FOOD CHOICE IN *CAENORHABDITIS ELEGANS*: DIFFERENCES IN A *CEH-36* MUTANT AND NATURAL HAWAIIAN ISOLATE

by

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THESIS ABSTRACT

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By understanding the biological mechanisms of food choice—a behavior that strongly impacts the evolutionary fitness of animals, from worms to humans—we can begin to understand the biological underpinnings of decision making in general and use such knowledge to better understand how this faculty fails in addiction, mental illness, and other disorders. Here, we analyze food choice in *Caenorhabditis elegans*, a convenient, genetically tractable organism. We describe how prior experience with high and low quality foods alters future food choice in three strains (N2, *ceh-36*, HW). We also provide evidence that chemosensory neurons AWC are required for altering food choice after experience. Additionally, we gather support for the use of HW and N2 in quantitative trait loci mapping, a method that would allow us to identify genetic loci that contribute to the heritable variation in food choice.

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To my mother, sister, and godmother, for providing me with unconditional love and support. And to Jake, for reminding me that I'm all out of gum.

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CHAPTER I

INTRODUCTION

Every day, we need to make decisions. Our survival and reproductive success depends on the ability to effectively weigh options and make the choices that benefit us most. By learning about the biological underpinnings of choice behavior, we can better understand how this faculty fails in addiction, mental illness, and other disorders—and devise appropriate treatments for restoring this faculty. But how do we begin to tease apart this complex behavior? We may benefit from analyzing decision making in the context of food choice. The ability to make effective food choices strongly impacts evolutionary fitness, and so these decisions have likely played a key role in shaping relevant neural circuits and other biological mechanisms (Pearson, Watson, & Platt, 2014). These mechanisms are likely to be shared across a wide variety of animals, from worms to humans. Here, we analyze food choice in *Caenorhabditis elegans*, a convenient, genetically tractable organism with only 302 neurons (Schafer, 2005; White, Southgate, Thomson, & Brenner, 1986).

Experience can shape future choices. Animals often face several food options in their natural environment and need to choose foods that effectively support survival and reproduction (Pearson et al., 2014). Prior experience with a food can provide valuable information about how to treat that food option in the future. Indeed, good decision making requires animals to assess factors such as pathogenicity and nutritive quality and incorporate such information into future decisions, so that they can make more efficient choices (i.e. those that maximize benefit while minimizing cost).

In the economic literature, consumers are said to assign value, or *utility*, to each item in a choice set (Tversky & Kahneman, 1986). In the context of food choice, one can imagine that the information gained during exposure to a food option can influence the utility assigned to that food option. For example, when encountering a novel food, many animals exhibit neophobia—or aversion to that food (Rozin, 1976). We may say that, initially, an animal assigns low utility to the food because the option poses more risk (e.g. pathogenicity) than benefit (e.g. high nutrient density). However, after repeated sampling, the animal may realize that the food causes no illness, is easy to eat, and promotes satiety. Thus, the animal increases the utility assigned to that option, so when it encounters that food in the future, it acts differently than it would have if it had never experienced that food before. Perhaps now the animal actively seeks out the food, remains in the same area as that food for a longer period of time, or consumes it more robustly when it is available. Indeed, when given the choice between a novel food and familiar food, animals tend to seek out and more robustly consume the familiar food (Katzen, unpublished raw data; Shtonda & Avery, 2006; Song, Faumont, Lockery, & Avery, 2013). The phenomenon of animals responding more so to familiar rather than unfamiliar stimuli has been referred to as the "mere exposure effect" or the "familiarity breeds liking effect" (Peskin & Newell, 2004; Pliner, 1982; Zajonc, 1968). However, in some cases, animals respond less so to familiar versus unfamiliar stimuli, a phenomenon referred to as the "familiarity breeds contempt effect" (Kelly, Graves, & Magurran, 1999; Norton, Frost, & Ariely, 2007). Animals may respond less so to familiar versus unfamiliar foods in cases of aversive over-satiation, or a need to seek novel foods to achieve nutritional balance

(Stang, 1975*a*; Stang 1975*b*; Wang & Provenza, 1996). In any case, the above example illustrates that prior experience with a food option can alter future food choice.

The bacteria-eating roundworm *Caenorhabditis elegans* appears to incorporate past experience with food options into future decisions—for example, by avoiding foods that previously caused illness (Zhang, Lu, & Bargmann, 2005). *C. elegans* can also distinguish between high quality and low quality foods—higher quality foods being those that better promote worm growth (Shtonda & Avery, 2006). When given two food options, worms seek out the higher quality food, and this behavior is enhanced in worms that have previously experienced that higher quality food (Shtonda & Avery, 2006). Recent studies have analyzed animal behavior through specific economic lenses (see Rosati and Stevens, 2009 for review). For our study, we chose to analyze worm behavior through the lens of rational choice theory (RCT). RCT asserts that choice designates preference and that consumers act in a way to maximize utility (Rosati & Stevens, 2009; Tversky & Kahneman, 1986). *C. elegans* appears to abide by RCT by choosing to feed on higher quality food that better supports growth rate, thus maximizing utility. Since worms choose those higher quality foods, we say that they *prefer* those foods.

We should note that preference is not always exhibited in absolutes (i.e. choosing option A over option B), but rather degrees (i.e. choosing option A more often than option B). Two animals may both prefer option A over option B, but one may choose option A 60% of the time, while the other chooses option A 90% of the time. In this case, the latter animal exhibits *greater preference* for option A than the former animal.

Now recall that *C. elegans* eats bacteria; Shtonda and Avery (2006) previously rated the quality of particular species of soil bacteria in terms of how well each species

supported worm growth. The authors categorized *comamonas sp.* as a "good food" (G), and *Bacillus simplex* as a "mediocre food" (M). They observed that, when given these two food options, the majority of worms in a population accumulated on G versus M within 3 h. If we define preference at the population level, we may say that worms quickly exhibited *preference* for G versus M. Additionally, the authors found that the proportion of worms on G increased over time, so we may say that worms exhibited *greater preference* for G versus M at later time points (e.g. 9-27 h).

Shtonda and Avery (2006) performed these experiments with hatchlings that had never before experienced food, so they seemed to observe worms' naïve preferences for G and M. Differences in naïve preferences are interesting in that they influence decision making, and given the heritability of decision making patterns (Simonson & Sela, 2011), we can perhaps identify genes associated with different naïve preferences. However, naïve preferences are also flexible and change with experience. For example, the authors observed enhanced seeking behavior towards high quality foods in worms that were previously conditioned on high quality food. Our lab seeks to elucidate the biological underpinnings of naïve preference and changes in preference, specifically in the context of food choice.

To make an efficient choice, worms must be able to sense each food, compare the utility of the two, and choose the option that offers more utility. Previous work in our lab has identified a potential neural locus for this comparison, namely chemosensory neurons AWC, which have previously been implicated in chemotaxis to volatile food odors and transitions between dwelling (moving slowly or remaining in a food patch) and roaming (moving rapidly across a food patch) states (Arous, Laffont, & Chatenay, 2009;

Bargmann, Hartwieg, & Horvitz, 1993). AWC neurons are described as "odor-OFF" neurons because they are activated by odor removal and inhibited in the continued presence of odors (Chalasani et al., 2007). We measured calcium transients in AWC in experienced adult worms that were previously exposed to G and M, and naïve adult worms that never experienced either food. Experience altered the response of AWC to the switch from G to M. Specifically, experienced worms exhibited a greater AWC response than naïve worms (Figure 1). Perhaps this change in AWC response underlies experienced worms' enhanced preference for G.

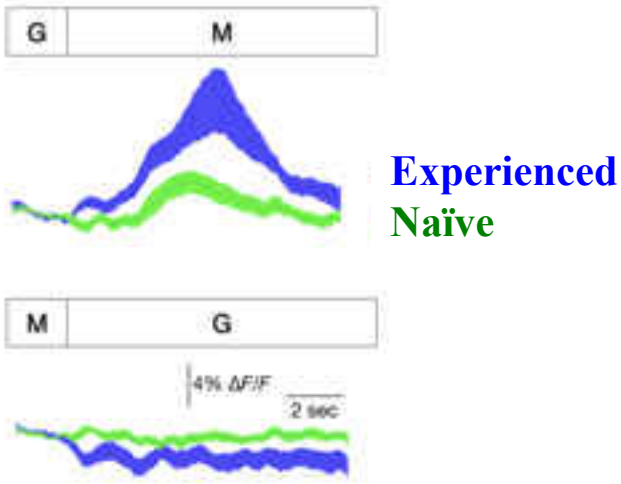

Figure 1. Calcium response of AWC neuron. Upper bars indicate the switch from good food (G) to mediocre food (M). Graph shows the relative change in G-CaMP fluorescence (ΔF/F) for Experienced (blue) and Naïve (green) worms over time. Center line represents mean value and shaded region denotes SEM; $N = 7$ animals per condition (Katzen, unpublished).

Considering these findings, we sought to determine if worms defective in AWC function exhibited altered future food choice after experience with G and M. The gene *ceh-36* encodes a homeobox transcription factor that controls gene expression of AWC neurons (Koga & Ohshima, 2004), and worms with the null allele *ky646* exhibit severely impaired chemotaxis to all volatile odors sensed by AWC, despite normal position and

morphology of these neurons (Lanjuin, VanHoven, Bargmann, Thompson, & Sengupta, 2003). In the current study, we found that experienced *ceh-36 (ky646)* worms failed to alter preference in our food choice assay, providing further support for the role of AWC in altering preference between previously experienced food options.

Our lab is also interested in the genetic underpinnings of preference variation and food choice. Natural variations in preference likely result from interactions among multiple genes and the environment. Quantitative trait locus (QTL) mapping is a method used to identify gene regions that contribute to the heritable variation of a complex quantitative trait, such as thermal preference (Gaertner, Parmenter, Rockman, Kruglyak, & Phillips, 2012; Gaertner & Philips, 2010). The method utilizes recombinant inbred lines (RILs), and several studies have found success in using reference strain N2 and genetically distinct Hawaiian isolate, CB4856 (HW), for the creation of such lines (Gaertner et al., 2012; Gutteling et al., 2007; Gutteling, Riksen, Bakker, & Kammenga, 2007). However, to be useful in QTL mapping, the strains must exhibit sufficiently distinct variation in the phenotype of interest—in this case, preference between G and M. Thus, the current study sought to determine if HW met this criterion. We found that HW exhibited substantially different preference from N2 in the choice between G and M, justifying the pursuit of QTL mapping to identify genetic loci that contribute to the heritable variation in food preference.

CHAPTER II

MATERIALS AND METHODS

Animals

We used reference strain *C. elegans* Bristol (N2), *ceh-36 (ky646)*, and a natural Hawaiian isolate (CB4856). Strains were provided by the CGC, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440). Nematodes were grown in mixed-stage cultures at room temperature (22-25^{o}C) on standard nematode growth medium (NGM) plates seeded with *Escherichia coli* strain OP50 as described (Stiernagle, 2006). Two to three days before experiment day, approximately ten L4 stage worms were picked to another seeded NGM plate. Adults were killed the following day, after laying a sufficient number of eggs. Once the synchronous population reached young adulthood, worms were used for experiments. All experiments were performed at room temperature (22-25^{o}C).

Bacteria and Plate Preparation

We used *Escherichia coli* strain OP50, *Comamonas sp*. strain DA1877, and *Bacillus simplex* strain DA1885. Quality of bacterial strains has previously been described in terms of nematode growth rate; *Comamonas sp.* was rated as a good quality food (G), while *B. simplex* and OP50 were rated as mediocre quality foods (M) (Shtonda & Avery, 2006). Bacterial suspensions were brought to an optical density of 1.0 immediately prior to seeding on 60 mm x 15 mm nematode growth medium (NGM) plates with 200 μg/mL of streptomycin and no bactopeptone to slow bacterial growth during the experiment. Plates used for the 3 h pre-exposure period consisted of 16 food patches in a 4 x 4 spot pattern; we deposited 10 μL of bacterial suspension to create each

food patch. In the experienced condition, plates consisted of alternating patches of *Comamonas sp*. and *B. simplex*. In the naïve condition, all patches consisted of standard worm food OP50. Plates used for the food choice assays (testing conditions) consisted of a single patch of *Comamonas sp.* (G only), a single patch of *B. simplex* (M only), or one patch of each strain (GM); we deposited 60 μL of bacterial suspension to create each food patch. On GM plates, bacterial patches were separated by 1 cm. On the underside of G only and M only plates, a circle was drawn 1 cm away from the food patch, equivalent in diameter to the food patch. Worms in this area, or blank spot, were counted so that the same preference index could be used across food choice assays. Plates were prepared in advance and used within one week.

Accumulation Index

We defined preference at the population level, with greater worm accumulation in a given patch indicating greater preference for that patch versus the other. The accumulation index was defined as follows:

Index value = $(A-B)/(A+B)$

For the G only and M only conditions, A was the number of worms in the food patch, while B was the number of worms in the blank spot. The index could range from -1 to +1, with -1 indicating that all worms were in the blank spot, +1 indicating that all worms were in the food patch, and 0 indicating an equal distribution between the two areas. For the GM condition, A was the number of worms in the good food patch, while B was the number of worms in the mediocre food patch. Again, the index could range from -1 to +1, with -1 indicating that all worms were in mediocre food, +1 indicating that all worms were in good food, or 0 indicating that worms were equally distributed between the two

food patches. We should note that in the GM condition, differences in accumulation index value could not be definitively attributed to attraction or aversion towards a particular food. For example, if a worm population exhibited an index value of 0.80 at time one (T_1) and an index value of 0.60 at T_2, we cannot definitively attribute this change to enhanced attraction towards M, enhanced aversion towards G, or both mechanisms.

Pre-Exposure Period

Once worms reached young adulthood, they were washed three times in buffer solution [96.5% distilled water, 0.1% 1M MgSO4, 1.0% 1M HEPES, ~ 2.4% 100% glycerol; goal osmolarity 350-360 mmol/kg] and allowed to settle to the bottom of a 1.5 mL polypropylene microcentrifuge tube. After settling, 2 µL of worms in buffer solution—at least 150 worms—were deposited onto a pre-exposure plate, either for the experienced condition (alternating G and M patches) or the naïve condition (all OP50 patches). Worms were left to feed on the plates for 3 h at room temperature. In a single experiment, there were 6 plates of worms running simultaneously, 3 in the experienced condition and 3 in the naïve condition.

Food Choice Assays (Test Conditions)

After pre-exposure, worms were washed three times with buffer solution in a 1.5 mL glass vial. Worms were then transferred via glass Pasteur pipette to a custom-made glass tube that tapered down to a 1.2 mm diameter. This tube allowed worms to settle into a tight clump, minimizing worm loss in the transfer procedure. After settling, 10 uL of worms in buffer solution (approximately 150 worms) were deposited onto a G only, M only, or GM plate. In a single experiment, there were 6 plates of worms running

simultaneously, 3 plates of experienced worms (G only, M only, GM) and 3 plates of naïve worms (G only, M only, GM). Worms were counted every 15 min for 1 hr. For the G only and M only plates, worms were counted in the food patch and blank spot. For the GM plates, worms were counted in the G patch and M patch. At the end of the experiment, preference index values were calculated for each time point: 15, 30, 45, 60 min. Eight experiments ($N = 8$) were conducted for each worm strain. The experimental design is summarized in Figure 2.

Statistics

For each strain, we sought to determine how experience affected food choice. For each test condition (G only, M only, GM), we conducted a two-way ANOVA to test for main effects of experience and time, and to test for an interaction between these two factors ($\alpha = 0.05$). For each strain (N2, *ceh-36*, HW) and each test condition (G only, M only, GM), we conducted four unpaired two-sample *t* tests to test the *a priori* hypotheses that naïve and experienced worms would differ in accumulation index values at each time point (15, 30, 45, 60 min). The significance level at each time point was adjusted using Bonferroni's correction to compensate for the fact that multiple comparisons increase the false positive rate. We used a Bonferroni adjusted alpha level of 0.0125 per test (0.05/4), though significant effects were also noted using a Bonferroni adjusted alpha level of 0.0025 per test (0.01/4). Given the conservative nature of the Bonferroni correction, significant effects in uncorrected *t* tests were also shown ($\alpha = 0.05$).

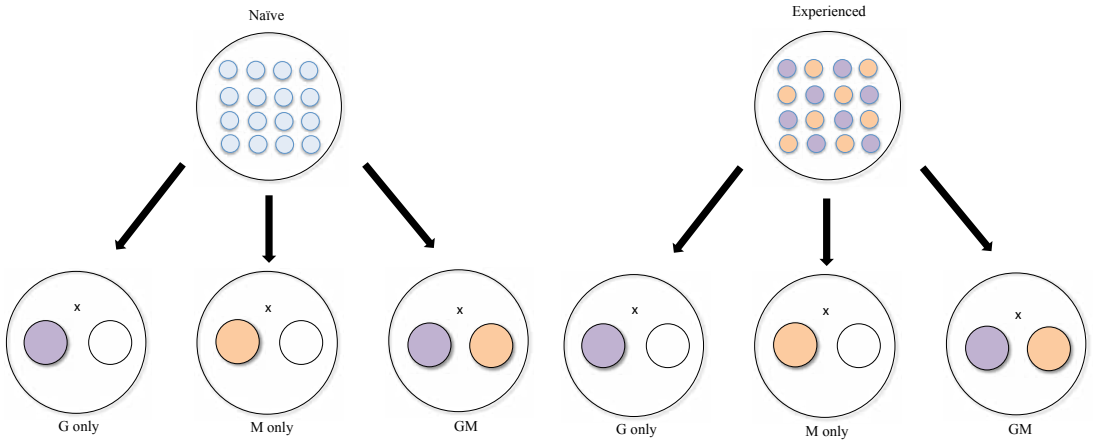

Figure 2. Summary of experimental design. Worms were raised from birth on standard lab plates seeded with OP50. Once worms reached young adulthood, a group of ~ 150 worms was transferred to one of two pre-exposure plates. For the Naïve group, worms were placed on a plate with 16 patches of standard lab food OP50; for the Experienced group, worms were placed on a plate with 16 food patches, alternating between G (purple) and M (peach). In a single experiment, there were 6 plates of worms running simultaneously, 3 Naïve and 3 Experienced. After 3 h of exposure, worms were transferred to one of three testing plates: G only, M only, or GM. The 'x' designates where worms were deposited. Worms in the G only condition were exposed to one patch of G; worms in the M only condition were exposed to one patch of M; and worms in the GM condition were exposed to one patch each of G and M. A reference circle equivalent in size to the food patch was drawn on the bottom of G only and M only plates, so that the same accumulation index could be calculated for each group. In a single experiment, 6 plates of worms were running simultaneously, 2 plates each (one Naïve, one Experience) for the G only, M only, and GM conditions. During testing, worms were counted in food patches and reference circles every 15 min for 1 h. After the 1 h testing period, the accumulation index was calculated for each time point. The accumulation index ranged from -1 to +1. For the G only and M only conditions, a value of +1 indicated that all worms were on food, -1 indicated that all worms were off food in the reference circle, and 0 indicated that worms were evenly distributed between the two areas. For the GM condition, a value of +1 indicated that all worms were on G, -1 indicated that all worms were on M, and 0 indicated that worms were evenly distributed between G and M. Eight experiments were conducted for each strain (N2, *ceh-36*, HW).

We also sought to compare N2 to *ceh-36* and N2 to HW. For each test condition (G only, M only, GM), we conducted a two-way ANOVA to test for main effects of strain and time, and to test for an interaction between these two factors ($\alpha = 0.05$). For each test condition (G only, M only, GM), we conducted eight unpaired two-sample *t*

tests to test the *a priori* hypotheses that *ceh-36* and HW would differ from N2 in accumulation index values at each time point (15, 30, 45, 60 min). We performed this procedure for both naïve (naïve N2 vs. naïve *ceh-36* and naïve HW) and experienced (experienced N2 vs. experienced *ceh-36* and experienced HW) worms. We used a Bonferroni adjusted alpha level of 0.0063 per test (0.05/8), though significant effects were also noted using a Bonferroni adjusted alpha level of 0.0013 per test (0.01/8). Given the conservative nature of the Bonferroni correction, significant effects in uncorrected *t* tests were also shown for alpha levels of 0.05 and 0.01. All *t* tests were conducted in Matlab and all ANOVAs were conducted in Igor.

CHAPTER III

RESULTS

Within Strains

For each strain (N2, *ceh-36*, HW), we sought to determine how experience affected food choice. We hypothesized that experienced and naïve worms would accumulate differently on G and M, whether presented with foods individually (G alone, M alone) or jointly (GM).

N2. We sought to replicate the finding that experienced worms increased accumulation on G when presented with both G and M (Shtonda & Avery, 2006). Indeed, we found that experienced worms accumulated more on G than naïve worms during joint presentation (GM) at the 45 and 60 min time points (Figure 3c). Previous work had not examined experience effects on accumulation during individual presentations of familiar foods, so we were unsure how experience would affect accumulation in the M only and G only conditions. In the M only condition, experienced worms exhibited greater accumulation on M than naïve worms at the 15 and 30 min time points (Figure 3b). Conversely, experience had no effect on accumulation in the G only condition (Figure 3a). However, we may not have been able to detect an effect because naïve worms already approached the upper limit of the accumulation index. In summary, experience affected accumulation in the M only and GM conditions, but not in the G only condition. We conclude that experience alters food choice between G and M, and that experienced worms exhibit detectable food familiarity learning in solo presentations of M, but not G.

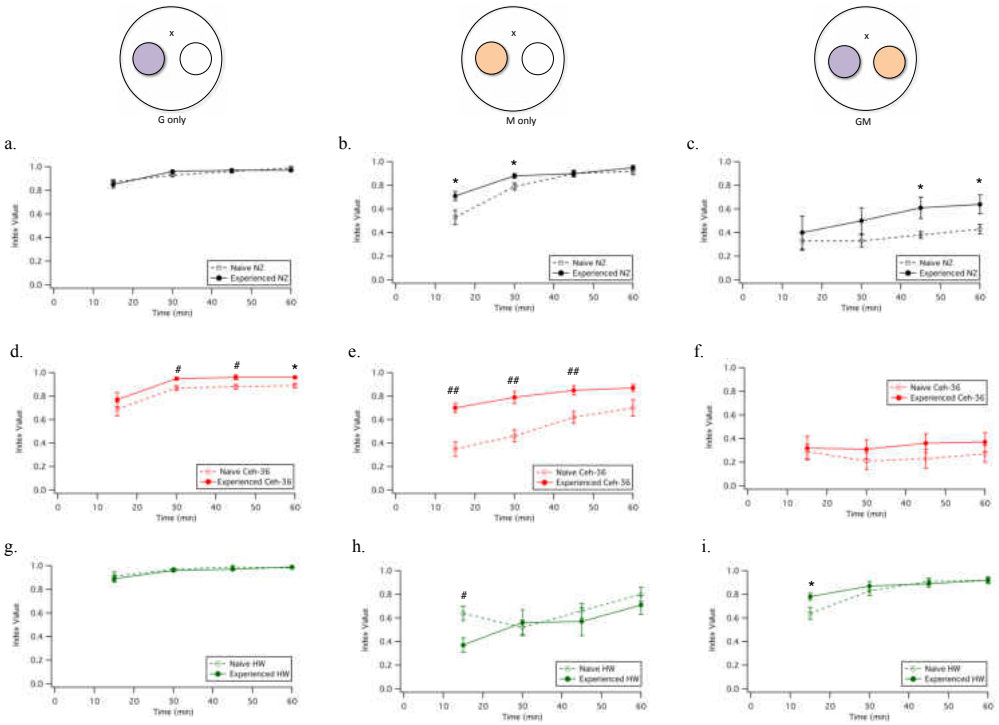

Figure 3. Effects of experience on food choice. Results for naïve (dashed line) and experienced (solid line) N2 (black, a-c), *ceh-36* (red, d-f), and HW (green, g-i) worms. Each data point represents mean index value for 8 replicates. Error bars denote SEM. Each column shows results for a particular test condition: G only (left), M only (center), GM (right). **a-c.** In N2, experience affects food familiarity learning (b) and choice between G and M (c). **a.** Effect of experience may be undetectable because naïve worms approached upper limits of accumulation index. **b.** Experienced N2 accumulated more on M than naïve worms [main effect, Experience, $F(1,56) = 5.81$, $p = 0.03$; Experience (E) x Time (T) interaction, $F(1,56) = 3.34$, $p = 0.03$; 15 min, $t(14) = -2.39$, $p = 0.03$; 30 min, $t(14) = -2.33$, $p = 0.04$]. **c.** Experienced N2 accumulated more on G than naïve worms in joint presentation [45 min, $t(14) = -2.28$, $p = 0.04$; 60 min, $t(14) = -2.50$, $p = 0.03$]. **d-f.** In *ceh-36*, experience affects food familiarity learning (d, e) but not choice between G and M (f), suggesting a role for AWC in adapting food choices after experience. **d.** Experienced *ceh-36* accumulated more on G than naïve worms [main effect, Experience, $F(1,56) = 5.15$, $p = 0.04$; E x T interaction, $F(1,56) = 0.03$, $p = 0.007$; 30 min, $t(14) = -3.14$, $p = 0.01$; 45 min, $t(14) = -3.24$, $p = 0.01$; 60 min, $t(14) = -2.83$, $p = 0.01$]. **e.** Experienced *ceh-36* accumulated more on M than naïve worms [main effect, Experience, $F(1,56) = 15.59$, $p = 0.001$; E x T interaction, $F(1,56) = 3.34$, $p < 0.001$;15 min, $t(14) = -4.63$, $p < 0.001$; 30 min, $t(14) = -4.32$, $p < 0.001$; 45 min, $t(14) = -3.75$, $p = 0.002$]. **f**. Experience had no effect on accumulation between G and M**. g-i.** In HW, experience weakly affects food familiarity learning (h) and choice between G and M (i). **g.** see (a**). h**. Experienced HW accumulated less on M than naïve worms [15 min, $t(14) = 3.25$, $p = 0.005$]. **i**. Experienced HW accumulated more on G than naïve worms in joint presentation [E x T interaction, $F(1,56) = 3.54$, $p = 0.02$;15 min, $t(14) = -2.39$, $p = 0.03$]. Significant differences noted at * $\alpha = 0.05$ uncorrected; # $\alpha = 0.05$ Bonferroni corrected; ## $\alpha = 0.01$ Bonferroni corrected.

ceh-36. Our lab observed that experience changed the response of AWC neurons during the switch from G to M, and we wondered if this altered neural response was required for the experience effect on food choice observed in N2. We sought to determine if the AWC-deficient *ceh-36* strain would alter accumulation behavior after experience. We observed that experienced and naïve worms accumulated similarly in the GM condition (Figure 3f), but differently in the G alone (Figure 3d) and M alone (Figure 3e) conditions, with experienced worms accumulating more on food than naïve worms. That is, experienced *ceh-36* exhibited food familiarity learning in solo food presentations, but did not alter food choice between G and M. We conclude that AWC neurons are not required for food familiarity learning, but that they are required for the form of learning that alters food choice between G and M.

HW. Since wild strain HW possesses functioning AWC neurons, we expected that HW would exhibit experience effects on food choice. However, considering that HW has emerged as the natural isolate most genetically distinct from N2 (Wicks, Yeh, Gish, Waterston, & Plasterk, 2001), we did not expect experience effects on behavior to be identical to those observed in N2. We observed that experience had a small effect on accumulation in the M only (Figure 3h) and GM (Figure 3i) conditions, but not in the G only condition (Figure 3g). In the GM condition, experienced HW accumulated more on G than naïve worms, but only at the first time point. In the M only condition, experienced HW accumulated less on M than naïve worms, but only at the first time point. Experience had no effect on accumulation in the G only condition, but as was the case with N2, we may not have been able to detect an effect because of a ceiling effect. We

conclude that, for HW worms, experience weakly affects food choice in GM and familiarity learning in M alone.

Between Strains

We sought to determine (1) if mutant strain *ceh-36* altered food choice after experience in the same way as N2, and (2) if wild strain HW exhibited substantially different food choice behavior from N2, making it suitable for use in QTL mapping of food preference. For both N2 vs. *ceh-36* and N2 vs. HW comparisons, we explored differences among naïve and experienced worms. However, we were more interested in differences among experienced worms in N2 vs. *ceh-36* comparisons, since we sought to determine if experience effects on AWC response underlied previously observed experience effects on food choice (i.e. enhanced preference for high quality food when presented simultaneously with high and low quality food options). Conversely, we were more interested in differences among naïve worms in N2 vs. HW comparisons, since we sought to determine if natural variation in food choice existed between the two strains.

N2 vs. *ceh-36*. Naïve *ceh-36* worms showed significantly weaker accumulation than N2 in response to G and M alone (Figure 4a, b). We may attribute the solo presentation results to the impaired ability of *ceh-36* to sense particular volatile odors [benzaldehyde, butanone, isoamyl alcohol, 2,3-pentanedione, and 2,4,5-trimethylthiazole] (Lanjuin et al., 2003), which may impair chemotaxis towards a lone food patch. However, the two strains exhibited similar accumulation in joint presentation (Figure 4c), so detection of particular odors was not necessary for naïve *ceh-36* and N2 to make similar choices between G and M. In contrast, experienced worms exhibited the opposite pattern. Experienced *ceh-36* worms behaved similarly to N2 in solo presentations (Figure

4d, e), but exhibited weaker accumulation on G than N2 in joint presentation (Figure 4f). That is, experience abolished the accumulation differences between *ceh-36* and N2 in solo presentations, but enhanced accumulation differences between the two strains in joint presentation. Since N2 altered food choice between G and M after experience, but *ceh-36* did not, we conclude that AWC neurons are required for that behavioral change. In summary, our results suggest that (i) AWC is required in naïve animals for normal levels of accumulation when food is presented alone, but not for normal levels of accumulation when foods are presented jointly, and (ii) AWC is *not* required in experienced worms for normal levels of accumulation when food is presented alone, but *is* required for normal levels of accumulation when foods are presented jointly.

Differences in sensory integration. The behavioral differences between experienced *ceh-36* and N2 suggest that, after the 3 h exposure to G and M, the strains differed in sensory integration of the two cues (G and M), rather than an inability to sense either cue (Figure 4d-f). Our G alone/M alone/GM design was inspired by Shinkai et al. (2011), in which they observed if mutants responded similarly to N2 when copper (repellent) and diacetyl (attractant) were presented individually, but differently when the two stimuli were presented together. The authors argued the behavioral choice in joint presentation reflected worms' relative preference between the contradictory sensory cues. We observed that *ceh-36* responded similarly to N2 during solo food presentation (G alone, M alone), but differently from N2 during joint presentation (GM). Such a set of responses suggests that differences in accumulation in the GM condition stemmed from differences in *integration* of sensory cues from G and M, rather than differences in

sensation of the individual foods. However, we cannot exclude the possibility that ceiling effects obscured differences between the strains in the G only and M only conditions.

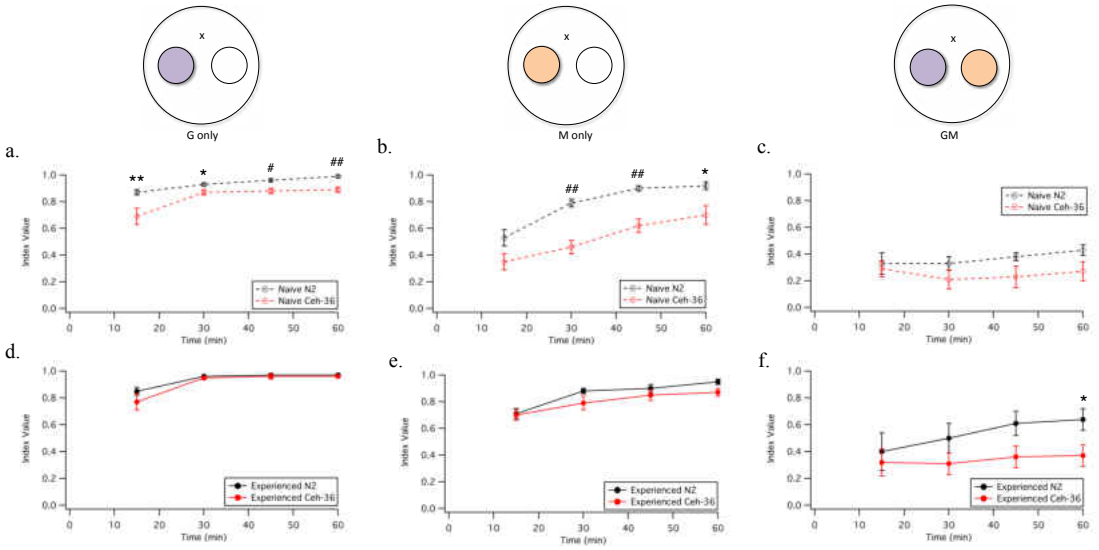

Figure 4. N2 and *ceh-36* comparisons. Data replotted from Figure 3a-f. Results for naïve (dashed line, a-c) and experienced (solid line, d-f) N2 (black) and *ceh-36* (red) worms. Each data point represents mean index value for 8 replicates. Error bars denote SEM. Each column shows results for a particular test condition: G only (left), M only (center), GM (right). **a.** Naive *ceh-36* exhibited weaker accumulation than N2 on G alone [main effect, Strain, $F(1,56) = 14.42$, $p = 0.002$; Strain x Time interaction, $F(1,56) = 3.58$, $p = 0.02$;15 min, $t(14) = 2.99$, $p = 0.01$; 30 min, $t(14) = 2.42$, $p = 0.03$; 45 min, $t(14) = 3.65$, $p = 0.002$; 60 min, $t(14) = 4.51$, $p < 0.001$] and **(b)** on M alone [main effect, Strain, $F(1,56) = 16.67$, $p = 0.001$;30 min, $t(14) = 5.04$, $p < 0.001$; 45 min, $t(14) = 5.26$, $p < 0.001$; 60 min, $t(14) = 2.76$, $p = 0.02$], which may be due to an inability of *ceh-36* to detect particular olfactory cues. **c.** However, naïve N2 and *ceh-36* accumulated similarly on G and M in joint presentation. **d,e.** Experienced *ceh-36* and N2 worms respond similarly to G and M alone, perhaps because experienced *ceh-36* learned to rely on other olfactory cues to locate lone patches, but **(f)** experienced *ceh-36* exhibited weaker accumulation than N2 in joint presentation [Strain x Time interaction, $F(1,56) = 3.78$, $p = 0.02$; 60 min, $t(14) = 2.45$, $p = 0.03$]. AWC-deficient *ceh-36* did not alter choice between G and M as N2 did, suggesting that AWC is required for this behavioral change. Significant differences noted at * $\alpha = 0.05$ uncorrected; **$\alpha = 0.01$ uncorrected; # $\alpha = 0.05$ Bonferroni corrected; ## $\alpha = 0.01$ Bonferroni corrected.

N2 vs. HW. We sought to determine if N2 and HW differed substantially in food choice, which would justify future pursuit of QTL mapping for that trait. Our results

showed that, in general, HW accumulated similarly to N2 in the G only condition (Figure 5a, d), but differently from N2 in M only (Figure 5b, e) and GM (Figure 5c, f) conditions regardless of experience. More specifically, naïve and experienced HW exhibited weaker accumulation than N2 on M alone and greater accumulation than N2 on G in joint presentation. Experience had little effect on these accumulation differences, except that in the M only condition, experienced HW exhibited weaker accumulation than N2 at two additional time points.

We conclude that natural variation in food choice exists between the HW and N2 strains, and that experience has little effect on the naïve accumulation differences that exist between them. We note that, while both strains changed behavior in joint presentation after experience, HW did so to a lesser degree (Figure 3c, i). These results suggest that HW worms are less influenced by prior experience with G and M than N2 worms. Recall that worms' choice in the GM condition reflects worms' relative preference between G and M; N2 worms may alter relative preference of G and M more drastically after experience than HW worms. In any case, the substantial differences in food choice that exist between N2 and HW, particularly in the GM condition, justify the pursuit of QTL mapping to identify genetic loci that contribute to the heritable variation in food preference.

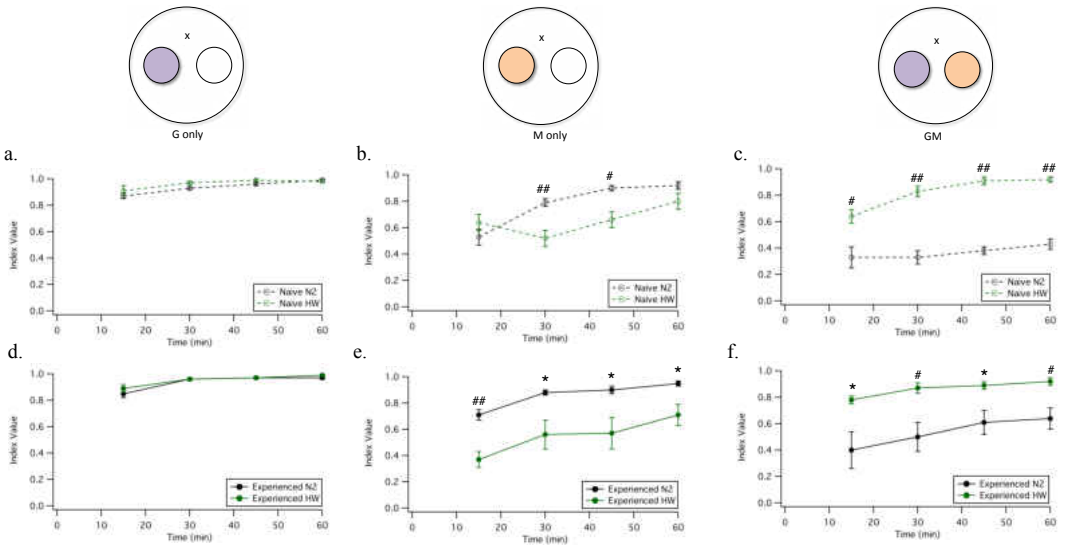

Figure 5. N2 and HW comparisons. Data replotted from Figure 3a-c, g-i. Results for naïve (dashed line, a-c) and experienced (solid line, d-f) N2 (black) and HW (green) worms. Each data point represents mean index value for 8 replicates. Error bars denote SEM. Each column shows results for a particular test condition: G only (left), M only (center), GM (right). Regardless of experience, HW accumulated differently from N2 in M only (b,e) and GM (c,f) conditions, but similarly in the G only condition (a,d). **b,e.** HW exhibited weaker accumulation than N2 on M alone [naïve: main effect, Strain, $F(1,56) = 13.14$, $p = 0.003$; Strain x Time interaction, $F(1,56) = 6.09$, $p = 0.002$;30 min, $t(14) = 4.07$, $p = 0.001$; 45 min, $t(14) = 3.83$, $p = 0.001$; experienced: main effect, Strain, $F(1,56) = 16.53$, $p = 0.001$;15 min, $t(14) = 4.84$, $p < 0.001$; 30 min, $t(14) = 2.98$, $p = 0.01$; 45 min, $t(14) = 2.76$, $p = 0.02$; 60 min, $t(14) = 2.96$, $p = 0.01$]. **c,f.** HW exhibited greater accumulation on G than N2 in joint presentation [naïve: main effect, Strain, $F(1,56) = 84.34$, $p < 0.001$; Strain x Time interaction, $F(1,56) = 4.85$, $p = 0.005$;15 min, $t(14) = -3.36$, $p = 0.004$; 30 min, $t(14) = -8.32$, $p < 0.001$; 45 min, $t(14) = -12.52$, $p < 0.001$; 60 min, $t(14) = -10.84$, $p < 0.001$; experienced: main effect, Strain, $F(1,56) = 9.88$, $p = 0.007$;15 min, $t(14) = -2.67$, $p = 0.02$; 30 min, $t(14) = -3.29$, $p = 0.005$; 45 min, $t(14) = -2.86$, $p = 0.01$; 60 min, $t(14) = -3.48$, $p = 0.004$]. Since worms' choice in the GM condition reflects worms' relative preference between G and M, and HW differs substantially from N2 in this choice, we may pursue QTL mapping to identify genetic loci that contribute to the heritable variation in food preference.

CHAPTER IV

CONCLUSIONS AND FUTURE DIRECTIONS

We sought to determine (i) how experience altered food choice/preference in three strains (N2, ceh-36, HW), (ii) if AWC was necessary for behavioral changes in food choice after experience, and (iii) if HW exhibited substantial differences in food choice from N2, justifying the pursuit of QTL mapping of food preference. We found that experience altered accumulation behavior in all three strains, but to varying degrees in particular test conditions; we found evidence that AWC neurons are required for alterations in food choice between G and M after experience; and we found that HW and N2 differed substantially in food choice, making them suitable strains for use in QTL mapping of food preference.

Prior Experience with Food Options Has Varying Degrees of Influence on Future Preference

If we consider accumulation as an indicator of preference, we observed that all strains altered future food preference after experience in particular ways. For *ceh-36*, preference changes manifested in solo presentations of food. For N2 and HW, preference changes manifested in M only and GM conditions. We may not have observed preference changes in the G only condition for N2 and HW because index values approached the upper limit for naïve worms, and there was little room to exhibit enhanced preference after experience. While both N2 and HW altered preference after experience, experienced N2 seemed to alter preferences more than experienced HW, since significant effects were observed over a longer portion of the time course. However, in the case of the GM condition, N2 had more room to change behavior; naïve HW worms already approached

upper index limits in joint presentation, and so, HW had little room to exhibit enhanced preference after experience. We cannot apply this argument to the M only case, however. Index values did not approach upper limits, so there was room to exhibit a preference shift. Yet experienced HW exhibited a difference in preference for only a brief time, before matching that of naïve worms. Taken together, these results seem to suggest that experience had less of an effect on future preference in HW than N2. Perhaps then, HW has preferences for G and M that are less flexible than the preferences of N2 for G and M.

It would be of interest to measure calcium transients in AWC in HW worms that experience a switch from G to M. Perhaps experience alters the AWC response in HW as it does in N2, but to a lesser degree, which would reflect the small preference shifts exhibited by HW in the current study.

AWC as a Neural Locus of Comparison

We observed that experienced *ceh-36 (ky646)* exhibited substantially different preference from experienced N2 in the GM condition, but not the G only and M only conditions. These results suggest that the difference in preference was not due to an inability to sense one food or the other, or both—or even an inability to alter the utility assigned to each food individually—but rather a difference in the integration of the two sensory cues, or comparison of the two foods. Considering that *ceh-36 (ky646)* worms are defective in AWC function, these results suggest that AWC neurons may act as comparators of utility between food options. We should emphasize that the ability to compare foods seems to be what was affected—or more specifically, the ability to alter that comparison after experience with each food. *Ceh-36* worms seemed capable of

altering food utility after experience in that they exhibited greater preference than naïve worms during solo presentations, in line with the familiarity effect. Thus, information gained about each food option could have been stored neurally. Perhaps AWC neurons are involved in this storage of food information, or utility assignment, but the greater role of AWC neurons appears to be in changing relative preference between food options after experience.

Previous work in our lab showed that experience altered the response of AWC to the switch from G to M. We should repeat this experiment with *ceh-36 (ky646)* and compare calcium transients in AWC with those exhibited by experienced and naïve N2 worms. Considering the behavior of *ceh-36* in our food choice assays, we would expect that naïve and experienced *ceh-36* worms exhibit similar calcium responses, reflecting the inability to alter comparison between food options, while naïve and experienced N2 worms exhibit different calcium responses.

Whereas experienced *ceh-36* worms' enhanced preference for M and G alone could be explained by an ability to alter utility after experience, we cannot exclude the possibility that worms merely became better at locating these foods. Perhaps worms initially experienced difficulty in locating foods because of the inability to sense volatile odors detected by AWC [benzaldehyde, butanone, isoamyl alcohol, 2,3-pentanedione, and 2,4,5-trimethylthiazole] (Lanjuin et al., 2003). Then, after a time, they were better able to locate foods by associating them with cues detected by other neurons, such as AWA, which detect other volatile odors [diacetyl, pyrazine], or ASE, which detect water-soluble compounds often present in bacterial patches (Bargmann et al., 1993). We should note that certain *ceh-36* mutants (e.g. alleles *ky640*, *ks86*) exhibit defects in ASE

function, but *ky646* does not appear to suffer from such defects (Koga & Ohshima, 2004; Lanjuin et al., 2003). While successfully locating a food option does not necessitate changing its utility, it does present the opportunity to sample the food more often, gain information, and alter utility accordingly. In the future, we hope to identify a worm mutant not defective in chemotaxis that exhibits the same phenotype as *ceh-36*. Such a mutant may be a more effective pawn than *ceh-36* for the argument that worms altered utility assignments after experience in G only and M only conditions.

Justification for QTL Mapping of Natural Variation in Preference

To be successful for QTL mapping, strains used to create RILs should differ substantially in genotype and the phenotype of interest. HW has emerged as the strain most genetically distinct from N2 (Wicks et al., 2001), and we observed that HW exhibited substantially different naïve preference from N2 in M only and GM conditions. Thus, QTL mapping may be worth pursuing to identify genetic loci that contribute to the heritable variation in food preference.

Compared to N2, HW appears to exhibit weaker preference for M alone, and greater preference for G during joint presentation. However, we cannot definitively say that this preference is innate since worms experienced OP50 before G or M, and this exposure to OP50 could have affected preference towards G or M. Like *B. simplex* (M2), OP50 is categorized as a mediocre quality food (M1). We can imagine a scenario in which worms develop familiarity for M1, and so exhibit enhanced preference for M1-like foods (i.e. M2), rather than their truly innate preference for M2. Alternatively, experience with M1 may make higher quality foods (i.e. G) more enticing, so worms exhibit greater preference for G than they would innately. In either case, experience with M1 alters the

preference towards M2 or G. In order to say that the HW preferences we observed are actually innate, we would need to repeat this experiment with hatchlings that have never experienced any other food. For rigor, we should repeat this experiment with N2 hatchlings as well, though the preferences observed in our current study do align with those exhibited by hatchlings in the Shtonda and Avery (2006) experiments.

Locomotor differences between N2 and HW may contribute to the differences in accumulation on M alone. Compared to N2, HW moves faster on food and slower off food (Bono & Bargmann, 1998). Perhaps then, HW appeared to exhibit weaker preference for M alone than N2, when in actuality, HW worms merely moved more quickly than N2 through the food patch. While we cannot exclude this possibility, we would expect HW to exhibit weaker accumulation than N2 in G alone as well, if locomotion differences were responsible for accumulation differences. However, HW accumulates almost identically to N2 in G alone.

Other Limitations

In these experiments, we cannot distinguish between attraction and aversion in the GM condition. For example, if a worm population exhibited an index value of 0.80 at time one (T_1) and an index value of 0.60 at T_2, we cannot say if the change was due to increased attraction of M, or increased aversion of G, or both mechanisms. One could also argue that attraction and aversion cannot be distinguished in G only and M only conditions. We may interpret increased accumulation on food as an indication of increased attraction and decreased accumulation on food (i.e. increased accumulation off food) as an indication of increased aversion. However, these interpretations may not be valid. For example, an odor gradient exists on an agar plate with a single patch of food;

odors are strongest at the food patch and weaker farther away. Decreased accumulation on food may be due to increased attraction to lower concentrations of odor (off food) rather than increased aversion of the food itself. So we cannot easily distinguish between attraction and aversion, even in G only and M only conditions. Additionally, throughout these experiments, we define greater accumulation on a food patch as greater preference of that food, since worms choose to dwell there versus elsewhere. However, dwelling in food does not necessitate eating the food. It could be the case that worms spend more time in G, but never consume food while they are there, and spend less time in M, but consume food for the entire time that they are there. This scenario seems intuitively bizarre, but is technically possible.

Ideally, we would be able to measure pharyngeal pumping (i.e. consumption) while simultaneously tracking worms. By tracking worm movement and pharyngeal pumping simultaneously, we could verify that worms are actually consuming more of the food that they spend more time in. We could also code movements (e.g. dwelling, roaming, reversals) and record the number of patch-leaving events. Increased number of reversals could be an indicator of increased aversion to a particular food, helping us to distinguish between the mechanisms responsible for preference shifts. The transition between dwelling and roaming appears to involve ciliated sensory neurons, including AWC (Arous et al., 2009). Considering that prior experience with food options alters AWC response, it would be interesting to see how experience alters the dwelling:roaming ratio. Adding a tracking system to our design would also allow us to verify that worms in the experienced condition actually sample both G and M prior to transfer to test plates. However, we are currently in the process of developing a microfluidic device in which

we can tightly control worms' exposure to G and M, thus ensuring that each worm experiences each food the same amount.

Final Remarks

In the current study, we have identified neurons (AWC) that may be responsible for altering food choice after experience. We have also gathered support for using HW and N2 in QTL mapping to identify genetic loci that contribute to the heritable variation in food choice. By understanding the biological mechanisms of food choice—a behavior that strongly impacts the evolutionary fitness of animals, from worms to humans—we can begin to understand the biological underpinnings of decision making in general. We can also use such knowledge to help us better understand how decision making processes are affected in cases of addiction, mental illness, and other disorders.

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