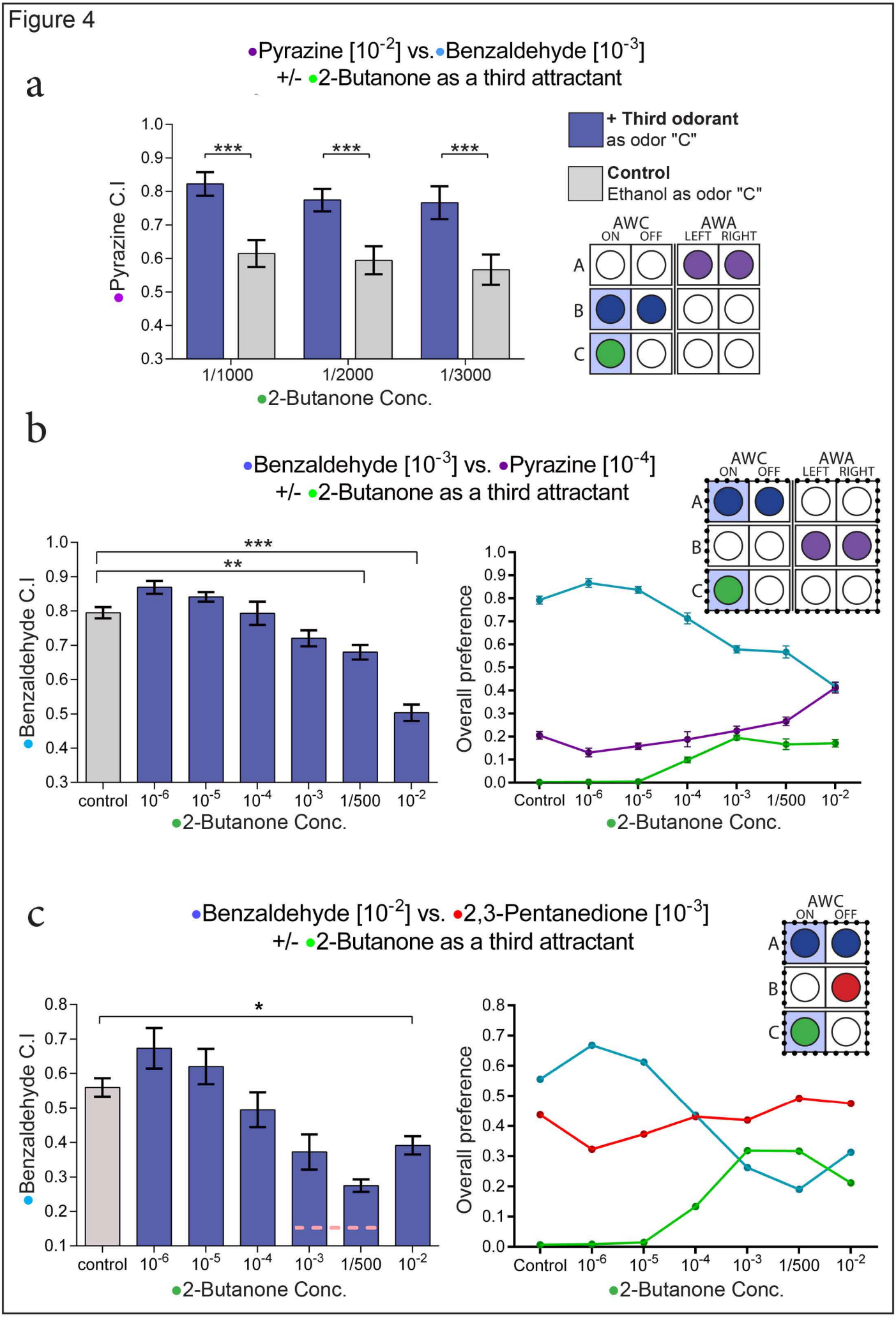


Figure 4. The effect of 2-butanone on the relative preference for benzaldehyde in WT animals

(a) Presenting 2-butanone as a third attractant significantly changes the relative preference between pyrazine (10^{-2}) (AWA) and benzaldehyde (10^{-3}) (AWC) ($C=10^{-3}$: W=193, p<0.000 ; C=1:2000: W=184, p<0.000 ; C=1:3000: W=169 , p<0.000; n=4, Each plate contained 200-400 worms). Bars represent the C.I of Pyrazine **(b)** The effect of 2-butanone as a third attractant on the relative preference between benzaldehyde (10^{-3}) and pyrazine (10^{-4}), and the overall preference of each attractant point in every condition ($C=10^{-6}$: W=66, p<0.019; $C=10^{-5}$: W=54, p=0.2 ; $C=10^{-4}$: W=43, p=0.758; $C=10^{-3}$: W=17, p=0.059 ; C=1/500: W=5, p<0.003 ; $C=10^{-2}$: W=0, p<0.000 ; n=6, Each plate contained 200-400 worms). **(c)** The effect of 2-butanone as a third attractant on the relative preference between benzaldehyde (10^{-2}) and 2,3-pentanedion (10^{-3}), and the overall preference of each attractant point in every condition. ($C=10^{-6}$: W=15, p=0.166; $C=10^{-5}$: W=16, p=0.38 ; $C=10^{-4}$: W=6, p=0.547; $C=10^{-3}$: W=0, p<0.023 ; C=1/500: W=0, p<0.023 ; $C=10^{-2}$: W=0, p<0.023 ; n=3, Each plate contained 200-400 worms). Bars represent the C.I of benzaldehyde. Error bars represent standard error of the mean C.I. Colors correlate between an odor and the specific neuron recognizing it. Dashed red line indicate the point where the third odor point (2-butanone) is too attractive for our purposes, i.e. rendering 2,3-pentanedione irrelevant to the choice task.

Figure 5

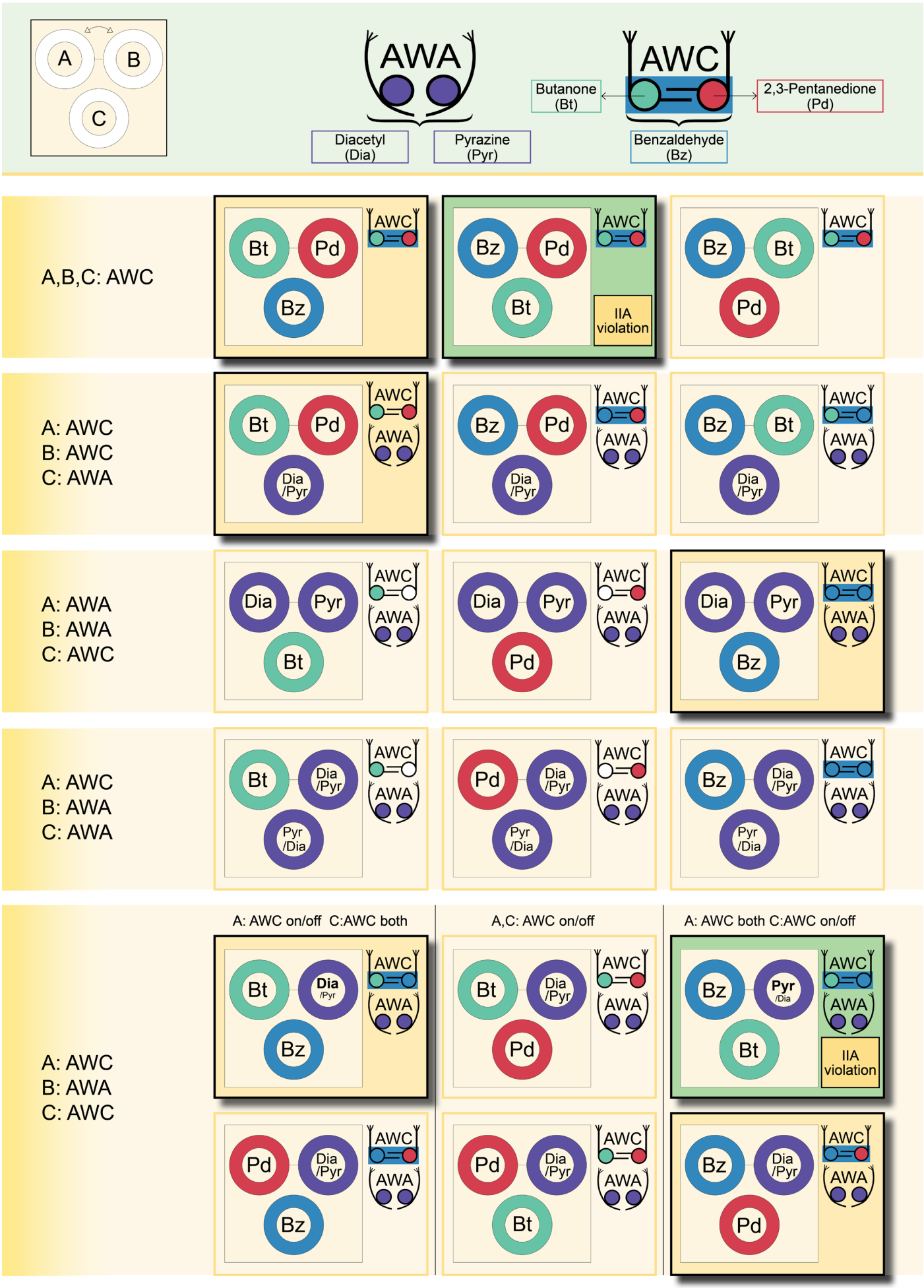


Figure 5. Different possible odor combinations to test the constrains of specific neuronal sensation architectures.

A schematic diagram which depicts different possible setups for activation of the worm's sensory neurons using attractive odorants. The two pairs of olfactory sensory neurons, AWC and AWA, which are required for chemotaxis toward volatile odors, are depicted. AWC detects at least five attractive odors, three of them are benzaldehyde (blue), 2-butanone (green) and 2,3-pentanedione (red). AWA detects at least three volatile compounds, two of which are diacetyl and pyrazine (purple). In this scheme, the possible combinations of odors that can be used to involve different sensory neurons were divided into groups, each group shares a common architectural principle (described on the left of each row). For example, the first group includes all the possible experiments in which all of the attractants are sensed by the AWC pair of neurons (among the 5 odorants that were used at this point of the paper). Each color represents a specific odor, and the locations of the odors on the plate, indicate which odors serve as A, B, or C (as marked above).. Bold frames depict options that have been tested, up to this point of the paper. Experiments conducted until now, which yielded IIA violations, are shown in green. Additional experiments that test new odor combinations, as inspired by these schematics, are described later in the manuscript.

Figure 6

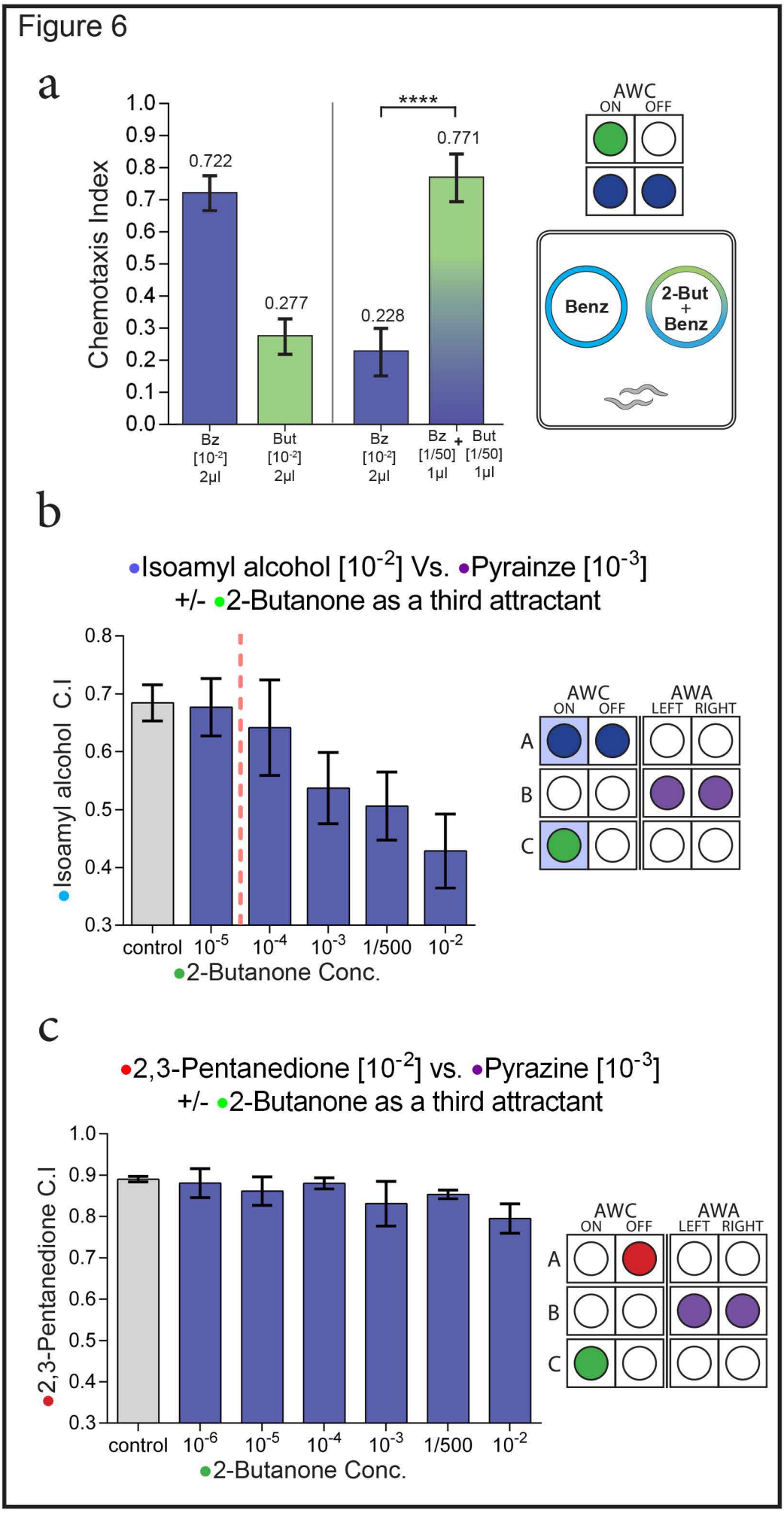


Figure 6. The violation observed are not chemical-specific (not limited to interactions between 2-butanone and benzaldehyde).

In order to examine whether the IIA violations that we found stem from specific interactions between 2-butanone and benzaldehyde, two experiments were conducted **(a)** 2-butanone and benzaldehyde together are as attractive as would be expected based on the simple summation of the attractiveness of each of the odors alone. **(b)** 2-butanone (AWC^{ON}) as a third attractant significantly reduced the relative preference for isoamyl-alcohol (10^{-2}) (AWC) over pyrazine (10^{-2}) (AWA). Bars represent the C.I of isoamyl-alcohol ($C=10^{-5}$: W=12, p=0.787 ; $C=10^{-4}$: W=10, p=0.527; $C=10^{-3}$: W=6, p=0.163 ; C=1/500: W=3, p<0.042 ; $C=10^{-2}$: W=1, p<0.012 ; n=4, Each plate contained 200-400 worms). Dashed red line indicates the point where the third odor point (2-butanone) is too attractive for our purposes, i.e. rendering both pyrazine irrelevant to the choice task. **(c)** The effect of 2-butanone (AWC^{ON}) as a third attractant on the relative preference between 2,3-pentanedione (10^{-2}) (AWC^{OFF}) and pyrazine (10^{-3}) (AWA). Bars represent the C.I of 2,3-pentanedione ($C=10^{-6}$: W=12, p=0.342; $C=10^{-5}$: W=11, p=0.485 ; $C=10^{-4}$: W=12, p=0.342; $C=10^{-3}$: W=11, p=0.870 ; C=1/500: W=8, p=1 ; $C=10^{-2}$: W=1, p=1 ; n=4, Each plate contained 200-400 worms). Error bars represent standard error of the mean C.I. Colors correlate between an odor and the specific neuron recognizing it.

Figure 7

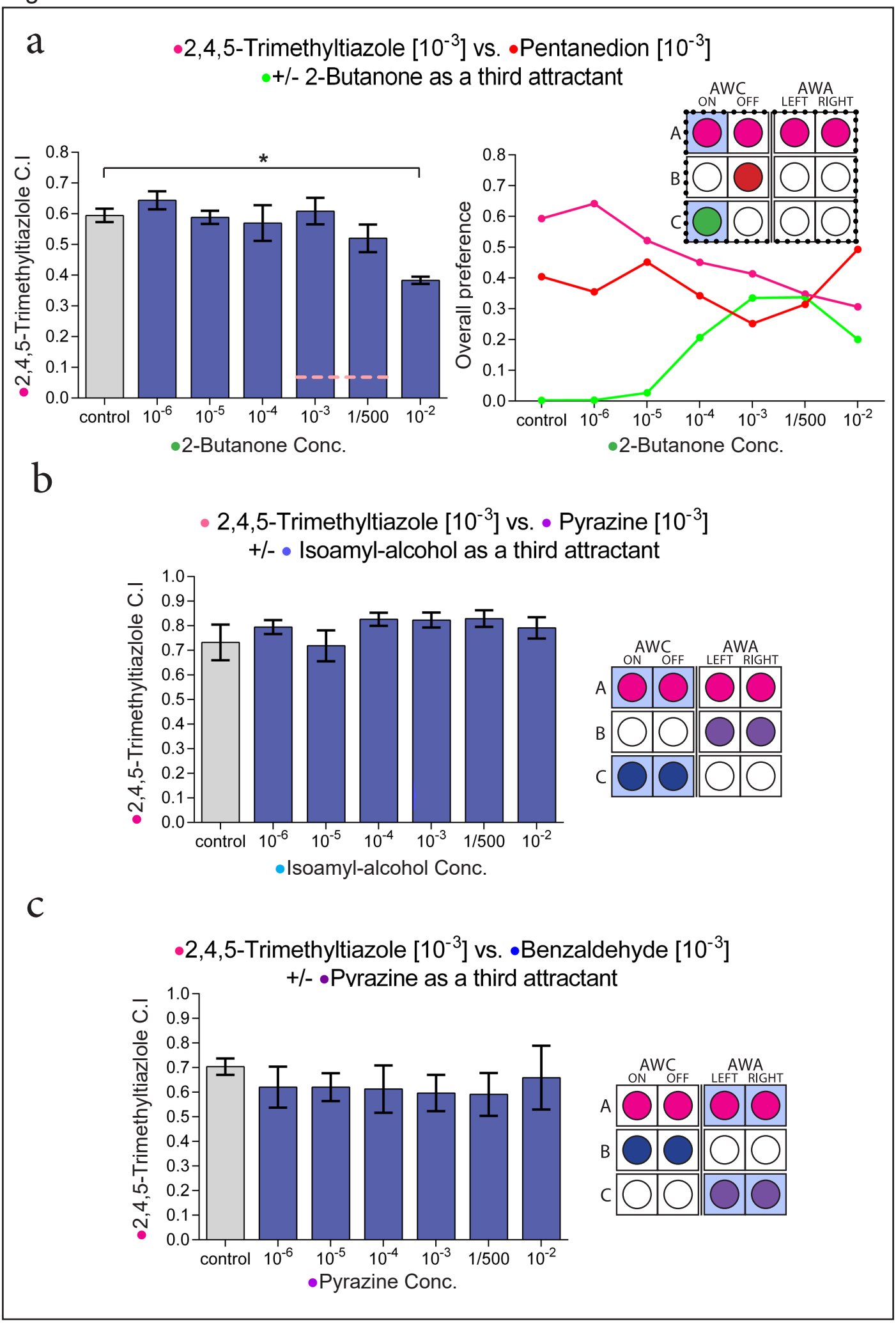
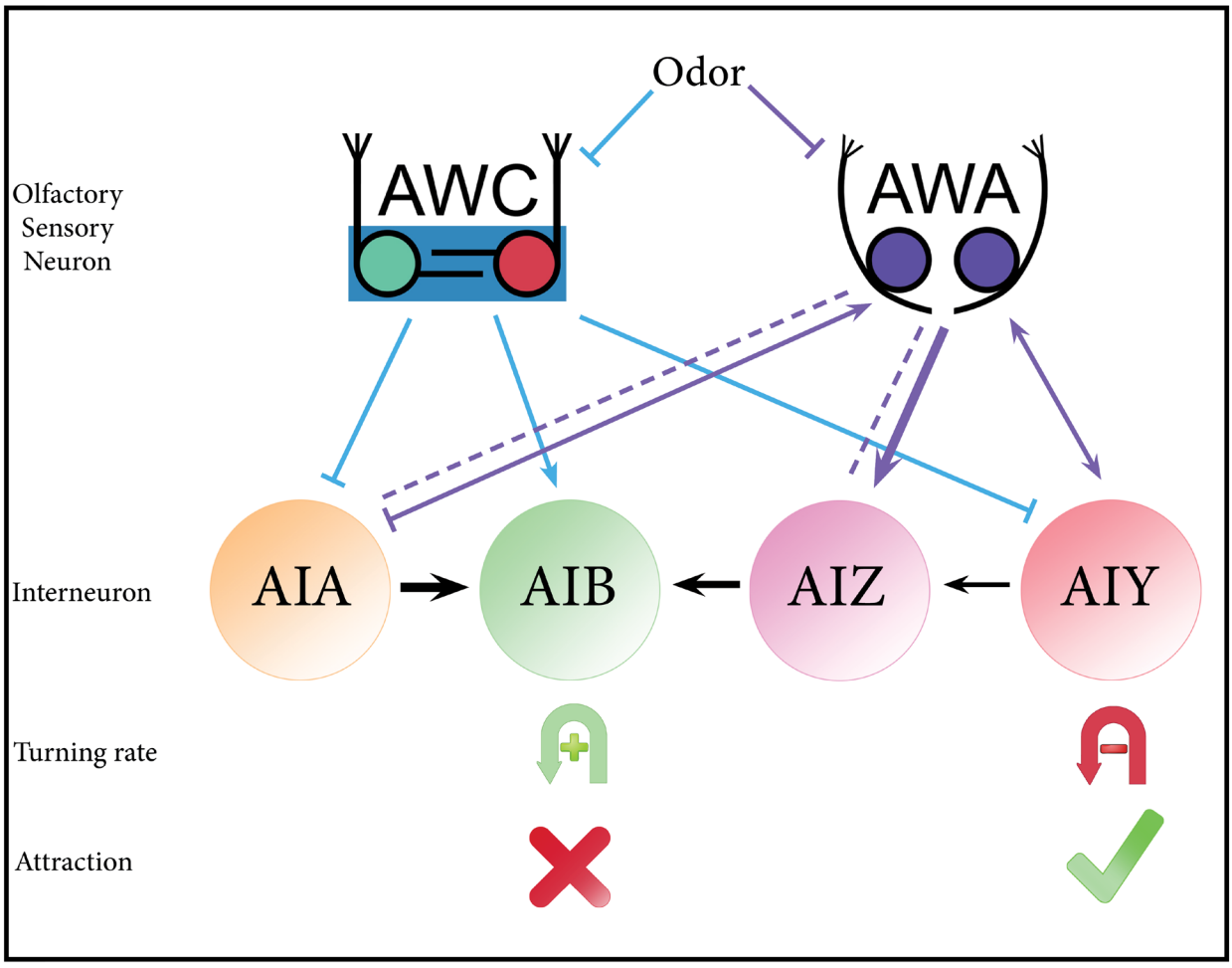
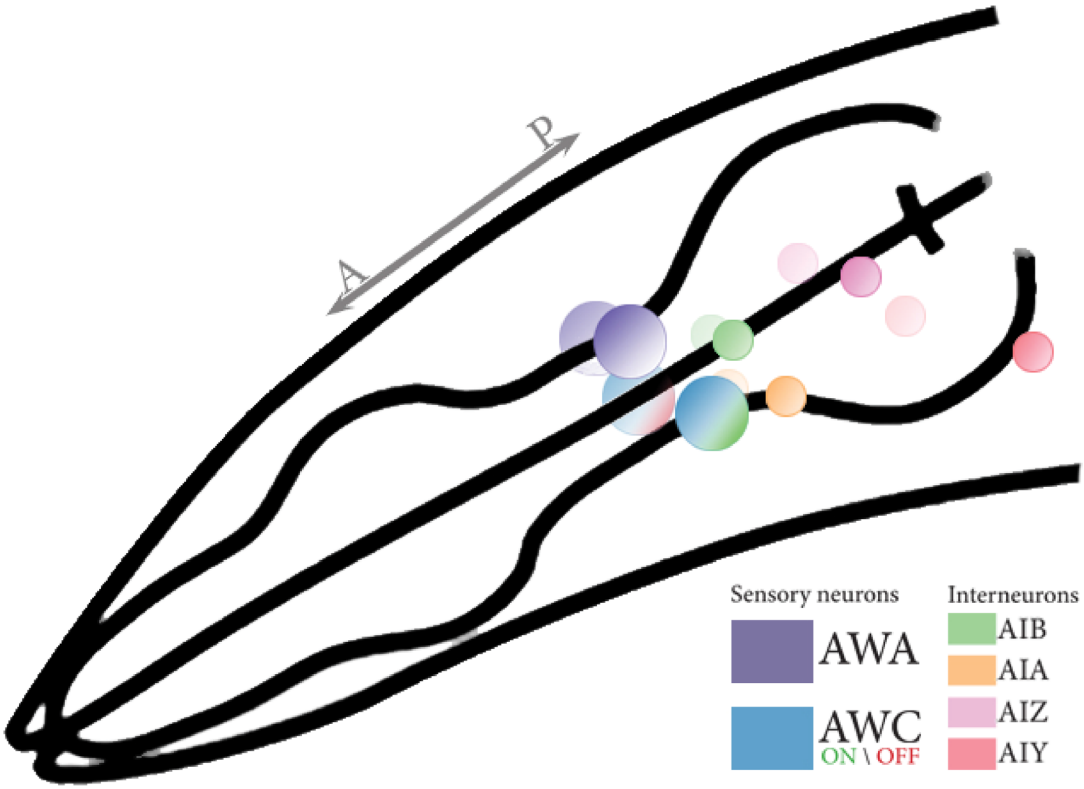


Figure 7. Examining setups in which more neurons are involved. Increasing the sensation "bandwidth" of odor "A" does not prevent IIA violations **(a)** The influence of 2-butanone (AWC^{ON}) as a third attractant on the relative preference between 2,4,5-trimethyltiazole (10^{-3}) (AWC&AWA) and 2,3-pentanedione (10^{-3}) (AWC^{OFF}), and the Overall preference of each attractant point in every condition ($C=10^{-6}$: W=20, p=0.428; $C=10^{-5}$: W=9, p=0.329 ; $C=10^{-4}$: W=10, p=0.428; $C=10^{-3}$: W=21, p=0.329 ; C=1/500: W=7, p=0.177 ; $C=10^{-2}$: W=0, p<0.004 ; n=6, Each plate contained 200-400 worms). IIA violations are not observed when a larger neural circuit is involved, despite an *asymmetric overlap* setup **(b)** Isoamyl-alcohol (AWC) as a third attractant does not change the relative preference between 2,4,5-trimethyltiazole (10^{-3}) (AWC&AWA) and pyrazine (10^{-3}) (AWA) ($C=10^{-6}$: W=19, p=0.536; $C=10^{-5}$: W=13, p=0.792 ; $C=10^{-4}$: W=20, p=0.428; $C=10^{-3}$: W=16, p=0.93 ; C=1/500: W=20, p=0.428 ; $C=10^{-2}$: W=21, p=329 ; n=6, Each plate contained 200-400 worms). **(c)** Pyrazine (AWA) as a third attractant does not change the relative preference between 2,4,5-trimethyltiazole (10^{-3}) (AWC&AWA) and benzaldehyde (10^{-3}) (AWC) ($C=10^{-6}$: W=9, p=0.547 $C=10^{-5}$: W=9, p=0.547 ; $C=10^{-4}$: W=8, p=0.42; $C=10^{-3}$: W=7, p=0.309 ; C=1/500: W=9, p=0.547 ; $C=10^{-2}$: W=13, p=1 ; n=5). Bars represent the C.I of 2,4,5-trimethyltiazole. Error bars represent standard error of the mean C.I. Colors correlate between an odor and the specific neuron recognizing it. Dashed red line indicate the point where the third odor point (2-butanone) is too attractive for our purposes, i.e. rendering 2,3-pentanedione, pyrazine or benzaldehyde irrelevant to the choice task.

Supplementary Figure 1

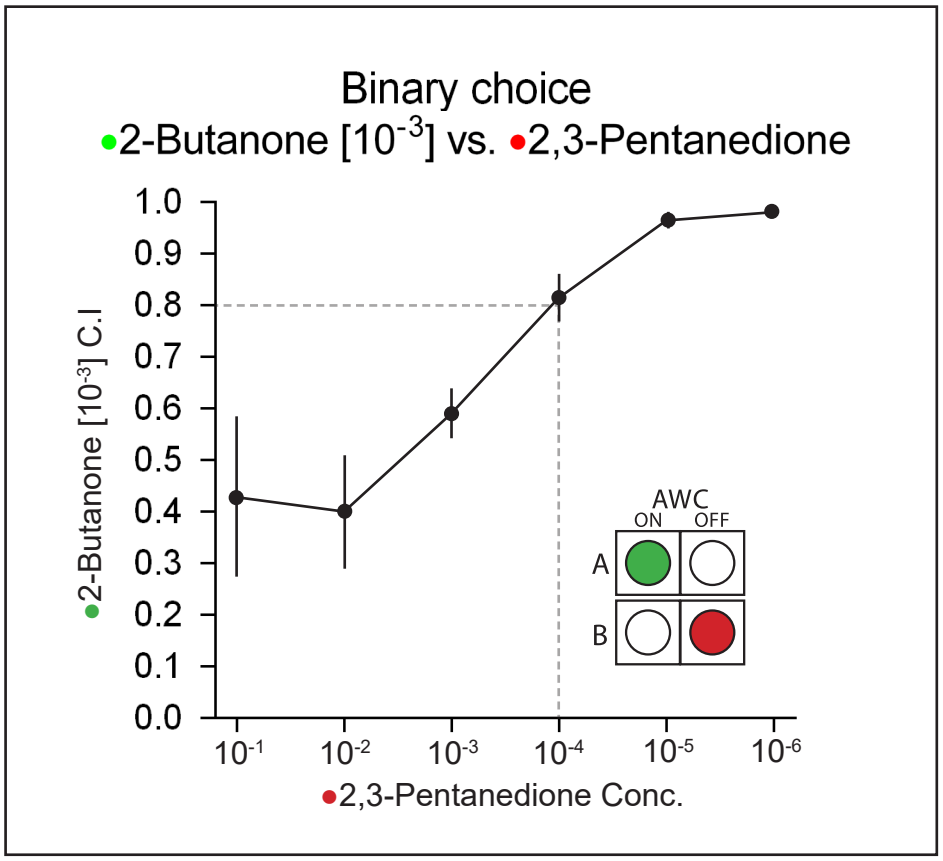




Supplementary Figure 1. Neural circuit architecture of volatile odor sensation in *C. elegans*.

(a) A partial diagram of the neural level architecture and connections inducing olfaction chemotaxis (Inspired by drawings from Wormbook). Each of the two neurons of AWC and AWA are represented as small colored circles. The colors of the circles depict sensation by specific odors: 2-butanone (green) is sensed only by AWC^{ON}, 2,3-pentanedione (red) is sensed only by AWC^{OFF}, while benzaldehyde (blue area) is sensed by both AWC neurons. In the AWA neurons, diacetyl and pyrazine activate both neurons (purple). Full lines represent connections via chemical synapses, while dashed lines represent connections via gap junctions (electrical synapses).Arrowheads represent excitatory synapses, while bar ends represent inhibitory synapses. Lines' weight indicates the connection's strength. The *first layer* of *interneuron* pairs downstream to AWA and AWC (AIA, AIB, AIZ, AIY) are shown in large circles. *AIB* enhances (green arrow) and *AIY* suppresses (red arrow) turning rate*, thus enabling the worm to move up* the *odor gradient. **(b)** An illustration of the location of AWC, AWA and the four interneurons in the head of the worm. A – anterior, P-posterior*.

Supplementary Figure 2



Supplementary Figure 2. WT Relative preference between butanone and 2,3-pentanedione

The relative preferences between 2-butanone (10^{-3}), and ascending concentrations of 2,3-pentanedione. n=3, each plate contained 200-400 worms, error bars represent SD.