ESTABLISHING HERITABILITY FOR VALUE-BASED DECISION MAKING QUALITIES IN ANANDAMIDE TREATED *CAENORHABDITIS ELEGANS* NATURAL ISOLATE STRAINS

by

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Value-based decision making plays a significant role in the lives and functioning of many organisms and is impacted by drug use often resulting in negative outcomes. Marijuana's active chemicals mimic the existing neurochemicals in the endocannabinoid system to elicit altered decisions. One of the most well-known alterations in decision making caused by cannabinoids is an increased appetite for nutrient dense foods, which is referred to as hedonic feeding. Understanding cannabinoid signaling pathways can aid in illuminating how drugs alter food preferences and decision making. This study investigates whether genetic screens for hedonic amplification in *C. elegans* is possible by establishing broad sense heritability of the trait.

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INTRODUCTION

Value-based decision making exists throughout nature, exhibited not only by humans, but many other organisms. It is described as making a choice from different options dependent upon the subjective value of each option. Foraging, stock trading, or whether to buy chips or soda at a convenience store are examples of value-based decision making.

Decision making is thoroughly ingrained within our everyday lives. Understanding this crucial phenomena is necessary for understanding what it is to be cognizant, to understand individuality. However, decision making is a large and cumbersome concept to comprehend. The field which studies and seeks to understand human decision making is Neuroeconomics, a cross disciplinary field of neuroscience and model economics.

BACKGROUND

Neuroeconomics

Neuroeconomics' goal is to provide the biological explanations underpinning human behavior that is applicable within the natural and social science fields. Neuroscience has developed a wealth of knowledge of the workings of the brain and provided tools and studies to examine the neural mechanisms that compose decision making. While psychology has detailed accounts of animal behavior regarding learning and decision making under varying conditions, economics and computer science has provided the computations necessary to link the fields and provide models for decision

making. Neuroeconomics as a field attempts to bring together these levels of understanding to fully comprehend choice.

To study choice and value-based decision making, economists often look towards Paul Samuelson, an American economist who proposed the Revealed preference theory[1]. This theory states that the preferences of an individual, or consumer, can be understood through their purchasing habits which have the goal of maximizing the consumer's utility. The theory assumes that the consumer has a budget constraint, and that if goods are affordable preferring a combination of goods over another combinations reveals preference. It further assumes that preferences are stable across an observable time period. If a consumer chooses one bundle of items over another, the first is revealed preffered to the second, and that the first bundle will always be preferred over the second, unless its price becomes unaffordable[2].

Revealed preference theory is useful to determine whether an organism is capable of value-based decision making and culminates in three revealed preference axioms which test the utility of modeled preferences. Weak Axiom of Revealed Preference (WARP) is where a choice has the utility that is equal to or higher than any other possible and available choice[2]. A violation of WARP reveals an organism is irrational, indifferent or that the decision has contextual effects. Strong Axiom of Revealed Preference (SARP) is where chains of choices are compared to one another and that any choice made within that chain must have greater or equal to utility than those after it. An example of which is if A is greater than B, and B is greater than C, A must also be greater than C[2]. However, WARP and SARP and not suitable for empirical research as they are designed for single-valued utility maximization. For empirical

work, we must take a look to the third revealed preference axiom. Pioneered by Afriat's analysis of finite sets of values and choices, this axiom culminates in an explicit algorithm to construct a utility function: the generalized axiom of revealed preference (GARP)[2].

GARP is a revealed preference axiom which is sufficient and necessary for well-behaved preferences, when linear budget constraints are applied[3]. A linear budget constraint is a representation of all of the services and/or goods combinations that a consumer may purchase given their income or other budget. A budget constraint is linear if all goods may be purchased at the same set price to the maximal total of the budget. GARP is structured upon cyclical consistency and covers choice cases in which for a certain value, there is more than one decision which maximizes utility. Indifference curves produced by GARP can be 'flat'[4], where at any point on the curve, a person is willing to give up a small amount of one good for another. An indifference curve is the graphical representation of goods combinations at which the consumer has no preference for one combination over another. 'Flat' indifference curves allow for empirical analysis across individuals.

Heritability

When considering any phenotypic trait, one ponders whether observed variation of that trait is due to genetics. This is due to the understanding that development is rooted heavily in genes, however the variation between individuals may not necessarily be so. For example the variation in human height is rooted in genetics, but the variation in

which language people speak is not. For a trait to be heritable, similarity must arise from shared genotypes[5].

Broad sense heritability (H^2) is the ratio of genetic variance in relation to phenotypic variance[6,7]. This was formalized by Wright and Fisher by stating that the whole of phenotypic variance must be the sum of genetic and environmental variance[8]. Where a trait with H^2 of zero has no genetic variance accounting for the phenotypic variance, and the phenotypic variance of a trait with $H^2 = 1$ is fully due to genetic variance.

Establishing heritability is incredibly important for any trait for which future genetic studies are considered. Without a significant amount of genetic variation accounting for the phenotype, determining genes, or the biological mechanisms underpinning the trait, is difficult. By establishing heritability, genome wide association studies and mappings become a possibility.

Biological basis of decision making

Dopamine is a monoaminergic neurotransmitter which likely plays a multi-faceted role in decision making. Dopamine plays an important role in positive reinforcement in value-based decision making and learning. Dopamine encodes expected and received rewards to form neural predictions of the outcome of choices[9]. Subsecond dopamine release concentrations modulate cost-benefit analyses undergone by an animal via encoding information regarding the reward value of a choice[10]. These secondary bursts increase in concentration in relation to the reward expected and are thought to strengthen choices which result in larger reward[11].

The endocannabinoid system can modulate dopaminergic systems in a manner which is significant to value-based decision making. Receptor agonists modulate the subsecond dopamine bursts by uninhibiting dopamine neurons and increasing the subsecond burst[9].

The endocannabinoid system is characterized by endocannabinoids which bind to cannabinoid receptors, as well as their receptor proteins which are expressed through the nervous system. This system is involved in many physiological functions and cognitive behaviors. One of the most widely expressed of its receptors is Cannabinoid receptor type 1 (CB1) which inhibits the release of GABA-mediated neurotransmission[12]. It is this receptor's agonists that modulate the subsecond dopamine concentrations through their decrease in GABA release[9].

Caenorhabditis elegans

Caenorhabditis elegans is a small bacteria eating roundworm and model organism. *C. elegans* is a hermaphroditic species that is self-fertile and produces approximately 300 progeny per generation after 3 to four days. *C. elegans* has a 97-megabase genomic sequence with over 19,000 genes all of which have been sequenced. The whole cellular lineage of the hermaphrodite, 959 somatic cells, have been mapped and the nervous system wiring has been diagramed. *C. elegans*has also been shown to exhibit value-based decision making via GARP[13].

The *C. elegans* life cycle is comprised of four larva stages (L1-L4) and an adulthood stage. Only adult *C. elegans*are capable of egg laying.

C. elegans also has a large pool of natural isolates or strains. Each strain is a natural population of genetic variation within the species, and can be examined to understand the genetics underlying phenotypic variation across strains[14].

The quick generation of individuals and their observance of GARP allow for robust testing of broad sense heritability. *C. elegans* also has an orthologue to the human CB1 receptor: npr-19. *npr-19*-null animals can have function rescued by CB1[15]. The npr-19 receptor modulates monoaminergic signaling which effect nociception, locomotion, and feeding behaviors[15]. An agonist of the npr-19 receptor is Anandamide (AEA) also known as N-arachidonoylethanolamine, which is also an agonist for the CB1 receptor[16].

C. elegans feeding behavior

C. elegans feeds via filtration utilizing the pharynx, a neuromuscular organ that joins the mouth and intestine. This organ 'pumps' food via electrically stimulated contractions[17]. It is formed of a long thin lumen surrounded by three triangular bands of muscle and marginal cells. The anterior of the pharynx is the corpus, connected to the posterior terminal bulb via the isthmus. Food is brought into the lumen via near-simultaneous contraction of the three parts, and liquid is expelled via near-simultaneous relaxation. The small differences in contraction/relaxation timing accounts for the grinding and posterior peristalsis of the bacteria[18]. This unique feeding mechanism limits the food preference of *C. elegans* not only by nutritional value, but particulate size. It has thus been observed that *C. elegans* prefer *Comamonas*species DA1877

which promotes swift developmental growth[19]. Whereas *C. elegans* when offered a *Bacillus* strain 1885, they experienced less growth[19].

Feeding behavior is characterized by periods of roaming and dwelling. During roaming *C. elegans* undergoes bursts of movement to seek food. Dwelling is composed of slower movement, as well as frequent stops and reversals of locomotion direction. During dwelling *c. elegans* is able to remain in a patch of food until completely consumed or until satiation.[23]

Genome Wide Association Studies

Genome wide association studies (GWAs), also known as whole genome association studies (WGAs) are powerful tools for the investigation of genetics in humans. GWAs map genome-wide variants among individuals to potentially identify variants which are responsible for a trait. They focus on single-nucleotide polymorphisms (SNPs) which are single base-pair differences in DNA sequences that occur at a high frequency[21]. SNPs are often used as genetic markers in the genome, and although they largely have minimal impact on biological function, some may have important consequences to an individual's traits such as disease risk or appearance.

Prior to the invention of GWAs, traits were often examined through genetic linkage among first degree relatives. This limited the scope of identifying potential causes to single gene disorders[22]. GWAs, by examining allele frequency of the genetic variant, may be able to provide information on detecting weaker genetic effects[24].

In humans GWAs are commonly used in clinical populations. Variations of the case-controlapproach of GWAs are usually quantitative analysis of phenotypic data

such as height. However, evidence has shown that interactions between many SNPs, or genes, can contribute and influence these factors including complex diseases. The multifaceted causes usually require further experimentation and analysis such as protein-protein interactions. Experimenters must also consider possible variables that can confound the results of association between genes and phenotype. These variables include sex and age which are common confounding variables. Geographic and historical populations that can give rise to mutations responsible for phenotypes must also be considered (e.g. common ancestry of populations). Thus human studies must understand the ethnic and geographic background of their participants to control for population stratification which can add difficulty to these studies[24].

Human GWAs are also difficult, as all individuals involved in the study must have the majority of their commonly known SNPs genotyped, which are typically numbered in the millions. From there the allele frequency of each of these SNPs among all participants is examined for significant differences between the case and control groups. Utilizing human subjects means that there is a very large number of genomes that must be sequenced. Although genome sequencing has become more affordable in recent years, the expenditure and number of humans required is high. To narrow costs, a smaller library of SNPs are genotyped depending on the technology and methodology.

Few studies have utilized GWA mappings across different *C. elegans* natural isolates, as the approach is new[14]. However, with the introduction of the*Caenorhabditis elegans*Natural Diversity Resource (CeNDR), GWA studies across multiple populations is accessible. CeNDR has collected and provides the whole-genome sequence and variant data of the natural isolates within its database. This eliminates the need to

genotype and sequence each of the strains of *C. elegans* used[14]. *C. elegans* are also asexual; their offspring are exact genomic copies of their parents. Therefore the number of groups can be expanded allowing for further ease of narrowing candidate genes that underpin value-based decision making.

Considering the difficulty of discovering candidate genes in humans for value-based decision making, using the model organism *C. elegans* may facilitate the narrowing of possible human genes.

MATERIALS AND METHODS

Behavioral assay

The behavioral stage involvedquantifying a shared phenotype across naturally isolated strains of *Caenorhabditis elegans*. 12 strains of *C. elegans* were chosen from theCeNDR, comprising of the divergent set, a genotypically different set of strains that allow for heritability testing. This set includes the following strains: CB4856, CX11314, DL238, ED3017, EG4725, JT11398, JU258, JU775, LKC34, MY16, MY23, and N2. The behavioral stage was composed of 7 different steps: worm synchronization, food concentration, plate preparation, worm washing, drug incubation, experiment loading and running, and worm counting.

Bacterial Name	Bacterial Species	Growth Temperature
OP50-1	*E. coli*	37
DA1877	*Comamonas sp.*	37
DA1885	*B. simplex*	37

Table 1. Bacterial strains used.

Worms weresynchronized by transferring 30-40 adult worms from a mixed stage population plate onto a second **NGM plate** that is seeded with OP50 bacteria. This bacteria provided a rich environment for the worms to generate on. This wasrepeated for 8 plates per strain alongside 8 plates of N2 strain as an experimental control. The plates were incubated for 4 hours at 22 degrees Celsius at which point the adult worms were removed from the plate. These plates had approximately 100 eggs on them and were incubated for 3 days until adulthood was reached. This ensured that all worms used in experimentation were of approximately the same age and stage.

After the 3 day incubation the next 5 stages of behavioral testing commenced. Food was concentrated using a centrifuge. Two 50 mL corning tubes were filled with 40 mL of bacterial solution. One tube was filled with ‘G’ bacteria: Comamonas DA 1877, and the other was filled with ‘M’ bacteria: Comamonas DA1885 bacteria as shown in table 1. G bacteria referred to preffered or ‘good’ food and M bacteria referred to mediocre or less preferred food. Each tube was spun in a centrifuge for 7 minutes at 5,000 rpm to form a bacteria pellet. After which the supernatant was dumped into a waste receptacle without jostling the pellet. This process was to purify desired bacteria from the rest of the LB broth. 10 mL of 0mMol NaClbuffer solution was added to each

tube using and vortexed until the pellet was well incorporated into the buffer creating a solution. Both tubes were vortexed again with the supernatant dumped afterwards. 10 mL of buffer was added to each tube and weighed for later calculations. Both tubes were returned to the centrifuge after which the bacteria wasresuspended to an optical density (O.D.) of 8 for the M food and O.D. of 0.5 for the good food.

The resuspension volume for each bacteria was calculated using the dilution calculation.300 uL of buffer solution and 100 uL of bacteria solution was added to two 2 mL Eppendorf tube and vortexed. Using a spectrophotometer a sample of each tube was measured. Between each reading the sample was vortexed to prevent sedimentation. Bacterial concentration optical density was calculated using the information from Table 2.

Variable	Calculation	Description
OD_2	$\frac{V_1 \times OD_1}{V_2}$	Where V_1 is the volume of the bacterial sample. OD_1 is the measured O.D. from the spectrophotometer. V_2 is the volume of the measured solution.
V_f	$\frac{OD_2 \times V_i}{OD_f}$	Where V_i is the initial volume of bacterial solution measured after the second centrifuging. OD_f is the desired O.D of the bacterial solution. G: 8; M: 0.5

Table 2. Calculations for bacterial concentrations. V_1 is always 100 uL and V_2 is always 400 uL for the purposes of this experiment.

After the food is prepared, both tubes were added to a rack for storage alongside four 50 mL corning tubes, one filled with buffer, one filled with deionized water, one with 5 mL bleach for waste, and 1 filled with 70%EtOH.

28 NGM plates were added into a dehydrator set for 45 minutes at 113 degrees Fahrenheit. After 45 minutes the plates were removed and placed covered to cool for 30 minutes. Once cooled, each plate had a 2 mm foam laser cut T-maze, shown in Figure 1was added to its surface and pressed flat with the plunger end of a syringe without damaging the agar. This process was completed to discourage worms from escaping from the assay zone of the T-maze. 4.5 uL of each food mixture will be placed into each end of the T-maze without breaking the surface tension of the food droplet. G food was placed on the left and M on the right.

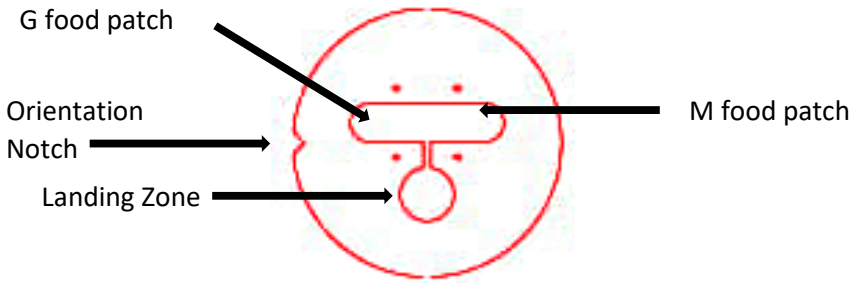


Figure 1. Foam T maze diagram depicting landing site of worms as well as locations of food. The four points surrounding the T maze indicate interior marker where worms will stop being counted as in food patch.

Worms were washed to prevent lingering in the landing zone of the maze, as a high accumulation of bacteria on the worms would cause them to remain and eat. Each plate of worms that had been incubated for 3 days was cleaned with 1000 uL or 1 mL or buffer solution and poured into a 2 mL Eppendorf tube. Each tube was spun in a small centrifuge for 30 seconds at 4,000 rpm. After which the supernatant from each tube was

removed down to the 0.1 mL mark. This process was repeated four more times to ensure all bacteria and eggs from the worms were removed.

2 glass concentration tubes were used per strain and cleaned with bleach and then deionized water. Each tube was labeled with the strain name and whether it contained AEA or not. Worms were allowed to incubate in the drug for 20 minutes.

After the 20 minutes 2.5 ul of worms were placed into the landing zone of the T-maze on each plate, for about 50 worms or less per plate. 12 plates were loaded with each condition of worms. A scan upon loading of worms was done to ensure fidelity of scanner. Every 15 minutes each tray was scanned for a total of four data scans. The temperature of the room wasrecorded. At high temperatures the behavior of the worms was erratic and all data taken above 23 degrees Celsius was neglected.

The counting of the worms included the number of worms in the good food, mediocre food, and not in either of the food patches. The index of worms, their food preference, was calculated by dividing the difference between the number of worms in G and M by the sum of worms in G and M.

G vs M assay

The cause of differences between AEA and control condition food preferences was assayed by measuring the proportion of worms in the G food versus the M food. Increase in the index value is impacted by two factors: increase in worms in G or decrease of worms in M. This was calculated by dividing the number of worms in each spot by the total number of worms. Contrasting the G and M proportions in the AEA

and control conditions showed whether the AEA effect increases preference for G, decreases preference for M or both.

Broad sense heritability

Broad sense heritability (H^2) was calculated to determine the proportion of phenotypic behavior that is due to genotypic variance. The calculations to determine H^2 are shown in Table 3.

Variable	Calculation	Description
$\bar{A}_i$	$\sum_{J=1}^{N} A_{ij}$	Mean of replicates (j) of one strain (i)
V_i	$\frac{\sum_{J=1}^{J=N}(A_{ij}-\bar{A}_i)^2}{N-1}$	Variance of $\bar{A}_i$
V_e	$\frac{\sum_{i=1}^{N} V_i}{N}$	Mean of all multi-replicate strain variances
$\bar{\bar{A}}$	$\frac{\sum_{i=1}^{N} \bar{A}_i}{N}$	Grand mean of hedonic amplification across strains.
V_p	$\frac{\sum_{i=1}^{N}(\bar{A}_i-\bar{\bar{A}})^2}{N-1}$	Variance of $\bar{\bar{A}}$
H^2	$\frac{V_p-V_e}{V_p}$	Broad sense heritability of hedonic amplification

Table 3. Calculations for broad sense heritability.

RESULTS

Food Preference Data

In order to determine the preferences of each *C. elegans* strain, groups of worms were offered a bilateral choice between good and mediocre food. By dividing the difference between the number of worms in G and the number of worms in M over the total number of worms that made the food choice, the index of food preference may be concluded. The N2 strain was used as the control strain and run in tandem with all other natural isolates. Figure 2.Illustrates N2 preference index over a one hour period, measured every 15 minutes.Preference index remains constant across time points. However, at the 60 minute measurement, depression of the preference index in certain data replicates could be observed due to starvation of worms in the good food. Analysis of the 45 minute time mark measurements was conducted to ensure fidelity of preference index and high number of worms in food.

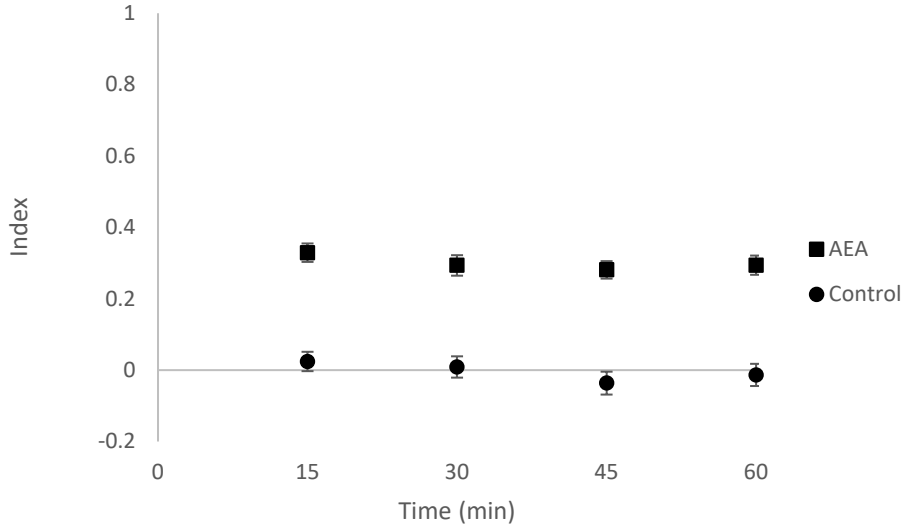


Figure 2. N2 strain control and AEA data at 15, 30, 45, and 60 minute time intervals. Temperature of agar did not exceed 23 degrees.

The index preference of each strain in AEA treated and control conditions are shown in Figure 3. Both AEA and control treated conditions were administered the same optical densities of food to choose between, 0.5 for G and 8 for M. Preference indexes were largely positive, preferring good food, except for JU775 in control conditions. CX11314, CB4856, LKC34and DL238 had insignificant differences between preference indexes in AEA treated and control conditions. This could imply possible absence or lowered activity of the genes underpinning value-based decision making or alterations to the npr-19 receptor.

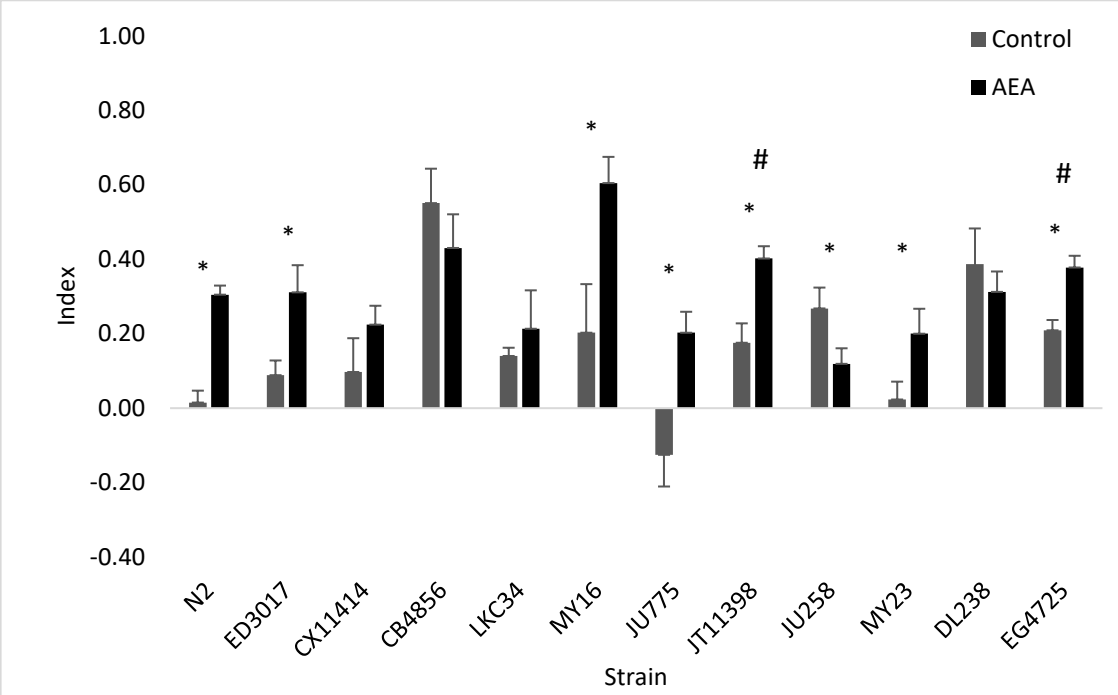


Figure 3. Food preference index values for AEA and control treated strains as 45 minutes into assay. Temperature of agar did not exceed 23 degrees. * significance values are for an alpha of 0.05. # significance values refer to the Bonferoni correction alpha value of 0.004.

Hedonic amplification

The difference between the AEA treated and control condition preference indexes was calculated to determine hedonic amplification. This measurement shows the variable effect of AEA treatment and is shown in figure 4.

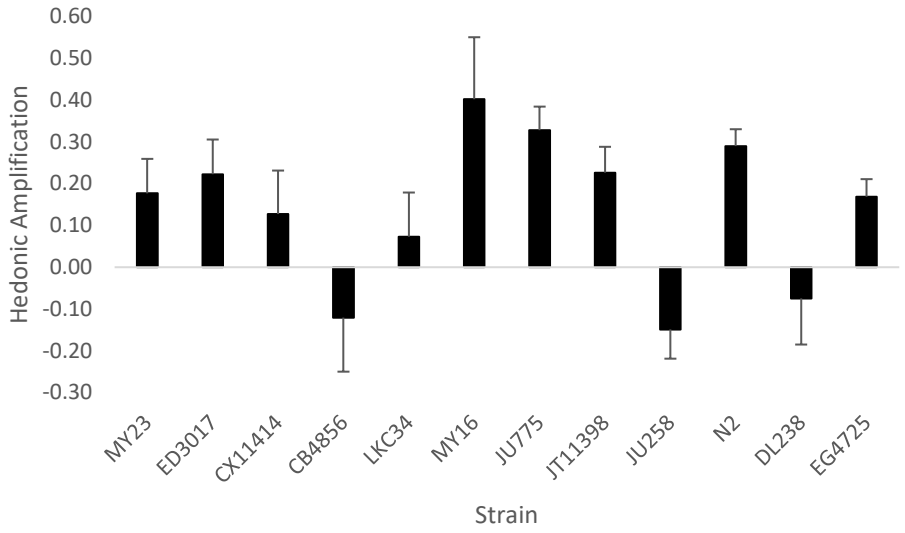
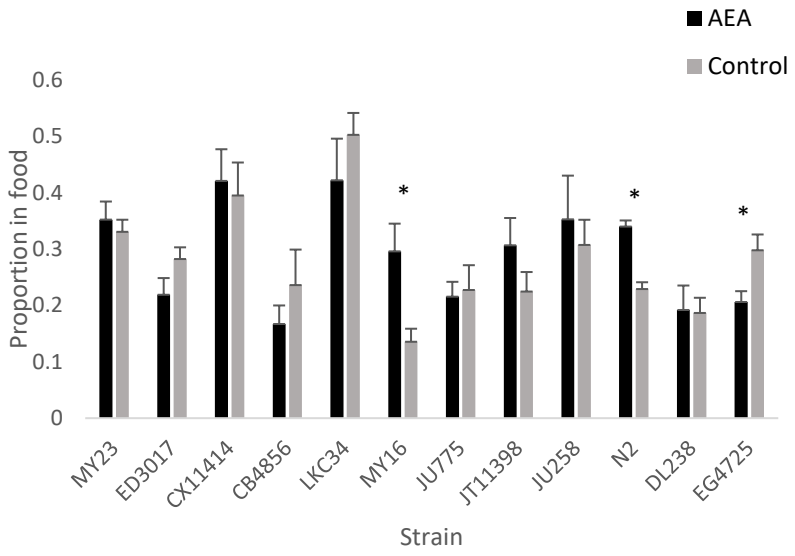


Figure 4. Hedonic amplification for each natural isolate. Data was collected at 45 minutes.

G vs M assay

G and M worm proportion data was calculated and displayed in Figure5. MY16 experienced an increase in G preference Fig. 5A. MY23, ED3017and JU775 experienced a decreased preference for M Fig 5A. N2 and EG4725 experienced both an increase in G preference and decrease in M preference. These differences suggest *C. elegans* genes which affect both increased good preference and decreased M preference.

A



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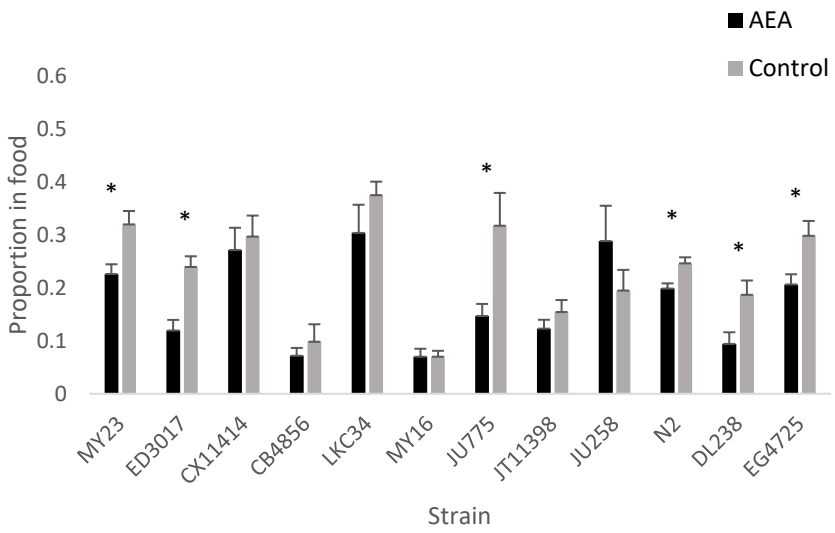

Figure 5. Ratio of worms in food patch to total worms introduced to plate. **A.** Proportion of worms in DA1877 at 45 minute time point. **B.** Proportion of worms in DA1885 at 45 minute time point.

Broad sense heritability

Variance	Value
V_E	3.44×10^{-4}
V_p	1.18×10^{-3}
H^2	0.708

Table 4. Values of V_E V_P and H^2

Broad sense heritability was calculated for hedonic amplification inheritance at 0.708 therefore approximately 70.8% of phenotypic variation of hedonic amplification is due to genotype. The values of environmental and phenotype variation are displayed in Table 4.

DISCUSSION

AEA effect

The treatment of AEA either had no effect or significantly increased the preference index of natural isolate strains of *C. elegans* with the exclusion of JU258. This data supports existing literature of NPR-19 receptor modulation of *C. elegans*feeding behavior as well as the increase in human reward reinforcement due to endocannabinoid agonists.

The negative AEA effect displayed by JU258 may have large implications for the cannabinoid signaling pathway in *C. elegans*. Investigation into the genome of

JU258 and comparing to N2 may be revealing of potential mutations. Further investigation of JU258 npr-19 modulated behavior may be revealing of possible mutations.

The absence of significant food preferences differences due to AEA treatment may be rooted in mutations or absence of *npr-19* modulated monoaminergic signaling. CX11314, C4856, LKC34, and DL238 lack characteristic changes in food preference upon AEA treatment. Evaluation of possible mutations in NPR-19 or pumping behavior may explain the difference in preferences and may be a source of natural knockout or partial knockout mutants.

AEA effect can reinforce rewarding behaviors and decrease un-preffered reward behaviors

The increase in preference index across significantly changed preferences is explained by increasing the preference towards G food or decreasing preference for M food. This gives insight into the cannabinoid signaling pathway in *C. elegans*. In the N2 strain, the preference for G is significantly increased, and the preference for M is significantly decreased. This effect is also mirrored in EG4725 strain. Potential inhibitory and excitatory effects for dopaminergic signaling is a likely cause of the increase in G preference. However, the decrease in M preference is not necessarily explained through the dopaminergic and endocannabinoid signaling pathways.

This interesting divide is further exemplified by the remaining strain which had G preference increased with AEA treatment: MY16. This strain does not have simultaneous decrease of M preference. There is potential that this strain exemplifies

the currently understood reward reinforcement due to increase in dopamine signaling. However, it may lack whatever pathway that the canonical N2 strain possesses. Whereas MY23, ED3017, JU775, and DL238 experienced decreased M preference due to AEA treatment, without a significant increase in G preference. Examination of the npr-19 signaling pathway may reveal intricacies in reward seeking behavior. Where decisions are not only enforced through increased preference of rewards, but decreased preference of reward lacking or detrimental choices.Strains exhibiting isolated behavior modulation pathways may be useful tools to look at signaling pathways individually.

JU258 does not have significant difference between either G or M food preference under AEA treatment. However, the error of each value is largeenough that an increase of N may reveal whether M preference is increased as is implied in Figure 5B.

These different phenotypes of the divergent set of *C. elegans* may have lasting impact on future studies surrounding the effects of the npr-19 receptor. Modulations of the receptor, which may be responsible for different reward seeking behaviors, may influence nociception and locomotion behaviors as well. This could deepen our understanding of multiple behaviors and their mechanisms all by utilizing *C. elegans*.

Future Directions

This paper has established that the broad sense heritability of hedonic amplification is 70.8 percent in *C. elegans*. Therefore 70.8 percent of variation in this trait is statistically associated with genetic variation within the different *C. elegans*natural isolates. This provides a basis for future genome wide association studies

to examine the possible genes that underpin hedonic amplification in *C. elegans* and eventually humans. This data can be utilized for future studies in that it lays the framework of 12 phenotyped strains shaving the potential scale of future studies.

APPENDIX

The human nervous system

The nervous system is the necessary combination of organs responsible for perception, thought, behavior, and feelings. Composed of two parts: the central nervous system (CNS) and the peripheral nervous system (PNS), the focus of this paper is the CNS. The CNS is anatomically separate from the PNS, although both are deeply interrelated, and is composed of the brain and spinal cord.

Function of the central nervous system is characterized in five stages[1]. The first of which is internal (visceral) and external (peripheral) receptors sensing changes in their environment. Secondly these signals are sent to the spinal cord or brain. Thirdly the information is integrated and processed in various sections of the brain depending on the type of information encoded. The number and interconnectedness of the regions of the brain which process this information is dependent on the complexity and type of information. Fourthly the brain sends commands to the peripheral systems such as motor commands often through the autonomic nervous system (ANS). Lastly the system's effectors are signaled to alter activity or state of the target organs to lead to potential behavioral changes.

The cell of the nervous system is the neuron. Unlike most other cells of the body, neurons do not undergo mitosis. The neuron is an electrically excitable cell which receives, processes, and sends information to other neurons or tissues. A neuron is typically composed of a cell body or soma, has one long projection called the axon, and many branching projections called dendrites. The activity of a neuron is electrical,

however communication between neurons and cells is chemical: through neurotransmitters. Processing at a neuron occurs at the cell body, where the nucleus resides. Information uptake occurs at the dendrites, while information transmission occurs at the axon. These systems of neurons are often referred to as neural networks. The human brain is estimated to have approximately 100 billion neurons, where each neuron is connected to around 10,000 other neurons[1].

Axons often come into close contact with the ends of dendrites forming a synapse. The end of axons are terminal buttons, the ends of which are calledpresynaptic clefts, which receive the electrical signal of the axon and release neurotransmitters. Neurotransmitters are the messengers of the nervous system and are taken up by the dendrite via receptors that correspond to the released chemical at the postsynaptic cleft. Examples of neurotransmitters are acetylcholine, dopamine, gamma-Aminobutyric acid (GABA) and serotonin. Neurotransmitters can be inhibitory or excitatory at the dendrite depending on the action of their receptor. Excitatory signals activate the neuron, and inhibitory signals deactivate the neuron or hyperpolarize it. Both of these signals cause a change in the electrical potential of the neuron.

Neurons can be active or resting. When a neuron is resting there is a larger ratio of negative ions to positive ions inside the neuron than outside the neuron which causes it to have a resting membrane potential. When a signal activates a neuron, the ratio of ions changes causing an electrical signal to be sent down the axon, this signal is called an action potential. However, action potentials are called all-or-none, the signal cannot be a partial signal. A certain level of membrane potential must be reached for an action potential to be fired.

Most neurons are part of three basic types: sensory, motor, and interneurons. Sensory neurons are responsible for detecting information from the external environment and synapsing with the spinal cord and are often called afferent neurons. Motor neurons are directed to the muscle from the spinal cord to elicit contraction or relaxation to generate movement; they are often called efferent neurons. Interneurons form local circuits among other neurons, and do not travel as long of distances as sensory or motor neurons.

Neurons can release different neurotransmitters for varied effects[2]. For example acetylcholine has many important functions including motor control, learning, and memory. These effects are modulated by what receptors are found at the postsynaptic cleft[3]. However, these receptors can be activated or blocked by different substances which are called agonists and antagonists. Agonists are chemicals which enhance or activate the receptor mimicking or promoting the neurotransmitter effect. Antagonists blocks the receptor and decreases or ceases the effect of the neurotransmitter. The method of agonist and antagonist effects are varied[1]. Agonists can increase neurotransmitter release, block neurotransmitter reuptake, or mimic the neurotransmitter. Antagonists can block the release of neurotransmitters, destroy neurotransmitters, or mimic the neurotransmitter without activating the receptor.

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