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Behavioral Economic Demand: Investigation of Sex Differences in Four Core Genotype Model of Oral Oxycodone Self-administration

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Abstract

Opioid misuse has been identified to be related to problematic release of dopamine in the brain that is otherwise satiated by other alternative rewards. As mediated by patterns of positive and negative reinforcement, prolonged opioid use may transition into misuse and development of opioid use disorder. In human populations, men and women differ in their acquisition of drug use and escalation to drug misuse. Women quickly surpass their male counterparts in their transition from use to misuse in what is coined the *telescoping effect.* In this four core genotype model of oral oxycodone self administration, males were hypothesized to consume more total oxycodone in comparison to females during acquisition of drug behavior. Additionally, XX mice, regardless of gonadal sex, were hypothesized to be less sensitive to price increases as summarized in demand value alpha, as compared to XY mice. Subject consumption and price were used to create demand curves and parameters using the package in R. Average consumption in price were differentiated for every combination of sex, chromosome, complement, and gonadal sex as represented in the mouse model. This allows the generation of four averaged demand curves to reflect alpha value. There were no observable signifivcant differences in total consumption. Largely no main effect differences were observed to be due to gonadal sex or sex chromosome complement in referenc to total oxycodone consumption. When observing mean values of alpha, there is no main effect significance between male and female subjects. However, there is a difference between XX and XY females where there is a two-fold increase in XX females (sig 0.053). Within the limitations of the timeline of this present model, further research would need to be conducted to preserve the findings of atypical female consumptions at beginning stages of problematic use.

Introduction

Addiction is a phenomenon that occurs in a subset of individuals who initiate use with a potentially addictive substance. It is presented as a cycle of spiraling dysregulation of brain reward systems that progressively worsens, resulting in compulsive use and loss of control over drug-taking (Koob & Le Moal, 2001). In general, drugs can act through positive reinforcement, such as producing euphoria, or as negative reinforcement, through alleviation of withdrawal or dysphoria (Camí & Farré, 2003). According to the U.S. Department of Health and Human Services, approximately 92,000 individuals in the United States have experienced fatal drug-related overdose in 2020. In the same timeframe, death from synthetic opioid overdose, excluding methadone, soared to 56,516 individuals, accounting for a substantial amount of the total casualties due to drug overdose within the United States (NIDA, 2022). These drugs, including fentanyl and carfentanil, are much stronger than their natural counterparts and can cause rapid onset of addiction, tolerance, and overdose.

Opioids are commonly used for pain management in the clinical setting, however, a subset of individuals go on to misuse prescription or illegal opioids. A proportion will develop opioid use disorder (OUD), characterized by periods of chronic use followed by attempts to discontinue and devastating relapse, and/or opioid dependency, characterized by physical or psychological dependence; oftentimes, individuals may experience both (Dydyk et al., 2022). Popular opioids, such as morphine, heroin, and prescription analgesics such as Vicodin and OxyContin all have unacceptably high misuse liability due to their potential to cause euphoria and manage pain (Veilleux et al., 2010).

In the brain, opioids evoke dopamine release into reward structures through disinhibition. Opioids inhibit the activity of GABA-inhibitory interneurons in the ventral tegmental area (VTA), which results in enhanced dopamine release in the nucleus accumbens (NAc). Repeated opioid exposure elicits compensatory neuroadaptations that leave individuals vulnerable to the effects of tolerance, in which more opioids are needed to produce the same release of dopamine within the NAc, and dependence, causing negative symptoms of withdrawal in those who discontinue opioid use after opioid neurons eventually become less responsive to excitatory stimulation (Kosten & George, 2002). To counteract drug tolerance, individuals may increase the dose from what they have taken before or reduce the time between subsequent doses (Camí & Farré, 2003). As a consequence of chronic opioid abuse, many individuals find themselves at the crossroads of jeopardizing their financial, interpersonal, and individual priorities (Dydyk et al., 2022).

Addiction

Drug addiction is characterized by a chronic pattern of compulsive drug-seeking and taking despite the existence of negative consequences (Camí & Farré, 2003). Positive reinforcement occurs when the previous experience of a drug increases the probability that an individual will attempt to obtain the drug again as associated with the drug's pleasurable effects (Koob & Le Moal, 2001). Negative reinforcement occurs when an aversive state, such as drug withdrawal, is alleviated by taking the drug again (Koob & Le Moal, 2001). Even when the drug is unavailable, environmental cues can induce drug craving and withdrawal causing conditioned responses from the affected individual (Camí & Farré, 2003). Through the initiation phase, drugs often induce euphoric effects on the body, which reinforces continuous use (Camí & Farré, 2003). Through continuous use, the brain is hypersensitized

to the direct effect of the drug, as well as the environmental cues which provoke drug craving during times of drug absence (Camí & Farré, 2003).

Pharmacologic properties of drugs may influence the onset and severity of drug misuse and dependence by factors such as liposolubility, time to onset of action, intensity, and half-life (Roset et al., 2001). Dependence is a hallmark criterion for opioid addiction, causing individuals to lose pleasure in otherwise rewarding activities such as eating or sex as well as leading to compulsive drug-seeking behaviors (Kosten & George, 2002). Chronic drug misuse causes changes within the brain that lead to craving and the possibility of relapse even years after detoxification as mediated by environmental stressors and drug-related cues (Camí & Farré, 2003). With acute opioid use, the risk of tolerance and dependence is relatively low, as the drugs are typically used for a short period of time. However, tolerance and dependence can develop with chronic use, leading to the need for higher doses of the drug to achieve the same pain relief (Hooten et al., 2015). Another key difference is the potential for opioid-induced hyperalgesia (OIH), which is a paradoxical increase in pain sensitivity that can occur with chronic opioid use. OIH can make chronic pain worse and can lead to increased opioid use, which can exacerbate the problem (Wilson et al., 2021).

Opioid Use Disorder

Opioids are defined as any substance that binds to opioid receptors within the body, and it can be composed of both natural and synthetic opioids. Endogenous opioids, or those that are naturally produced by the body, are vital to reinforcing rewarding habits for survival (Veilleux et al., 2010). Opioids are capable of producing pain-relieving effects without producing subsequent feelings of anesthesia or altering level of consciousness (Lopresti

2020). Opioids have the potential to produce euphoric effects through the inhibition of GABA neurons which disinhibit dopamine projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) and prefrontal cortex (Lopresti 2020). Opioid addiction is characterized by dysregulation of the mesolimbic reward system resulting in compulsive use and 'loss of control' or loosely defined *involuntary* drug consumption by affected individuals (Koob & Le Moal, 2001) who are further unlikely to cease drug-seeking and taking behaviors after initiation despite the greatest efforts, causing an increased risk of relapse back into addictive behavior. As a result, critical brain regions responsible for reinforcing pleasurable experiences are left sensitized to drug and drug-linked stimuli (Robinson & Berridge, 2000).

The point at which pleasure is achieved, or the *hedonic set-point*, arguably readjusts depending on the cycles of administration of a drug, in which the individual is inevitably counteracting the effects of the drug through positive and negative reinforcement (Koob & Le Moal, 2001). Symptoms of opioid withdrawal include hypertension, tachycardia, abdominal pain, agitation, shaking, and diarrhea. Individuals suffering from opioid intoxication may experience confusion, pupil constriction, nausea, constipation, and hypoalgesia. In the severest of cases, individuals who are suffering from an opioid overdose may become unresponsive, hypothermic, or bradycardic (Dydyk et al., 2022). One of the main ways that opioids cause overdose is by depressing the respiratory system, leading to a decrease in breathing rate and depth. This can result in a lack of oxygen to the brain and other vital organs. Opioids can be particularly dangerous when combined with other drugs, such as benzodiazepines or alcohol, which can increase the risk of respiratory depression and overdose (Palkovic et al., 2020).

Polysubstance abuse is a rising hurdle in the treatment of opioid use disorder, as many affected individuals demonstrate comorbid nicotine dependence at rates upward of 92% and often also meet criteria for alcohol, cannabis, stimulant, and sedative abuse disorders as well (Veilleux et al., 2010). Comorbidity of opioid abuse with affective disorders such as depression and bipolar disorder, while also especially prevalent in those suffering from post-traumatic stress disorder (PTSD) and antisocial personality disorder (APD) (Veilleux et al., 2010). Individuals affected by personality and psychiatric disorders which affect risk-taking or novelty-seeking behavior favor drug-taking (Helmus et al., 2001). Genetic factors are also heavily indicated in the rate of metabolism and the effect of drugs within the body which may affect an individual's likelihood of developing a substance use disorder (Camí & Farré, 2003). Medication-assisted treatment options, such as methadone or buprenorphine clinics, offer decreased withdrawal effects and may show limited efficacy, especially when combined with behavioral therapy (Lopresti 2020). Opioids are medically prescribed for pain relief, commonly including morphine, codeine, and oxycodone.

Dopamine Reward Systems

Rewards are goals that an individual will allocate time, energy, or effort to seek. The behavioral definition of reward also includes those which do not support elementary processes such as nutritional satiation or reproduction, such as gambling (Arias-Carrión et al ., 2010). Studies utilizing select lesioning, dopamine agonists or antagonists, and electrical self-stimulation have shown dopamine projections to the striatum and frontal cortex are essential for mediating reward and learning. (Arias-Carrión et al., 2010). Motivation to return to previous rewards and the environment in which previous rewards were received is largely

learned and reinforced by dopaminergic action. (Arias-Carrión et al., 2010). Once associations of reward and stimulus have been established, these motivations can still remain responsible for behavior despite efforts of devaluation (Arias-Carrión et al., 2010). The dopamine reward system includes the mesolimbic and mesocortical pathways. The mesolimbic pathway arises from the ventral tegmental area (VTA) and projects to the nucleus accumbens (NAc) and the olfactory tubercle. Natural rewards, such as sex, stimulate the mesolimbic pathway to produce euphoria and reinforcement of this pleasurable behavior (Camí & Farré, 2003). The mesocortical pathway also includes the VTA which then projects to the prefrontal, cingulate, and perirhinal cortex. Collectively, these pathways are known as the mesocorticolimbic system (Arias-Carrión et al., 2010). Five G protein-coupled receptors are responsible for the physiological actions of dopamine. Adenylyl cyclase is activated through D1A-1D and D5 (D1-like) receptors. D2, D3, and D4 (D2-like) receptors are Gi protein-coupled receptors that inhibit adenylyl cyclase and activate potassium channels . doi: (Arias-Carrión et a;., 2010l) The concentration of D2-like receptors is higher in the nucleus accumbens as opposed to D1-like receptors (Arias-Carrión et al., 2010l) Dopamine is released outside the synaptic cleft where it then diffuses in the extracellular fluid (Arias-Carrión et a;., 2010l).

Short-term administration of opioids causes the inhibition of adenylyl cyclase, cyclic adenosine monophosphate levels, and subsequent cAMP-dependent protein kinase A activity. Meanwhile, chronic administration causes upregulation of cAMP pathways, increased activity of adenylyl cyclases, and increased cAMP-dependent protein kinase activity. In the NAc, upregulation of cAMP following chronic opioid abuse causes downstream activation of kappa-opioid receptors in the VTA, striatum, and NAc and decrease of DA release,

contributing to dysphoric effects when the individual attempts to discontinue drug-taking (Nestler, 2001). In response to opioid tolerance, the body downregulates the number of opioid receptors and continuous use will lead to the internalization of opioid receptors (Nestler & Landsman, 2001). Opioids cause structural changes to the dendrites and soma of dopamine neurons inside the VTA by decreasing their size (Sklair-Tavron et al., 1996).

Sex Differences

Men are more likely than women to use and misuse drugs overall. However, women tend to progress more quickly from initial use to addiction, and may experience more severe physical and psychological consequences of drug use (Serdarevic et al., 2017) . Women are at greater risk for the development of substance use disorders in comparison to men due to the rapid escalation in drug use and shorter periods of abstinence during recovery (Harp et al 2020). Social and cultural factors can play a role in drug misuse among men and women, with gender-specific norms and expectations influencing patterns of use and help-seeking behavior (Serdarevic et al., 2017). The effects of stimulant use may vary across the menstrual cycle due to varying levels of estradiol (E2) (Harp et al., 2020). Pregnancy can also be a significant factor in drug misuse among women, as substance use during pregnancy can have serious adverse effects on the developing fetus (Krans & Patrick 2016). In cocaine studies, females todents acquire cocaine self-administration faster, at lower doses, and are more motivated to use cocaine versus males (Harp et al., 2020). In further studies, the reinforcing effects of cocaine are heightened with levels of E2 and progesterone are low, such as during adolescence and proestrus (Harp et al., 2020). Acquisition of cocaine self-administration in rats is markedly reduced by ovariectomy and restored by E2 replacement. (Harp et al., 2020). In electrophysiology studies, female mice show increased firing of dopamine (DA) neurons

in the ventral tegmental area (VTA) during estrus/proestrus when E2 levels are the highest (Harp et al., 2020). After gonadectomy, female mice still acquire cocaine self-administration more rapidly than their male counterparts (Harp et al., 2020), suggesting circulating hormones alone do not fully explain sex differences noted across the literature.

In comparison to men, women increase their rates of consumption of drugs of abuse more than men in the following substances, marijuana, cocaine, opioids, and alcohol (Becker & Hu 2008). In past literature, sex differences in the brain were thought to be due to gonadal hormone differences during development or varying levels in adult animals. However, sex chromosome complements (XX versus XY) have recently shown their potential in explaining cases in which sex differences exist prior to the onset of sex-specific gonadal secretions (Becker & Hu., 2008). The escalation from drug use to meeting criteria for a substance use disorder is influenced by innate characteristics such as biological sex and individual sensitivity to drug. Other social and environmental factors also have an immense impact including socioeconomic status, education, history of abuse or trauma, social constructs, and gender expectations (Becker & Hu 2008). As affected individuals transition from drug initiation to compulsive drug use, women have a higher tendency to escalate to compulsive drug behavior in what is coined as the *telescoping effect*. (Becker & Hu 2008). Women transition more rapidly from opioid use to dependence and experiences of withdrawal and cravings are often stronger as opposed to their male counterparts (Lopresti 2020) Hormones including progesterone, testosterone, and estradiol play vital roles in regulating ovulation, stimulating spermatogenesis, and sperm production. These hormones are released in response to pituitary hormones luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in males and females (Becker & Hu 2008). Organization of neurocircuitry during development

is mainly affected by circulating hormones, chromosome differences, and sex differences in the placenta. However, sex differences may also be due to activational effects of readily circulating gonadal secretions (Becker & Hu 2008). Women are more likely to feel more depressed, anxious, and have more past medical problems than men who are opioid-dependent (Manubay et al., 2015). Women are more likely to use prescription opioids to cope with interpersonal stress while men are more likely to abuse opioids due to legal and behavioral problems (Manubay et al., 2015). According to pharmacy records, women are more likely to be prescribed opioids than men and likely to take higher doses than their male counterparts (Manubay et al., 2015). Intake of opioids is also different for men and women. While women are more likely to receive their opioids from a prescription, men are more likely to receive from family and friends or purchase them. Men are also more likely to alter their routes of administration, such as inhalation or intravenous injections versus women who are prone to administer as-advised (Manubay et al., 2015).

The translocation of the testis-determining *Sry* gene off of the Y chromosome and to an autosome allows for sex chromosome complement and gonadal sex to be independent of eachother in the four core genotype model (Becker & Hu 2008). Gonadal secretions were identified as causal agents of sexual dimorphism following experimental studies which interfered with such secretions (Arnold & Chen 2009). In the 1959 experiment of Pheonix et al., organizational, or permanent, effects of gonadal excretions were observed to produce differentiating effects on the brain (Arnold & Chen 2009). Activational effects, or acute effects of gonadal steroids, are observed throughout the lifetime and may be manipulated in adulthood by gonadectomy (Arnold & Chen 2009). The organizational-activational concept has grown as a popular explanation for sex differences (Arnold & Chen 2009).

Organizational effects may be studied through intentional masculinization through injection of the hormone testosterone or feminization through deprivation of naturally-produced testosterone (Arnold & Chen 2009). Activation effects may be studied through acute manipulation of circulating gonadal secretions, such as gonadectomy or hormone replacement. To date, the primary approach to studying activation effects is the alteration of gonadal hormone levels, either directly or indirectly through receptor or enzyme manipulation (Arnold & Chen 2009). Much previous research has used only male subjects, some have failed to report the sex of their subjects entirely. It is now known that sex may have influence on neural and behavioral phenotypes (Arnold & Chen 2009). Translation of experimental research conducted in only male subjects confounds results that may not be generalizable to female subjects. Not all sex differences may be attributed to gonadal hormones as evidenced by sex differences that exist before differences in levels of gonadal hormones during development or when traditional hormonal manipulations failed to cause sex reversal (Arnold & Chen 2009). Sex steroids play a major role in organizational and activational effects through three major classes. Progestins, mainly progestin in mammals, androgens, including testosterone and dihydrotestosterone (DHT), and estrogens, such as estradiol (Lopresti 2020). Activational effects are largely seen during development, in which the presence or absence of sex hormones leads to permanent changes while continuously circulating hormones throughout development or into adulthood are responsible for semi-permanent activational effects (Lopresti 2020). Dopaminergic nigrostriatal neurons are influenced directly by the Y-linked gene *Sry* which regulates tyrosine hydroxylase levels in the mesencephalon and indirectly influences dopamine levels (Arnold & Chen 2009).

L-Tyrosine is converted to L-3,4-dihydroxyphenylalanine (L-DOPA) which is the precursor to dopamine.

The laboratory mouse offers a readily available model for human biology and disease. Much of this is owed to their physiological similarity and the ability to manipulate genetic strains for scientific research. Animal models of research provide similarities with human neurochemical and neuroanatomical influences involved in addictive behavior. The propensity to develop medications or translate into the human clinical setting is seen through the validity of animal model use. The translation of findings from animal models to human addiction is not always straightforward, and there may be differences in the pharmacology, behavior, and genetic background between mice and humans that limit the applicability of results. Firstly, the efficacy of conducting behavioral economic demand analysis using the four core genotypes (FCG) model in the analysis of oral oxycodone self-administration will offer the baseline from which the influence of intermittent drug access on incentive motivation through time. The FCG model allows a unique opportunity to further implicate differences in gonadal sex and sex chromosome complement (XX and XY) (Arnold & Chen 2009).

Four core genotype (FCG) mouse model have been previously used in sex differences studies examining stroke, immunity, obesity, and cardiovascular function (Harp et al 2020). In food reward studies of FCG mice, XX mice continue responding for food even when the response is associated with an aversive stimulus as opposed to XY mice, regardless of gonadal sex, adding that sex chromosome complement may have an impact on habit formation (Arnold & Chen 2009). In previous studies of food restriction in the FCG model, XX mice with and without gonads acquired the task to receive food reward faster than XY

mice. Even after the devaluation of the food reward with the pairing of an aversive stimulus, in this case, lithium chlorite, the XX mice continued working for food (Harp et al., 2020). In the FCG model, DA receptor D2 (*DRD2*) and prodynorphin (*Pdyn*) were expressed higher in the adult striatum in XX mice versus XY mice (Harp et al 2020). Independent of gonadal sex, XX mice are found to acquire food-reinforced habit faster than XY mice implicating independent effects between sex chromosome complement and gonadal hormone secretions (Becker & Hu 2008). Drug-naive animals, both male and female acquire oxycodone self-administration behavior at comparable rates but males are more likely to consume more drug during the acquisition of drug behavior (Becker & Hu 2008).

Demand

Behavioral economics is a term used to describe the mix between traditional economic concepts and behavioral psychology (Reed et al., 2013). Consumption refers to the overall amount of a commodity obtained within a session. Cost refers to the number of responses to obtain a commodity. Unit price is defined as the relationship between cost and benefit typically reported as a ratio (Reed et al., 2013). The law of demand states that consumption will typically decline when the unit price of a given commodity increases (Reed et al 2013). A demand curve summaraizes consumption (Q) as a function of price (P) (Newman & Ferrario 2020). Consumption which is stable across price changes is considered to be inelastic demand while elastic demand demonstrates changes in consumption as a function of price (Reed et al., 2013). Elasticity of demand (E) varies with changes in price and is also widely used in human economics as a measure of the price sensitivity of goods (Newman & Ferrario 2020).

Hypotheses

1. Males, regardless of sex chromosome complement, were hypothesized to consume more total oxycodone.
2. XX mice, regardless of gonadal sex, were hypothesized to be less sensitive to increases in price, operationalized as α.

Methods

Subjects

To examine the contributions of sex-chromosome complement (SCC) and gonadal sex (GS), the four core genotype (FCG) mice were used as a genetically modified strain of C57BL/6J background. Offspring were generated by crossing an XY mouse with a deleted endogenous *Sry* gene and an autosomal *Sry* transgene to XX females. No subjects were single-housed and mice were grouped based on the same gonadal sex. All subjects were cared for according to standard practice protocols set by the Institutional Animal Care and Use Committee at Binghamton University. Prior to the training protocol, all subjects had free access to food and water within their home cages. All subjects underwent earclipping during weaning and genotypes were determined by processing earclipped samples through Transnetyx. All subjects were houses according to a 12-hour light and dark cycle.

Training Protocol

All subjects were acclimated to an operant training schedule which allowed them to learn how to respond for rewards within the operant chamber. Water was used as the reward

during all operant training. The first phase is magazine training, which allows the subject to become familiar with the typical stimuli during the delivery of a reward including sounds, smells, lights, etc. During this phase, the active lever extends into the chamber, and a reward is delivered to the magazine 5 seconds later (regardless of lever activation; i.e., no lever press is required for rewards). During the delivery of a reward, the house light turns off, the magazine light illuminates, and 25 µL of water is delivered into the magazine. A reward is considered to be collected when the infrared beam across the magazine is broken by a head entry from the mouse, which also causes the active lever to retract, the magazine light to turn off, and the house light to turn on. The cycle begins again with lever extension 25 seconds after a reward is collected.

During the second phase of training, the active lever is extended at the beginning of the session and remains available throughout. Pressing the lever results in the delivery of a reward according to a FR1 schedule. Presses made when a reward is currently available are recorded, but do not produce an additional reward delivery. During the third phase of training, again only the active lever is available and results in the delivery of a reward on an FR3 schedule. The FR3 schedule is maintained for the rest of the training protocol and kept during further demand analysis. During the fourth and last phase of training, both the active and inactive levers are available. Active lever presses produce a reward on an FR3 schedule, and inactive lever presses have no programmed consequences. From here, the subjects learn to differentiate between the active and inactive levers within the operant chamber. Subjects continue to respond within a specific training phase until 30 rewards are delivered. Throughout these phases of training, mice were food restricted to approximately 85% of their starting weights; this encouraged the subjects to respond to water as a reward. Post-prandial

induction can impact responding for rewards, especially *like-rewards*. In this case, there is a difference between responding for water during the training procedure versus saccharin during the demand protocol.Once all subjects have succeeded within the training protocol, they are passed onto the demand assessment.

Demand Protocol

All subjects transitioned from the training protocol to the demand protocol. The demand assessment occurs in two sessions (the morning session and afternoon session) which elapses for two hours each. Between the morning session and afternoon session is a two-hour break where animals are returned to their home cages and again have access to water. The timeline for the demand assessment lasts for eighteen consecutive days with two sessions each day. During the morning session, subjects only have access to saccharin as a reward; a sweet, non-caloric output. During the afternoon session, subjects have access to oxycodone or water which is previously determined and remains the same rewards tested throughout the duration of the demand protocol. During the two hour break each day, subjects do not have access to sahharin or oxycodone. The scope of this experiment will only focus on oxycodone consumption and demand parameters. The concentrations of oxycodone used were 0.05, 0.1 and 0.5 mg/mL as abbreviated as low, medium, and high concentration respectively. The concentrations of saccharin used were 0.01, 0.1 and 1 mg/mL as also abbreviated as low, medium, and high concentrations respectively. Prices, or epxenditure of work from the subject by pressing the response lever, were adjusted by changing the reward volume and concentration of reward. The reward volume is altered by adjusting the speed of the reward pump and minute changes based on the subject's daily body weight were also considered. As a result, nine different prices were examined as summarized in *Table 1.* Each

price is examined twice during each cohort and also tested on subsequent days (ex. Days 1-2 would encompass once price, days 3-4 would encompass a different price.) Prices were not given in ascending or descending order but were randomly assigned. Individual consumption of oxycodone and price were used to create demand curves for each subject using the *beezdemand* package in R (Kaplan et al., 2018) as well as visually-representative demand curves for averaged consumption per price for each genotype group.

Statistical Analysis

A 2x2 ANOVA was analyzed with sex chromosome complement and gonadal sex as the independent variables, and individual subject alpha and total consumption as the dependent variables. Main effects and interactions were evaluated.

Results

No main effect on total oxycodone consumption was observed as a function of gonadal sex [F(1,16)=0.218, p=0.0647], sex chromosome complement [F(1,16)=2.467, p=0.0136], or interaction between SCC*GS [F(1,16)=3.178, p=0.094]. Hypotheses one is not supported since no significant differences in total oxycodone were observed between males and females, XX versus XY mice, nor any interaction between SCC*GS. This is graphically represented in *Figure 1*.

When observing mean values of alpha, there is no main effect of gonadal sex [F(1,16(-0.017, p=0.897]. However, there is a SCC*GS interaction in alpha [F(1,16)-5.285, p=0.035]. Post-hoc comparisons show a higher alpha value in XXF than in XY F (p=0.053), indicating that XXF mice are more sensitive to price increases than XYF mice. This is

graphically shown in *Figure 2*. Hypothesis two is also not supported as there is no evidence to suggest that XX SCC pairs with a significantly lower alpha value. Figures 3-6 visualize the demand curves created for each genotype using the averaged values of total oxycodone consumption. It is important to note that the ANOVA was run on each individual subject (n=20) and the corresponding parameters reported (alpha) are also based on individual subjects.

Discussion

The philosopher and economist, John Stuart Mill, assumes that humans will exhibit behavior which maximizes their gain and that they are fully aware of the costs, benefits, and alternatives to their decisions (Reed et al., 2013). Behavioral economists assume irrational behavior in decision making as well and may serve to explain *why* these decisions are made (Reed et al., 2013). Undoubtedly, real-life differences between men and women exist in terms of their drug acquisition, experience of withdrawal symptoms, and likelihood of transition into problematic use. Men are more likely to report higher lifetime and past-year use of all opioid drugs, however, women are more likely to report non-medical use of prescription opioids as their primary drug of abuse (Kokane & Perroti 2020). Women are more likely than men to transition more rapidly from casual non-medical use of prescription opioids to developing opioid dependence (Kokane & Perroti 2020).

In the present study, gonadally-intact female mice were observed to consume more oxycodone than their male counterparts suggesting that perhaps there is evidence against the typical report of higher overall consumption in males. However, these findings were not statistically significant. Within this model of drug acquisition, it was not hypothesized that females would escalate their consumption as compared to males in the beginning stages of

substance use. The confluence of information between reproductive and pain literature suggests that circulating gonadal hormones, opioid receptors, and sex chromosome complement are all indicated in observed sex differences in opioid addiction (Kokane & Perroti 2020). The present model targets early stages of drug use, so further research would need to be conducted to evaluate atypical female consumption at different stages of problematic use to fully investigate the neurobiological mechanisms of the *telescoping effect*.

Price Sensitivity

The success of treatment programs to address substance use disorders are largely dependent on the funding that they recieve from public and private means. Now, the funding for these programs is linked with cost and performance measures in an effort to continue reliable funding. There is some evidence to suggest that women are more likely than men to decrease their drug use in response to increases in the price of drugs (Zarkin et al., 2004). Another student corroborates the reduction in drug use in women experiencing economic constraits in comparison to men (French et al., 1997). In the present model, although a statistically significant interaction effect was not expected, a difference in alpha value adds to the importance of differentiation between SCC and GS to corroborate further studies of sex differences. In human studies, we see that monetary expenditure may have great impacts on an individual's decision to engage in drug consumption. For example, women are more likely than men to reduce smoking in response to changes in the price of a pack of cigarettes (Ross et al., 2003). Even when both men and women are enrolled in treatment programs, women are more likely to decrease use of illicit drugs when the price increases (Zarkin et al., 2004).

Present Sex Differences

Women are more likely to experience aversive drug reactions to approved medications than men. Adverse drug reactions in women are anywhere from 50-75% higher in women than men (Gavins et al., 2021). Women are more likely than men to report more past medical history and be unemployed. Naturally, the approach to substance use disorders needs to encompass a multifaceted problem (Back et al., 2011). There is concern that perhaps women are given inappropriate, often increased doses than needed or polymedicated which leads to about 60% of all hospitalizations due to adverse drug reactions being attributed to women (Gavins et al., 2021). Women are more likely than men to report current and past history of psychiatric problems such as depression and anxiety (Back et al., 2011). Women are more likely to have higher rates of comorbidities associated with substance use disorders, many of which precede initiation of drug use. This suggests the possibility of coping with negative affect consistent with the self-medication hypothesis (Back et al., 2011). Disaggregation of data according to sex differences hopes to strengthen the scientific rigor to propose individualized treatment plans. Underrepresentation of women in human and animal studies is reported across the field of neuroscience (Gavins et al., 2021). With these findings, it is evident that behavioral neuroscience research will need to encompass more considerations for the possibility of sex differences to attenuate major challenges faced by women who suffer from substance use disorders. An ode to the social differences which between men and women are also important to acknowledge as powerful mediators of drug acquisition, drug experience, and treatment success.

Severe Addiction Phenotype

Substance use disorders are a complex and multifaceted condition which may ultimately affect an indivudual in unique ways. Not all individuals will transition to the

development of a severe addiction phenotype which may be mediated by many factors. The present study is limited in the capacity to produce a severe addictive-state not only by the duration of access to oxycodone, but also by the concentrations and volumes delivered to each subject. An interesting avenue for further discussion on validity of the oral oxycodone self-administration model in the FCG group may be necessary for defining subject-specific physical attributes to opioids. Evidence of objective physical findings in humans and the mouse model are different, such as the *straub tail response* in mice. One step further, an investigation of precipitated withdrawal may help researchers address the possibility of sensitivity differences within the FCG within this model. Notably, female mice are noted to exhibit more severe withdrawal symptoms such as agitation, hyperactivity, shakiness, etc. In mice, precipitated opioid withdrawal has been studied using the hosplate test before and after naloxone administration.

Adolescent Vulnerability

Additionally, the adolescent population arises as an important topic of study due to their vulnerability to opioid use. Current drug treatment programs, such as medication-assisted programs, are limited to adults. It may be true that the approach to adolescent drug misuse is different than adults and represents an understudied topic. To date, there are stark limitations in addressing possible sex differences in the adolescent popuation. Use of the FCG model along with adolescent mice may prove to be a worthy future direction. In humans, treatment programs may require that the patient is able to consent to treatment, whereas an adolescent would not maintain their rights to treatment consent unless medically emancipated. This highlights an important social caveat where adolsecences may not feel ready to share that they would like treatment for a substance use to their parents or guardians

or instances where these connections are nonsupportive. Despite these challenges, drug treatment opteions fortunately do exist for adolescents and provide a valuable opportunity for parental invovlement that may prove to be successful towards the path of recovery.

Table 1.

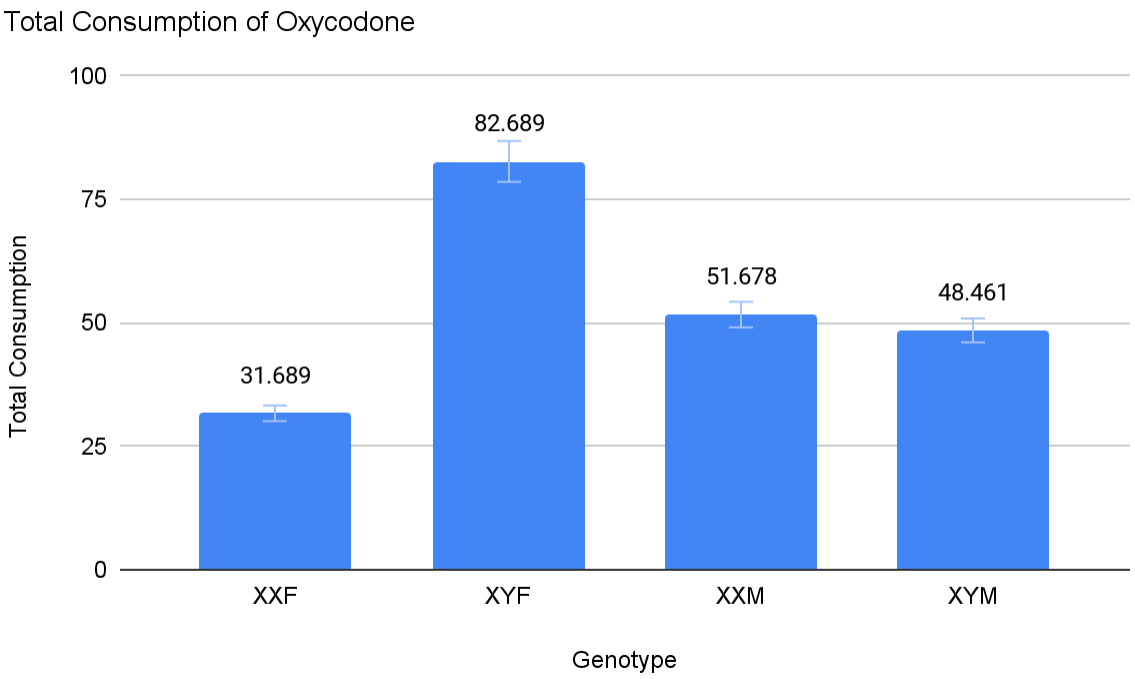
Concentrations and Volume Manipulations of Oxycodone

PRICE (responses/mg/kg)	1.9	4.8	9.6	12.4	19.6	24.5	48.8	61.2	121.8
VOLUME VOLUME (ml/reward)	0.05	0.025	0.05	0.01	0.05	0.025	0.025	0.01	0.01
CONCENTRATION (mg/ml)	0.5	0.5	0.1	0.5	0.05	0.1	0.05	0.1	0.05

Note. Prices are adjusted by changing the reward volume and concentration of oral oxycodone. Each price is examined twice during each cohort and prices are not presented in asencind or descending prices. Each price was evaluated on consecutive days acrossan 18-day experiment.

Figure 1.

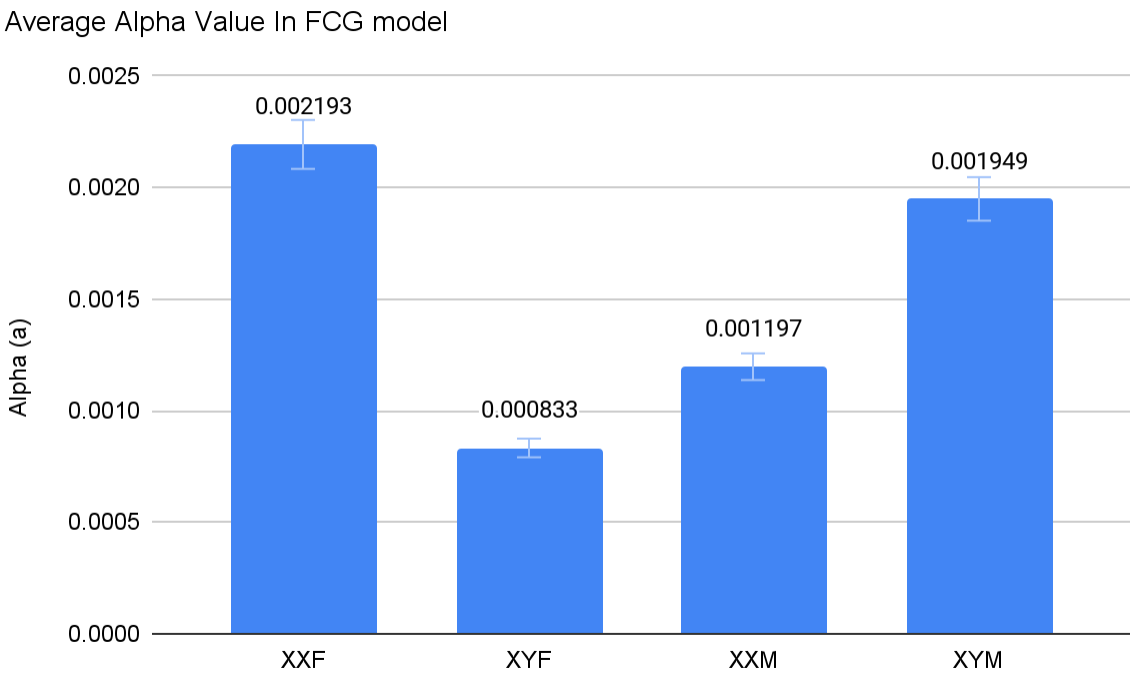
Average Total Consumption Across FCG Genotypes



Note. Number of subjects per group =5. No main effect on total oxycodone consumption was observed as a function of gonadal sex [F(1,16)=0.218, p=0.0647], sex chromosome complement [F(1,16)=2.467, p=0.0136], or interaction between SCC*GS [F(1,16)=3.178, p=0.094].

Figure 2

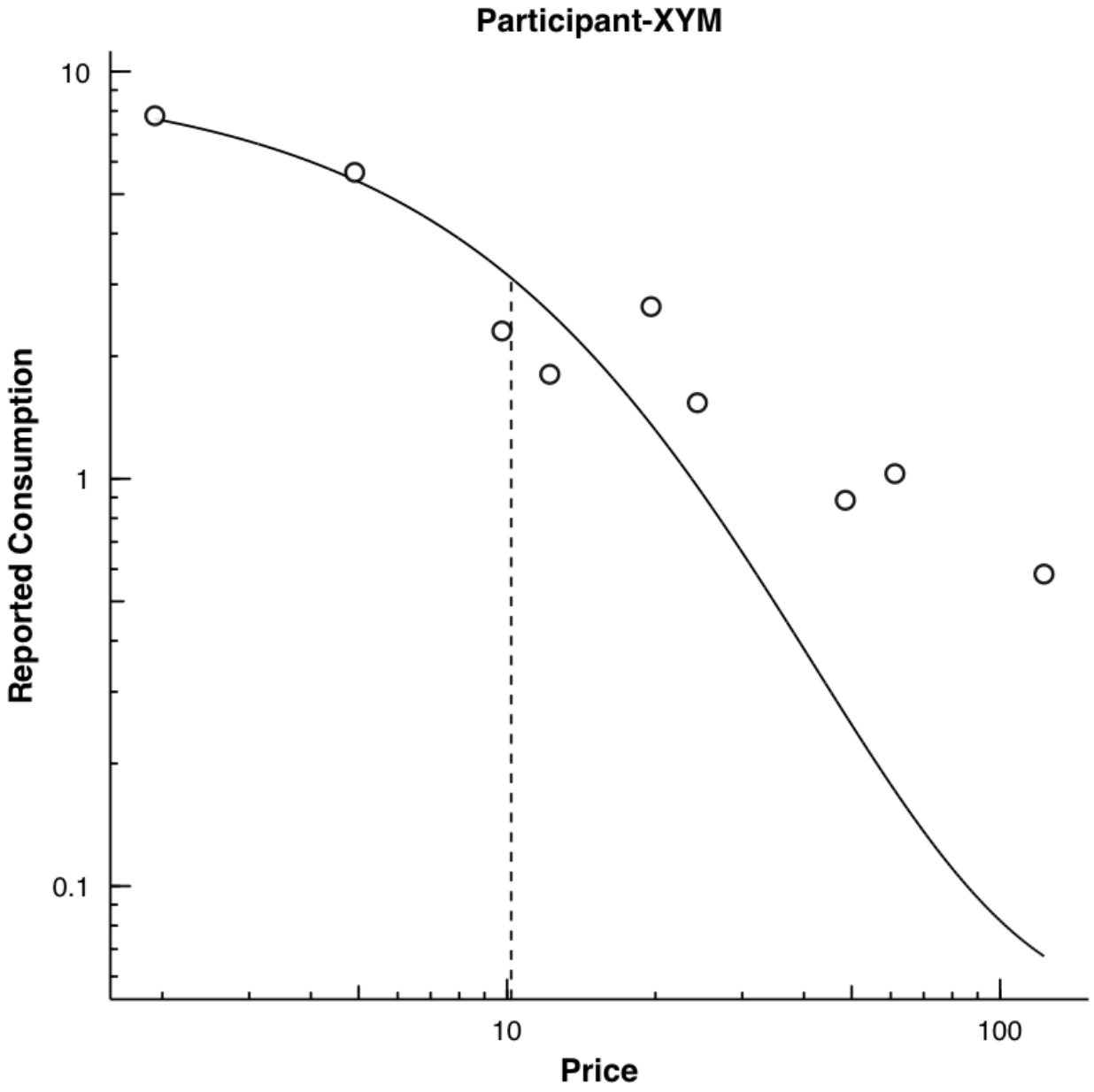
Average Alpha Value Across FCG Genotpyes



Note. Number of subjects per group = 5. When observing mean values of alpha, there is no main effect of gonadal sex [F(1,16(-0.017, p=0.897]. However, there is a SCC*GS interaction in alpha [F(1,16)-5.285, p=0.035]. Post-hoc comparisons show a higher alpha value in XXF than in XY F (p=0.053), indicating that XXF mice are more sensitive to price increases than XYF mice.

Figure 3.

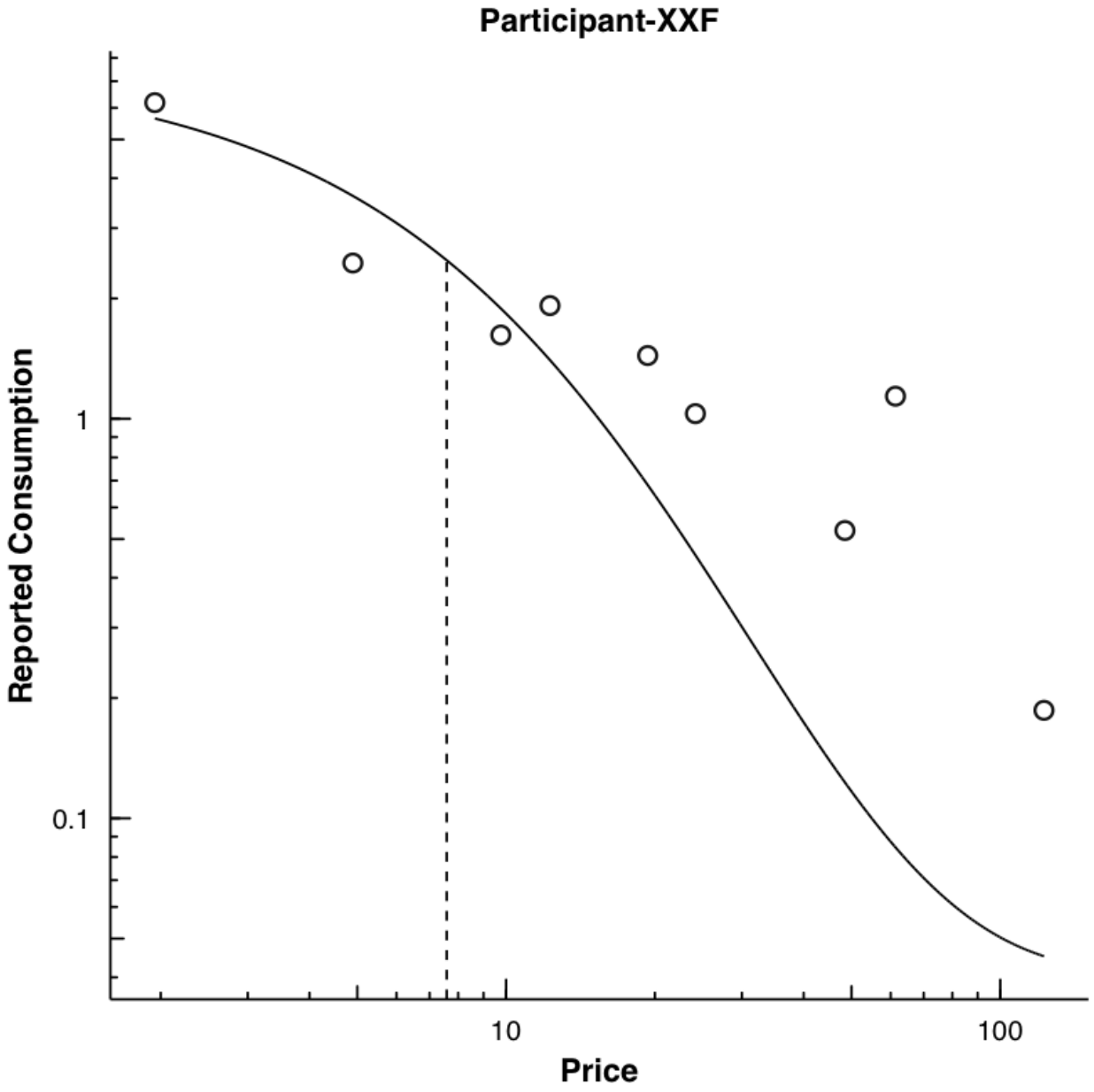
Demand Curve: XYM Averaged Consumption



Note. Demand curve analysis of averaged total consumption of the XYM genotype. Alpha value for XYM = 0.001197 averaged.

Figure 4.

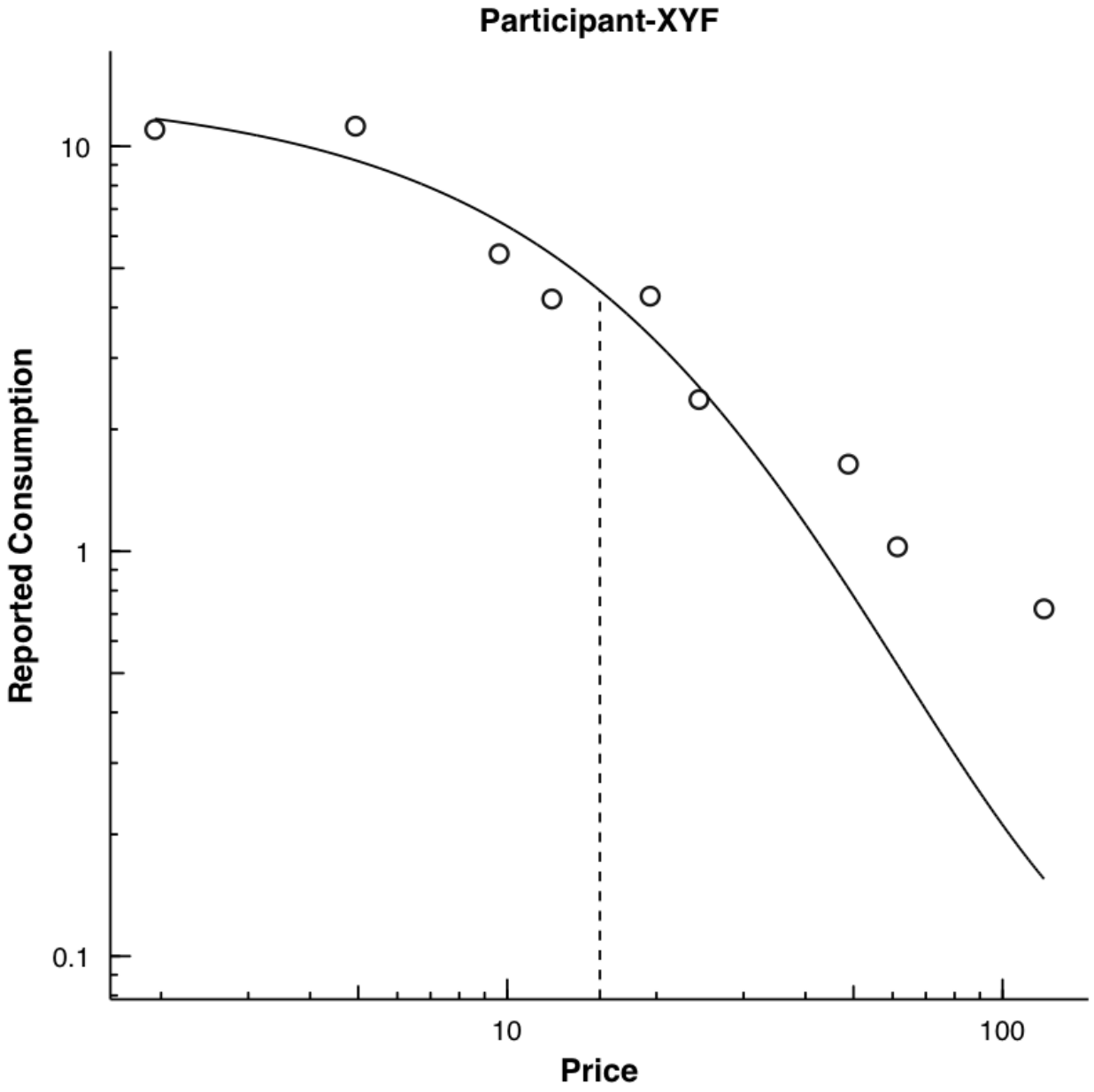
Demand Curve: XXF Averaged Consumption



Note. Demand curve analysis of averaged total consumption of the XXF genotype Alpha value for XXF= 0.00219 averaged.

Figure 5.

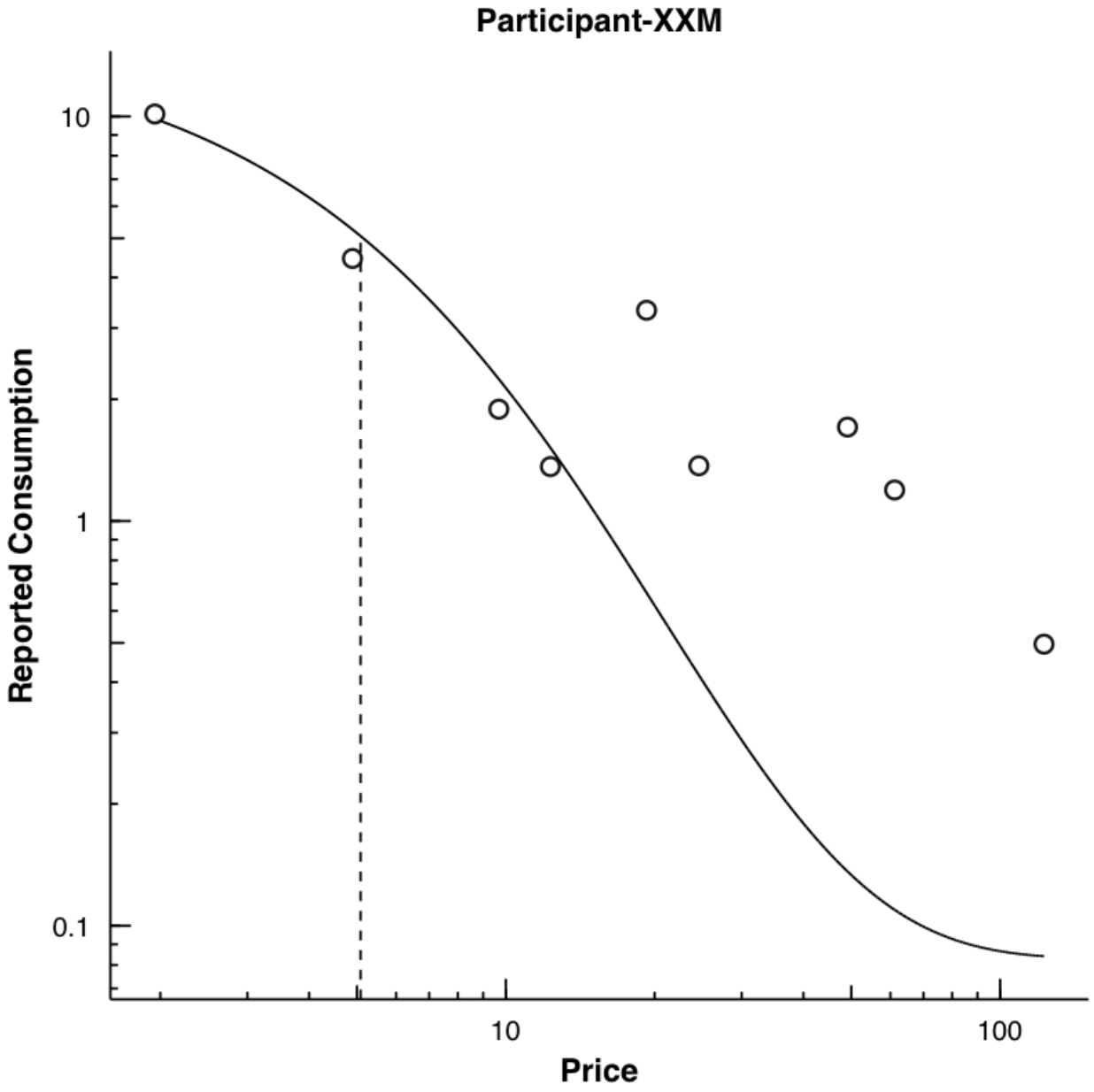
Demand Curve: XYF Averaged Consumption



Note. Demand curve analysis of averaged total consumption of the XYF genotype Alpha value for XYF = 0.00083 averaged.

Figure 4.

Demand Curve: XXM Averaged Consumption



Note. Demand curve analysis of averaged total consumption of the XXM genotype. Alpha value for XXM = 0.001949 averaged.

References

Arias-Carrión, O., Stamelou, M., Murillo-Rodríguez, E., Menéndez-González, M., & Pöppel, E. (2010). Dopaminergic reward system: A short integrative review. International Archives of Medicine, 3(1), 24. https://doi.org/10.1186/1755-7682-3-24

Arnold, A. P., & Chen, X. (2009). What does the "four core genotypes" mouse model tell us about sex differences in the brain and other tissues? Frontiers in Neuroendocrinology, 30(1), 1–9. https://doi.org/10.1016/j.yfrne.2008.11.001

Back, S. E., Payne, R. L., Wahlquist, A. H., Carter, R. E., Stroud, Z., Haynes, L., Hillhouse, M., Brady, K. T., & Ling, W. (2011). Comparative profiles of men and women with opioid dependence: Results from a National Multisite Effectiveness Trial. The American Journal of Drug and Alcohol Abuse, 37(5), 313–323. https://doi.org/10.3109/00952990.2011.596982

Camí, J., & Farré, M. (2003). Drug addiction. New England Journal of Medicine, 349(10), 975–986. https://doi.org/10.1056/nejmra023160

French, M. T., Dunlap, L. J., Zarkin, G. A., McGeary, K. A., McLellan, A. T., & Gordon, A. J. (1997). A structured instrument for estimating the economic cost of drug abuse treatment: The Drug Abuse Treatment Cost Analysis Program (DATCAP). Journal of substance abuse treatment, 14(5), 445-455.

Harp, S. J., Martini, M., Lynch, W. J., & Rissman, E. F. (2020). Sexual differentiation and substance use: A mini-review. Endocrinology, 161(9). https://doi.org/10.1210/endocr/bqaa129

Hooten, W. M., St Sauver, J. L., McGree, M. E., Jacobson, D. J., & Warner, D. O. (2015). Incidence and Risk Factors for Progression From Short-term to Episodic or Long-term Opioid Prescribing: A Population-Based Study. Mayo Clinic proceedings, 90(7), 850–856. https://doi.org/10.1016/j.mayocp.2015.04.012

Kaplan, B. A., Gilroy, S. P., Reed, D. D., Koffarnus, M. N., & Hursh, S. R. (2018). The R package beezdemand: Behavioral Economic Easy Demand. Perspectives on behavior science, 42(1), 163–180. https://doi.org/10.1007/s40614-018-00187-7

Kokane, S. S., & Perrotti, L. I. (2020). Sex differences and the role of estradiol in mesolimbic reward circuits and vulnerability to cocaine and opiate addiction. Frontiers in Behavioral Neuroscience, 14. https://doi.org/10.3389/fnbeh.2020.00074

Koob, G. (2001). Drug addiction, dysregulation of reward, and Allostasis. Neuropsychopharmacology, 24(2), 97–129. https://doi.org/10.1016/s0893-133x(00)00195-0

Krans, E. E., & Patrick, S. W. (2016). Opioid Use Disorder in Pregnancy: Health Policy and Practice in the Midst of an Epidemic. Obstetrics and gynecology, 128(1), 4–10. https://doi.org/10.1097/AOG.0000000000001446

Lopresti, N. M., Esguerra, M., & Mermelstein, P. G. (2020). Sex differences in animal models of opioid reward. Current Sexual Health Reports, 12(3), 186–194. https://doi.org/10.1007/s11930-020-00266-4

Madla, C. M., Gavins, F. K. H., Merchant, H. A., Orlu, M., Murdan, S., & Basit, A. W. (2021). Let's talk about sex: Differences in drug therapy in males and females. Advanced Drug Delivery Reviews, 175, 113804. https://doi.org/10.1016/j.addr.2021.05.014

Manubay, J., Davidson, J., Vosburg, S., Jones, J., Comer, S., & Sullivan, M. (2015). Sex differences among opioid-abusing patients with chronic pain in a clinical trial. Journal of Addiction Medicine, 9(1), 46–52. https://doi.org/10.1097/adm.0000000000000086

Newman, M., & Ferrario, C. R. (2020). An improved demand curve for analysis of food or drug consumption in behavioral experiments. Psychopharmacology, 237(4), 943–955. https://doi.org/10.1007/s00213-020-05491-2

Nestler, E. J. (2001). Molecular basis of long-term plasticity underlying addiction. Nature Reviews Neuroscience, 2(2), 119–128. https://doi.org/10.1038/35053570

Palkovic, B., Marchenko, V., Zuperku, E. J., Stuth, E. A. E., & Stucke, A. G. (2020). Multi-Level Regulation of Opioid-Induced Respiratory Depression. Physiology (Bethesda, Md.), 35(6), 391–404. https://doi.org/10.1152/physiol.00015.2020

Reed, D. D., Niileksela, C. R., & Kaplan, B. A. (2013). Behavioral economics: a tutorial for behavior analysts in practice. Behavior analysis in practice, 6(1), 34–54. https://doi.org/10.1007/BF03391790

Ross, H., Chaloupka, F., & Wakefield, M. (2003). Differential effects of cigarette price on smoking behavior in men and women. Drug and Alcohol Dependence, 72(3), 255-264. doi:10.1016/S0376-8716(03)00128-5

Serdarevic, M., Striley, C. W., & Cottler, L. B. (2017). Sex differences in prescription opioid use. Current opinion in psychiatry, 30(4), 238–246. https://doi.org/10.1097/YCO.0000000000000337

Substance Abuse and Mental Health Services Administration. (2021). Key substance use and mental health indicators in the United States: Results from the 2020 National Survey on Drug Use and Health (HHS Publication No. PEP21-07-01-003, NSDUH Series H-56). Rockville, MD: Center for Behavioral Health Statistics and Quality, Substance Abuse and Mental Health Services Administration. Retrieved from https://www.samhsa.gov/data/

Veilleux, J. C., Colvin, P. J., Anderson, J., York, C., & Heinz, A. J. (2010). A review of opioid dependence treatment: Pharmacological and psychosocial interventions to treat opioid addiction. Clinical Psychology Review, 30(2), 155–166. https://doi.org/10.1016/j.cpr.2009.10.006

Wilson, S. H., Hellman, K. M., James, D., Adler, A. C., & Chandrakantan, A. (2021). Mechanisms, diagnosis, prevention and management of perioperative opioid-induced hyperalgesia. Pain management, 11(4), 405–417. https://doi.org/10.2217/pmt-2020-0105

Zarkin, G. A., Dunlap, L. J., Homsi, G., McKay, J. R., & Jamison, R. N. (2004). The effects of prices on alcohol use and its consequences. Journal of Drug Issues, 34(2), 333-356. doi:10.1177/002204260403400208