THE INFLUENCE OF A POSITIVE ENVIRONMENT ON NICOTINE SELF-ADMINISTRATION. A GENE-ENVIRONMENT INTERACTION STUDY

BY

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Abstract

Genetic vulnerabilities can predispose individuals to develop psychological disorders. One of these disorders is a tobacco use disorder characterised by dependency on nicotine. A polymorphism in the serotonin transporter gene can result in a greater likelihood of developing psychological disorders. This polymorphism can be mimicked in rats by disrupting the serotonin transporter gene. Using an animal model allows us to examine the influence of factors such as the environment. It is suggested that housing rats in an enriched environment can protect against disorders. In the current study, serotonin knockout and wild type rats were housed in either environmental enrichment or standard housing. Self-administration acquisition was assessed, followed by extinction, reinstatement, and progressive ratio testing. Environmental enrichment exhibited protective effects during the acquisition stage in knockout rats genetically predisposed to nicotine dependence, but not in wild type rats. Rats in standard housing extinguished cue-induced reinstatement responding to a greater extent than rats in enrichment, but also showed a marginal increase in responding during extinction. Contrary to predictions, rats in standard housing were not more likely to self-administer nicotine compared to rats in enrichment, nor were their break points higher in progressive ratio. The findings of this study help to understand the mechanisms underlying genetic and environmental vulnerabilities of nicotine dependence, suggesting a gene x environment interaction in nicotine dependence. Thus, it is important to not only treat the dependence, but also consider environmental effects and psychological disorders to find an effective treatment, whether it be through therapy, pharmacology, or a combination.

Contents

Introduction

Prevalence and Health Effects

Tobacco is one of the most widely consumed substances, with 1.1 billion people consuming tobacco worldwide (World Health Organisation, 2019). 8 million people died of tobacco related disease in 2017. Tobacco use is declining however, with global smokers reducing from 33.3% in 2000 to 24.9% in 2015. 14.2% of New Zealanders smoked in 2018, with an average of 10 cigarettes a day or half a pack (Ministry of Health, 2019). The risk of developing lung cancer increases to 15% if you continually smoke cigarettes or to 6% if you stopped by age 50 (International Agency for Research on Cancer, 2004). Some of the carcinogenic effects of smoking arise from the presence of tobacco-specific N-nitrosamines (TSNA) such as n N′-nitrosonornicotine (NNN) (First detected in cigarette smoke by Hoffman and colleagues (1974)) and 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK) (detected by Hecht (1998)). Hecht (1999, 2002) found NNK to be a pulmonary carcinogen that induced lung tumours in mice through mutations of the Kirsten rat sarcoma viral oncogene homolog (KRAS) gene (Hecht, 1989; Belinsky et al., 1989; Devereux et al., 1991). The KRAS gene is an oncogene that is mutated in 30% of human cancers. When mutated, the gene becomes constitutively activated which results in an overproduction of cells and consequently, tumours (Bos, 1989; O'Hagan & Heyer, 2011).

Current initiatives to help with tobacco reduction include monitoring tobacco use, protecting people from tobacco smoke, offering help to quit tobacco use, warning about the dangers of tobacco use, enforcing bans on advertising, and raising taxes on tobacco. With these initiatives in place, 116 of the 136 countries that have implemented at least one of these have seen reductions in tobacco consumption (World Health Organisation, 2019). In New Zealand $61.7 million is spent on tobacco control programmes. Tobacco advertising is banned, cigarette tax rates are increased annually, plain packaging with images showing the

harmful effects are used, and by 2025 the government set a goal of less than 10% of New Zealanders smoking. Interventions such as Quitline support, nicotine replacement therapies and community-based smoking reduction services are the most effective in New Zealand. Quitline alone has a 21% quit rate amongst users after one year (Ministry of Health, 2016).

Nicotine

The psychoactive component in tobacco that plays a role in the desire and addiction of smoking is nicotine. Nicotine acts on nicotinic acetylcholine receptors in the brain which subsequently result in dopamine release, the principal neurotransmitter in reinforcement and drug use (Koob, 1989). Smokers report experiences of head-rush, increased heart rate, euphoria, and increased arousal upon consumption of nicotine, especially when nicotine deprived. Subjective experiences of nicotine vary depending on many factors such as time since last consumption, other substances consumed such as alcohol, baseline mood ratings, psychological disorders, pre-consumption mood and the environment. Compared to smokers, non-smokers report more negative effects of nicotine such as increased tension and reduced alertness (Kalman D., 2002; Perkins et al., 2003).

Dependence

Dependence on nicotine is characterised by two main features – withdrawal and tolerance. Dependence on nicotine means that people are more likely to be exposed to the hazardous effects of smoking as they continue use even when the effects are adverse. Dependence involves interactions between individual vulnerability and the degree and amount of drug exposure, which can result in a loss of control. Individuals with vulnerabilities such as hyperactive dopamine systems find drugs more rewarding and may be more likely to start drug use. Two theories of dependence have been hypothesised, either drug focused or individual focused. In drug focused, transition to dependence only occurs after long term repeated exposure to the substance. In individual focused, dependence is only

seen in some drug users that are more vulnerable. Some report dependence from first use, and others can go months of use without. It is more likely a combination of these theories that may explain transition to dependence (Piazza & Deroche-Gamonet, 2013).

Nicotine dependence fits under the DSM definition of a tobacco use disorder which falls under the category of substance use disorders. According to the DSM-5, a substance use disorder envelops the continued use of a substance to the point of impairment in everyday life including functioning and relationships. Under a tobacco use disorder, the problematic substance is tobacco. For an individual to be diagnosed with a tobacco or substance use disorder, they must display 2 of 11 symptoms outlined in the DSM-5. These include but are not limited to: Consuming more than originally planned, craving the substance, building a tolerance to the substance, experiencing withdrawal symptoms and repeated use despite the negative effects (American Psychiatric Society, 2013). In the context of this paper, a tobacco use disorder is discussed more broadly as a substance use disorder due to the many overlaps in brain pathways between substances that fall under this disorder, and the range of literature that covers other substances than tobacco that are still relevant.

Withdrawal

With continued use of substances including nicotine, withdrawal symptoms can present themselves if one goes without the substance for a long period of time. Nicotine withdrawal is classified as cessation of nicotine accompanied by at least four symptoms including irritability, anxiety, difficulty concentrating, increased appetite, restlessness, depressed mood, and insomnia (American Psychiatric Society, 2013). Withdrawal occurs as the body tries to maintain homeostasis or remain stable. Upon the consumption of a substance such as nicotine the balance of chemicals in the body changes – chemicals such as dopamine are released in higher quantities than normal. With frequent use of substances and consequently chronic exposure of the extra chemicals, the body alters its function to counter

the effects. For example, by desensitising the associated receptors for nicotine when nicotine exposure is high. This returns the body to homeostasis as it was before nicotine consumption. When the substance is abruptly stopped, the body is again out of homeostasis due to desensitisation of the nicotinic receptors. The period in which the body is out of homeostasis after cessation of the drug is the withdrawal period, and the negative side effects are seen due to this imbalance (Barr et al., 2011).

Relapse

Withdrawal symptoms can make it harder to abstain from smoking. Many smokers who quit relapse (Fiore, 2000; Shiffman et al., 1998) and many will return to regular smoking (Kenford et al., 1994). One factor that may drive this is that the environmental cues associated with tobacco or nicotine consumption are often hard to avoid - such as home, work, or social environments. In a study conducted by Brandon et al. (1990), 92 of 129 participants reported smoking again in the 2-year period after completing a smoking cessation program. Another study by Chornock et al., (1992) examined 67 subjects who were long-term smokers. Participants abstained from smoking for 3 days before being assigned to smoking 5 cigarettes in the normal smoking environment or remain abstinent. All subjects assigned to smoke 5 cigarettes had relapsed within 2 days and smoked more than the allocated amount, while only 16% of the abstinent participants relapsed. This study provides support that relapse occurs more frequently when participants are in an environment that would normally cue their smoking behaviour and having a few cigarettes when trying to quit hinders the success (Chornock et al., 1992). The neurobiological effects that contribute to relapse of nicotine are not greatly understood and blockage of one of the systems important to nicotine dependence has effects on reinstatement. Blocking D1 and D2 receptors with antagonists prevents reinstatement of nicotine even when given a nicotine priming dose (Fattore et al., 2009).

Brain Effects

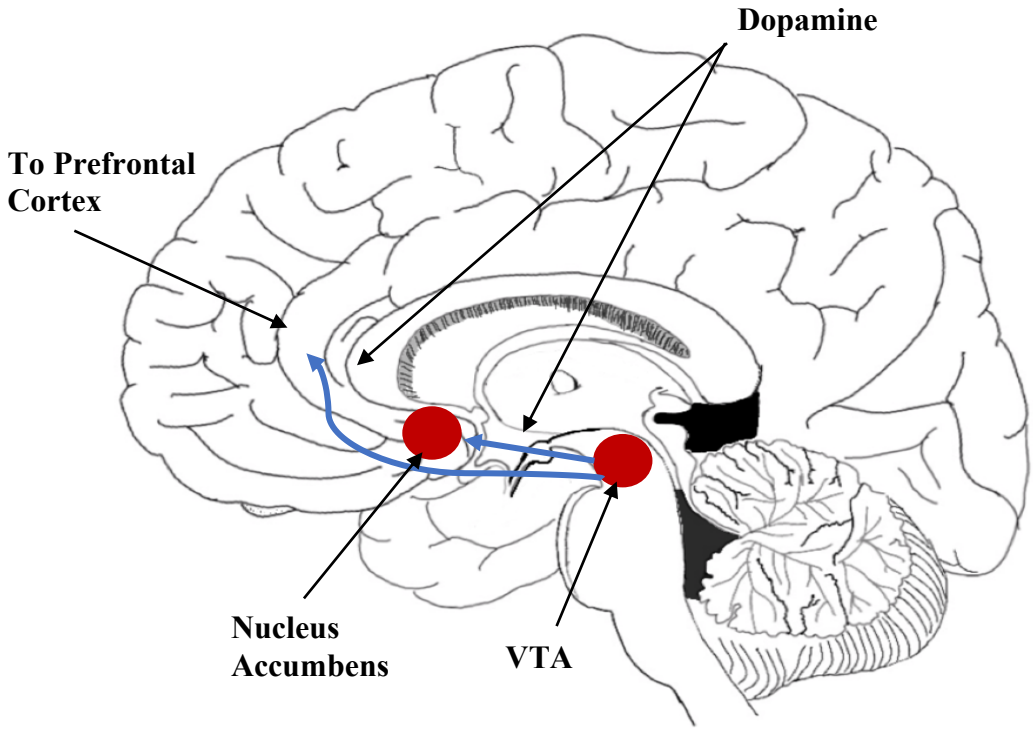
Nicotinic acetylcholine receptors (nAChRs) bind nicotine which opens an ion channel allowing for the transmission of positively charged ions into the cell, leading to excitation. After acute exposure to nicotine, the receptor remains activated until the nicotine unbinds. Chronic exposure to nicotine, in contrast, results in down-regulation and desensitisation of the receptors which inactivates them (Calabresi, Lacey & North, 1989).

Nicotinic receptors are widespread in the central and peripheral nervous system, including on dopaminergic cell bodies in the ventral tegmental area (VTA). Nicotine has been shown to act on this pathway as nAChRs are present on VTA neurons and terminals of the nucleus accumbens (Nisell, Nomikos & Svensson, 1994; Sziraki et al., 2002). The dopamine pathway starts in the VTA and projects to other brain regions such as the prefrontal cortex (PFC) and nucleus accumbens (NAcc) (Figure 1*)*. Dopamine release in the NAcc is associated with the initial rewarding effects of drugs. The hypothesis that nicotine's rewarding properties are mediated (at least in part) by this pathway is supported by the findings that lesions of the dopaminergic system in the NAcc reduces nicotine self-administration as do dopamine agonists (Corrigall et al., 1992; Sziraki et al., 2002). It is worth noting although this is not explored in the current study, that there are other substances present in tobacco smoke that alter its reinforcing value. Reduced amounts of monoamine oxidase B known to break down dopamine are found in the brains of smokers compared to non-smokers (Fowler et al., 1996). Increasing research is providing evidence that monoamine oxidase inhibitors which block the breakdown of dopamine (Khalil et al., 2000), are present in tobacco and enhance the reinforcing properties of nicotine (Kapelewski et al., 2011; Smith et al., 2016). It is well established that with chronic nicotine use nAChRs are desensitised, but the number and density of receptors in nicotine abusers is increased as shown by brain imaging studies (Mukhin et al., 2008; Cosgrove et al., 2009; Sharma & Brody, 2009; Bartal

2001). The increase in receptors is induced by the nicotine itself (Brody et al., 2009) as supported by increased amounts in the brains of laboratory animals exposed to nicotine (Pauly et al., 1996; Pistillo, 2016).

Figure 1

The mesolimbic and mesocortical system in the brain



As stated, chronic exposure to nicotine inactivates some nAChRs. After a period of abstinence, these receptors become active again. As even more receptors are active than normal, the first re-exposure of nicotine may be more rewarding than previously experienced. Smokers reported this effect when having their first cigarette of the day (Russell 1989) which may account for the high rates of relapse seen in smokers. Once nicotine is reintroduced the receptors may become inactivated again, reducing the withdrawal effects and providing smokers with the "relief" they feel after smoking (Dani & Heinemann, 1996). This hypothesis

is supported by findings that nAChR antagonists that block nAChRs reduce drug seeking behaviour (Corrigall & Coen, 1989).

Activation of nAChRs leads to release of dopamine, noradrenaline (Clarke & Rueben, 1996), acetylcholine (Wilkie et al., 1993), glutamate (McGhee et al., 1995) and GABA (Yang et al., 1996) and thus has effects in many brain regions and pathways, including those related to anxiety, learning and memory. Nicotine stimulates the release of glutamate through nAChR activation on glutamatergic terminals (Fu et a., 2000). Alongside dopamine, glutamate is involved in the reinforcing effects of nicotine and related dopamine release in the NAcc. This release is blocked by glutamatergic NMDA receptor antagonists which reduces nicotine self-administration (Kosowski et al., 2004; Kenny et al., 2009). Fu et al., (2000) hypothesised that glutamate NMDA receptors in the VTA are responsible for stimulating and speeding up the release of dopamine in the NAcc. The NMDA receptor antagonist decreased dopamine secretion in the NAcc when administered in the VTA, but not when administered in the NAcc directly, suggesting that NMDA receptors in the VTA are involved in the secretion of dopamine in the NAcc following nicotine administration.

The serotonin system is also believed to play a role in nicotine reinforcing effects, by having an inhibitory effect on dopamine release, and serotonin2C receptor agonists reduce nicotine self-administration (Di Matteo et a., 1999). Thus, disruptions in the serotonergic system may impact nicotine self-administration, potentially through reduced inhibitory control on the dopamine system, increasing the reinforcing effect of nicotine.

Self-Administration Studies

Nicotine use and its abuse and dependence properties can be examined causally by using animal models of drug use. Using animal models allows for factors that may potentially play a role in drug use to be manipulated, and the resulting effect on drug use examined. Self-administration paradigms are a commonly used method to examine the reinforcing effects of

substances under different conditions. In a self-administration experiment, animals are trained to press a lever for a dose of a substance. In most cases, the substance is administered intravenously to ensure rapid delivery to the brain. Self-administration paradigms provide information on the abuse potential of a drug by showing how quickly an animal can acquire self-administration and maintain this responding over time, how responding changes after removal of the substance (extinction), and how quickly responding starts again when an associated cue is presented (reinstatement) (Schuster & Thompson, 1969). Self-administration studies have shown that nicotine can act as a reinforcer resulting in robust administration by rats even when responding in a progressive ratio (Donny et al., 1999). Upon removal of nicotine, withdrawal symptoms are seen (Le Foll & Goldberg, 2005) and self-administration is easily reinstated once nicotine is provided again (Shaham, Adamson, Grocki & Corrigall, 1997).

One limitation of some self-administration studies in rats is that compulsive drug-seeking can take months to manifest, and most studies often have too short of a period to accurately relate withdrawal and relapse behaviours to humans of the same extent. When given the opportunity and 23hr access, rats will self-administer nicotine to the point of dependence as shown by extinction resistance, food intake and somatic signs of withdrawal such as body shakes, eye-blinks, and abdominal writhes (O'Dell et al., 2007). In one study, extended access to nicotine did not increase self-administration, but did result in dependence as shown by increased withdrawal behaviours. Interestingly, groups allowed access to 1hr of nicotine and 23hrs of nicotine 7 days a week showed similar levels of withdrawal, whereas rats who were only given access for 5 days, with 2 days abstinence had delayed and reduced withdrawal symptoms. Rats exposed to 1hr per day as opposed to 23hr per day elicited higher rates of responding (Paterson & Markou, 2004). These findings suggest that the frequency of exposure rather than length has the strongest effects on nicotine dependence, which aligns

with hypotheses that dependence is partially due to the habit of daily use (Piazza & Deroche-Gamonet, 2013).

Progressive Ratio Schedule of Reinforcement

In a PR schedule of reinforcement, the number of responses required to obtain the reinforcement increases after each reinforcement. The number required increases until it reaches a point at which the reinforcing properties of a stimulus do not outweigh the effort required to obtain the reinforcement - known as the "break point". The more reinforcing a stimulus is, the higher the effort will be to obtain the reinforcement and thus the higher the break point. One aspect that is interesting when examining drug self-administration is the reinforcing efficacy of a drug. How reinforcing a drug is depends on more than just the physical properties of a drug infusion and includes the pharmacological effects within an individual which can be influenced by factors such as the environment, previous exposure, and genetics. For example, when self-administering cocaine on a progressive ratio (PR) schedule, food deprivation increased the break point for cocaine (Comer et al., 1995) as does stress induced by a foot shock (Shaham et al., 1993). These results further support that it is not just properties of the drug itself that influence the reinforcing efficacy. For this reason, progressive ratio schedules of reinforcement are becoming more abundant in drug testing either as stand-alone schedules or to compliment the standard fixed ratio (FR) schedules often used. One advantage of using PR over FR is that PR does not measure the rate of responding, which may be a weak indicator of reinforcing efficacy. It is also suggested that drug dependence is associated with higher reinforcing efficacy and consequently higher break points when tested on a PR schedule of reinforcement. Limitations of PR include variations of criterion between laboratories and research groups. For example, some use shorter times before a break point is defined (e.g., a few minutes) while others may use longer (e.g., 48 hours). Differing pause lengths within responding may allow for the drug to wear off and

influence the likelihood for a subject to seek to obtain another reinforcer (Stafford et al. 1998). Some have suggested that FR measures the hedonic reinforcer effects, while PR measures craving (Donny et al., 1999). For this reason, PR will be used alongside FR in this study as a measure of craving and reinforcing efficacy between groups over direct drug effects. This distinction is supported by the finding that 65% of the variance in PR is not accounted for by FR infusions (Donny et al., 1999) and differences found across PR where they were not apparent in FR (Roberts et al., 1989). In a study by Donny et al., (1999), where nicotine self-administration usually peaks at 0.03mg/kg then decreases producing an inverted U, this was not seen in PR. This may be because the reinforcing efficacy or craving is higher for a higher dose while reducing the effects of satiation with longer times between doses.

Extinction Experiments

Extinction is the process of reducing associations between a stimulus and a cue such as between a drug and a location or a light in the case of laboratory animal experiments. After an association has been made between the cue and the drug, extinction training begins. To eliminate this association the cue is presented but no drug or stimulus is provided (Millan et al., 2011). Many studies examine factors that may contribute to the difficulty that smokers experience when trying to quit. As mentioned, many smokers relapse when trying smoking cessation and researchers can use extinction experiments to examine why this may occur and what influences have stronger effects on relapse. Cue-induced craving has previously been found to increase within the first 35 days of cessation, while self-reported levels of craving decreased (Bedi et al., 2011). To further investigate this effect, Markou et al., (2016) examined the effects of nicotine-associated cues on extinction responding throughout the timeline of abstinence. Highest rates of cue-induced responding were found between 7-21 days of abstinence, producing an inverted U curve. This contrasts to the higher cue-induced craving at 35 days seen in the study conducted by Bedi et al., (2011) on human participants.

When cues were repeatedly presented with no reinforcement, the effect of the cue to induce nicotine-seeking was reduced due to extinction of the conditioned response and extinction responses declined after 7 days. The effects were present for at least 42 days after abstinence. Rats who only experienced withdrawal without extinction training produced higher responses than those that were trained to extinguish cue associations. The results from these studies suggest that exposure to nicotine-related cues may be more effective in reducing relapse potential in humans compared to withdrawal alone. In the current study, all rats will undergo extinction training, thus the effect that this may have on responding compared to humans may be stronger.

Brain pathways associated with extinction have been examined and unsurprisingly include areas of the PFC due to the presence of dopamine systems and consolidation of learning-related memories. An area of interest within the PFC is the infralimbic region (IL) which has projections to the nucleus accumbens. In a study by Peters et al., (2008), rats were trained to self-administer cocaine followed by extinction of the behaviour. When the IL was inactivated using GABA agonists, the drug-seeking behaviour previously extinguished was reinstated. These findings suggest that the activation of IL plays a role in the extinction or suppression of drug-seeking behaviour. Another brain region associated with extinction of drug-seeking behaviour is the NAcc. As previously mentioned, the NAcc plays an important role in reinforcement associated with drug-taking and the consequent dependence. In a study by Sutton et al., (2003), rats that were extinction trained for cocaine self-administration showed an upregulation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors for glutamate in the NAcc compared to those that only underwent withdrawal, or those that were extinction trained for sucrose self-administration. The authors suggest that extinction training may aid in reversing changes in the NAcc following drug exposure and

thus reduce relapse potential. Overall, drug or smoking cessation programmes that focus on associated cues and extinguishing this association may produce lower rates of relapse.

Reinstatement Experiments

After a behaviour has been extinguished, it can be reinstated through presentation of previously paired cues. Drug reinstatement procedures in animals are used as a model of relapse behaviour seen in humans. In drug reinstatement paradigms, the responding on the active lever begins again after the pairing of a cue or a dose of the drug which is a measure of drug seeking behaviour. Importantly, responses on the active lever are not reinforced during reinstatement experiments thus the responding starts and is maintained by the cue alone (Erb & Placenza, 2011). In its simplest form, drug reinstatement paradigms are asking "when reminded of a previously administered drug, how much do you want it?"

Reinstatement studies often measure responses after a priming injection of the drug, exposure to a paired cue, or stress - factors that lead to relapse in humans (Martin-Garcia et al., 2009). The availability of nicotine following extinction reinstates nicotine self-administration (Shaham et al., 1997). When paired with visual cues such as a light prior to availability, nicotine self-administration is increased compared to if the nicotine was available absent of a cue. Interestingly, after 17 days of having nicotine + cue replaced with saline + cue, only partial extinction of responding is seen. In the absence of nicotine, visual cues alone were able to maintain responding higher than that seen when no cue is present, suggesting that cues associated with nicotine delivery and/or smoking are also a strong reinforcer of the behaviour and can maintain this behaviour for a period without nicotine present (Cagguila et al., 2001).

Nicotine-associated cues can induce responding after extinction training even after 41 days of abstinence as reported by Xiu et al., (2007). The authors also found the robustness of the cue to induce responding over multiple reinstatement experiments depended on the dose

of nicotine used during training. Rats that were given a higher dose of nicotine more readily responded during the three reinstatement experiments on 0, 15 and 30 days after extinction compared to rats trained on a lower dose that had lower responding over the final two reinstatement experiments. The finding that cue-induced reinstatement was associated with training dose suggests that more long-term neuroadaptive changes may be taking place when exposed to higher doses compared to lower doses of nicotine.

When considering why relapse or reinstatement occurs, it is important to consider what brain changes may be influencing the desire or craving for the drug. Schroeder et al., (2001) examined *Fos* levels in the brain after being exposed to a nicotine-associated cue. *Fos* is an intermediate-early gene used as a marker of activation in neurons. The authors hypothesised that cues associated with drugs may activate pathways associated with learning and memory due to the role of dopamine and glutamate systems in both learning and reward. When rats were exposed to an environment previously associated with nicotine administration, *Fos* levels increased in areas such as the prefrontal cortical and limbic areas. These findings suggest a neural mechanism in the cortical and limbic pathways responsible for relapse and craving when cues are present, likely through associated reward expectancy and impairment of rational decision making - also associated with those who abuse substances (Watanabe, 1996; London et al., 2000). These findings have also been found in studies with morphine (Schroeder et al., 2000) and cocaine (Brown et al., 1992) associated cues. Brain activation for nicotine was similar to activation for a food reward, suggesting that drugs can recruit the same activation and pathways as evolutionarily advantageous rewards. Similarly, Schiltz et al., (2005) found increased levels of another intermediate-early gene, activity-regulated cytoskeleton-associated protein (arc), in the prefrontal cortex, cingulate cortex, sensory cortex, ventral striatum and amygdala of rats exposed to nicotine-associated cues compared to controls. These findings may help to explain why drug-associated cues are

able to induce drug-seeking behaviour so robustly on their own and why relapse or reinstatement can occur even after months of withdrawal through activation of memory circuits.

Genetic Effects

Some individuals can be more prone to dependence due to vulnerabilities such as genetics as per the individual focused approach to dependence mentioned prior (Piazza & Deroche-Gamonet, 2013). While many genetic factors influence vulnerability, the current study will focus on serotonin-transporter related vulnerabilities.

5-HTTLPR

The serotonin transporter (5-HTT) is responsible for regulation of serotonin levels through the re-uptake of extracellular serotonin back into the pre-synaptic membrane. The human serotonin transporter is encoded by a single gene, *SLC6A4* (solute carrier family 6 member 4). 5-HTTLPR is a genetic variation in the promoter region of this gene that regulates gene activity (Blakely et al., 1994). Insertions or deletions of repeat sequences within this region are associated with two different alleles. An insertion results in the long (L) allele and the deletion results in the short (S) allele. The short allele of this gene (leading to reduced 5-HTT protein expression) is associated with many psychiatric disorders including affective disorders (Kenna et al., 2012) and substance use disorders (Cao, Hudziak & Li, 2013), although the data are far from consistent (Villlalba et al., 2015; Culverhouse et al., 2003). This may, in part, be due to allelic variations within the L allele which is normally associated with a higher expression of the 5HTT protein (Hu et al., 2005). One of which is the single nucleotide polymorphism (SNP) rs25531 near 5-HTTLPR which results in an A→G change, leading to lower activity of SERT analogous to the S allele, when compared to the L_A allele. This variation within the L allele may explain some of the conflicting results found when comparing the S and L alleles as these higher risk, low expression L_G allele

variants are included within the L group in these studies. To further support the idea that L_G is analogous to the S allele, Hu et al. (2005) found that while the high expressing L_A allele was more associated with a low response to alcohol, both the L_G and S alleles which had similar expression levels were less associated. A following study by Hu et al. (2006), found evidence to suggest that the SNP rs25531 of A→G leads to the creation of an activating protein 2 (AP2) transcription factor binding site. AP2 is responsible for activation or suppression of transcription and binding at this site suppresses transcription of SERT leading to similar expression to the S allele. Other allelic variants have also been discovered near the 5-HTTLPR region (Nakamura et al., 2000). One of these is a SNP rs25532 which results in a C→T change that influences the expression of *SLC6A4* in combination with other polymorphisms, such that the L_A SNP rs25531 with the C SNP rs25532 results in a L_{AC} allele with high expression of *SLC6A4* (Wendland et al., 2008). Another polymorphic region in intron 2 of *SLC6A4* known as STin2 (Serotonin Transporter intron 2) contains a variable number of tandem repeats of 16-17bp in length. Three alleles STin2.9, STin2.10 and STin2.12 carry either 9, 10 or 12 repeats respectively and the expression of *SLC6A4* increases in proportion with the number of repeats (MacKenzie & Quinn, 1999). The influence of many alleles that act on the 5-HTTLPR region and the ability of the alleles to work in combination with one another contributes to the complexity of *SLC6A4* studies in humans and the conflicting results that are often found (Shadrina et al., 2018) which can make it hard to fully understand the role of the serotonin transporter in relation to psychological disorders based on human studies alone.

Serotonin is synthesised in serotonergic terminals from the precursor tryptophan. Serotonin mediates the neurotransmission of many chemicals which contribute to functions such as food intake, sleep, motor activity, reproduction, cognition, and emotional states. Thus, the function of the serotonin transporter is important in regulating serotonin responses

and the consequent effects on function and development (Lesch et al., 1996). The fact that serotonin is important in modulating many chemicals in the brain helps to link why changes in regulation of serotonin from birth and particularly during neurodevelopment may result in disorders. To further support this hypothesis, perinatal exposure to selective serotonin reuptake inhibitors (SSRIs) results in similar effects as seen alongside in rats with the serotonin transporter gene disrupted (SERT-/-) (Oberlander, Gingrich & Ansorge, 2009).

Foetal brain development occurs over specific timelines. While many genes control brain development, neurotransmitters such as serotonin play a role in managing the length and degree of changes at each stage such as cell proliferation, migration, differentiation, and apoptosis (Vitalis & Parnavelas, 2003). Previous research conducted in the lab has found an increase in neurogenesis corresponding to inactivity of the serotonin transporter in SERT -/- rats suggesting that increased extracellular serotonin levels are impacting brain development and consequently increasing vulnerability to disorders (Kidwell, 2019). SERT -/- has also been associated with a decrease in dendritic spine density (Chaji et al., 2021). Brain-derived neurotropic factor (BDNF) is an important growth factor that supports existing neurons and growth of new neurons and connections. SERT -/- rats show reduced BDNF mRNA levels, suggesting that the prolonged blocking of this transporter has flow on effects to other brain regions such as the hippocampus and prefrontal cortex. Reductions in BDNF can impair brain plasticity, and impaired neuronal plasticity has been associated with disorder vulnerability (McClung & Nestler, 2008). In humans, BDNF mRNA levels in leukocytes are reduced in S allele carries, and more so if they were homozygotes (Molteni et al., 2010).

5-HTTLPR and Psychiatric Disorders

As with many genetically linked disorders, it is likely that substance use disorders are not only the combination of the environment and a gene, but also the combination of many risk genes that increase the likelihood of developing a substance use disorder. While those

with the 5HTTLPR polymorphism may be genetically predisposed to substance use disorders, only some of those who have the higher linked risk allele (the S allele) have other genetic or environmental combinations that increase their risk to show significant differences above normal populations. In addition, many genetic disorders are comorbid with each other, such as links between smoking and depression, anxiety, and schizophrenia (Dani & Harris, 2005). Genes that influence dopamine systems, such as serotonin, norepinephrine, glutamate, and GABA are likely candidates in genes that may contribute to substance use disorders, due to dopamine's link in drug use, abuse and dependence (Koob, 1989). Alongside many other neurotransmitter-related genes, serotonin genes are candidates that may be linked to nicotine dependence, both because of serotonins' role in dopamine release, and the fact that nicotine increases serotonin release (Tyndale, 2003).

Some studies find homozygous S allele carriers have an elevated risk of developing a substance use disorder (Navarro-Mateu et al., 2019) and are associated with alcohol, heroin, cocaine, and methamphetamine dependence (Cao et al., 2013; Rubens et al., 2016). Those with an S allele are 15% more likely to be diagnosed with alcohol dependence than those with LL alleles (McHugh et al., 2010). In contrast, a meta-analysis examining 25 studies with 8,800 participants found no link between the polymorphism and alcohol dependence (Villlalba et al., 2015), but this could be due to L_A alleles in the L population which show similar effects to S alleles.

In the USA, people who are nicotine dependent or suffer from a psychiatric illness make up about 70% of the smokers, suggesting a high comorbidity between smoking and psychiatric illnesses (Grant et al., 2004). Around 60% of individuals with a mental illness are smokers, compared to 25% of the general population that smoke (Leonard et al., 2001). Nicotine is also suggested to regulate deficits in schizophrenia (Harris et al., 2004). Those with depression have a high prevalence in smoking and more severe depression is associated

with a higher likelihood to suffer from dependence (Breslau et al., 1993). It is suggested that this may be due to increased adverse reactions to stressful stimuli, which may increase the drive to relieve it with the use of drugs such as nicotine, or that nicotine has antidepressant effects (Balfour & Ridley, 2000). The antidepressant effect hypothesis has been backed up by findings that nicotine patches have increased mood of depressed patients (Salin-Pascual et al., 1996), nicotine has similar effects on sleep-wake patterns in rats as another antidepressant fluoxetine (Vazaques-Palacios et al., 2010), and chronic nicotine exposure reduces failed escape attempts in learned helplessness paradigms (Semba et al., 1998) as do nAChR agonists (Ferguson et al., 2000). These antidepressant effects may suggest why those with mental illnesses are more likely to turn to nicotine.

SERT -/- Rats

The 5-HTTLPR polymorphism can be mimicked in animal models by selecting for rats that have the serotonin transporter gene disrupted (SERT) through a nonsense mutation induced by N-ethyl-N-nitrosourea (ENU) mutagenesis (Smits et al., 2006).

Homberg et al., (2007) examined the functional changes of disruptions to the serotonergic system in SERT -/- rats. SERT is the target of many SSRI treatments and thus is of interest in depression. The authors found that extracellular serotonin levels increased when serotonergic neurons were activated in the raphe nuclei, projecting to most brain regions and extracellular serotonin levels were 9-fold higher in the hippocampus of SERT -/- rats. Activity of the tryptophan hydroxylase (TPH) enzyme involved in serotonin synthesis and monoamine oxidase activity was no different between genotypes suggesting the effects were not due to changes in serotonin synthesis. As reduced uptake may affect intracellular levels, Homberg et al., (2007) also measured serotonin tissue levels and found reductions in the hippocampus, cortex, and amygdala of SERT -/- rats. Electrically evoked serotonin release was reduced in the hippocampus, hypothalamus, and cortex of SERT -/- rats. Noradrenergic

and dopaminergic transporters were not different between groups. The reduced serotonin release may be explained by reduced availability of intracellular serotonin or an adaptation to counteract high extracellular serotonin levels.

Heterozygous SERT -/+ rats exhibit a 50% reduction in SERT, similar to humans with the s allele (Homberg et al., 2007), however, very few studies have investigated behavioural changes in these animals. Therefore, to investigate the causal effects of genetic reduction in SERT on drug use, we will have to rely on studies that investigate SERT -/- animals. Indeed, SERT -/- rats have shown greater responding in self-administration of cocaine (Homberg et al., 2008) and MDMA (Oakly et al., 2014), but not heroin (Brox et al., 2018). No research exists on the effect of nicotine self-administration on serotonin knockout rats; however, nicotine has been found to have antidepressant effects by increasing brain serotonin levels (Bhalsinge et al., 2017). Given that SERT-/- rats show increased sensitivity to some (especially stimulant type), but not other drugs of abuse, it is of interest to examine the reinforcing properties of nicotine in these animals.

Genetic and Environmental Interactions

Very rarely do genes and environments act in isolation of one another. The focus of recent research has started to shift away from trying to identify one gene or environment that contributes to disorders, and instead considers the interaction between a gene and different environmental conditions and in which instances disorders are more likely to occur. Thus, it is important not only to consider the genetic susceptibilities that may result in substance use, but also to examine environmental effects. Stress is a major environmental vulnerability related to drug use (Solinas et al., 2010). Factors such as low socioeconomic class and poor parental or social support are associated with chronic stress and are associated with a higher likelihood to self-administer substances (Volkow & Li., 2005).

The hypothalamic-pituitary-adrenal (HPA) axis in the brain is responsible for the release and regulation of hormones in response to stressful stimuli. Stress leads to the release of glucocorticoids which act on dopamine pathways to increase activation and dopamine release (McEwen et al., 1986) and dopamine levels are increased in response to foot shocks in rats (Takahashi et al., 1998; Thierry et al., 1976). Dopamine release in relation to stress may explain why drugs are used more in response to stressful situations. Oswald et al., (2005) tested the hypothesis that glucocorticoids are associated with drug reinforcement in response to amphetamine. The authors found that cortisol - a glucocorticoid - was positively associated with dopamine release and cortisol responses were positively correlated with positive subjective experiences, in which the greater the positive experience, the higher the cortisol levels. These findings suggest a link between HPA axis activation and drug use vulnerability. To further support these findings, another study by Piazza et al., (1991) found that injecting corticosterone increased self-administration of cocaine. Furthermore, blocking glucocorticoid release by removal of adrenal glands or administering inhibitors reduces self-administration of cocaine (Goeders et al., 1998; Goeders & Guerin., 1996). Marinelli & Piazza., (2002) hypothesise that these effects may occur as the increase in dopamine in response to stress may function to reduce the aversiveness of stress, while increasing sensitisation of the reward system. This increase in sensitisation could lead to more positive effects of drug use through the dopamine system.

Early life and chronic stress impair development of the PFC. The PFC can activate the HPA axis in response to stress and aids in regulation of the stress response and the HPA axis. Impairment in the ability of the PFC to regulate the HPA axis has been found to be associated with an overactive response to stress and an increase in glucocorticoids and reduction in behavioural and cognitive control which may make an individual more vulnerable to drug use and abuse (Gratton et al., 2005). This influence of stress hormones on drug use also works in

the other direction, where drug use leads to activation of the HPA axis as demonstrated by increased plasma cortisol levels when exposed to nicotine (Wilkins et al., 1982; Mendelson et al., 2005) and cocaine (Baumann et al., 1995).

While factors such as increased stress can increase vulnerability to addiction (Gratton et al., 2005), it is conceivable that other factors can also be preventative in reducing the likelihood that one will develop a substance use disorder, although unfortunately this is much less investigated. By using animal models, we can manipulate these factors independently of each other to see the result it has on a function, such as drug use. One of these factors is having a positive life experience which reduces stress.

Environmental Enrichment

We can mimic a positive life environment in animals through environmental enrichment. In environmental enrichment, animals are raised in a larger enclosure than usual, which is equipped with many toys such as running wheels, ropes, tubes, and other animals. The enclosure layout is changed frequently so that the toys and locations are frequently novel (Figure 2). The stimulating environment that environmental enrichment produces has shown to have positive effects on some neurological and psychiatric diseases (Laviola et al., 2008), increase brain plasticity (Nithianantharajah & Hannan, 2006), and increase learning and memory (Rosenzweig & Bennett, 1996). It has also been found that behavioural sensitisation to amphetamine, morphine and nicotine is reduced in rats housed in EE (Bardo et al., 1995; Bardo et al., 1997; Green et al., 2003), acquisition of cocaine self-administration is diminished (Puhl, et al., 2012) and amphetamine self-administration is reduced (Bardo et al., 2001).

Figure 2

Enrichment housing and setup

Environment and SERT -/-

Many studies have examined the interaction between SERT -/- and the environment with a large body of literature finding SERT -/- rats are more sensitive to the influence of a negative environment compared to SERT +/+ rats (Homberg & Van den Hove, 2012). Belsky et al. (2009) proposed that certain genes, among others the 5-HTTLPR, are so-called plasticity genes, which increases the individual's sensitivity to all environments rather than just negative environments. This theory of plasticity may account for the otherwise contradictory results seen in SERT -/- rats in both positive and negative environments.

The Current Study

In summary, genetic mutations such 5-HTTLPR can increase the likelihood of developing a tobacco use disorder in humans and SERT -/- rats have shown to produce greater self-administration responding to several drugs of abuse in animal models. In contrast, environmental enrichment has been shown to reduce self-administration, acquisition, and reinstatement for drugs such as cocaine and amphetamine. This study investigates the effect environmental enrichment will have on predisposed SERT -/- rats.

Firstly, this study aims to examine potential differences in responding in a nicotine self-administration paradigm between SERT-/- and SERT+/+ rats to examine if high responding seen for other drugs applies to more available substances such as nicotine. If SERT-/- rats are more vulnerable to nicotine dependence, then the number of responses from SERT-/- rats would be higher than that of the SERT +/+ rats, particularly in the progressive ratio paradigm.

This study further aims to examine the effect that environmental enrichment has on self-administration. As SERT-/- is known to be more sensitive to environmental stimuli, both negative and positive, then the positive environment may be more beneficial for SERT-/- compared to SERT+/+ rats. Furthermore, if a positive environment overall can act as a protective factor against tobacco use disorders, then it is expected that this reduces the self-administration of nicotine in enrichment housed rats compared to standard housed rats.

Finally, if rats without a serotonin transporter gene are predisposed to tobacco use disorders, then they will be more resistant to extinction, more vulnerable to reinstatement and have higher break points than SERT +/+ rats. Furthermore, if a positive environment can protect against substance use disorders, then rats housed in environmental enrichment will be less resistant to extinction, more resistant to reinstatement and have lower break points for nicotine than those in standard housing.

Method

Experimental Design

The independent variables were genotype (SERT -/- and SERT +/+), housing condition (enriched and standard) both manipulated between subjects, and nicotine dose (0.01 mg/kg, 0.03 mg/kg and 0.06 mg/kg) manipulated within subjects. Dependent variables were self-administration acquisition rate, progressive ratio break point, extinction responses and reinstatement responses. Ethics approval was obtained from the animal ethics committee (AEC 28383).

Subjects

SERT +/+ and SERT -/- male Sprague-Dawley rats from different litters were used. SERT +/- rats were not used in this study due to the number of extra rats needed compared to the available time and because this study aimed to start with the extremes in which the outcome could be used to determine if it is worthwhile examining SERT +/- rats. A minimum of 8 rats were allocated to each of the four groups (genotype x environment) based on previous experiments which found sufficient power with these numbers and the awareness that some rats may be lost during surgery or post-operative care. Given the expected larger variance in rats housed in enrichment, attempts were made to maximize these groups. A total of 58 rats were used for this study, but some did not make it to self-administration.

Table 1

N Per self-administration Condition. More enriched rats were used due to low acquisition rate and to ensure large enough sample sizes for the experiments following acquisition.

Condition	N
SERT -/- Enriched	19
SERT -/- Standard	9
SERT +/+ Enriched	20
SERT +/+ Standard	10

Housing

At post-natal day 21, offspring were weaned, and each genotype was divided and randomly assigned to standard housing or enriched housing (Table 1) where they remained until surgery in week 8. There were two rats per standard housing condition to minimise the confound of stress that may be introduced when rats are housed in isolation. Environmental enrichment had approximately 6 rats per cage and toys. Rats were kept on a 12-hour reversed light dark cycle (light during 19:00-07:00), and experiments were conducted between 07:00 and 19:00 during the dark cycle. Food and water were available ad libitum until self-administration training started, after which the rats were food restricted on 20g of pellets per day after experiments.

Nicotine

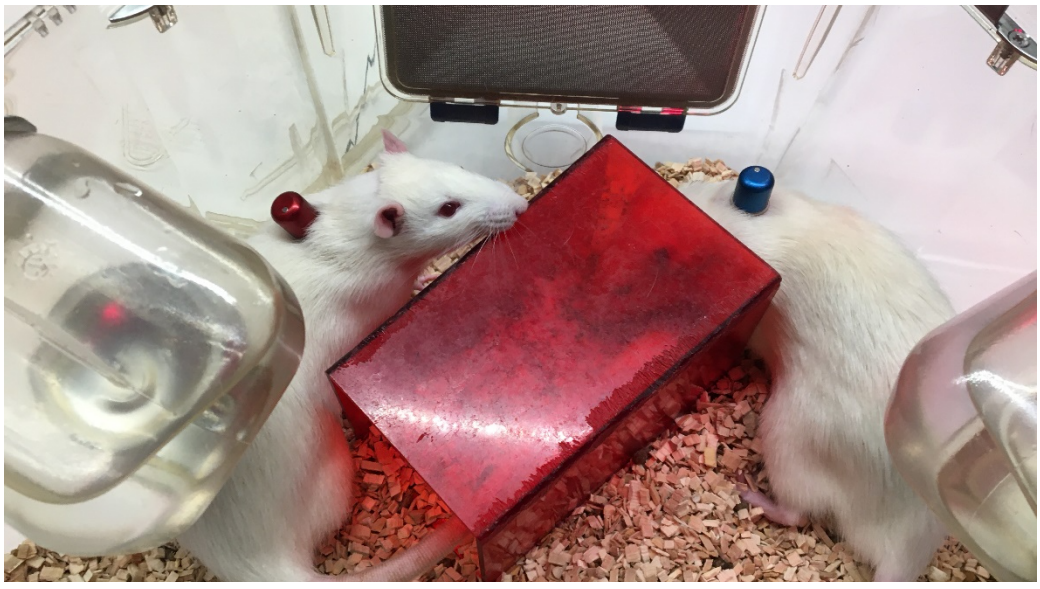
Nicotine hydrogen tartrate salt was dissolved in 0.9% sterile saline. The nicotine solution was further diluted with saline to produce three doses of pure nicotine (0.01 mg/kg, 0.03mg/kg and 0.06mg/kg as used previously in other studies).

Surgery

When rats reached at least 300 grams bodyweight in week 8, they underwent surgery to implant a catheter for drug infusions into the blood stream. Surgical procedures followed standard protocol used in the animal lab and involved the insertion of a catheter into the jugular vein, secured between the shoulder blades using a one channel Vascular Access Button from Instech Laboratories as shown in figure 3 (Boeri & Horsmon, 2021). The analgesic Carprofen was given for the two days post-surgery and catheters were flushed twice daily with 0.2 ml of a sterile/heparinized saline solution containing 0.4mg/mL of the antibiotic enrofloxacin. Catheter functionality was tested weekly, and in the event of failure, rats underwent repair surgery.

Figure 3

Vascular Access Button used for surgery



Self-Administration

These experiments followed protocol previously used in the lab (Brennan et al., 2015).1-week post-surgery, the rats were individually placed in an operant chamber with two levers (one active and one inactive) and a stimulus light. The light was illuminated when the

lever was pressed, and drug-reinforcement was infused. Rats were placed in the operant chamber for self-administration training. Responding on the active lever resulted in an infusion of nicotine through the intravenous catheter. Responding on the inactive lever had no result but served to eliminate the alternative explanation that rats are pressing the lever regardless of the drug infusion as responses were higher on the active lever but not the inactive lever. The session started with an experimenter administered prime. FR1 (days 1-15), followed by FR2 (days 16-20) and FR5 (days 21-30) was used to establish self-administration (training dose 0.03 mg/kg/infusion). Nicotine self-administration was considered acquired when, during the final FR5 sessions, responses were higher on the active than inactive lever and an animal responded at least 20 times/session on the active lever for a minimum of three consecutive days.

Extinction and Reinstatement

Animals that acquire self-administration were placed in an operant chamber for six 2-hour extinction experiments wherein responding on the active lever did not result in an infusion of nicotine or illumination of the cue light. Behaviours were deemed extinguished when a maximum of 25 lever presses were recorded on the active lever for two consecutive days. This examined the effects of drug extinction on responding. The rats then underwent four 2-hour reinstatement sessions which were started with a cue light that was illuminated upon each lever press. Responses were not reinforced but were measured, this examined reinstatement of drug-seeking behaviour or relapse potential of the drug in the groups.

Progressive Ratio testing

After extinction and reinstatement, the rats underwent 3 FR5 training sessions to bring responding back to baseline. Progressive ratio sessions ran until a break point was reached, defined by no responses for 30 minutes. 3 doses of nicotine were used (0.01 mg/kg, 0.03mg/kg and 0.06mg/kg) for 3 days each dose, randomly assigned. Progressive ratio trials

were separated by a FR5 day for a return to baseline. The number of responses required for each dose increased using the equation $5e(0.2 \times \text{Infusion\#}) - 5$ (rounded to the nearest integer) resulting in response requirements of 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, etc. until a break point was found (Brennan et al., 2015). The break points for each condition were plotted to provide a measure of the reinforcing strength of nicotine.

Results

Acquisition

A repeated measures ANOVA with genotype and housing condition as between subject factors and average fixed ratio session responses (FR1, FR2 and FR5) as within subject factors found a main effect of session $F(2, 64), = 186.31$, $p < .001$, $\eta_p^2 = .85$ where responses significantly increased from FR1 ($M = 7.72$, $SD = 4.38$), FR2 ($M = 18.70$, $SD = 10.5$), and FR5 ($M = 44.1$, $SD = 16.5$). A session x genotype interaction was also observed $F(2, 64), = 6.1$, $p = <.05$ $\eta_p^2 = .16$ where SERT -/- rats in FR5 had significantly less responses ($M = 37.5$, $SD = 11.9$) compared to SERT +/+ rats in FR5 ($M = 51.4$, $SD = 18.2$) as shown in post hoc comparisons with Bonferroni corrections $t(68.5) = 3.32$, $p < .05$. Further analysis of the session x genotype interaction revealed that SERT -/- rats in enrichment pressed significantly less ($M = 34$, $SD = 12.9$) than SERT +/+ rats in enrichment ($M = 53.4$, $SD = 13.4$) as shown by post-hoc tests with Bonferroni corrections $t(68.5) = 3.98$, $p = <.05$). No other significant main effects or interactions were observed, however, planned post-hoc analyses showed marginal significance between SERT +/+ rats in enrichment ($M = 34$, $SD = 12.9$) and SERT -/- rats in standard housing ($M = 49.2$, $SD = 23.2$) in FR5 $t(18) = 1.89$, $p = 0.07$, $d = 0.86$. Planned post-hoc analyses also showed marginal significance between SERT -/- rats in enrichment ($M = 34$, $SD = 12.9$) and SERT -/- rats in standard housing ($M = 43.6$, $SD = 7.29$) in FR5 $t(17) = 1.81$, $p = 0.09$, $d = 0.86$. Further planned post-hoc analyses on FR1 responses showed a significant difference between SERT -/- rats in enrichment ($M = 6.22$, $SD = 3.26$) and SERT -/- rats in standard housing ($M = 10.3$, $SD = 3.06$) $t(17) = 2.68$, $p < .05$, $d = 1.27$.

Figure 4.

Average daily infusions for condition, overall genotype and overall housing on FR1, FR2 and FR5 sessions. Error bars represent standard error of the mean (SEM). (*) indicates significance, (#) indicates marginal significance.

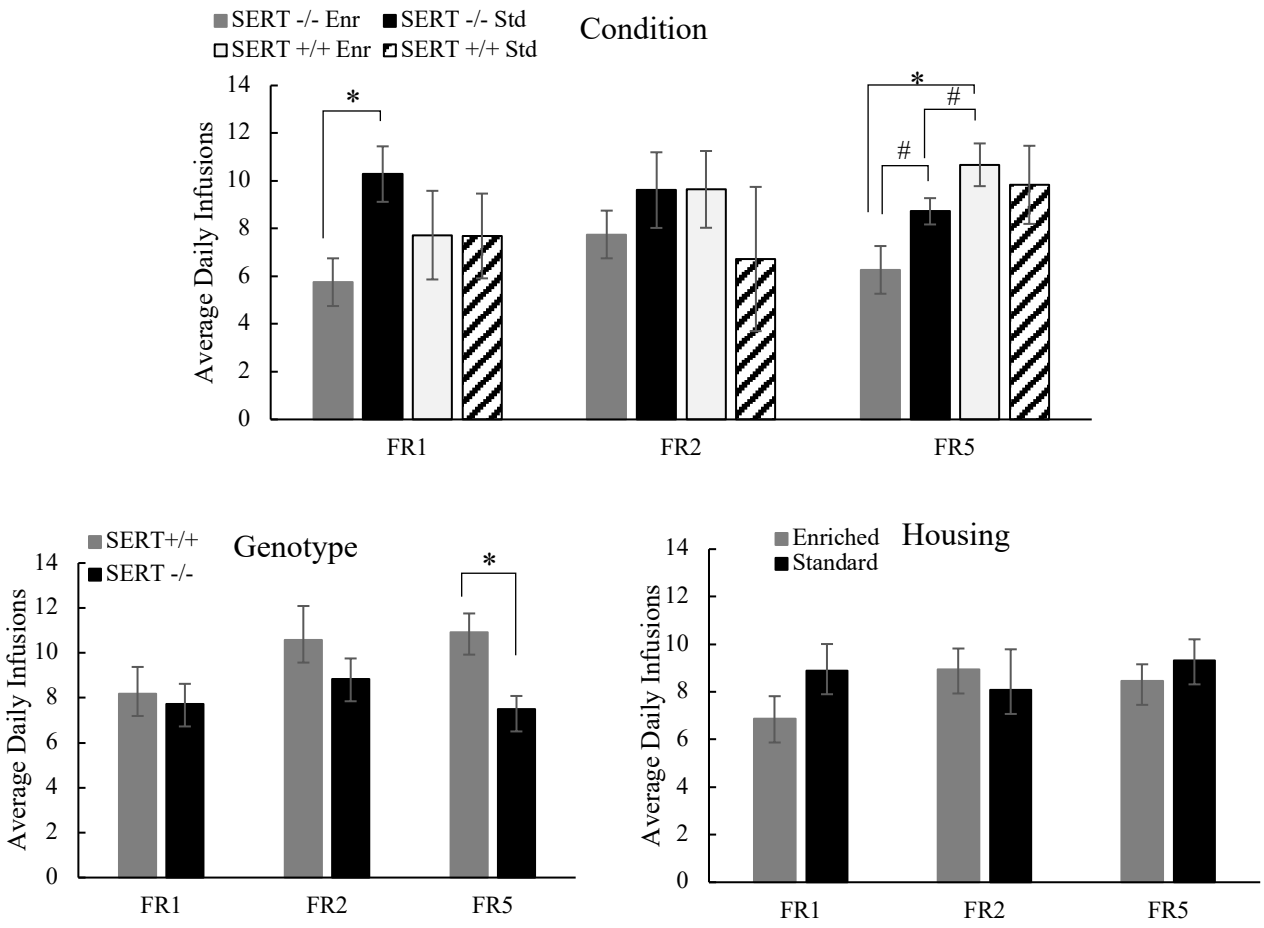
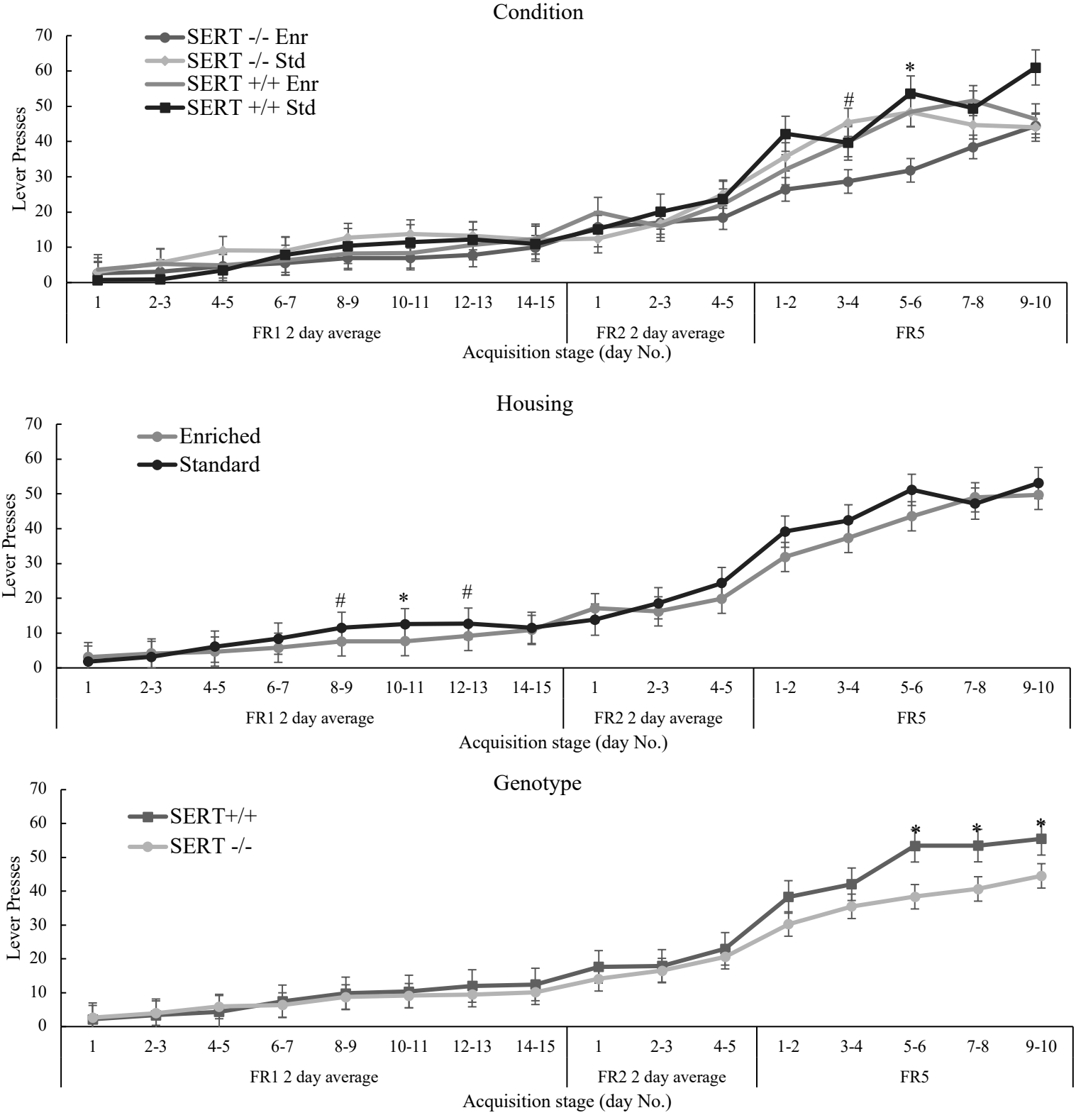


Figure 5.

Line graph showing the increases in average responses for condition, overall genotype and overall housing in FR1, FR2 and FR5 sessions. X axis shows average lever presses. (*) indicates significance. (#) indicates marginal significance.

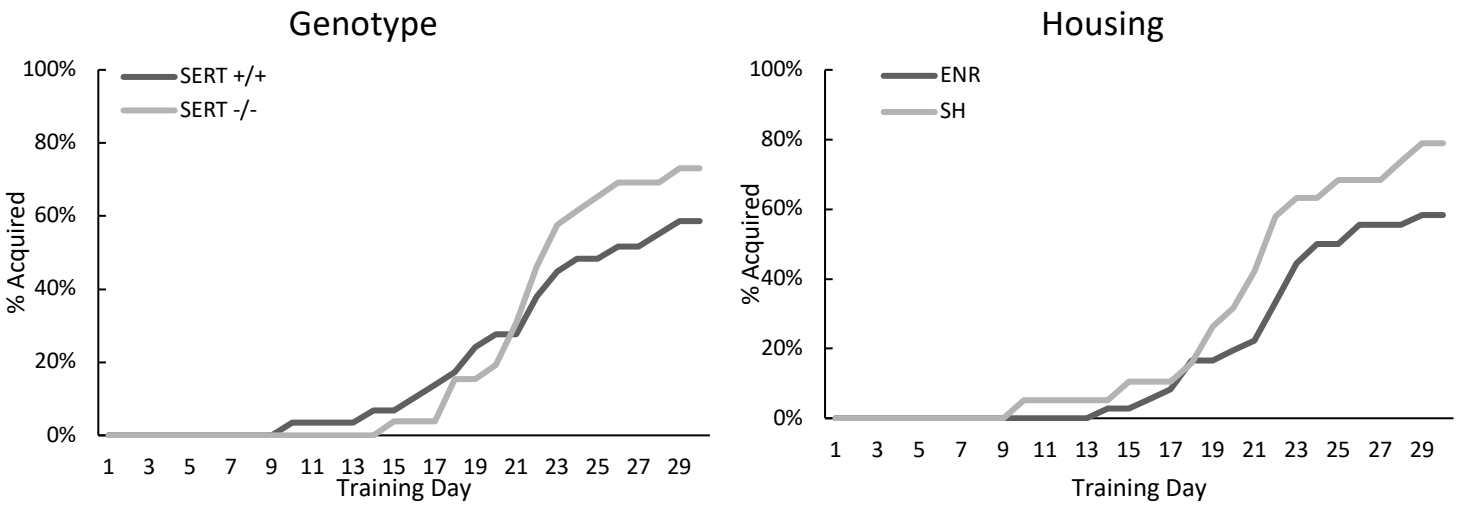


Rate of Acquisition

The day that acquisition criteria was met was recorded and plotted to produce a graph showing the rate of acquisition (Figure 6). An ANOVA on day acquired with genotype $F(1,31) = 0.82$, $p = .37$, $\eta_p^2 = .03$) and housing $F(1,32) = 0.48$, $p = .49$, $\eta_p^2 = .02$ as between subject factors showed no significant main effects and no interactions $F(1,32) = 0.72$, $p = .40$, $\eta_p^2 = .02$ where all rats acquired self-administration at the same rate.

Figure 6.

Percentage of rats each condition that reached acquisition criteria on a given session day.



Active Lever Responses

A repeated measures ANOVA examining average active and inactive lever responses with genotype and housing as between subject factors, and lever as within subject factors was used to ensure preference was for the active lever. A significant main effect of lever was found $F(1,29) = 265.34$, $p < .001$, $\eta_p^2 = .90$ where responses on the active lever ($M = 1424$, $SD = 501$) were higher than responses on the inactive lever ($M = 79.2$, $SD = 71.2$). No significant

main effects of genotype and housing and no significant interactions were observed, such that the difference between left and right lever responses was the same between conditions.

Percentage of Acquisition

The percentage of rats allocated to a condition that acquired self-administration was recorded and presented in Table 2. Rats in enrichment (M = 0.56, SD = 0.50) appeared less likely to acquire self-administration than rats in standard housing (M = 0.79, SD = 0.42). SERT +/+ rats (M = 0.59, SD = 0.50) also appeared less likely to acquire self-administration than SERT -/- rats (M = 0.73, SD = 0.45), however, a logistic regression with housing and genotype as predictors for acquisition found no significant effects as presented in Table 3.

Table 2.

Percentage of rats per condition that reached the criteria for self-administration acquisition.

Condition	Total Run	Total Acquired	%
SERT +/+ total	29	17	58.6
SERT -/- total	26	19	73.1
Enriched total	36	21	58.3
Standard total	19	15	78.9
SERT -/- Enriched	17	12	70.6
SERT -/- Standard	9	7	77.8
SERT +/+ Enriched	19	9	47.4
SERT +/+ Standard	10	8	80.0

Table 3.

Logistic regression results with genotype and housing as predictors of acquisition. Adjusted R-squared was reported as -0.04.

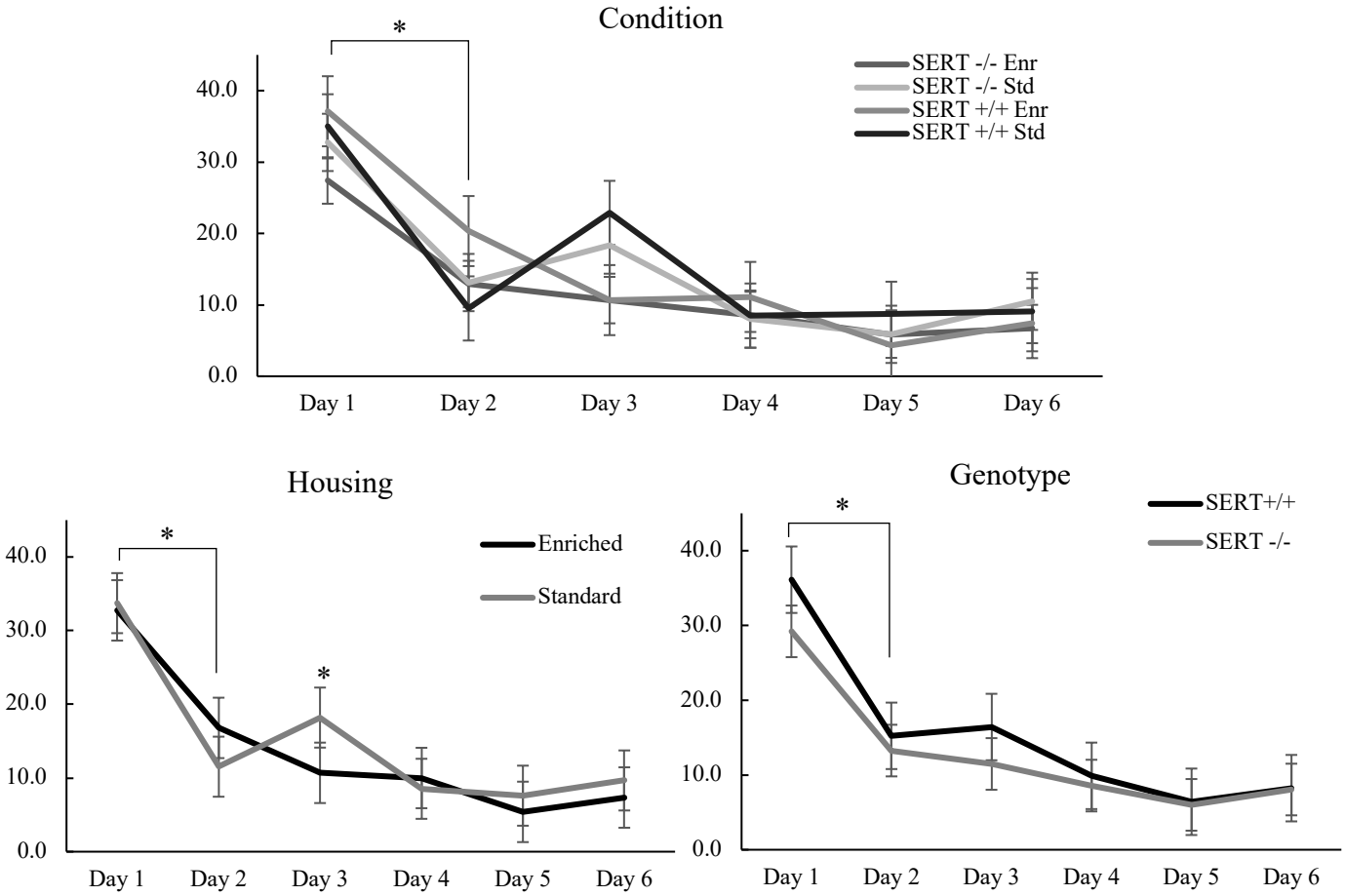
	Coefficient	Odds Ratio	Std. error	p-value
Intercept	0.036		0.29	0.77
Genotype	0.62	1.86	1.08	0.28
Housing	0.82	2.28	1.31	0.19

Extinction

A repeated measures ANOVA examining extinction responses, with genotype and housing condition as between subject factors, and active lever presses as within subject factors found a main effect of day $F(5,160) = 41.25$, $p < .001$, $\eta_p^2 = .56$ where responses on the active lever significantly reduced from day one ($M = 32.5$, $SD = 17.2$) to day two ($M = 14.2$, $SD = 8.92$). No significant interactions were observed. Planned post-hoc analyses found a main effect of housing on day three $F(1,32) = 4.93$, $p < .05$, $\eta_p^2 = .13$ where rats in standard housing showed increased responding ($M = 18.2$, $SD = 14.0$) compared to rats in enrichment ($M = 10.7$, $SD = 5.25$). No other significant effects were observed.

Figure 7.

Active lever presses over the 6 days of extinction. Error bars represent SEM. (*) indicates significance.



Reinstatement

A repeated measures ANOVA examining reinstatement responses, with genotype and housing condition as between subject factors, and active lever presses as within subject factors found a main effect of day $F(3,96) = 2.98$, $p < .05$, $\eta_p^2 = .09$ and a day x housing interaction $F(3,96) = 2.78$, $p < .05$, $\eta_p^2 = .08$ where standard housing rats on day four responded less ($M = 5.27$, $SD = 4.68$) than enrichment rats ($M = 12.19$, $SD = 11.31$) as shown by post-hoc t-tests $t(34) = 2.23$, $p < .05$, $d = 0.75$. No other significant effects were observed (Figure 8). All conditions showed a significant increase in responses from the final two days

of extinction (*M* = 7.17, *SD* = 4.25) to the first two days of reinstatement (*M* = 13.3, *SD* = 6.15) when the cue light was re-introduced absent of an infusion as shown by a repeated measures ANOVA examining session, with genotype and housing condition as between subject factors and session as within subject factors $F(1,32) = 32.65$, $p < .001$, $\eta_p^2 = .50$. Individual test results for each condition are presented in Table 1A of the appendix and graphed in figure 9.

Figure 8.

Active lever presses over the four days of reinstatement. Error bars represent SEM. (*) indicates significance.

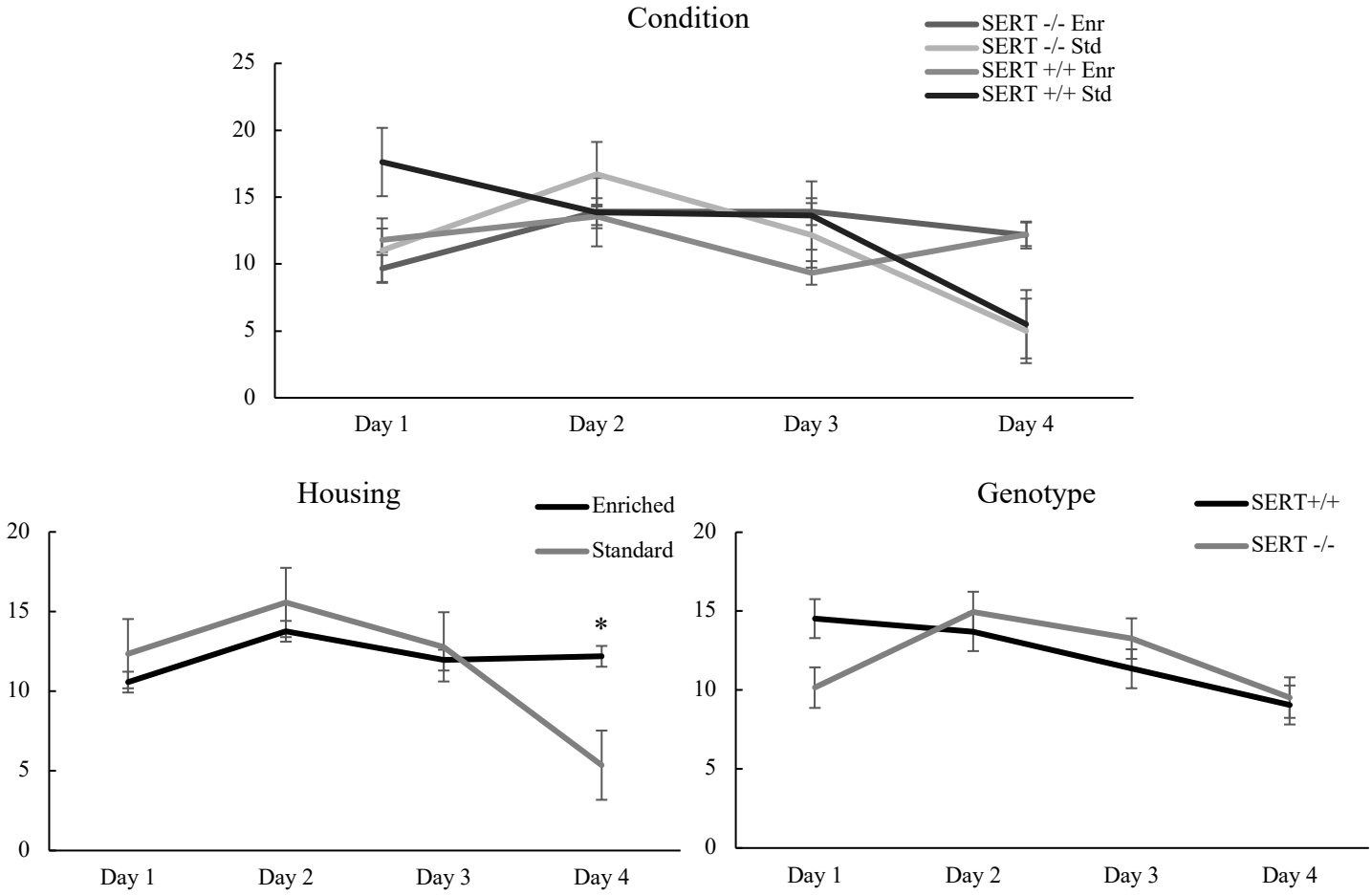
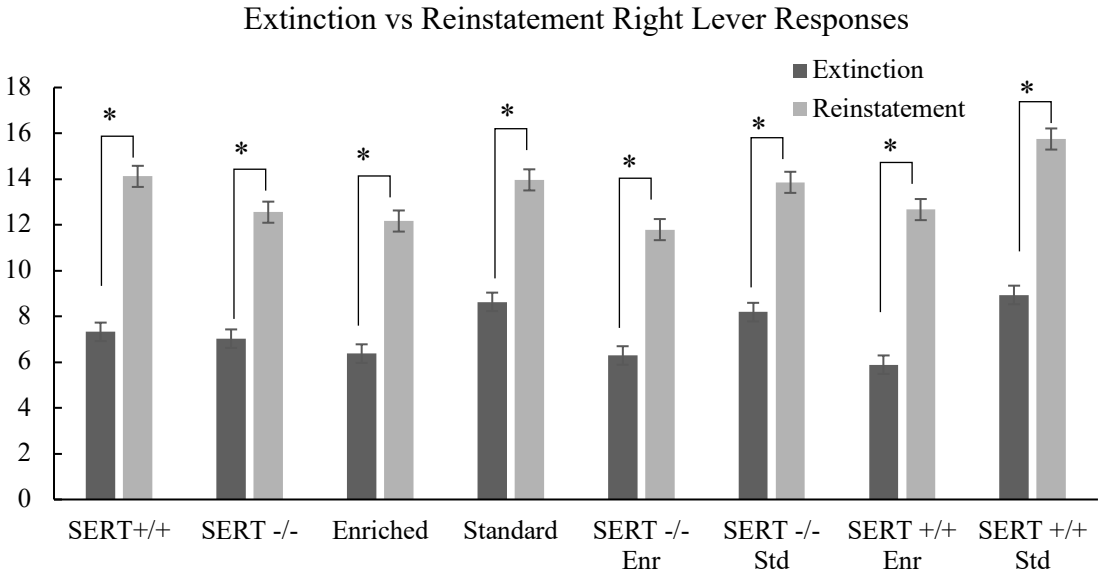


Figure 9.

Average right lever presses of the final two extinction days vs the first two reinstatement days. The final two extinction days are used as these better reflect extinction of the conditioned behaviour and are used as extinction criteria. Error bars represent SEM. (*) indicates significance.



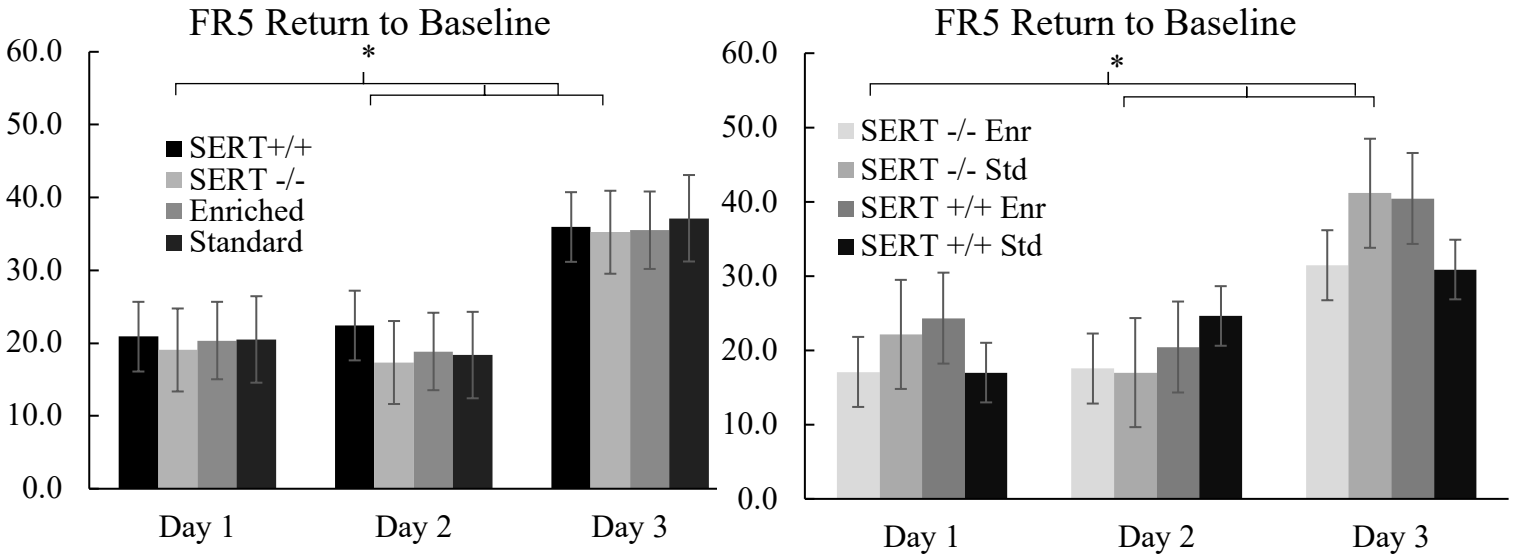
Return to Baseline

Prior to progressive ratio, the rats completed three FR5 sessions to assist a return to baseline. A repeated measures ANOVA with FR5 day as within subject factors and housing and genotype as between subject factors found a main effect of FR5 day $F(2, 62) = 11.27$, $p < .001$, $\eta_p^2 = .27$. Post Hoc tests showed a significant difference when comparing day three ($M = 35.6$, $SD = 26.8$) to day two ($M = 19.8$, $SD = 17.0$) $t(62) = 4.14$, $p < .001$) and to day one ($M = 19.8$, $SD = 19.1$) $t(62) = 4.08$, $p < .001$. No other significant main effects or interactions were observed across the three sessions, such that once the drug was reintroduced, responses between conditions did not significantly differ. It is worth noting that on day three, SERT -/-

in enrichment (M = 31.5, SD = 21.2) performed similar to SERT +/+ in standard housing (M = 30.9, SD = 26.5) and SERT -/- in standard housing (M = 41.1, SD = 32.6) performed similar to SERT +/+ in enrichment (M = 40.4, SD = 31.3)

Figure 10.

Active lever responses across three FR5 sessions preceding progressive ratio to assist a return to baseline responding. Error bars represent SEM. (*) indicates significance.

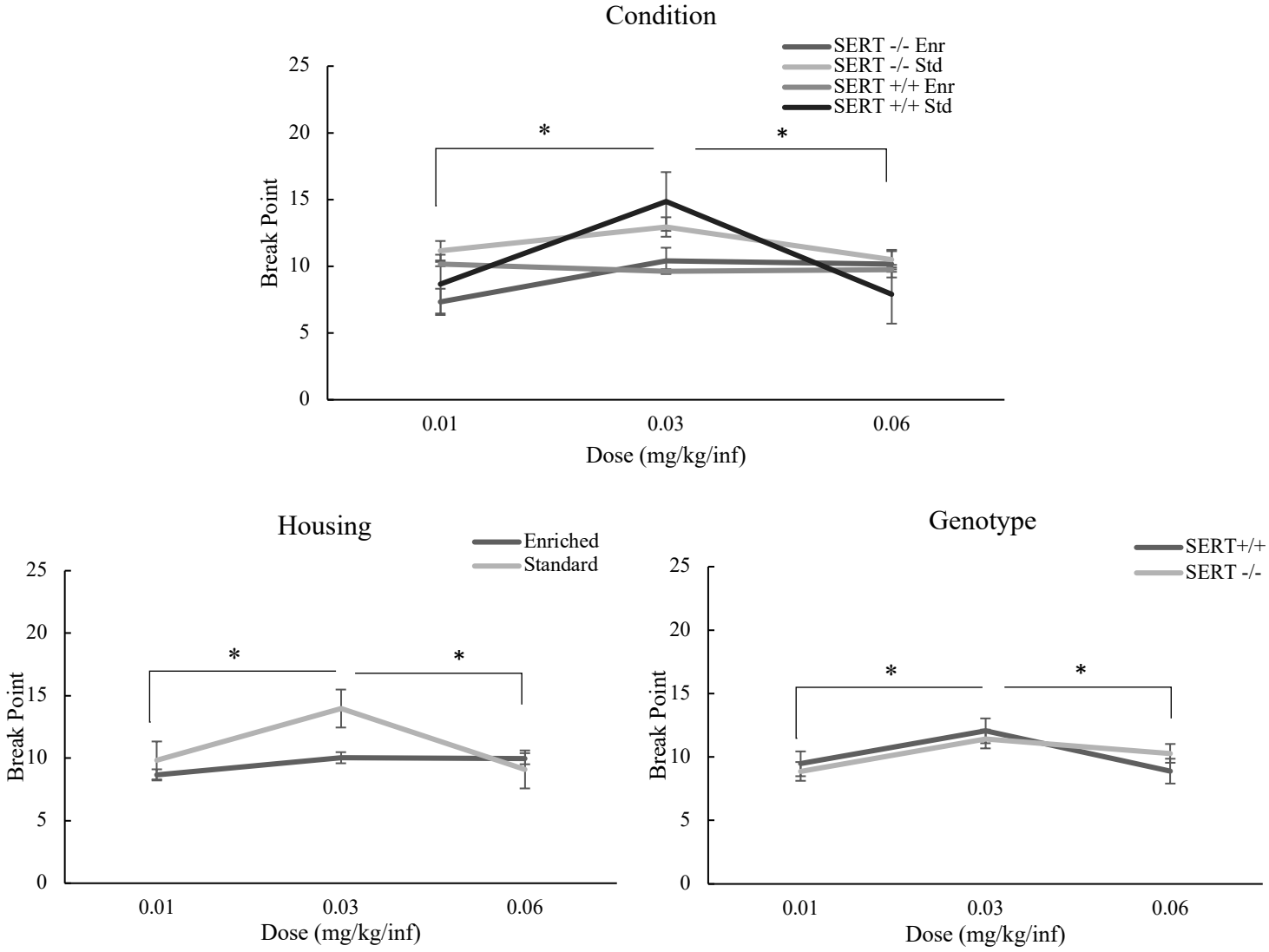


Progressive Ratio

A repeated measures ANOVA examining the break point for each dose, with genotype and housing condition as between subject factors, and dose as within subject factors found a main effect of dose $F(2,52) = 3.53$, $p < .05$, $\eta_p^2 = .12$ where the break point for 0.03 mg/kg/infusion was higher (M = 11.7, SD = 7.69) than the break point for 0.01 mg/kg/infusion (M = 9.17, SD = 5.74) $t(52) = 2.4$, $p = .05$). There was a marginally significant difference between the 0.06 mg/kg/infusion dose (M = 9.60, SD = 5.77) and the 0.03mg/kg/infusion dose $t(52) = 2.17$, $p = .09$. No other main effects or significant interactions were observed.

Figure 11.

Average break point across doses (0.01, 0.03 and 0.06 mg/kg/infusion). Each dose was tested three times in a randomly assigned order. Error bars represent SEM.



Discussion

In the present thesis, the role of a genetic reduction in the SERT and subsequent environmental enrichment on nicotine self-administration was investigated. Based on the existing literature, we hypothesised if rats without a serotonin transporter gene are predisposed to tobacco use disorders, then they will more readily self-administer nicotine, be more resistant to extinction, more vulnerable to reinstatement and have higher break points in progressive ratio than SERT +/+ rats. Furthermore, if a positive environment can protect against substance use disorders, then rats housed in environmental enrichment will less readily self-administer nicotine, be less resistant to extinction, more resistant to reinstatement and have lower break points for nicotine than those in standard housing. Finally, because SERT-/- is known to be more sensitive to environmental stimuli, both negative and positive, then the positive environment may be more beneficial for SERT-/- compared to SERT+/+ rats.

The results showed that as hypothesised, environmental enrichment exhibited protective effects during the acquisition stage in SERT -/- rats genetically predisposed to nicotine dependence, but not in SERT +/+ rats. In addition, rats in standard housing extinguished cue-induced reinstatement responding to a greater extent than rats in enrichment, but also showed a marginal increase in responding during extinction. Contrary to predictions, rats in standard housing were not more likely to self-administer nicotine compared to rats in enrichment, nor were their break points higher in progressive ratio. Below the findings of the different parts of the study (acquisition and maintenance, extinction and reinstatement and progressive ratio) will be discussed in more detail.

Acquisition of Self-Administration

This study sought to further understand the underlying neurobehavioural mechanisms that influence vulnerability to addiction. A positive environment during development and a genetic reduction in serotonin transporter function were examined. Contrary to the hypothesis that SERT -/- rats are more vulnerable to the reinforcing properties of nicotine, there were no significant differences overall in genotype, as the number of lever presses from SERT -/- rats in all experiments (acquisition, maintenance and progressive ratio) were not significantly higher than those of the SERT +/+ rats. However, although not significant, there was a trend that a larger percentage of SERT-/- rats acquire (fig 6 and table 2). This finding aligns with studies that show around 60% of individuals with a mental illness are smokers, compared to 25% of the general population that smoke (Leonard et al., 2001), as SERT is associated with increased vulnerability to other mental illnesses such as affective disorders (Kenna et al., 2012). On the other hand, SERT +/+ had significantly higher responses than SERT -/- during the FR5 sessions (Figure 4).

Overall, rats in environmental enrichment were hypothesised to self-administer nicotine to a lesser extent. However, rats in environmental enrichment did not differ in the number of lever presses for nicotine compared to rats in standard housing in self-administration or progressive ratio. There was a trend in the number of animals that acquired, where 58.3% of rats in enrichment acquired self-administration compared to 78.9% of rats in standard housing, suggesting that standard housed rats were more vulnerable to nicotine dependence. This finding aligns with studies that show that stress is a major environmental vulnerability related to drug use (Solinas et al., 2010), as the enriched environment is theoretically less stressful than standard housing.

SERT +/+ rats in enrichment showed increased self-administration compared to SERT +/+ rats in standard housing during FR5 sessions (figure 4). The increase for SERT

+/+ rats in enrichment counters any main housing effects we may expect to see and turns the focus to the gene x environment interactions. SERT -/- rats were hypothesised to be more sensitive to their environment thus the positive environment would be more beneficial for SERT -/- compared to SERT +/+ rats, based on the so-called plasticity hypothesis of Belsky discussed in the introduction. In alignment with the hypothesis, environmental enrichment exhibited protective effects in rats genetically predisposed to nicotine dependence, but not in SERT +/+ rats that were not predisposed especially during acquisition (Figure 4). Increases in self-administration between SERT +/+ in enrichment compared to standard housing were not predicted but could be due to increased stress from changing environments. Nader et al. (2012) found that removing rats from enrichment to standard housing increased cocaine CPP in mice, higher than that of those in standard housing that did not move. The findings of the current study alongside previous research on removal from environmental enrichment suggests that discontinuation of enrichment may increase vulnerability to drug addiction in SERT +/+ rats, but not in SERT -/- rats that still show reduced self-administration. As hypothesised, the increased protection of enrichment in SERT -/- rats could be due to greater environmental plasticity, or the concept of "for better and for worse" in which SERT -/- rats show higher negative behaviours in negative environments, but higher positive behaviours in positive environments, compared to controls (Homberg & Van den Hove, 2012).

One drawback of other gene x environment studies is that most focus on comparing a more- or-less neutral environment with a negative environmental challenge, and consequently the absence of adversity, is then considered to be the "good" environment (Belsky et al., 2009). Considering what is more likely a control as a "good" condition could limit any beneficial effects that are produced by genes such as the ones observed in this study. The current instead compares a more neutral environment (i.e. social housing in standard cages) to a positive environment (i.e. social housing in enriched cages). That is, comparing standard

housing to enriched housing to examine the influence of positive environments on "vulnerable" genes in support of instead referring to these as "plasticity" genes. These findings align with the current study in which the positive environmental enrichment reduced self-administration of nicotine in SERT -/- rats compared to SERT +/+ rats. In the more neutral environment, standard housed SERT -/- rats had increased self-administration of nicotine compared to SERT +/+ rats which suggests that SERT -/- rats may be more sensitive to their environments compared to SERT +/+ rats thus, a gene × environment interaction was observed. In support of this concept, SERT +/- mice show lower levels of depressive-like symptoms in a control maternal environment compared to SERT +/+. In contrast, when exposed to prenatal maternal stress, SERT +/- mice showed increased levels of depressive-like symptoms compared to SERT +/+ (Van den Hove et al., 2011). Furthermore, human studies show that stressful early family environments increase depressive symptoms in s allele carriers, but in a supportive early family environment, s allele carriers have reduced depressive symptoms (Taylor et al., 2006).

While not significant, the number of rats that acquired self-administration was higher in standard housing than enrichment, suggesting these rats more readily acquire self-administration. With a larger sample size, this effect may become significant which would suggest that environment can influence the likelihood of drug use developing to dependence.

Extinction and Reinstatement

It was hypothesised that rats in standard housing would be more resistant to extinction and more vulnerable to reinstatement. However, responses between housing conditions were the same for extinction, with the exception that standard housed rats showed increased responding on day 3 which was not present in enriched rats. This increase in responding could reflect greater withdrawal effects or drug seeking behaviour in standard housed rats, however, as it is only present on one of the extinction days the overall effect is marginal.

Increased dopamine can reduce withdrawal effects as seen in the administration of bupropion for smoking cessation. Therefore, an increase in baseline dopamine induced by enrichment may reduce withdrawal effects of nicotine. As this study only examined behavioural effects and not neurotransmitter levels in the brain, it cannot be confirmed that all enrichment rats had an increase in dopamine after the discontinuation of enrichment, as SERT +/+ rats in enrichment did have higher self-administration responses than SERT +/+ rats in standard housing.

During reinstatement sessions, no significant differences were observed until the final day (day 4) where enrichment rats maintained high responding while standard housed rats reduced their responding, contrary to the hypothesis. If, with a longer time-course this trend continues, it suggests that standard housed rats are more resistant to cue induced reinstatement and therefore may have a higher protection for relapse. If true, we would expect to see differences during the FR5 return to baseline when the drug was reintroduced, which was not observed ion the present study (Figure 10). This could imply that standard housed rats, although somewhat resistant to cue induced reinstatement, were not more resistant to drug induced reinstatement. Previous researchers examining the effects of bupropion on nicotine drug seeking observed an increase in reinstatement responding in rats pre-treated with bupropion. The authors suggest that bupropion facilitates dopamine pathways when presented with the cue which aids in reinstatement of the cue-induced response. As bupropion increases extracellular dopamine, the increase in dopamine produced by enrichment could mimic some of the effects of bupropion and induce similar effects (Liu et al., 2008). The researchers also suggest that higher responses during self-administration training would result in higher responses in reinstatement due to the cue being presented with the drug on more occasions (Liu et al., 2008). If this were the case, then we would expect to see differences between housing groups during the training sessions, which were not observed (Figure 4).

Along a similar line of thought, the lack of a reduction in reinstatement responses by enrichment rats could be due to greater PFC function and consequent conditioned stimulus association and motivational salience, allowing a more strongly paired cue and drug association compared to standard housed rats (McLaughlin & See, 2002). This could result in stronger memory connections for the cue that take more sessions than standard housed rats to be extinguished, however we would then expect extinction responses to differ which was not the case. Further research into the brain regions and neurotransmitters associated with self-administration, extinction, and reinstatement of nicotine in these conditions could help to discern why the differences in extinction and reinstatement were observed.

Progressive Ratio, Motivation, and Impulsivity

No significant differences in progressive ratio were observed, contrary to the hypothesis, however there was a trend towards significance in standard housed rats for the 0.03 mg/kg/infusion dose as well as SERT +/+ standard housed rats for the same dose (Figure 11) that could be significant with a larger sample size. The return to baseline responses showed no overall significant differences, but marginally SERT -/- in enrichment performed similar to SERT +/+ standard housing and SERT -/- in standard housing performed similar to SERT +/+ in enrichment, after extinction and reinstatement, suggesting a gene * environment interaction. As the responses differed from initial self-administration this may have disrupted progressive ratio responding and dampened the prior effects. Data from the FR5 sessions that came between each progressive ratio session also showed no significant differences, nor reflected the initial trends observed in the acquisition stage (Appendix figure 1C). The finding that both progressive ratio and fixed ratio sessions showed no significant differences suggests that any differences we would expect to observe in progressive ratio may have been eradicated after extinction and reinstatement, so no strong claims about a lack of difference in progressive ratio can be made. Further, it is unlikely that a lack of difference was observed

due to altered sensitivity to nicotine after abstinence, as baseline FR5 sessions prior to progressive ratio (figure 10) aimed to reduce this. We also see no significant increases in FR5 responses that were interspersed between the progressive ratio sessions that you would expect to see if the rats are pressing less due to heightened sensitivity to nicotine (Appendix table 1B). Progressive ratio is suggested to examine the motivation for a substance. While differences in motivation were not observed in progressive ratio, SERT +/+ rats in enrichment showed greater responding in FR5, surpassing SERT -/- rats in standard housing which had the highest number of infusions in FR1. This increase in infusions with a required increase in responses may reflect differences in motivation that were not observed in progressive ratio due to disruptions from prior experiments.

Progressive ratio experiments in this study produced an inverted U curve that is often seen when examining responses to drugs at different doses. In this experiment, both 0.01mg/kg/infusion and 0.06mg/kg/infusion doses produced reduced responding compared to the 0.03mg/kg/infusion training dose. Some authors find that both the low and high doses produce reduced responding during FR sessions due to the low dose not being as reinforcing, and the high dose producing increased satiation thus less of the drug is needed to experience optimal effects (Corrigall & Coen, 1989). Other authors, however, find that progressive ratio is more resistant to the inverted U curve as it is measuring motivation and is less impacted by satiation at higher doses (Donny et al., 1999). This was not found in the current study, where the higher dose produced a lower break point. This could be due to the time requirement for completing progressive ratio sessions being 30 minutes, where no infusions within this time end the session. Therefore, rats may not have enough time for the peak levels of nicotine to reduce enough to seek the drug again and thus acquire less infusions due to the half-life of nicotine being around 1 hour (Kyerematen et al., 1988) which is outside of the 30-minute

period used in this study. 30 minutes was used as per protocol previously used in the lab (Brennan et al., 2015).

As previously mentioned, the vulnerable allele of the 5-HTTLPR polymorphism is associated with many psychiatric disorders including affective disorders (Kenna et al., 2012) and substance use disorders (Cao, Hudziak & Li, 2013). These findings along with the reduced control seen in many psychiatric disorders suggest that alterations in the serotonin transporter may influence impulsivity (Dalley & Rosier, 2012) and increase the vulnerability to drug addiction. This could help to explain why SERT -/- rats in enrichment self-administered nicotine to a lesser extent than SERT -/- rats in standard housing due to a reduction in impulsivity encouraged by enrichment housing. However, this was not true for SERT +/+ rats in enrichment, possibly due to the stress of changing environments reducing the positive effects (Nader et al., 2012). These findings support the notion that impairments in control are a vulnerability associated with drug addiction (De Wit, 2009).

Impact of Environment

One caveat of many prior studies is the comparison to isolation housing. Most self-administration studies using a surgical technique that requires the animals to be singly housed. As rats are social animals, housing them in isolation creates substantial stress, often exacerbating the effect sizes (Lukkes et al., 2009). The current study aimed to avoid this caveat by housing all rats (when not in enrichment) with a cage mate, by using an alternative surgical technique. This prevented isolation stress and therefore reduced confounds introduced by combining many negative factors. As such, the social grouped standard housing provided a more appropriate control to compare to enrichment (Solinas et al., 2010). On the downside, this may have potentially reduced the differences between the two environmental challenges.

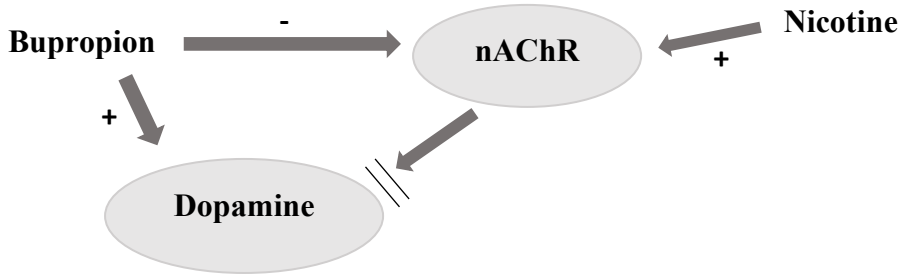
The current study examined the effects of early enrichment during development, rather than the effects of currently being in enrichment. This could give some insight into the impact of being raised in a healthy environment compared to being raised in a more vulnerable environment, as adverse childhood experiences are associated with a higher vulnerability to develop nicotine dependence (Elliott et al., 2014; Anda et al., 1999; Felitti et al., 1998). The impact of enrichment during early development but not during drug-taking may also be more applicable to clinical conditions, where the maintenance of a healthy environment throughout life is more difficult to control. The findings of this study and the association of adverse childhood experiences with mental health issues (Anda et al., 2006) sheds light on the importance of considering individual struggles and environments (past and present) when treating nicotine dependence, as there are likely underlying mental health issues that need to be treated alongside nicotine replacement therapies for a more successful outcome.

Treatment Options

Many smoking cessation options exist, and these include nicotine replacement therapies (NRTs) and antidepressants. As previously mentioned, the s allele of the 5-HTTLPR polymorphism is associated with mood disorders such as depression (Kenna et al., 2012). Furthermore, there is a high comorbidity between psychological disorders such as depression and tobacco use disorders (Grant et al., 2004; Leonard et al., 2001). Therefore, antidepressants for smoking cessation become of particular interest. One of the first used antidepressants for smoking session was bupropion after smokers who took the medication for depression also reduced their smoking (Ferry, 1999). As shown in figure 12, Bupropion reduces binding of nicotine (Slemmer et al., 2000), while still maintaining an increase in extracellular dopamine (Rau et al., 2005). The increase in dopamine can attenuate withdrawal effects that smokers normally experience due to the sudden drop in dopamine.

Figure 12.

The effect of Bupropion on dopamine and nAChRs.



While antidepressants for smoking cessation do not work for all people, such as those not actively trying to quit (Cousins et al., 2001), they are a good alternative to NRTs in individuals with affective disorders such as depression as it provides the option to treat both disorders simultaneously. As discussed, many individuals who suffer from a psychological disorder also smoke, so treatment considering the disorder should be used as a therapeutic option when other alternatives fail. If individuals are self-medicating with nicotine due its antidepressant effects (Balfour & Ridley, 2000), then identifying and treating the root problem could make smoking cessation more successful and reduce the likelihood of relapse. Further understanding the underlying mechanisms that environmental enrichment impacts could also benefit the design of medications that can mimic these mechanisms for treatment of nicotine and other drug dependence in genetically vulnerable individuals, as a positive environment had the greatest impact on these rats in the current study.

Limitations

Some limitations could have influenced the findings seen in the current study. For example, a large amount of handling in standard housing rats throughout the 60 days of experiments and 7 days post-surgery could have reduced the difference compared to the beneficial effects seen in enrichment rats as more handling could reduce the negative stress effects of standard housing (Campbell & Spear, 1999). After surgery, the rats were not placed

back into the main metal enrichment cages as the robustness of the magnetic buttons in free-roaming and climbing rats was unknown and standard surgeries in the lab are not suited for enrichment. Therefore, the enrichment was limited to the developmental phase of the rats. While this is, as discussed above, likely the most effective period for altering brain circuitry, the effects seen in the present study could be the effects of enrichment, the subsequent switch from enrichment to standard housing, or a combination of both. Moreover, given the plasticity gene hypothesis by Belksy and colleagues, these environmental changes may have had a stronger impact on SERT-/- than SERT+/+ rats, effects. Thus, it is conceivable that the higher self-administration of nicotine in enriched animals may be a response to the change from enriched to standard housing, rather than an effect of enriched housing perse, as discontinuation of enrichment can increase self-administration (Nader et al., 2012). Green and Greenough (1986) found that synaptic changes present in the hippocampus of rats housed in enrichment do not persist after they are placed into isolation housing, suggesting that some of the effects of enrichment may be reversible (although in the present study the animals were housed in pairs rather than alone). Some studies still find behavioural differences between enrichment housing and standard or isolation housing after removal, as did the current study, suggesting not all effects of rearing in enrichment are eradicated after removal from such an environment (Puhl et al., 2012). The rats removed from enrichment in Puhl et al. (2012) were housed four rats per cage after surgery to prevent stressors from isolation housing. In the current study, enrichment rats were housed two per cage after surgery and standard housing rats remained with two per cage which could help maintain some socialisation, but the effects may be smaller than if there were more cage mates.

Future Directions

In future, addressing some of the limitations by piloting the buttons in an enriched environment would allow investigation into the effects of continued enrichment on nicotine

self-administration. Considering this gene × environment interaction in relation to other drugs that SERT -/- rats are sensitive to could help to answer if this interaction is specific to nicotine, or if it holds true for other more addictive drugs such as cocaine, or more widely used substances such as alcohol. Further, in this study the protective effects of enrichment were examined. Thus, it would be beneficial to explore if a positive environment could also have curative effects in these conditions, where rats are exposed to enrichment housing after the formation of drug dependence and self-acquisition. This would further our understanding of the beneficial effects of environmental enrichment to discover if these benefits are only present during development and prior to the introduction of drugs, or if they can reduce responding in rats that are not drug naïve. Indeed, other research has provided evidence to suggest that enrichment can reduce CPP (conditioned place preference) and cocaine-induced CPP in mice when exposed to enrichment during a one-month withdrawal period (Solinas et al., 2008). Interestingly, the authors further found that extinction drug seeking, and cue and stress induced self-administration reinstatement were reduced in enrichment rats, but not drug induced reinstatement (Chauvet et al., 2009). Thus, it would be beneficial to consider the curative effects in relation to nicotine dependence to further develop successful smoking cessation programmes that consider the current environmental conditions and help aide removal from negative environments. As the half-life of nicotine is one hour (Kyerematen et al., 1988) which is longer than the pre-allocated 30-minute time-out used in the current study, extending this period to be longer than the half-life may provide significant results that are not limited by time. Further, conducting progressive ratio experiments separately from extinction and reinstatement experiments would combat any carry-over effects that the return to baseline sessions did not eradicate. It would also be beneficial to examine SERT -/- rats that are only exposed to enrichment during adulthood and not development, to examine if the benefits of a positive environment are time-sensitive.

Conclusion

Overall, environmental enrichment exhibited protective effects during the acquisition stage in SERT -/- rats genetically predisposed to nicotine dependence, but not in SERT +/+ rats. Rats in standard housing extinguished cue-induced reinstatement responding to a greater extent than rats in enrichment. Rats in standard housing were not more likely to self-administer nicotine compared to rats in enrichment, nor were their break points higher in progressive ratio.

The findings of this study help to understand the mechanisms underlying genetic and environmental vulnerabilities of nicotine dependence in terms of changes in dopaminergic pathways related to enrichment and the impact of chronic dysfunction in the serotonin transporter. Taken together, the findings of the current study combined with the currently available literature suggest both a genetic and environmental influence in nicotine dependence. This emphasises the importance of not treating the dependence alone, but also considering environmental effects such as childhood adversity or discontinuation of a positive environment, and the contribution to dependence to find an effective treatment, whether it be through therapy, pharmacology, or a combination.

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Appendix

Table 1A.

Increases from the last two days of extinction to the first two days of reinstatement for each condition, and genotype and housing overall.

	Extinction	Reinstatement	ANOVA Result
SERT -/-	(M = 7.03, SD = 5.13)	(M = 12.6, SD = 5.35)	(F (1,16) = 18.5, p<.001, η_p^2 = 0.54)
SERT +/+	(M = 7.32, SD = 3.15)	(M = 14.1, SD = 7.01)	(F (1,18) = 16.9, P<.001, η_p^2 = 0.48)
Enrichment	(M = 6.12, SD = 3.96)	(M = 12.2, SD = 5.49)	(F (1,20) = 20.3, P<.001, η_p^2 = 0.50)
Standard	(M = 8.63, SD = 4.34)	(M = 14.9, SD = 6.85)	(F (1,14) = 14.7, P<.05, η_p^2 = 0.51)
SERT -/- Enrichment	(M = 6.29, SD = 4.76)	(M = 11.8, SD = 5.22)	(F (1,11) = 9.54, p<.05, η_p^2 = 0.46)
SERT -/- Standard	(M = 8.29, SD = 5.87)	(M = 13.9, SD = 5.72)	(F (1,6) = 6.54, p<.05, η_p^2 = 0.52)
SERT +/+ Enrichment	(M = 5.89, SD = 2.80)	(M = 12.7, SD = 6.11)	(F (1,8) = 10.1, p <.05, η_p^2 = 0.56)
SERT +/+ Standard	(M = 8.84, SD = 2.83)	(M = 15.8, SD = 8.00)	(F (1,7) = 7.42, p<.05, η_p^2 = 0.51)

Table 1B.

Average daily FR5 responses for each condition across progressive ratio experiments

Condition	Day 40	Day 42	Day 44	Day 46	Day 48	Day 50	Day 52	Day 54
SERT+/+	40.9	37.3	41.9	42.1	45.6	47.8	43.1	41.7
SERT -/-	37.2	34.5	35.1	41.5	46.7	47.8	43.5	35.9
Enriched	40.6	36.2	38.2	37.4	42.1	41.7	39.8	32.7
Standard	37.0	35.5	38.8	47.6	51.4	55.8	47.8	46.7
SERT -/- Enr	36.6	32.1	33.0	33.7	38.2	43.3	41.3	29.3
SERT -/- Std	38.2	38.2	38.3	53.2	59.3	54.5	46.8	45.7
SERT +/+ Enr	45.1	40.9	44.1	41.5	46.5	39.9	38.1	36.5
SERT +/+ Std	36.0	33.1	39.3	42.9	44.6	56.9	48.7	47.6

Figure 1A.

Graph showing left (inactive) vs right (active) lever responses across all conditions.

Responses on the active lever resulted in illumination of the cue light and a nicotine infusion.

The left lever had no associated illumination or infusion. Error bars represent SEM.

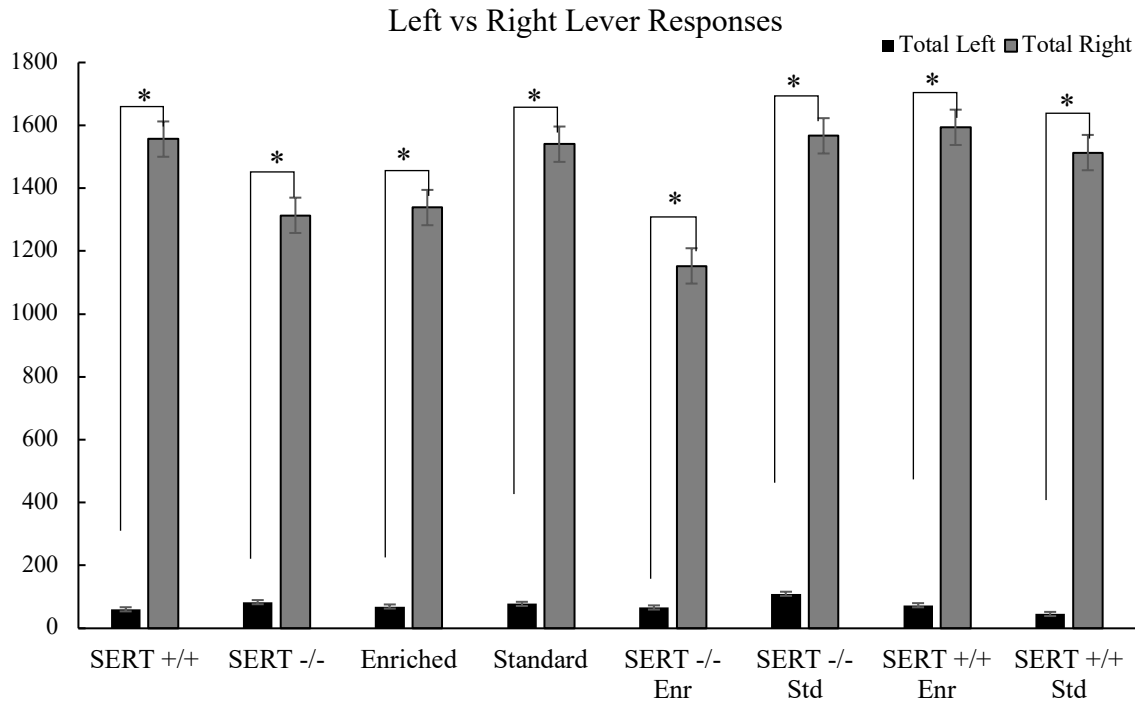

Figure 1B.

Average daily lever responses for each condition, broken down to each FR. Error bars represent SEM.

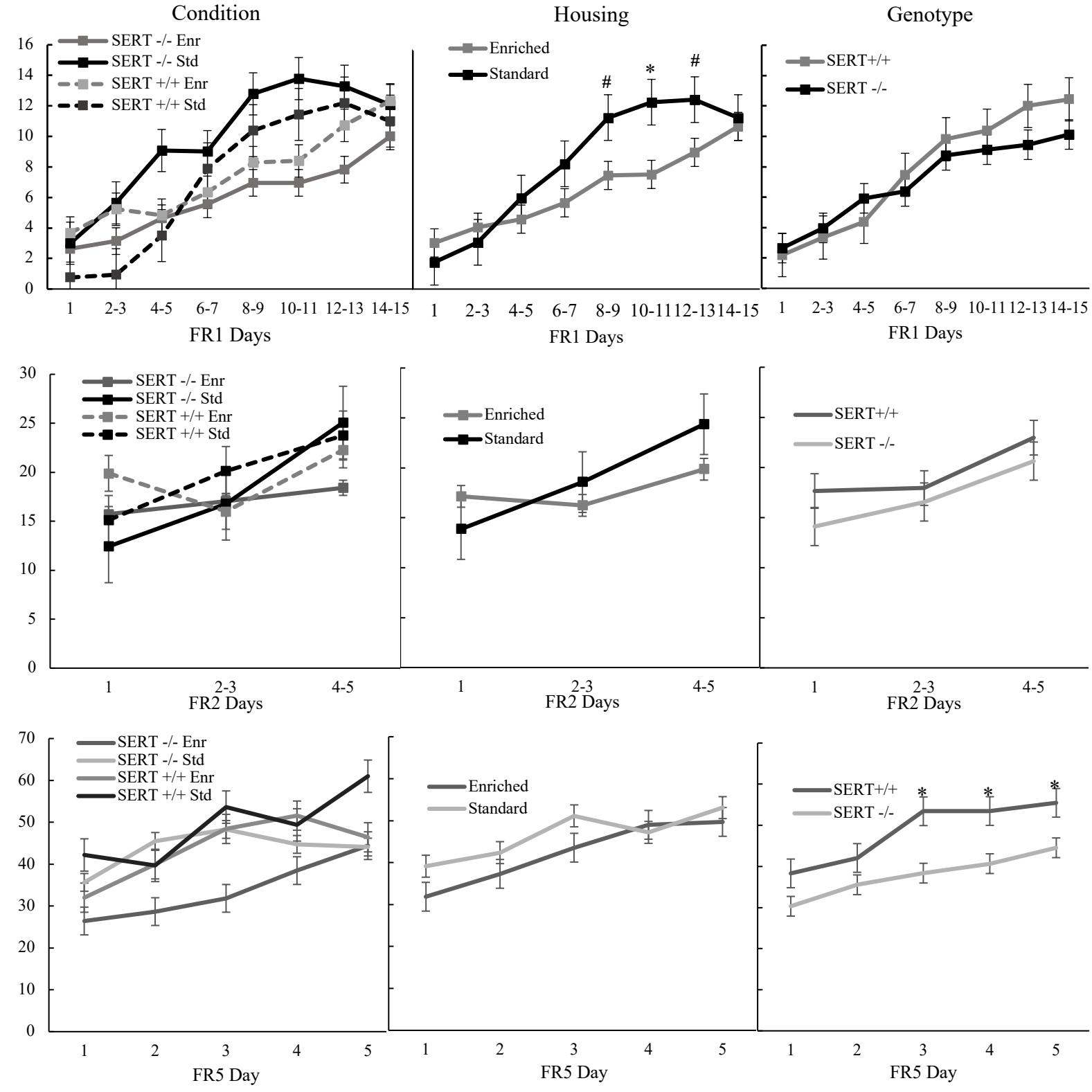

Figure 1C.

Average infusions for each condition during the FR5 sessions interrupting progressive ratio. (Error bars represent SEM).