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# Assessment of Attention in Vasopressin-Deficient Brattleboro Rats Using a Five-Choice Serial Reaction Time Task

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# ASSESSMENT OF ATTENTION IN VASOPRESSIN-DEFICIENT BRATTLEBORO RATS USING A FIVE-CHOICE SERIAL REACTION TIME TASK

By

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# ABSTRACT

## ASSESSMENT OF ATTENTION IN VASOPRESSIN-DEFICIENT BRATTLEBORO RATS USING A FIVE-CHOICE SERIAL REACTION TIME TASK

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Vasopressin is a neuropeptide that may influence behavioral and cognitive processes. The Brattleboro rat is a mutant variation of the Long Evans strain that exhibits no circulating vasopressin, resulting in a physiological state analogous to diabetes insipidus. Behaviorally, Brattleboro rats exhibit diminished fear conditioning and impairments in memory retention and sensory gating. The present study sought to further evaluate the cognitive profile of rats with vasopressin deficiency by studying attention in male and female Brattleboro rats, Long Evans rats, and heterozygous rats using a five-choice serial reaction time task. Sessions to meet criteria were significantly greater in the Brattleboro rats than Long Evans and heterozygotic rats; and, males required significantly more sessions than Long Evans and heterozygotic rats. Female Brattleboro rats displayed significantly poorer attention accuracy compared to Long Evans and heterozygotic rats. Premature responses were significantly greater in Brattleboro rats than Long Evans and heterozygotic rats. Taken together, the present findings add to previous literature suggesting that vasopressin deficiency diminishes cognitive functioning.

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TABLE OF CONTENTS

LIST OF FIGURES

# LIST OF ABBREVIATIONS

5-CSRTT: Five-Choice Serial Reaction Time Task

ACTH: Adrenocorticotropic hormone

BRATs: Brattleboro rats

CRF: Corticotrophin releasing factor

HZ: Heterozygous rats

LE: Long-Evans rats

PVN: Paraventricular nucleus

SCN: Suprachiasmatic nucleus

SON: Supraoptic nucleus

VP: Vasopressin

VPergic: Vasopressinergic

# INTRODUCTION

## *History of Vasopressin*

In 1895, medical doctor George Oliver and physiologist Sir Edward Albert Sharpey-Schäfer discovered that the administration of pituitary gland extracts would produce hypertension in animal subjects. Shortly following this observation, Howell (1898) concluded that the factor which exerted the pressor effects resided solely within the posterior lobe of the pituitary gland. Moreover, it was not until the mid-20$^{th}$ century that the two compounds of the posterior pituitary were identified as oxytocin and vasopressin, along with their individual roles in peripheral, physiological processes. Much of this work is credited to Du Vigneaud and his colleagues whom investigated the synthesis and characterization of these physiologically-active substances (e.g., Du Vigneaud, 1954-1955; Turner, Pierce, & Du Vigneaud, 1951; Katsoyannis & Du Vigneaud, 1958). Due in part to these series of experiments, Du Vigneaud received the Nobel Prize in chemistry in 1955. Since this seminal work by Du Vigneaud and his colleagues, investigations into vasopressin effects have expanded from peripheral functions towards central nervous system mediated effects— including cognitive and behavioral domains. The present work focuses on the cognitive effects of vasopressin and how these effects can be studied in the Brattleboro rat.

## *Vasopressin*

### *Properties*

Arginine vasopressin (VP) is a neuropeptide/neurohormone consisting of nine amino acids (CyS.-Tyr.Phe.Glu-$NH_2$.Asp-$NH_2$.CyS-Pro.Arg.Gly-$NH_2$), which form into a ring due to a disulfide bridge located between the two cysteine residues (e.g., Katsoyannis & Du Vigneaud, 1958; also see section in Caldwell, Lee, Macbeth, & Young III, 2008 for review of VP structure). The VP gene is structurally-similar to the oxytocin gene, but is oriented in the opposite transcriptional direction (Mohr, Schmitz, & Richter, 1988), and thus possesses biologically-distinct actions. Furthermore, the physiological activities of VP are centered around different target sites within the body; in this document, these sites will be referred to as "peripheral" (or outside of the central nervous system) or "central" (or inside the central nervous system). The present introduction will focus mainly on the contribution of VP in central processes.

VP is primarily synthesized in the magnocelluar cells of the hypothalamic paraventricular (PVN) and supraoptic nuclei (SON) (Buijs, Swaab, Dogterom, & van Leeuwen, 1978; Hou-Yu, Lamme, Zimmerman, & Silverman, 1986), and also within the parvocellular cells of the aforesaid PVN (e.g., de Souza & Franci, 2010), and the suprachiasmatic nucleus (SCN) (Sofroniew & Weindl, 1980; Buijs, van Eden, Goncharuk, & Kalsbeek, 2003). Large axons of the VP cells within the PVN and SON project to the posterior pituitary (e.g., see Brownstein, Russell, & Gainer, 1980) — where VP, along with its carrier protein, (denoted neurophysin) (see Zimmerman & Robinson 1976 for further information on neurophysins), can be released into circulation in

response to various physiological stimuli. The two most common peripheral actions of VP are: (1) to increase the reabsorption of water (hence VP is also called the "antidiuretic hormone") in the kidneys in response to osmotic stress (e.g., increased osmolality) (e.g., Dunn, Brennan, Nelson, & Robertson, 1973), and/or (2) VP can induce vasoconstriction in blood vessels in response to alterations in hemodynamics (e.g., hemorrhage of blood vessel) (e.g., Pittman, Lawrence, & McLean, 1982).

*Receptors*

Currently, three major receptor types of VP have been identified: arginine-vasopressin receptor 1a (Avpr1a or $V1_a$), Avpr1b ($V1_b$), and Avpr2 (V2); and all three receptor isoforms are shown to be g-protein coupled (see Michell, Kirk, & Billah, 1979; and Jard, Barberis, Audigier, & Tribollet, 1987 for review). Much progress has been made in exploring the properties of these receptor isoforms and in assessing the functional relevance of VP at these binding sites (e.g., see Barberis, Mouillac, & Durroux, 1998 for review). For example, studies have demonstrated that the V2 receptor isoform is responsible for mediating the antidiuretic properties in the kidneys (e.g., see Jard, Barberis, Audigier, & Tribollet, 1987 for review). Additionally, high-dose administration of VP to neonatal rats has been shown to disrupt adult kidney function (Handelmann, Russell, Gainer, Zerbe, & Bayorh, 1983); indicating the important role of VP in kidney diseases and normal physiological function. Further, following V2 receptor activation, the associated intracellular transduction cascade has been shown to involve coupling with adenylate cyclase (e.g., see Jard, 1983 for review). Identifying the intracellular signal transduction cascades associated with these receptors can provide

important information not only about the immediate physiological effects following receptor stimulation, but also about possible long-term, modulatory effects of various biochemical agents such as VP.

Different from the transduction cascade pathways associated with kidney V2 receptors, V1 receptors located in vasculature (i.e., receptors coupled to smooth muscles) (e.g., Nabika, Velletri, Lovenberg, & Beaven, 1985) and in the hepatocytes of the liver (e.g., Cantau, Keppens, De Wulf, & Jard, 1980; Michell, Kirk, & Billah, 1979), have been shown to be coupled with phospholipase; which initiates the phosphoinositol pathway(s) to elevate intracellular $Ca^{2+}$ concentrations (e.g., see Jard, Barberis, Audigier, & Tribollet, 1987), among other intracellular processes. Furthermore, previous literature suggests that the VP receptors in the brain predominantly consist of the V1 isoform (e.g., Kiraly, et al., 1986; Shewey & Dorsa, 1988). Thus, it is likely that central V1 receptors, rather than V2 receptors, mediate the putative behavior and cognitive effects of VP.

*Binding Sites and Distribution*

In addition to the intracellular properties associated with the VP receptors, numerous receptor binding sites have been identified in the brain. Many of these sites are observed to exist in areas throughout the central nervous system, (away from the majority of VP cell bodies found within the PVN, SON, and SCN), and within numerous accessory nuclei. For example, Caffe and van Leeuwen (1983) found binding sites to exist in the dorsomedial hypothalamus, the medial amygdala, and the locus coeruleus—structures which are involved in a number of behavioral domains. Shortly following this work, De Kloet, Rotteveel, Voorhuis, and Terlou (1985), found binding sites to exist

within such structures as the septum, central amygdala, dentate gyrus, olfactory nucleus, and the nucleus tractus solitarii. With these findings taken together, VP has thus been implicated in various domains affiliated with the aforementioned regions. Further, given the large number of VP binding sites found within these accessory areas, other research during this time began to identify numerous axonal projections of VP; which may offer important information on the functional relevance of these associated binding sites.

Early microscopy work identified a number of VP projections within the brain—some of which terminate at the binding sites noted previously. For example, Buijs (1980) identified distinct vasopressinergic (VPergic) projections which extended from the magnocellular nuclei of the PVN and/or SON, and/or from the parvocellular nuclei of the SCN. Specifically, magnocellular VPergic projections were found to extend to the amygdala, hippocampus, and spinal cord; and, parvocellular projections to the organum vasculosum of the lamina terminalis, the lateral septum, and the lateral habenular nucleus (Buijs, 1980). Additionally, during this time, Sofroniew (1980) using a immunoperoxidase method, found magnocellular projections (i.e., PVN) to extend towards the substantia nigra, the nucleus tractus solitarii, the nucleus commissuralis, and to the spinal cord; and, parvocellular projections (i.e., SCN) to extend towards the septum, thalamus, medial amygdala, ventral hippocampus, and a number of other regions (for review on VPergic projections, see section in De Wied, Diamant, & Fodor, 1993). Therefore, given the large number of VPergic projections found within the brain, it is thus possible that the neuropeptide may also serve central, neurotransmitter-like roles within the central nervous system which may ultimately modulate behavioral and/or cognitive processes.

### *Vasopressin Extensions*

#### *Suprachiasmatic Nucleus*

As mentioned previously, there are numerous projections of VP axons found within the brain—many of which been shown to stem from the SCN and project to a host of other target tissues (e.g., Buijs, 1980; Sofroniew, 1980). It has been known for some time that the SCN, or the "master clock" of an organism, is largely responsible for the circadian rhythmicity of a multitude of biochemical agents. The SCN performs this rhythmic control through acting upon various cellular compartments located throughout the body— such as along specific gene fragments within a cell's nucleus. Moreover, VP release has been shown to be entrained to such circadian rhythmicity with peak release occurring during the subjective day phase—although VP release may vary between species (Reppert Schwartz, Artman, & Fisher, 1983). Given this known relationship between the SCN and release of VP, physiological actions associated with its rhythmic release have also been found (e.g., see Buijs, van Eden, Goncharuk, & Kalsbeek, 2003, for review on the SCN and associated biochemical components) . For example, the circadian release of VP has been shown to exert a modulatory effect on the secretion of corticotrophin releasing factor (CRF) and adrenocorticotropic hormone (ACTH) (e.g., Raymond, Leung, Veilleux, & Labrie, 1985)—two compounds which are in part responsible for the excessive cortisol release during periods of chronic stress (e.g., Aguilera, 1994; Volpi, Rabadan-Diehl, & Aguilera, 2006). Furthermore, research has suggested that VP may function to amplify the excitatory effect produced by the SCN (Ingram, Snowball, & Mihai, 1996). With these findings taken together, the relationship

between VP and the SCN has stimulated research which further explores the utilization of the neuropeptide within circadian domains.

*Social Behaviors*

There are several areas of social behavior that may be modulated by the effects of VP. One such research area that has received much attention has been aggression. Aggressive behavior, for instance, has been shown to increase following microinjections of VP into the anterior hypothalamus of Golden hamsters (Ferris et al., 1997). Moreover, a direct relationship has been suggested to exist between the $V1_a$ receptor isoform and aggression as studied in Syrian hamsters— with a higher $V1_a$ receptor distribution in the anterior hypothalamus equated to increased observed aggression (Albers, Dean, Karom, Smith, & Huhman, 2006). Accompanying the $V1_a$ receptor studies which associate the receptor isoform with aggression, additional research has shown that mice lacking the $V1_b$ isoform, display reduced aggression, and alterations in social recognition as well (Wersinger, Ginns, O'Carroll, Lolait, & Young III, 2002). Based on these studies, VP may serve a modulatory role in aggressive behaviors and further research is thus necessary to elucidate the biochemical actions VP places within specific areas of the brain affecting such behavioral responses.

Other aspects of social behavior, such as pair-bonding and social recognition, have been examined as well. Perhaps among the most robust observations involving VP and social behaviors, has been through the examination of VP receptor distributions in the ventral pallidum that exist between different species of voles (see, Donaldson, & Young, 2008 for review). For example, a causal relationship has been shown to exist

between the high-VP receptor distribution located within the ventral pallidum of the prairie vole—which results in a monogamous behavioral phenotype— and, with the low-VP receptor distribution within the ventral pallidum of the nonmonagamous meadow vole (Donaldson & Young, 2008). Furthermore, by using a viral-vector method of transferring genes, previous studies have found that if the VP gene is taken from the monogamous prairie vole, and transferred to the nonmonagamous meadow vole, the meadow vole will then adopt the social behavior of the prairie vole (Donaldson & Young, 2008). Therefore, it seems that that the distribution levels of VP are largely responsible for inducing a specific behavioral phenotype within the vole species.

In addition to the aforementioned vole studies, the importance of VP and social behaviors has been suggested to be more relevant to males than females— as females do not appear to rely on VP for social recognition as much as males (Bluthe & Dantzer, 1990; Bielsky, Hu, & Young, 2005). Despite this robust observation, research using rodents has found relationships between VP neurotransmission and social behaviors. For instance, Engelmann, Ludwig, and Landgraf (1994), using a microdialysis procedure, found that following stimulation to the SON, a significant correlation existed between endogenous VP release in the SON and septum, and increased social memory (recognition) performance in male rats. Additionally, this improvement could be partially blocked by a V1 receptor antagonist (Engelmann, Ludwig, & Landgraf, 1994).

This association found between VP release and social memory would be further delineated with successive research using transgenic mice models. Current research has found that severe impairments in social recognition have been shown to exist in $V1_a$

knockout mice compared to wildtype controls (Bielsky, Hu, Szegda, Westphal, & Young, 2004; Egashira et al., 2007); and mild impairments in social recognition to exist in $V1_b$ knockout mice (Wersinger, Ginns, O'Carroll, Lolait, & Young III, 2002). Furthermore, by re-expressing the $V1_a$ gene in the lateral septum of $V1_a$ knockout mice, deficits in social recognition became completely restored (Bielsky, Hu, Ren, Terwilliger, & Young, 2005). These findings suggest that the $V1_a$ receptor in the lateral septum is necessary for normal social recognition function (at least in male rodents; see above). Taken together, these data suggest the relevance of VP in modulating social behaviors.

*Stress, Depression, and Anxiety*

VP has been previously demonstrated to have fibers projecting to the zona externa of the median eminence (e.g., Antunes, Carmel, & Zimmerman, 1977), and thus to the anterior pituitary (via a blood portal system). Given the terminating sites of these VPergic projections to the median eminence, it has been postulated that VP may affect the pituitary-adrenal-axis (e.g., Antunes, Carmel, & Zimmerman, 1977). It was later found that stress-responsive components, such as CRF (also referred to as "corticotropic hormone"), and ACTH, are physiologically-involved with the hypothalamic-pituitary-axis (HPA) (referring to the anterior pituitary) (e.g., Rivier & Vale, 1983; Aguilera, 1994; Volpi, Rabadan-Diehl, & Aguilera, 2006). Moreover, as mentioned above, VP has been shown to exert a modulatory role on the release of HPA-affiliated stress-response factors, such as CRF and ACTH (e.g., Rivier & Vale, 1983; Aguilera, 1994); and additionally, cell bodies of AVP and CRF have been found to co-localize in the external region of the median eminence (e.g., Whitnall, Smyth, & Gainer, 1987). These findings strongly

support a physiological relationship to exist directly between VP and the factors (e.g., CRF, ACTH, cortisol, etc.) included in mediating the effects of chronic stress. Furthermore, behavioral studies have provided additional evidence supporting the impact of VP in the stress response. For example, rats put into a 10-minute forced swim session were shown to have elevated levels of VP release in both the SON and PVN (Wotjak et al., 1998; and for review on VP and stress, see section in Caldwell, Lee, Macbeth, & Young III, 2008).

Accompanying the direct association between VP, CRF, ACTH, and the anterior pituitary, physiological interactions have been found between the $V1_b$ receptor isoform and the adrenal glucocorticoids. For example, recent evidence shows that $V1_b$ receptor up-regulation or down-regulation will occur depending upon circulating levels of glucocorticoids (Aguilera & Rabadan-Diehl, 2000). The release of glucocorticoids is directly proportionate to signals coming from the HPA, and once in circulation and thus reaching the brain, glucocorticoids exert numerous effects on functional neurochemistry—many effects which are strongly associated to stress, anxiety, and depression (for review, see Nestler et al., 2002). Moreover, elevated levels of VP have been found in the plasma of depressed patients compared to healthy controls (van Londen et al., 1997); and treatments for depression, such as the selective-serotonin reuptake inhibitor Fluoxetine, has been shown to increase VP afferent development in select brain regions (Ricci & Melloni Jr., 2012). These findings taken together suggest that VP shares a relationship with the mechanisms associated with depression, and thus may serve a useful role for therapeutic intervention.

In addition to the role of VP in the induction of stress and depression, the $V1_a$ receptor has received support for influencing anxiety-related measures as well. For example, $V1_a$ receptor knockout mice have been shown to exhibit reduced anxiety-like behavior in an elevated plus maze (Egashira et al., 2007); and, pharmacologically, V1 receptor antisense administration has been shown to reduce anxiety-related behavior in rats (Landgraf et al., 1995). Moreover, given the extensive literature supporting the role of VP in stress, depression, anxiety, and the structures affiliated with these ailments—which also contain VPergic projections, such as the amygdala (e.g., Willcox, Poulin, Veale & Pittman, 1992; Dorsa, Petracca, Baskin, & Cornett, 1984), and the hippocampus (Zhang & Hernandez, 2013)— VP has thus received much attention which attempts to delineate its functional relevance in these pathologies (for review see Scott & Dinan, 1998; De Wied & Sigling, 2002; Neumann & Landgraf, 2012).

### *Brattleboro Rats*

#### *History*

The Brattleboro rat was discovered as a useful model for studying possible relationships between VP and associated behaviors. The strain was identified during the 1960's in Brattleboro, Vermont. Dr. Henry Schroeder and his associate Mr. Tim Vinton noticed that some of the rat pups in their breeding colony were drinking excessive amounts of water, and with the administration of VP, this behavior could be corrected (Valtin, 1982; Mohring et al., 1978). After isolating these rats and reporting the behavior to several enthused colleagues, the Brattleboro rats as the "water-consumers" came to be called, became a successfully-bred, naturally occurring mutant rat strain to investigate

experimentally (Valtin, 1982). Moreover, the Brattleboro rats displayed polydipsic behavior (excessive water consumption) and polyuria (excessive urination), and therefore have been considered to be an animal model of congenital diabetes insipidus.

*Genetics and Characteristics*

It was later determined that the cause of the Brattleboro rats' excessive water consumption was due to a single guanisine residue deletion in the second exon of the arginine VP gene (Schmale & Richter, 1984). This deletion results in impaired secretion of the neuropeptide, which results in no circulating VP found in the animal (Schmale, Ivell, Brendl, Darmer, & Richter, 1984; Majzoub, Carrazana, Shulman, Baker, & Emanuel, 1987; Grant, 2000). Further, several alterations have been found in the Brattleboro rats in various hormonal factors (for review, see Sokol & Zimmerman, 1982) and neurotransmitter concentrations (Dawson, Wallace, King, 1990; Feenstra, Snijdewint, Van Galen, & Boer, 1990). Extensions of these alterations into various domains will be discussed later in this document.

In addition to the excessive drinking behavior found in the Brattleboro rats, body growth (measured in body weight), tail length and posterior pituitary gland weight were also found to be underdeveloped compared to wildtype controls (Arimura, Sawano, Redding, & Schally, 1968). Upon further examination, Arimura, Sawano, Redding, and Schally (1968) also found that growth hormone content in the posterior pituitary of Brattleboro rats was significantly lower than what was found in controls; however, growth-hormone releasing factor (the chief regulatory factor responsible for the synthesis and excretion of growth hormone) was not found to be different. This suggests that other

compounds beyond growth-hormone releasing factor may be responsible for the impaired somatic development observed in the Brattleboro rats (Arimura, Sawano, Redding, & Schally, 1968).

Along with the previously mentioned deficiencies in somatic development and concentrations of growth hormone found with the Brattleboro rats, the strain has also been shown to have smaller brains (4-9% smaller) compared to heterozygous controls (Boer, Van Rheenen-Verberg, & Uylings, 1982). Additionally, diminished proliferative growth of the cerebellum and medulla oblongata were also found in both male and female Brattleboro rats (Boer, Van Rheenen-Verberg, & Uylings, 1982). Accompanying these structural underdevelopments, numerous molecular components were found to be reduced as well—specifically, DNA content in the forebrain, cerebellum, and olfactory bulbs were found to be lower in Brattleboro rats compared to heterozygotic controls (Boer & Patel, 1983). Furthermore, expressed differences in monaminergic agents—including elevated levels of dopamine, serotonin, noradrenaline/norepinephrine, glutamine, and taurine in select brain regions—have been observed to exist in the Brattleboro rats versus wildtype controls (Dawson, Wallace, & King, 1990; Feenstra, Snijedwint, Galen, & Boer, 1990). These differences may exist due to the putative role of VP as a key neuropeptide which interacts with various factors in neurodevelopment (see Boer et al., 1980; and Ugrumov, 2002, for review on VP and neurodevelopment); such as modulating the noradrenaline neurotransmission pathways (e.g., Boer, Feenstra, Botterblom, Korse, & Te Riele; 1995).

*Behavioral Studies*

One of the first extensions of VP into the domain of mental processing came through the work of De Wied and colleagues during the mid-1900's. For instance, in a shuttlebox conditioned avoidance response paradigm—which can be suggested to measure contexutal memory (i.e., pairing an environment with a specific stimulus such as a shock) — it was found that animals with ablations to the posterior and intermediate lobes of the pituitary gland displayed impairments in the maintenance of an avoidance response (De Wied, 1965). Moreover, multiple doses and varied administration intervals of VP analogues were assessed in rats, and found to augment memory retention in active and passive avoidance tasks in a time-dependent manner (Bohus, Ader, & DeWied, 1972). This preliminary research stimulated the hypothesis that VP may subserve some modulatory role in memory processes.

Also during this time, the Brattleboro rats were becoming a model employed to investigate the effects of VP deficiency (see Valtin, 1982). Ostensibly due to the emergence and utilization of this mutant rat for research purposes, De Wied and colleagues were able to investigate the strain in several animal-memory paradigms. For example, it was noted that the Brattleboro strain exhibited deficits in learning, compared to heterozygotic rats with partial VP deficiency, using a one-trial step-through passive avoidance test (De Wied, Bohus, & van Wimersma Greidanus, 1975). Additionally, following administration of desglycinamide-8-lysine, a VP analogue, these deficits were ameliorated (De Wied, Bohus, & van Wimersma Greidanus, 1975).

The relationship between VP and shuttlebox behavior were expanded upon in Bohus, van Wimersma Greidanus, and De Wied (1975) where the Brattleboro rats were found to have deficits in acquisition of avoidance, extinction of behavior in pole jumping avoidance, and in the retention of passive avoidance response. In response to these findings and the conclusions made therein about the Brattleboro strain, other researchers such as Brito, Thomas, Gingold, and Gash (1980), sought to delineate other types of memory (e.g., reference-memory) that may be impaired in this strain and suggested that the Brattleboro rats exhibit deficits in reference-memory, and perhaps mild impairments in working-memory. Additionally, subsequent pharmacologic studies were also providing evidence supporting the role of VP in memory as well. For example, Strupp and colleagues (1990) noted that the administration of a VP metabolite was shown to improve memory retrieval in a rat social interaction, appetitive-motivated paradigm. These studies would provide assessments of memory performance in the Brattleboro rats, and further support the role of VP within this cognitive domain.

Conflicting with these findings in the shuttlebox behavior, Brito, Thomas, Gash, and Kitchen (1982) were not able to replicate these results; rather, there were no differences found between Brattleboro rats and controls in shuttlebox/shock-motivated assessments (i.e., approach-avoidance conflict, passive avoidance), in performance in a working memory task (alternating T-maze), in visual and olfactory discrimination (reference memory), and in a species-specific behavioral response (i.e., burying an aversive stimulus as a defensive response (see Pinel & Treit, 1978 for further information of this behavior)). Moreover, the researchers concluded that the Brattleboro rats exhibited differences in temperament (using measures of neophobia, timidity, etc.) which may

conflict with performance in these cognitive-behavioral tasks (Brito, Thomas, Gash, & Kitchen, 1982). This conclusion provides a relevant point to consider when speculating about Brattleboro rats' performance in cognitive-behavioral tasks, and will be considered later in this document. In addition, to add further robustness to experiments, researchers have incorporated rats with partial VP deficiency as well. For example, Aarde and Jentsch (2006) found delay-dependent deficits in choice accuracy in heterozygote rats (with partial deficiency in VP) compared to wildtype Long-Evans controls, using a delayed-non-to-match position paradigm— a model suggested to measure spatial working memory. These memory studies taken together add supporting evidence for a central, modulatory role of VP in cognitive domains, though conclusions about how VP exerts these effects differentially across paradigms remain unclear.

As mentioned previously, the Brattleboro rats have been suggested to exhibit different emotional profiles than other strains. To test this hypothesis, researchers examined the Brattleboro rats using animal measures of emotionality—one such measure is the freezing response. For example, one study found attenuated freezing responses in Brattleboro rats using a conditioned freezing paradigm (Stoehr, Cheng, & North, 1993). As a follow-up to this finding, similar attenuated freezing responses were found in normal rats following $V_1$ antagonism administration (Stoehr, Cheng, Serlin, Cramer, & North, 1993). Taken together, these studies lend support to the notion that the Brattleboro rats display an altered emotional response. Moreover, these results also suggest that VP plays a role the retention of this behavior— as the study using pharmacologic manipulation of VP indicated. Further, Colombo, Hansen, Hoffman, and Grant (1992) found that rats (the M520 strain) carrying the diabetes inspidus gene, expressed different

levels of timidity (measured by adaptability in a novel environment) in a t-maze paradigm, than what was found in Brito, Thomas, Gash, and Kitchen (1982); suggesting that the DI gene itself may not be responsible for the altered timidity found within the Brattleboro strain. Clearly, more research is necessary that evaluates the differences between the parameters set within these paradigms, and for potential differences in Brattleboro rats from different breeders—as rats from different colonies may display different behavioral and emotional profiles (e.g., noted in Laycock, Gartside, & Chapman, 1983; also see, Herman, Thomas, Laycock, Gartside, & Gash, 1986).

In addition to the shock-avoidance studies mentioned above, recent research has provided suggestions about the effects of maternal genotype on Brattleboro rats' stress response function and development (e.g., Zelena, Mergl, & Makara, 2009); adding further complexity to exploring the emotional profile of the Brattleboro strain. Further, Brattleboro rats have been shown to exhibit attenuated depression-like behavior in a forced swimming test (Mlynarik, Zelena, Bagdy, Makara, & Jezova, 2007), and prolonged elevation of plasma corticosterone and oxytocin levels following a 10-minute forced swim stressor (Zelena et al., 2009). Taken together, these findings suggest that the Brattleboro rats exhibit irregularities in stress-response activity; however, further research is necessary to delineate the neurophysiological causes of these irregularities, and to what impact they may have on performance in cognitive-behavioral tasks.

Along with previous studies indicating that the Brattleboro display altered stress responses compared to controls using a forced-swim stressor (Mlynarik, Zelena, Bagdy, Makara, & Jezova, 2007; Zelena et al., 2009), and deficits in assessments of memory

(Stoehr, Cheng, & North, 1993; Bohus, van Wimersma Greidanus, & De Wied, 1975; De Wied, Bohus, van Wimersma Greidanus, 1975), the strain has been found to exhibit alterations in other cognitive domains as well. For example, innate social recognition deficits in the Brattleboro strain have also been ameliorated following administration of synthetic arginine VP into the septum of the rats, and similar deficits can be induced in Long Evans rats following V1 antagonist administration into the same brain region (Engelmann & Landgraf, 1994). One other area of cognition examined in the Brattleboro strain, has been attention. Recently, the Brattleboro strain has been found to exhibit deficits in pre-pulse inhibition (e.g., Feifel & Priebe, 2001)—which is suggested to measure a pre-attention process referred to as "sensorimotor gating." Moreover, this finding has also been found in female Brattleboro rats (Feifel, Shilling, & Melendez, 2010); which, suggests the effects are due to some quality of the Brattleboro strain rather than a sexually-dimorphic characteristic. Moreover, the deficits are ameliorated in both sexes of the Brattleboro strain following antipsychotic administration (Feifel, Shilling, & Melendez, 2010) and novel experimental antipsychotic administration in male rats (Feifel, Melendez, Priebe, & Shilling, 2007). The implications of these findings will be discussed later in this document.

As mentioned previously, deficits in sensorimotor gating were observed in the Brattleboro rats; which suggest that the absence of VP could potentially modulate aspects of attention as well. Moreover, Jentsch, Arguello, and Anzivino (2003) found that Brattleboro rats displayed deficits in attentional engagement using a lateralized reaction time task. Briefly, this study required animals to correctly respond to visual stimuli via nose pokes in two apertures that randomly presented flashes of visual stimuli. Trials were

initiated via nose poke into a central aperture. Following this response, a visual stimulus was presented randomly in one of two apertures located on either side of the central aperture. Measures of accuracy, response times, premature responses, omissions, incorrect responses, and reinforcement retrieval times were collected (Jentsch, Arguello, & Anzivino, 2003). Furthermore, Jentsch (2003) found superior performance in a lateralized reaction time task using rats with partial VP-deficiency; which, overall, these studies suggest that VPergic tone may exert some effects on attention performance within the parameters of the lateralized reaction time task. Amid these prior studies using the lateralized reaction time task, further evaluations assessing the Brattleboro rats, VP, and attention must be examined. Therefore, further research is necessary to measure other aspects of attention and motivation not assessed in the lateralized reaction time task.

### *The Five-Choice Serial Reaction Time Task*

The operant five-choice serial reaction time task (5-CSRTT) has been commonly used to assess attention since the 1970's (for review, see Robbins, 2002). This method of assessing attention in animal models is more common than the aforementioned lateralized reaction time task and differs from the paradigm in several ways. Briefly, the 5-CSRTT requires animals to visually detect a stimulus light presented randomly in one of five apertures within a fixed-time period. The animals initiate the task by emitting a response on a stimulus (e.g., pressing a lever) within the chamber. This response then results in the appearance of the visual stimulus to occur randomly in one of five apertures and within a fixed-time period. Subjects must then nose-poke the illuminated hole within a fixed-time period in order to receive reinforcement, and initiate another trial. Failures to make a

nose-poke response (omission), a response before the onset of the visual stimulus (premature response), or a response into an incorrect aperture following visual stimulus presentation (incorrect response), results in a time-out period (e.g., typically a five-second period where the houselight is extinguished and response on the stimulus that typically initiates a trial has no outcome).

To vary the parameters associated with the 5-CSRTT, higher attentional demands can be placed on the subjects by altering the visual stimulus duration (e.g., changing the stimulus duration from 1.0 sec to 0.1 sec), and through varying the inter-trial interval (ITI) (i.e., instead of the visual stimulus appearing 5.0 seconds following head entry, have it randomized to appear 1.0, 5.0, and 10.0 seconds following entry). Additionally, distractors, such as bursts of white noise, can also be incorporated into the 5-CSRTT paradigm to add further attentional demands on the subjects.

The 5-CSRTT is regarded as an assessment of attention accuracy— including spatial attention, attention shifting, and inhibitory control— as well as other variables such as motivation, impulsivity, compulsivity, and cognitive flexibility (e.g., Robbins, 2002; Chudasama & Robbins, 2004;Amitai & Markou, 2010). These characteristics make the 5-CSRTT a robust animal model for evaluating multiple facets of behavior and mental processing. Moreover, although similar to the lateralized reaction time task in some ways, the 5-CSRTT may add further attentional engagement due to 1) an increase in task difficulty and 2) requiring the animal to shift attention from a stimulus that initiates a trial to a second stimulus located elsewhere in the experimental chamber (See Appendix A for photograph of chamber). Therefore, the 5-CSRTT adds additional

measurements of attention not addressed by the lateralized reaction time task, and may thus reveal relevant relationships between VP and attention.

# RATIONALE

Vasopressin has been implicated within various behavioral and cognitive domains, such as memory, social behaviors, and mental disorders, and is thus a relevant compound to consider for experimental examination. One of the challenges associated with experimental analyses is identifying a potential animal model which has high construct validity. The Brattleboro rat strain is uniquely relevant to addressing this challenge by possessing a genetic condition which completely impairs the animal's ability to synthesize vasopressin. Moreover, recent evidence has suggested that the Brattleboro rats exhibited deficits in such areas as memory (e.g., Stoehr, Cheng, & North, 1993), attentional engagement (Jentsch, Arguello, & Anzivino, 2003), sensorimotor gating (e.g., Feifel, Shilling, & Melendez, 2010), and social discrimination (Feifel et al., 2009). These findings provide further support for incorporating the Brattleboro strain in models that may address deficits associated with human mental disorders.

To date, no previous study has evaluated the Brattleboro rats in the five-choice serial reaction time task—a well-validated assessment of attention. Additionally, experimental studies are lacking that include female Brattleboro rats into their analyses, so much of the knowledge about potential sex differences within this strain are largely unknown. Therefore, the present study sought to assess attention performance in vasopressin-deficient rats by studying homozygous Brattleboro rats, heterozygous Brattleboro rats derived from breeding Long Evans and Brattleboro rats, and wildtype

Long Evans rats using a 5-CSRTT paradigm. Experiments also included both male and female rats to evaluate potential sexually-dimorphic effects of vasopressin deficiency. These findings expand upon the neurocognitive profile of the Brattleboro rats and suggest that levels of circulating vasopressin may influence aspects of attention, depending on sex. The goal of this study was to evaluate both male and female Brattleboro rats in a common animal model of attention, the five-choice serial reaction time task. This research further supports the utilization of the Brattleboro rat within cognitive and behavioral tasks that may ultimately provide support for modeling human disorders.

# METHODS

## *Subjects*

Male and female Brattleboro (BRAT) rats (N=10 and N=8, respectively) and heterozygotes (HZ) rats (N=10 and N=10) were obtained from a breeding colony at University of California, San Diego (San Diego, CA, USA), while male and female Long Evans rats (N=10 and N=10) were obtained from Charles River Laboratories (Portage, MI, USA). Animals were housed in a temperature and humidity-controlled vivarium kept on a 12 hour light-dark cycle with free access to water in their home cages. Food was restricted to a single daily feeding in order to maintain 85% of free-feed body weights. Rats were group housed within strain and sex, with the exception of male Brattleboro rats, which had to be separated due to aggressive behavior. Following training sessions, rats were individually housed for 30 min to provide a feeding period. Supplemental food pellets were subsequently provided in their homecages upon returning to group housing. All procedures were consistent with the *Guide for the Care and Use of Laboratory Animals* (2011) and were approved by the Institutional Animal Care and Use Committee at Northern Michigan University. Experimental sessions were conducted five days per week.

## *Apparatus*

These procedures used eight rat operant chambers contained in sound-attenuating cabinets equipped with fans for ventilation and masking noise (Med-Associates, St. Albans, VT, USA). Each chamber was equipped with a single retractable lever positioned

on the center of one wall, directly below the food pellet dispenser that delivered 45 mg dustless food pellets (BioServ, Frenchtown, NJ, USA). This wall also contained a houselight and receptacle for a 100ml water bottle, both located near the ceiling. The opposite wall contained five equally-spaced apertures equipped with stimulus lights and photo beam sensors. All experimental events and data collection was conducted using Med-PC version IV software (Med-Associates).

*Training procedures*

Prior to 5-CSRTT training, a 45 min acclimation session was conducted by placing rats in the experimental chambers with only the house light activated and the food-pellet dispenser set to fixed time 60 sec schedule. This session was included to habituate the animals to the experimental chambers and to the location and the sound of the food magazine. All animals moved onto training following this habituation session.

Training for the 5-CSRTT was conducted in five phases, and all training sessions ended after 100 trials or 30 min, whichever came first. During the first phase, all stimulus lights were activated and a nose-poke into any of the apertures resulted in the delivery of a food-pellet reinforcer. A nose-poke also deactivated all stimulus lights for a 5 sec period. First-phase training sessions were 30 min in length and the training criteria consisted of a rat evoking at least 10 nose-pokes for 1 session. This first phase aimed to train the animals to establish the nose-poke contingency.

During the second phase of training, rats were required to press the center lever in order to activate the five stimulus lights. A subsequent nose-poke into any aperture led

to the deactivation of the stimulus lights and the delivery of a food pellet. Rats moved on to the third phase of training after achieving at least 10 food pellets within a single session.

Phase three training procedures were similar to phase two except that a lever press led to the activation of only one stimulus light, which was selected randomly 5 sec later. Only a nose-poke into signaled aperture led to the delivery of a food-pellet, whereas no experimental event occurred after a nose-poke into a non-signaled aperture. However, failing to emit a nose-poke into the lit aperture within 5 sec (an "omission") led to deactivating the stimulus light and the houselight for a 5 sec period. After the timeout period ended, another lever press was necessary to activate a stimulus light. The goal of establishing this contingency was to train the rats of a temporal consequence following the absence of a response. In order to proceed to phase four, rats were required to omit no more than 10 percent of total trials for 2 out of 3 consecutive sessions.

Phase four was identical to phase 3 except that a nose-poke occurring during the 5 sec after the lever press, but before the activation of a stimulus light (a "premature response"), led to a timeout. This training phase introduced the animals to another condition (in addition to an omission) that would result in a timeout period. The goal of this phase was to minimize both premature and omission values during a training session. As such, the criterion for proceeding to the final phase of 5-CSRTT training was 2 out of 3 consecutive trials where omissions and premature values were no more than 10 percent of total trials initiated.

The final phase of 5-CSRTT training was identical to the fourth phase of training except that stimulus was shortened to 0.5 sec and nose-pokes occurring in the signaled aperture within 5 sec led to a food-pellet delivery. Moreover, incorrect responses led to a timeout. If the animal made a nosepoke in the correct aperture, a food pellet was immediately delivered. Training continued until correct choice accuracy had stabilized; which was defined as no more than 10% variation in correct choice accuracy with omitting no more than 20% total trials initiated for 6 out of 8 consecutive sessions. Once choice accuracy had stabilized for an individual rat, that rat was considered to have completed the 5-CSRTT training. (See Appendix B for box diagram detailing the procedure of the final 5-CSRTT training phase).

*Bodyweight and water intake*

Immediately following completion of 5-CSRTT training, all rats were free-fed for bodyweight and water consumption analysis. In addition to the homecage water consumption assessment, the free-fed rats were put into the experimental chambers supplied with a water bottle to assess water consumption for a 60 min period. The doors on the experimental chambers were left open during this session. Two measures were recorded during this 60-minute session: 1) the amount of water consumed from the 100ml bottle placed in the experimental chamber, and 2) the amount of licks made to the water bottle.

*Data Analysis*

The following dependent variables were measured for 5-CSRTT performance: percent accuracy, trial omissions, and premature responses. Data were reported as means (+/- the standard error of the mean [SEM]). Percent accuracy was calculated by dividing the number of correct responses by the sum of correct and incorrect responses, and then multiplying this value by 100. Each rat's stable percent accuracy value was reported as the mean percent accuracy across the 8 sessions where training criteria were met for the final phase of 5-CSRTT training. Means for premature responses and omissions were calculated in the same manner. All 5-CSRTT dependent variables were analyzed using a one-way between groups analysis of variance (ANOVA). Statistically significant differences were further assessed using the Unprotected Fisher's Least Significant Difference (LSD) multiple comparisons test. A chi-square analysis was conducted to assess differences in the number of rats meeting the final-phase training criteria between strains. A one-way ANOVA was conducted to assess differences in free-feed bodyweights, 24 hour homecage water consumption, or experimental dependent variables between strains in male or female rats during the 60-minute session. Again, statistically significant differences were further assessed using the Unprotected Fisher's LSD multiple comparisons test.

Given that sample sizes were reduced due to some rats failing to meet the training criteria, which subsequently reduced power, one-way between groups ANOVAs were also conducted in order to provide an assessment of strains between samples containing both male and female rats. Statistically significant differences were further assessed

using Unprotected Fisher's LSD comparisons tests. All analyses were conducted using GraphPad Prism for Windows version 6 (GraphPad Software, La Jolla, CA, USA).

# RESULTS

*Sessions to training criteria*

Rats were determined to have failed to learn the task if they exceeded the number of sessions to meet a phase by over two standard deviations, compared to the mean number of sessions met by the other rats of the same strain and sex. In addition, one LE male rat died prior to the final 5-CSRTT phase. The number of rats completing the final 5CSRTT training criteria consisted of the following: male Long Evans (LE) (6 out of the original 10), female LE (9/10); male heterozygotes (HZ) (9/10), female HZ (6/10); and male Brattleboro rats (BRAT) (6/10), female BRAT (5/8). As mentioned previously, given the relatively low samples sizes reached for many of the groups, a one-way between groups ANOVA was conducted to assess differences between strains in groups containing both male and female rats.

Figure 1 presents the number of male rats that completed all training. No statistically-significant differences were found in the number of males rats per strain that completed the training $\chi^2(2, N=29) = 2.468$, $p > 0.05$.

Figure 2 presents the number of female rats that completed all training. No statistically-significant differences were found in the number of female rats per strain that completed the training $\chi^2(2, N=28) = 2.643$, $p > 0.05$.

Figure 3 presents the number of male and female rats that completed all training. No statistically-significant differences were found in the number of male and female rats per strain that completed training $\chi^2(2, N=57) = 0.449, p > 0.05$.

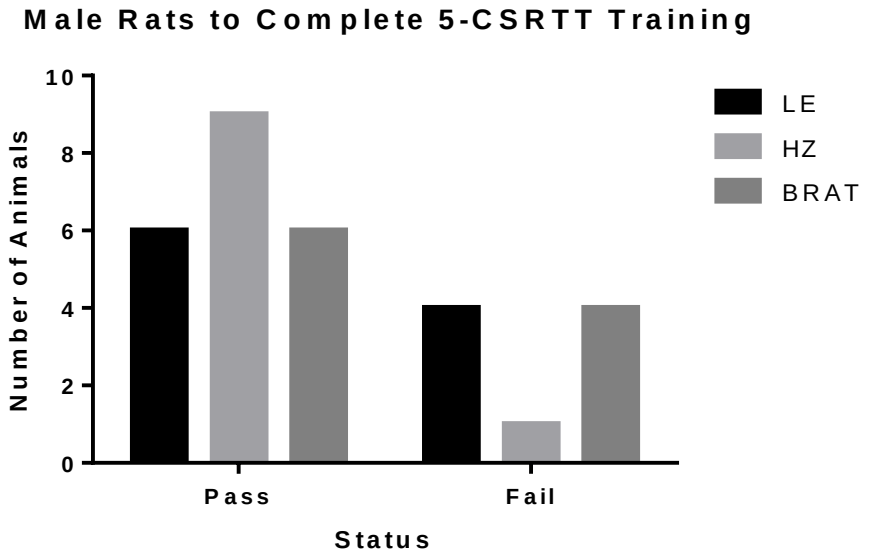


Figure 1 shows the number of animals as a function of status (pass or fail) and strain (LE, HZ, or BRAT) in the male rats completing the 5-CSRTT training.

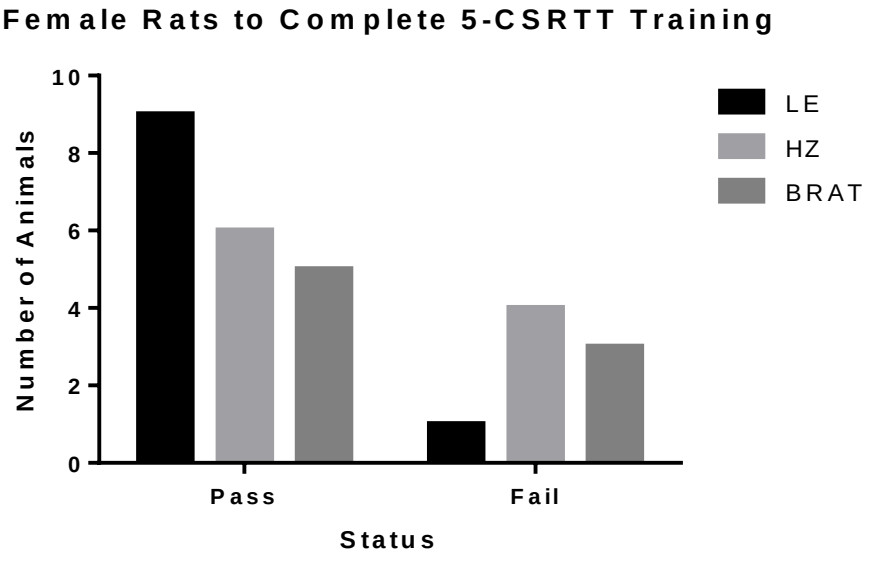


Figure 2 shows the number of animals as a function of status (pass or fail) and strain (LE, HZ, or BRAT) in the female rats completing the 5-CSRTT training.

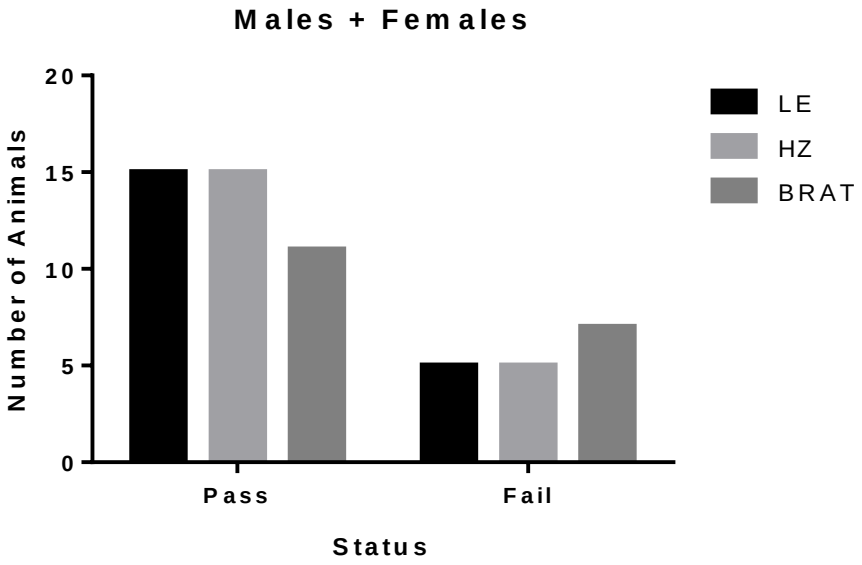


Figure 3 shows the number of animals as a function of status (pass or fail) and strain (LE, HZ, or BRAT) in the male and female rats completing the 5-CSRTT training.

Figure 4 presents the mean number of sessions to meet criteria for the male rats Statistically significant effects were found in males, $F(2,18) = 4.022$, $p < 0.05$, which were due to BRAT rats requiring significantly more sessions to meet the training criteria than LE and HZ rats. No statistically significant effects were found between LE and HZ rats.

Figure 5 presents the mean number of sessions to meet criteria for the female rats. No statistically-significant differences were found in female rats $F(2, 17) = 0.3587$, $p > 0.05$.

Figure 6 presents the mean number of sessions to meet criteria for the male and female rats. Statistically-significant differences in the number of sessions to meet the training criteria were observed between strains for male and female rats $F(2,38) = 3.339$, $p < 0.05$, which were due to BRAT rats requiring significantly more sessions to meet the training criteria than LE and HZ rats. No statistically significant effects were found between LE and HZ rats.

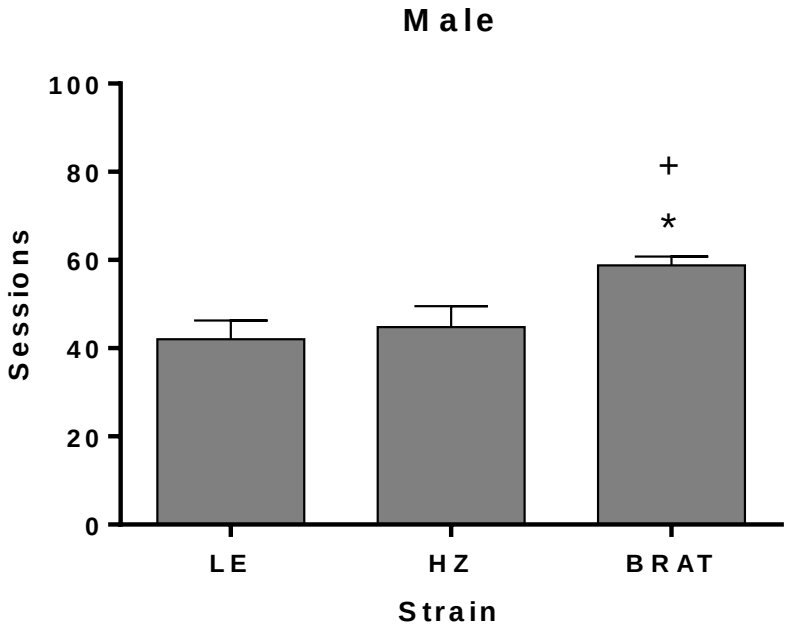


Figure 4 shows the mean number of sessions as a function of strain (LE, HZ, or BRAT) in the male rats, *$p < 0.05$ versus LE, +$p < 0.05$ versus HZ.

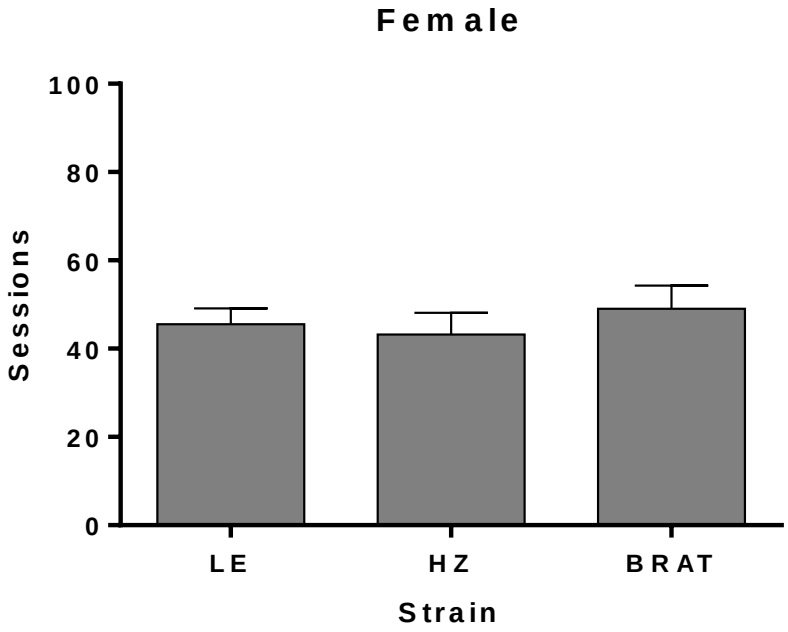


Figure 5 shows the mean number of sessions as a function of strain (LE, HZ, or BRAT) in the female rats.

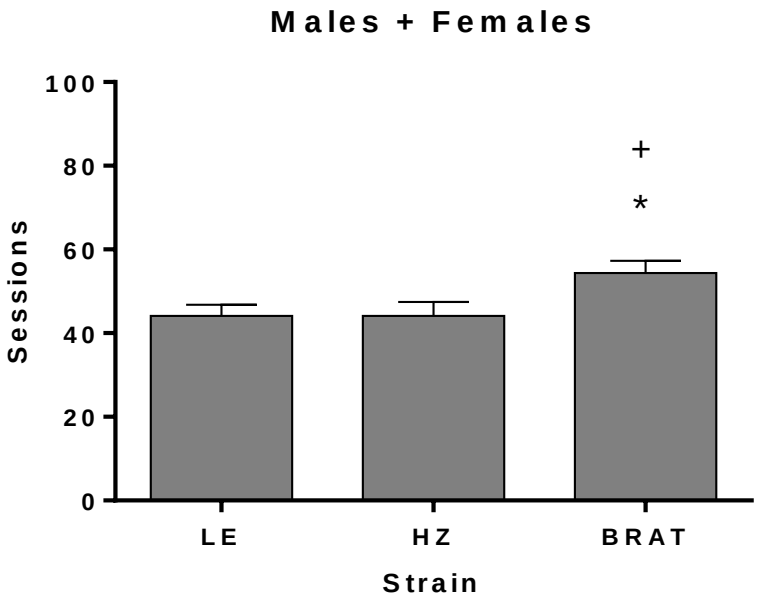


Figure 6 shows the mean number of sessions as a function of strain (LE, HZ, or BRAT) in the male and female rats, *$p < 0.05$ versus LE, +$p < 0.05$ versus HZ.

*Percent accuracy*

Figure 7 presents percent accuracy data during the sessions where training criteria were met for male rats. Percent accuracy for the male rats did not differ significantly $F(2,18) = 0.0629$, $p > 0.05$

Figure 8 presents percent accuracy data during the sessions where training criteria were met for female rats. Statistically-significant differences were observed between strains in female rats $F(2,17) = 4.330$, $p < 0.05$, which was due to female BRAT rats exhibiting significantly poorer accuracy than LE and HZ rats. No statistically significant effects were found between LE and HZ rats.

Figure 9 presents percent accuracy data during the sessions where training criteria were met for male and female rats. No statistically-significant differences were observed between strains in male and female rats $F(2,38) = 0.9221$, $p > 0.05$.


Figure 7 shows the percent accuracy as a function of strain (LE, HZ, or BRAT) in the male rats.

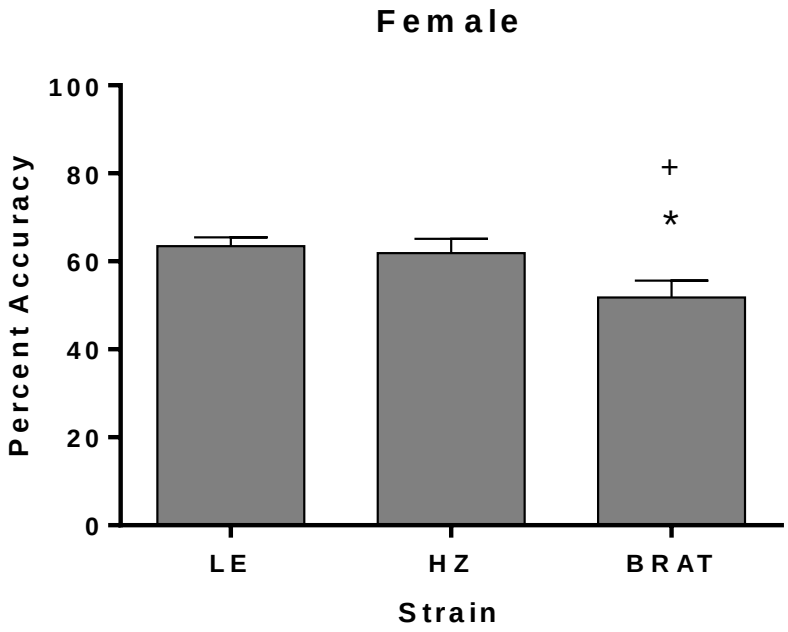


Figure 8 shows the percent accuracy as a function of strain (LE, HZ, or BRAT) in the female rats, *$p < 0.05$ versus LE, +$p < 0.05$ versus HZ.

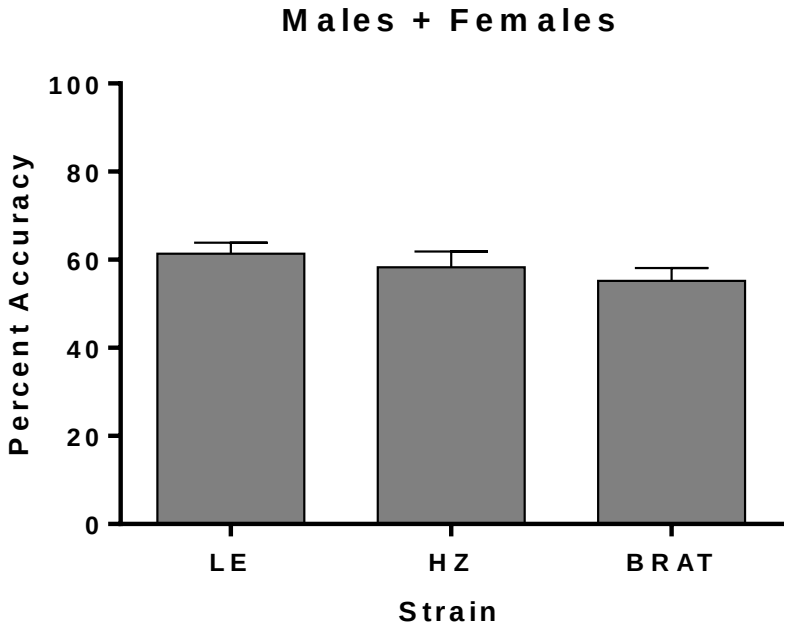


Figure 9 shows the percent accuracy as a function of strain (LE, HZ, or BRAT) in male and female rats.

*Premature responses*

Figure 10 presents the mean number of premature responses exhibited during the sessions where training criteria were met for male rats. No statistically-significant differences were observed between strains in male rats $F(2,18) = 1.986$, $p > 0.05$.

Figure 11 presents the mean number of premature responses exhibited during the sessions where training criteria were met for female rats. No statistically-significant differences were observed between strains in female rats $F(2,17) = 3.051$, $p > 0.05$.

Figure 12 presents the mean number of premature responses exhibited during the sessions where training criteria were met for male and female rats. Statistically-significant differences were observed between strains $F(2,38) = 4.865$, $p < 0.05$, which was due to BRAT rats exhibiting more premature responses than LE and HZ rats. No statistically significant differences were found between LE and HZ rats.

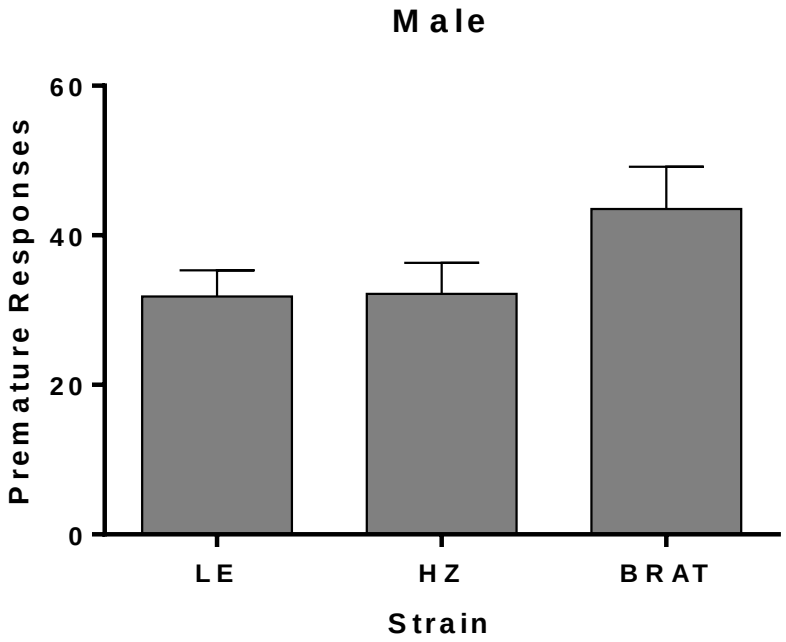


Figure 10 shows premature responses as a function of strain (LE, HZ, or BRAT) in male rats.

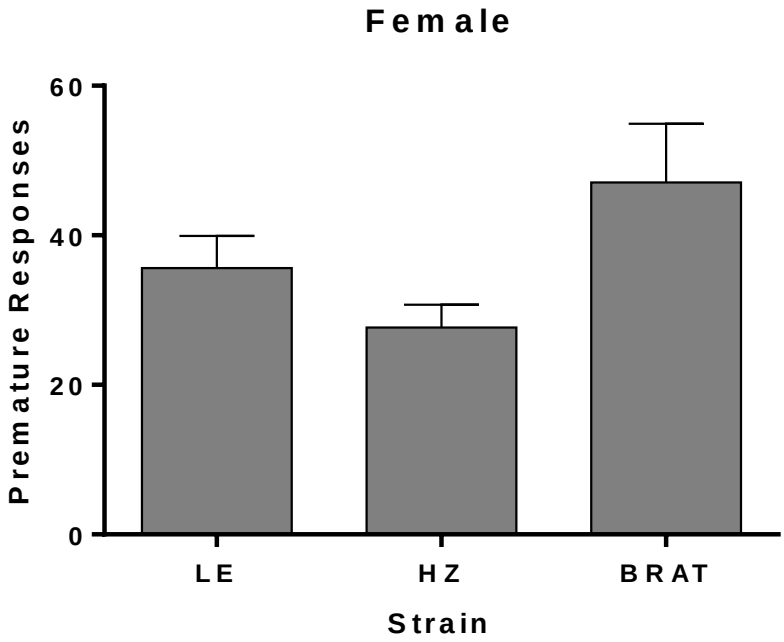


Figure 11 shows premature responses as a function of strain (LE, HZ, or BRAT) in female rats.

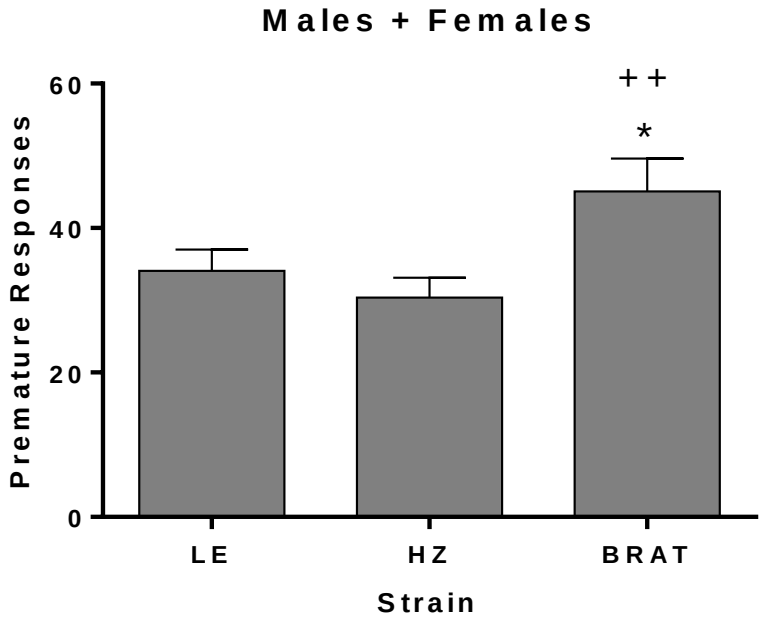


Figure 12 shows premature responses as a function of strain (LE, HZ, or BRAT) in male and female rats, *p < 0.05 versus LE, ++p < 0.01 versus HZ.

*Omissions*

Figure 13 presents the mean number of omissions exhibited during the sessions where training criteria were met for male rats. No statistically-significant differences were observed between strains in male rats $F(2,18) = 0.4552$, $p > 0.05$.

Figure 14 presents the mean number of omissions exhibited during the sessions where training criteria were met for female rats. No statistically-significant differences were observed between strains in female rats $F(2,17) = 0.5753$, $p > 0.05$.

Figure 15 presents the mean number of omissions exhibited during the sessions where training criteria were met for male and female rats. No statistically-significant differences were observed strains in male and female rats $F(2,38) = 0.035$, $p > 0.05$.

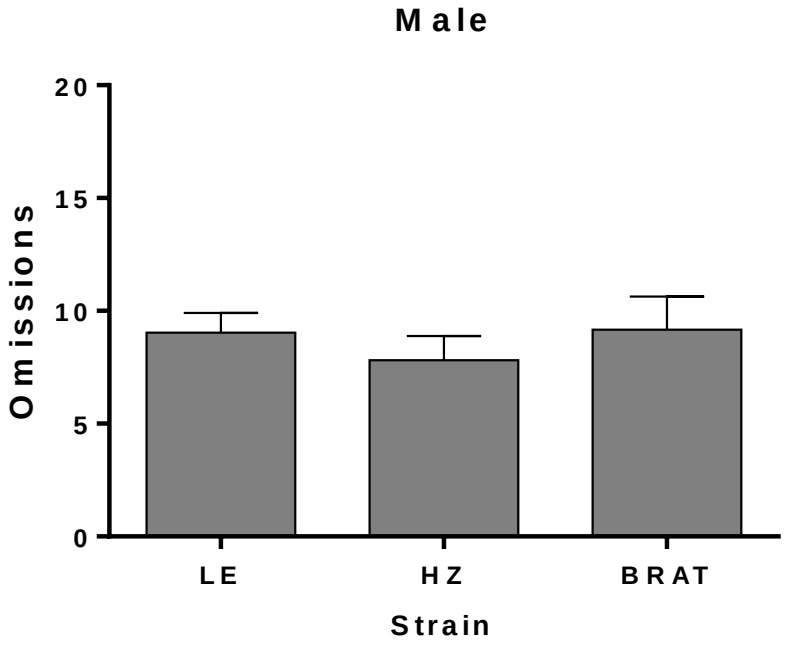


Figure 13 shows omissions as a function of strain (LE, HZ, or BRAT) in male rats.

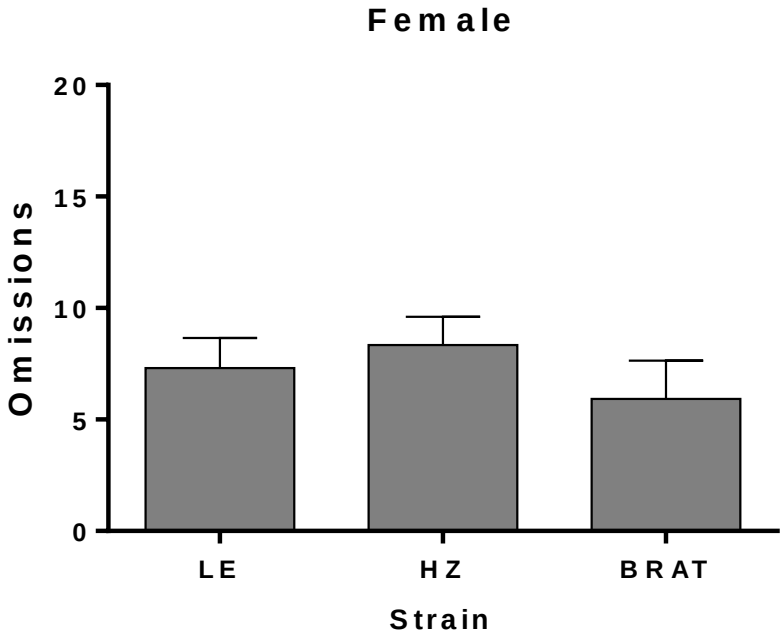


Figure 14 shows omissions as a function of strain (LE, HZ, or BRAT) in female rats.

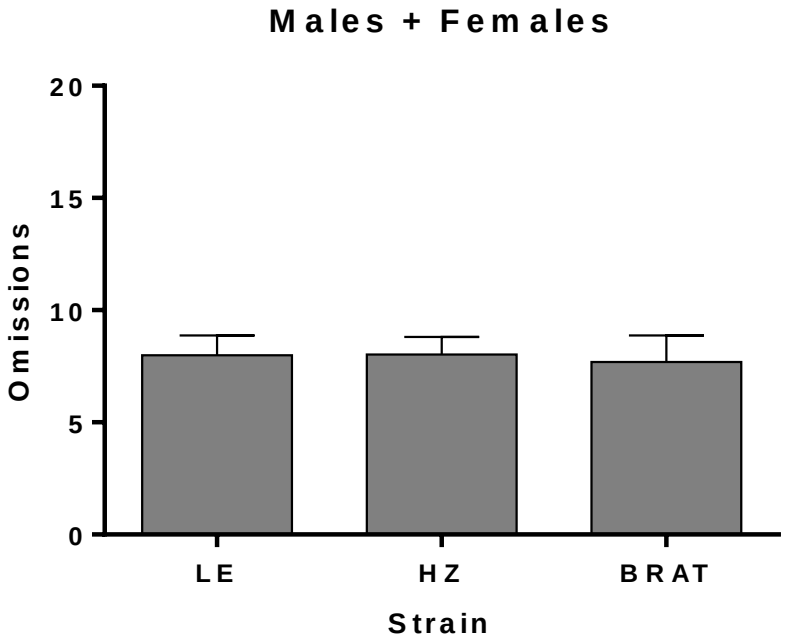


Figure 15 shows omissions as a function of strain (LE, HZ, or BRAT) in male and female rats.

*Bodyweight and water intake*

After completing these experiments, all rats were returned to free-feeding and assessments of bodyweights and amount of 24 hour home-cage water consumption were conducted.

Figure 16 presents the mean weights of the male rats. Statistically-significant effects were found in the male rats $F(2,26) = 28.3$, $p < 0.0001$, which were due to male HZ weighing more than LE and BRAT rats, and LE weighing more than BRAT rats. No statistically significant differences were found between LE and HZ rats.

Figure 17 presents the mean weights of the female rats. Statistically-significant effects were found in the female rats $F(2,25) = 16.07$, $p < 0.0001$, which were due to female HZ weighing more than LE and BRAT rats, and LE weighing more than BRAT rats. No statistically significant differences were found between LE and HZ rats.

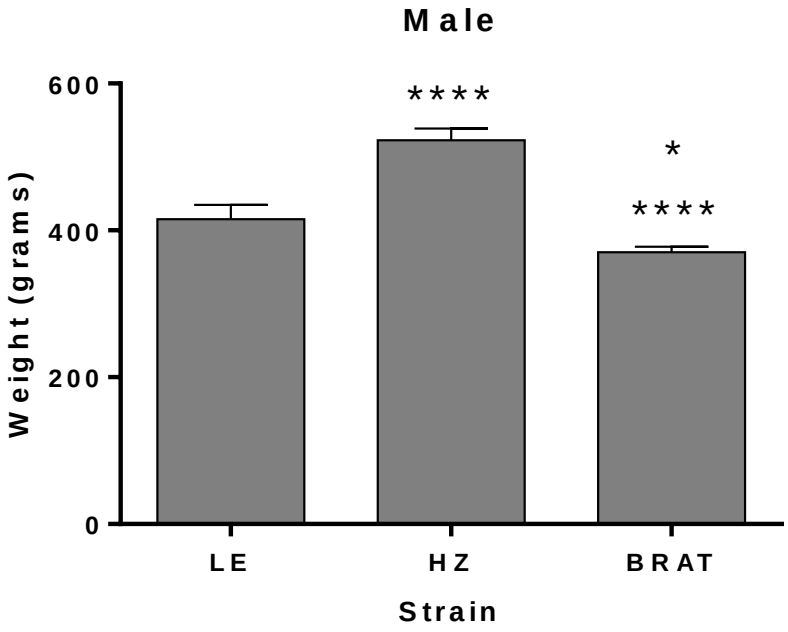


Figure 16 shows the mean weights as a function of strain (LE, HZ, or BRAT) in male rats, ****$p < 0.0001$ versus LE, *$p < 0.05$ versus LE.

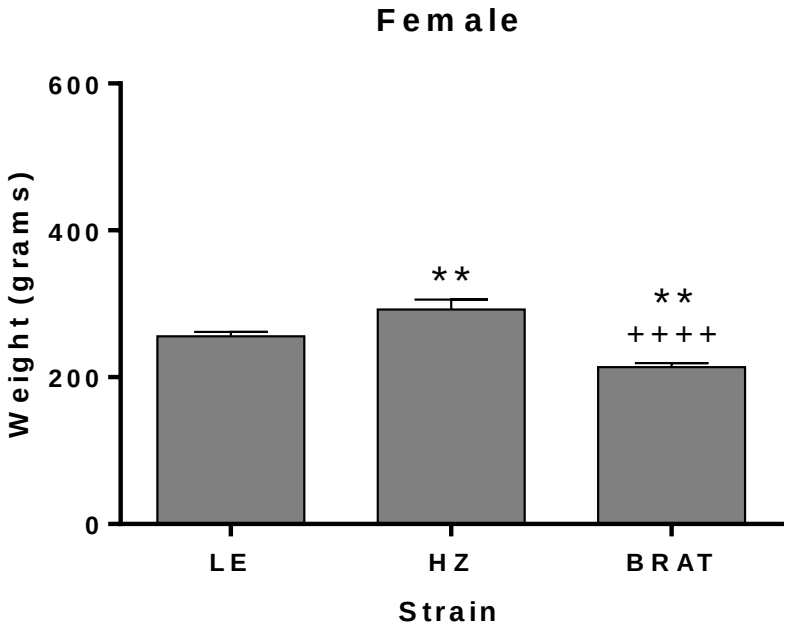


Figure 17 shows the mean weights as a function of strain (LE, HZ, or BRAT) in female rats, **p < 0.01 versus LE, ++++p < 0.0001 versus HZ.

Figure 18 presents the 24 hour water consumption in male rats. Statistically-significant effects were found in water consumption amounts in male rats $F(2,26) = 180$, $p < 0.0001$, which were due to the BRAT rats drinking more water than LE and HZ rats. No statistically significant differences were found between LE and HZ rats.

Figure 19 presents the 24 hour water consumption in female rats. Statistically-significant effects were found in water consumption amounts in female rats $F(2,25) = 295.7$, $p < 0.0001$, which were due to BRAT rats drinking more water than LE and HZ rats. No statistically significant differences were found between LE and HZ rats.

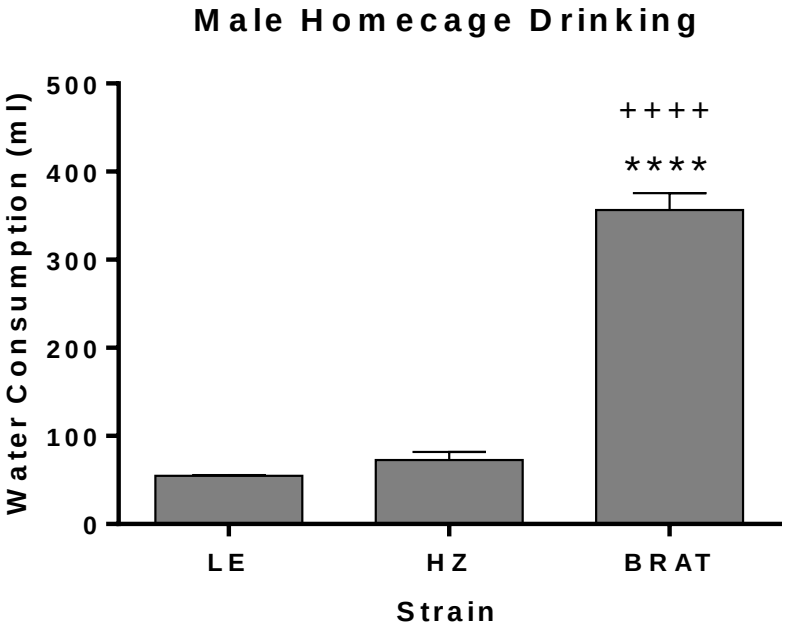


Figure 18 shows the 24 hour water consumption as a function of strain (LE, HZ, or BRAT) in male rats, ****$p < 0.0001$ versus LE, ++++$p < 0.0001$ versus HZ.

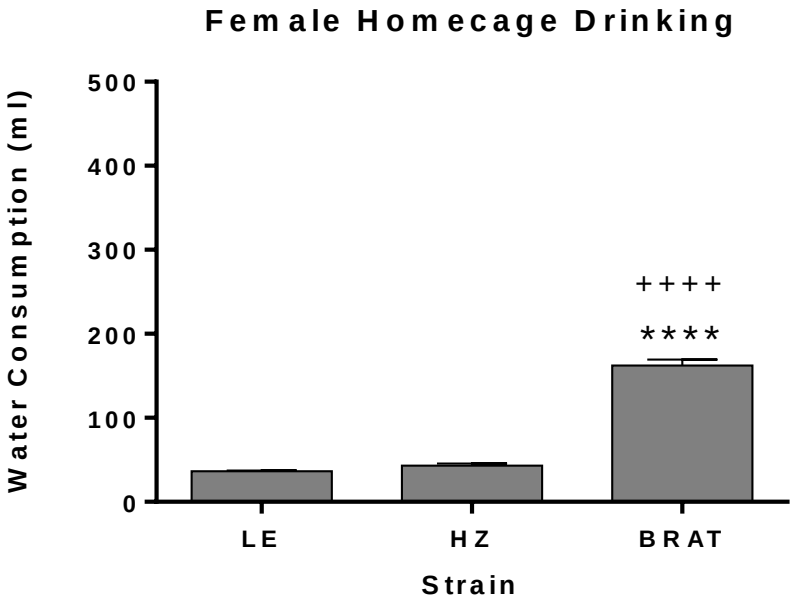


Figure 19 shows the 24 hour water consumption as a function of strain (LE, HZ, or BRAT) in female rats, ****$p < 0.0001$ versus LE, ++++$p < 0.0001$ versus HZ.

Figure 20 presents the amount of water male rats consumed during a 60 minute session. Statistically-significant differences in water consumption were found among the male rats $F(2,26) = 7.862$, $p < 0.01$, which were due to male BRAT rats consuming more water than LE and HZ. No statistically significant differences were found between LE and HZ rats.

Figure 21 presents the amount of water female rats consumed during a 60 minute session. Statistically-significant differences in water consumption were found among the female rats $F(2,25) = 15.73$, $p < 0.0001$, which were due to female BRAT rats consuming more water than LE and HZ. No statistically significant differences were found between LE and HZ rats.

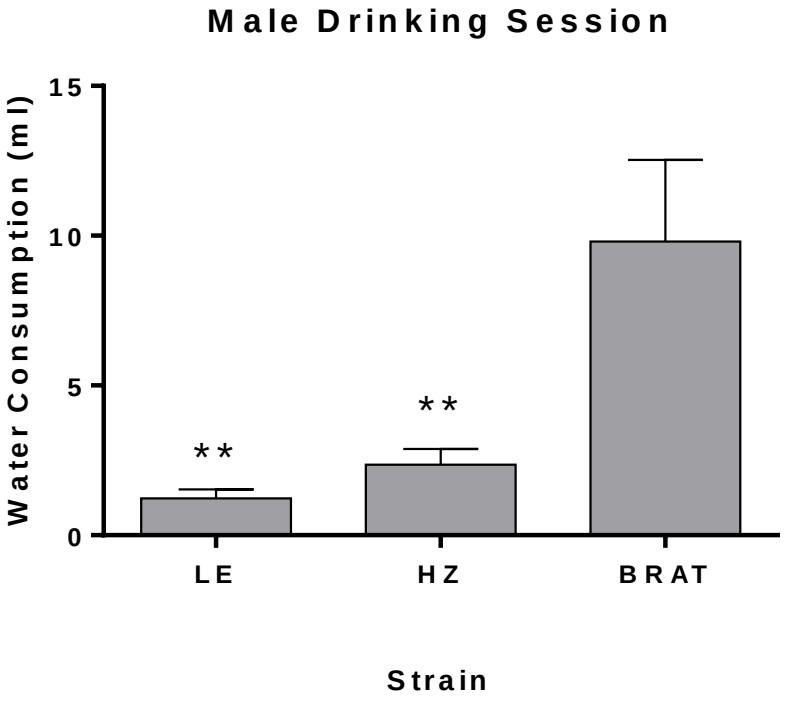


Figure 20 shows the amount of water consumed during a 60 minute session as a function of strain (LE, HZ, or BRAT) in male rats, **p < 0.01 versus BRAT.

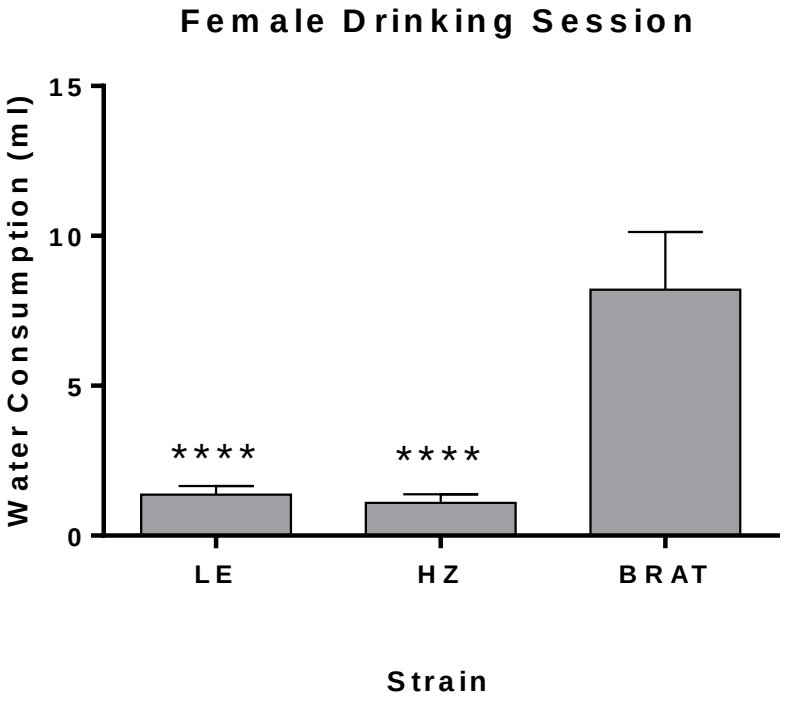


Figure 21 shows the amount of water consumed during a 60 minute session as a function of strain (LE, HZ, or BRAT) in female rats, p**** < 0.0001 versus BRAT.

Figure 22 presents the number of licks made during a 60 minute session in male rats. Two rats were excluded in this measure due to mechanical error with the lickometer equipment. Statistically-significant effects were found in the number of licks made in the male rats $F(2,24) = 10.13$, $p < 0.001$, which was due to male BRAT rats exhibiting more licks than LE and HZ. No statistically significant differences were found between LE and HZ rats.

Figure 23 presents the number of licks made during a 60 minute session in female rats. Statistically significant effects were found in the number of licks made in the female rats $F(2,25) = 38.32$, $p < 0.0001$., which was due to female BRAT rats exhibiting more licks than LE and HZ. No statistically significant differences were found between LE and HZ rats.

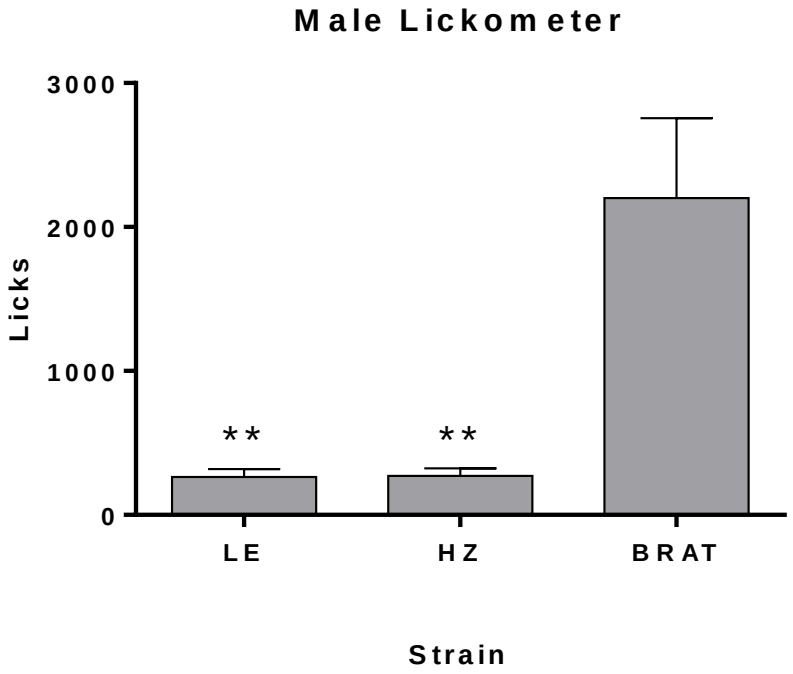


Figure 22 shows the number of licks made during a 60 minute session as a function of strain (LE, HZ, or BRAT) in male rats, **p < 0.01 versus BRAT.

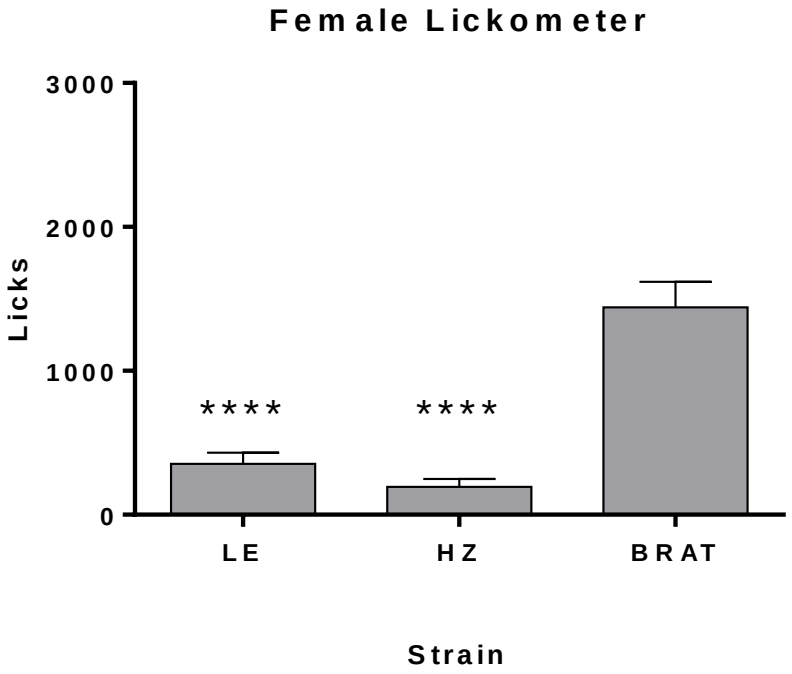


Figure 23 shows the number of licks made during a 60 minute session as function of strain (LE, HZ, or BRAT) in female rats, ****$p < 0.0001$ versus BRAT.

# DISCUSSION

The present study provides the first report on the assessment of both male and female BRAT rats using the 5-CSRTT. Male BRAT rats required significantly more trials to complete this task than LE rats (16.8% higher), while differences were not observed between strains for the female rats. During the sessions that rats met the training criteria, only female BRAT rats displayed a lower percent accuracy (51.8%) than the HZ (61.1%) and LE rats (63.5%). When males and females were combined, the BRAT rats required significantly more sessions to complete training than both the LE and HZ rats. Differences also were not found between strains in either male or female rats for omissions or the number of premature responses. However, when increasing the sample sizes by combining both male and female rats, a significant increase in premature responses was observed in the BRAT rats compared to LE and HZ rats. The HZ rats were found to have heavier free-feeding weights than LE and BRAT rats, while BRAT rats weighed less than LE and HZ rats—similar to findings reported elsewhere (e.g., Arimura, Sawano, Redding, & Schally, 1968). VP deficiency led to significantly greater home cage and experimental water consumption in BRAT rats compared to LE and HZ rats, while VP deficiency in HZ rats may not have been substantial enough to significantly enhance water consumption compared to LE rats. Furthermore, the BRAT rats made significantly more licks during the drinking session than both LE and HZ rats.

The training criteria used for the present study were developed in order to determine a steady state for attention accuracy for the purpose of making comparisons between different strains. These criteria differ from other 5-CSRTT studies, which usually set training criteria toward attaining some a priori level of accuracy, such as 80% (e.g., Auclair, Besnard, Newman-Tancredi, & Depoortere, 2009; Agnoli & Carli, 2012). From the approach used here, many rats, including the LE rats, failed to achieve greater than 60% choice accuracy, and most were below 80% choice accuracy. Moreover, some rats were removed from the study due to either failing to pass early phases of training or to exhibiting too much variability for day to day performance. These criteria suggest that for studies aiming for a high accuracy, rats may initially reach a steady state of lower accuracy, such as the 60% value found in the present study, prior to gradually meeting a higher level of accuracy.

In the present study, differences in attention were found in female BRAT rats but not in male BRATs. This is the first study to indicate a potential cognitive deficit in attention in female BRAT rats. Perhaps the present finding of impaired attention accuracy in the female BRAT rats can be attributed to the effects of hormonal cyclicity; the present study did not control for the rhythmic release of hormonal factors in the female strains. Additionally, it has been observed that the BRAT strain exhibits several alterations in a number of hormonal factors (Sokol and Zimmerman, 1982), and it is thus possible that these factors may be further disrupted in female versus male BRAT rats. Clearly, more research is needed to assess how sex differences may interact with VP to account for potential behavioral or cognitive effects. Moreover, aside from a previous assessment of pre-pulse inhibition (Feifel, Shilling, & Melendez, 2010) no other behavioral or cognitive

studies have been conducted between strains in female BRAT rats, and therefore it is difficult to suggest what type of unique behavioral or cognitive profile may exist for female rats with VP deficiency.

The closest comparisons between the present findings and previous studies are those conducted by Jentsch, Arguello, and Anzivino (2003) and Jentsch (2003), which both used a lateralized reaction time task (see introduction). In the study by Jentsch (2003), male HZ rats failed to exhibit differences in choice accuracy at 30, 5, and 2.5 sec durations but displayed improved choice accuracy at a 1 sec duration. In an another lateralized reaction time study, Jentsch, Arguello, and Anzivino (2003) reported that BRAT rats showed improved choice accuracy at 2.0 sec duration but displayed impaired choice accuracy at a 0.2 sec duration (Jenstch, Arguello, & Anzivino, 2003). Considering these findings together, Jentsch, Arguello, and Anzivino (2003) suggested that increases in VPergic tone may diminish attention accuracy. However, the present findings fail to support these conclusions, since accuracy changes were not observed in male BRAT or HZ rats, and that deficits, rather than improvements, in attention accuracy were observed in female BRAT rats.

These differences may, in part, owe to the approaches used to assess attention. The present study employed a 5-CSRTT, which utilizes five receptacles that must be surveyed by an animal in order to attend to a 0.5 second pulse of light; whereas the lateralized reaction time task includes only two choices, which are located adjacent to a central receptacle that an animal uses to begin each trial. Thus, the 5-CSRTT may provide greater task difficulty, although the differences between these two tasks have yet

to be empirically studied. Certainly, the present findings are more in line with previous investigations (see below) suggesting that VP deficiency impairs cognitive performance.

As mentioned previously, the BRAT rats made significantly more premature responses than the LE and HZ rats. In the 5-CSRTT premature responding is generally considered a measure of impulsivity (e.g., Robbins, 2002; Chudasama & Robbins, 2004; Amitai & Markou, 2010). For example, psychostimulants such as methylphenidate tend to dose-dependently increase premature responding (e.g. Paterson, Ricciardi, Wetzler, & Hanania, 2011; Navarra, et al. 2008), which may relate to enhanced locomotor activity at similar doses. Given this general interpretation for premature responding in the 5-CSRTT, the present findings may indicate greater impulsivity in BRAT rats. BRAT rats have also been shown to exhibit increased locomotor activity compared to LE controls (e.g.,Cilia, et al. 2010; Schank, 2009) and have lower increases in locomotor activity following d-amphetamine administration than LE controls (Cilia et al., 2010). The increases in impulsivity found in the present study and increased locomotor activity found in other studies might be caused by differences in the dopamine system of BRAT rats (e.g., see Dawson Jr., Wallace, & King, 1990; Feenstra, Snijdewint, Galen, & Boer, 1990; and Shilling et al., 2006, for altered neurotransmitter levels found in BRATs). In particular, Shilling et al. (2006) reported that BRAT rats displayed an upregulation of dopamine $D_2$ receptors in the nucleus accumbens shell and dorsomedial caudate/putamen compared to LE rats.

As noted above, the 5-CSRTT has been suggested to involve various subtypes of attention— such as selective and divided attention— in order to perform the task (e.g.,

Robbins, 2002). Much progress has been made delineating the substrates and functional neurochemistry between these subtypes of attention. For example, a study by McGaughy, Kaiser, and Sarter (1998) demonstrated a direct relationship of reduced acheylcholinesterase-fiber density and disrupted performance in a rat model of sustained attention— suggesting that cholinergic transmission is necessary for this particular subtype of attention. Moreover, perhaps one of the more intuitive mechanisms to occur before attention processes can begin is arousal. Among the many brain structures involved in arousal, the brain stem is particularly important for initiating this process (e.g., see Jones, 2003). As such, two substructures of the brain stem, the medulla and locus coeruleus, have been largely implicated in arousal and have been shown to involve neurotransmitters such as glutamate and noradrenaline (e.g., Jones, 2003; Sara, 2009). The BRAT strain has been shown to have developmental impairments within the medulla measured in neonatal BRAT rat pups (Boer, Van Rheenen-Verberg, & Uylings, 1982), and elevations in noradrenergic concentrations were also found in the strain (Dawson Jr., Wallace, & King, 1990). Although further research is necessary to evaluate these agents within the process of arousal, and particularly across sexes within the BRAT strain, we the investigators suspect that perturbations within these arousal mechanisms may also be related to the lower accuracy effect found in the present study.

Few other studies have evaluated cognitive differences in the BRAT strain— several of which have been briefly presented in the introduction of this document. As previously mentioned in the introduction of this document, impaired performance in shock-motivated memory paradigms have been suggested in the BRAT strain of rats (De Wied, Bohus, & Van Wimersma Greidanus, 1975; Stoehr, Cheng, & North, 1993).

BRAT rats also display an innate deficit in the pre-pulse inhibition response, which appears to measure sensory gating; a process implicated in early attention processing (e.g., Braff, Swerdlow, & Geyer, 1999). Moreover, current research has directly linked polymorphisms within the AVPR1a promoter region of the VP gene to altered pre-pulse inhibition responses (Levin et al., 2009). This involvement of VP in pre-pulse measures may be partially responsible for the pre-pulse inhibition deficits found in both male (e.g., Feifel & Priebe, 2001; Cilia et al., 2010; Feifel, Melendez, & Shilling, 2004) and female (Feifel, Shilling, & Melendez, 2010) BRATs; though, more research is necessary exploring this area. Nonetheless, such deficits in pre-pulse inhibition may compromise performance of BRATs in attention procedures, possibly resulting in a poorer ability to shift attention between stimuli. The findings, taken with those from the present study, suggest that diminished VP concentrations contribute to declines in attention, and potentially other domains of cognition.

In addition to exploring the role vasopressin may play in behavioral and cognitive processes, VP deficiency has been recently explored as a screening model for antipsychotic drugs. Both antipsychotic drugs (e.g., Cilia et al., 2010; Feifel & Priebe, 2001) and the putative antipsychotic drug and neurotensin $NT_1$ receptor agonist PD149163 (Feifel, Melendez, & Shilling, 2004) have been shown to attenuate pre-pulse inhibition deficits in BRAT rats. Further, PD149163 and the antipsychotic drug clozapine have both reversed social discrimination deficits displayed in BRAT rats (Feifel et al., 2009). In addition to these behavioral data, subchronic administration of the NMDA noncompetitive receptor antagonist and psychotomimetic phencyclidine has been shown to reduce vasopressin $V1_a$ receptor binding in the brain, which corresponds with

deficits in social interaction (Tanaka et al., 2003). Further, as noted earlier, Shilling et al. (2006) reported an upregulation of $D_2$ receptors, possibly suggestive of enhanced dopaminergic signaling. Taken together, BRAT rats may have utility for antipsychotic drug development.

Taking together these pre-pulse inhibition findings and social deficits observed in the BRAT strain, researchers have suggested that the strain may be a useful animal model of schizophrenia due to the behavioral responses following both established and novel antipsychotic drug administration (e.g., Feifel, 2010; Cilia et al., 2010; Feifel et al., 2009). Other research has also included VP as a potential biochemical candidate involved in austism spectrum disorder (e.g., see Carter, 2007; and, Lukas & Neumann, 2012 for review on the subject). As such, the BRATs have also been suggested to perhaps subserve a role in this area of research (e.g., Insel, O'Brien, & Leckman, 1999; Schank, 2009); however, further investigation is necessary which expands upon this possibility.

The present study adds to previous research investigating the behavioral-cognitive profile of the BRAT rat, indicating that VP deficiency may diminish attention in female, but perhaps not male, BRAT rats. More research is necessary which further evaluates the other perturbed factors in the BRAT strain (e.g., hormones, monoamines, etc.), and moreover, how these alterations can affect performance in behavioral and cognitive tasks. Additionally, the present findings and earlier investigations suggest that BRAT rats could be used for the screening of antipsychotic drugs; particularly in models sensitive to impairments in sociality or cognitive functioning. Further studies are needed to evaluate

the behavioral and cognitive profile of VP deficiency and to elucidate the neurodevelopmental impact of VP deficiency.

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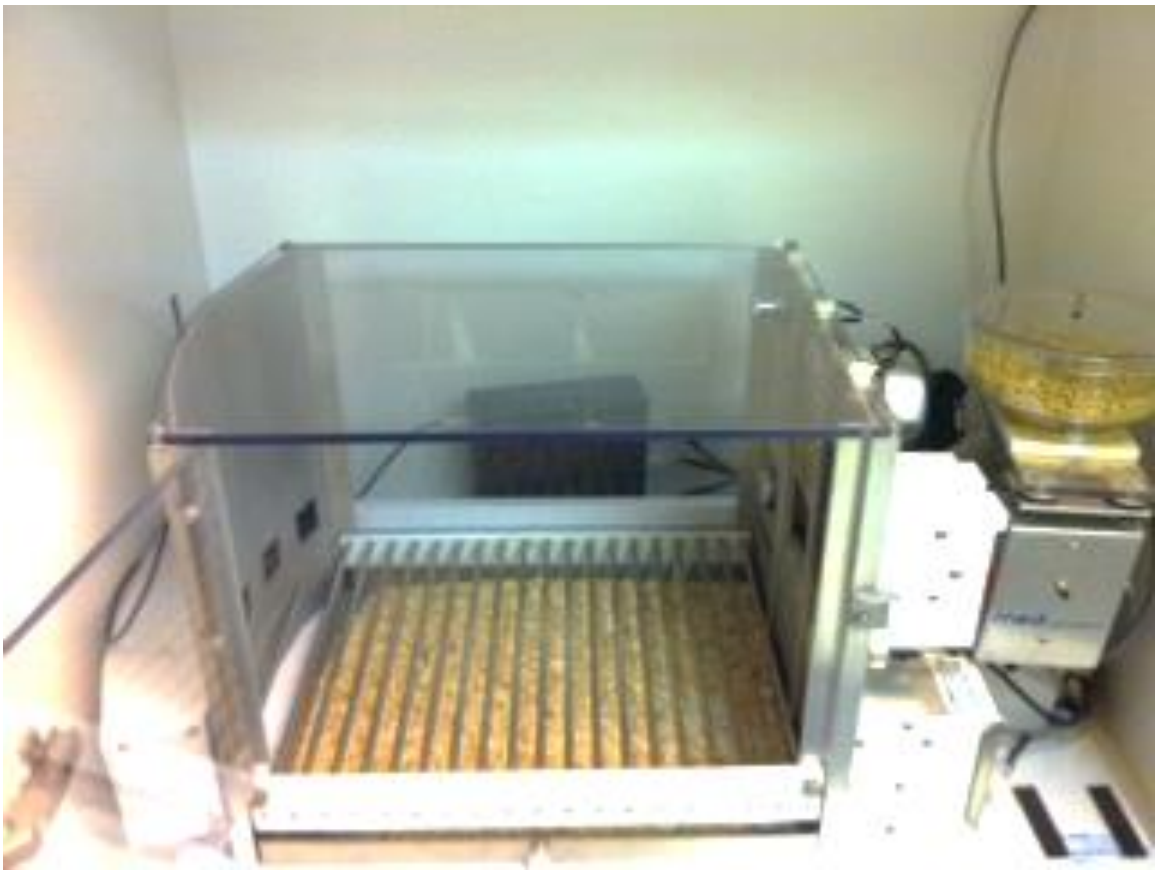
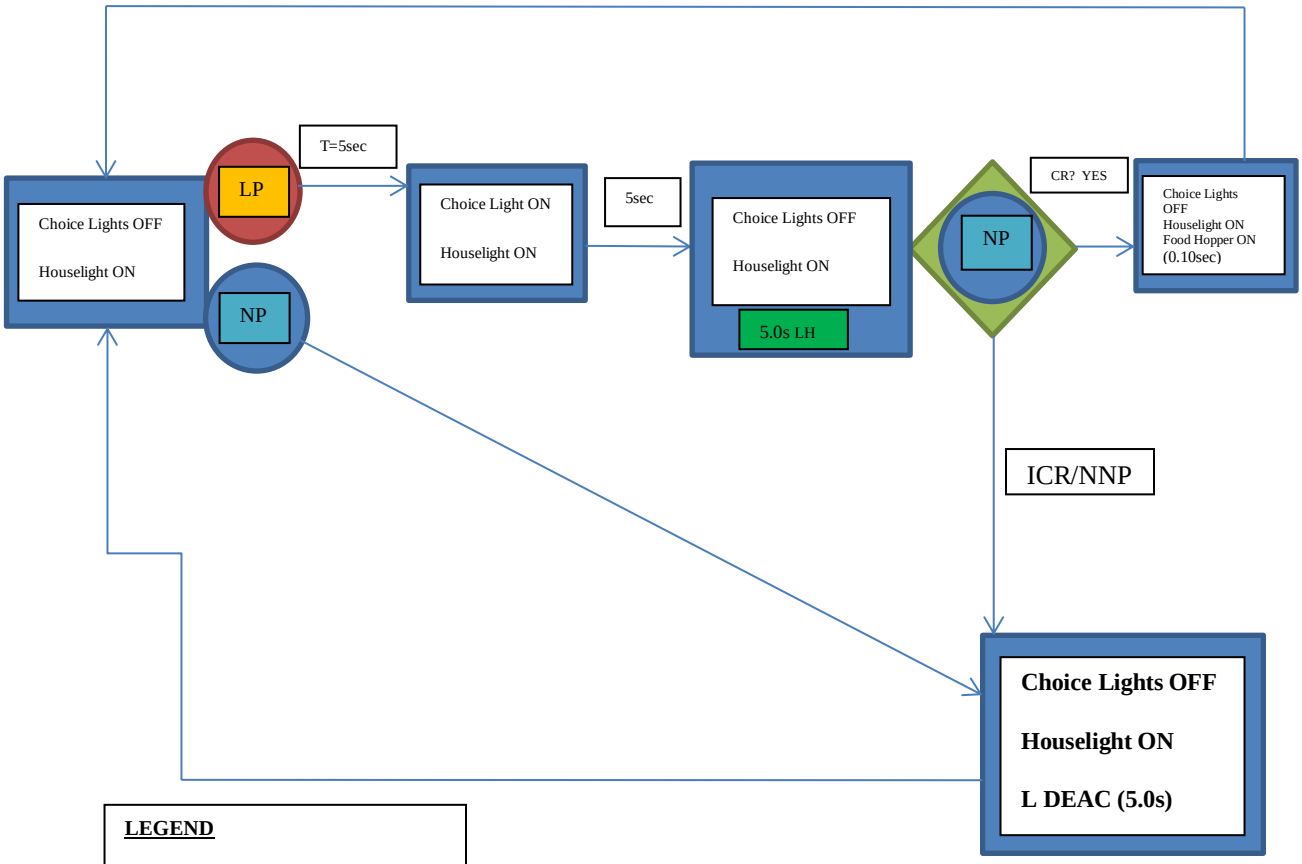
APPENDIX A

Five-Choice Serial Reaction Time Task Experimental Chamber



# APPENDIX B

## Box Diagram for Final Phase of 5-CSRTT Training




**LEGEND**

LP = Lever Press

NP = Nose Poke

T = Time Interval

LH = Limited Hold Period

CR = Correct Response

ICR = Incorrect Response

NNP = No Nose Poke

L DEAC = Lever Deactivated

APPENDIX C

IACUC Protocol Approval

Northern Michigan University

College of Graduate Studies
1401 Presque Isle Avenue
Marquette, MI 49855-5301
906-227-2300
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**MEMORANDUM**

February 2, 2012

**TO:** Dr. Adam Prus
Psychology Department

**FROM:** Brian Cherry, Ph.D. BC
Dean of Graduate Studies & Research

**RE:** **Application to use Vertebrate Animals**
**Modification Application # IACUC 174**
**Approval Period: 03/11/2011-02/21/2013**

The Institutional Animal Care and Use Committee, has approved your modified application by designated member review to use vertebrate animals in research for "Antipsychotic Drug Effects on Cognition in Brattleboro Rats".

If you have any questions, please contact me.

kjm

# APPENDIX D
## Habituation Session Programming

```
\Test 5csrtt
\FT60

^HOUSELIGHT = 15
^POKE1 = 4
^POKE2 = 5
^POKE3 = 6
^POKE4 = 7
^POKE5 = 8
^FAN = 16
^PELLET = 9

S.S.1,
S1,
  1":ON ^FAN--->S2
S2,
  #START: ON ^HOUSELIGHT, ^POKE1, ^POKE2, ^POKE3, ^POKE4, ^POKE5--->S3
S3,
  #R^POKE1:ADD A; OFF ^POKE1, ^POKE2, ^POKE3, ^POKE4, ^POKE5; ON ^PELLET; SHOW 1,
POKE1, A--->S4
  #R^POKE2:ADD B; OFF ^POKE1, ^POKE2, ^POKE3, ^POKE4, ^POKE5; ON ^PELLET; SHOW 2,
POKE2, B--->S4
  #R^POKE3:ADD C; OFF ^POKE1, ^POKE2, ^POKE3, ^POKE4, ^POKE5; ON ^PELLET; SHOW 3,
POKE3, C--->S4
  #R^POKE4:ADD D; OFF ^POKE1, ^POKE2, ^POKE3, ^POKE4, ^POKE5; ON ^PELLET; SHOW 4,
POKE4, D--->S4
  #R^POKE5:ADD E; OFF ^POKE1, ^POKE2, ^POKE3, ^POKE4, ^POKE5; ON ^PELLET; SHOW 5,
POKE5, E--->S4
S4,
  0.1":OFF ^PELLET; ADD F;SHOW 6, PELLET, F--->S5
S5,
  5":ON ^POKE1, ^POKE2, ^POKE3, ^POKE4, ^POKE5--->S3

S.S.2,
  S1,
   #START:--->S2
  S2,
   60":ON ^PELLET;ADD F;SHOW 6, PELLET, F--->S3
  S3,
   0.1":OFF ^PELLET--->S2

S.S.3,
  S1,
45':--->STOPABORTFLUSH
```

APPENDIX E
Phase 1 Training Programming

```
\Test 5csrtt
\FT60

^HOUSELIGHT = 15
^POKE1 = 4
^POKE2 = 5
^POKE3 = 6
^POKE4 = 7
^POKE5 = 8
^FAN = 16
^PELLET = 9


S.S.1,
S1,
  1":ON ^FAN--->S2
S2,
  #START: ON ^HOUSELIGHT, ^POKE1, ^POKE2, ^POKE3, ^POKE4, ^POKE5--->S3
S3,
  #R^POKE1:ADD A; OFF ^POKE1, ^POKE2, ^POKE3, ^POKE4, ^POKE5; ON ^PELLET; SHOW 1,
POKE1, A--->S4
  #R^POKE2:ADD B; OFF ^POKE1, ^POKE2, ^POKE3, ^POKE4, ^POKE5; ON ^PELLET; SHOW 2,
POKE2, B--->S4
  #R^POKE3:ADD C; OFF ^POKE1, ^POKE2, ^POKE3, ^POKE4, ^POKE5; ON ^PELLET; SHOW 3,
POKE3, C--->S4
  #R^POKE4:ADD D; OFF ^POKE1, ^POKE2, ^POKE3, ^POKE4, ^POKE5; ON ^PELLET; SHOW 4,
POKE4, D--->S4
  #R^POKE5:ADD E; OFF ^POKE1, ^POKE2, ^POKE3, ^POKE4, ^POKE5; ON ^PELLET; SHOW 5,
POKE5, E--->S4
S4,
  0.1":OFF ^PELLET; ADD F;SHOW 6, PELLET, F--->S5
S5,
  5":ON ^POKE1, ^POKE2, ^POKE3, ^POKE4, ^POKE5--->S3

S.S.2,
  S1,
    30':--->STOPABORTFLUSH
```

APPENDIX F
Phase 2 of Training Programming

```
\Test 5csrtt
\FT60

^HOUSELIGHT = 15
^POKE1 = 4
^POKE2 = 5
^POKE3 = 6
^POKE4 = 7
^POKE5 = 8
^FAN = 16
^PELLET = 9
^CENTERLEVER = 2


S.S.1,
S1,
  1":ON ^FAN--->S2
S2,
  #START: ON ^HOUSELIGHT,^CENTERLEVER--->S3
S3,
  #R^CENTERLEVER:ON ^POKE1, ^POKE2, ^POKE3, ^POKE4, ^POKE5--->S4
S4,
  #R^POKE1:ADD A; OFF ^POKE1, ^POKE2, ^POKE3, ^POKE4, ^POKE5; ON ^PELLET;
SHOW 1, POKE1, A--->S5
  #R^POKE2:ADD B; OFF ^POKE1, ^POKE2, ^POKE3, ^POKE4, ^POKE5; ON ^PELLET;
SHOW 2, POKE2, B--->S5
  #R^POKE3:ADD C; OFF ^POKE1, ^POKE2, ^POKE3, ^POKE4, ^POKE5; ON ^PELLET;
SHOW 3, POKE3, C--->S5
  #R^POKE4:ADD D; OFF ^POKE1, ^POKE2, ^POKE3, ^POKE4, ^POKE5; ON ^PELLET;
SHOW 4, POKE4, D--->S5
  #R^POKE5:ADD E; OFF ^POKE1, ^POKE2, ^POKE3, ^POKE4, ^POKE5; ON ^PELLET;
SHOW 5, POKE5, E--->S5
S5,
  0.1":OFF ^PELLET; ADD F;SHOW 6, PELLET, F--->S3


S.S.2,
  S1,
    30':--->STOPABORTFLUSH
```

# APPENDIX G
## Phase 3 of Training Programming

```
\Five choice serial reaction time task

\Written by Adam Prus and Mike Berquist

\January 18 2012

\0.5 sec light pulse in recepticle.  Nosepoke leads to food pellet.

\Session last 80 trials or 30 minutes
\incorrect or omission leads to 5 sec time out

\ Training steps = 1.  FT60 2.  Nosepoke = food pellet delivery (all receptacles lighted)  3.  Only light hole triggers pellet (stays lit until response - incorrect leads to timeout)

\4.  Lever press triggers light (light stays on) 5.  Lever press triggers light (light stays on for maybe 5 sec  6.  Same but reduce light duration until 0.5" responding occurs
\STEP 2.  Training Here


\Alias


^Poke1 = 4
^Poke2 = 5
^Poke3 = 6
^Poke4 = 7
^Poke5 = 8
^Houselight = 15
^CenterLever = 2
^Pellet = 9
^Fan = 16



\A=PREMATURE RESPONSE
\B=CORRECT RESPONSE
\C=OMISSIONS
\D=TRIAL
\E=CORRECT RESPONSE
\F=INCORRECT RESPONSE
\G=TOTAL RESPONSES
```

```
\H=FOR NO RESPONSE CALCULATION
\Y=CHOOSES LIGHT
\Z=LIST FOR RANDOM LIGHT

LIST Z = 4,5,6,7,8
DIM B = 6



\SHOW 1 = TIME
\SHOW 2 = NUMBER OF TRIALS
\SHOW 3 = OMISSIONS
\SHOW 4 = PREMATURE
\SHOW 5 =
\SHOW 6 = %CORRECT
\SHOW 7 = TOTAL RESPONSES

\-------------------------------------------------------------------------------

S.S.1,

S1,

  1":  ON ^FAN--->S2

S2,

  #START: ON ^HOUSELIGHT,^CENTERLEVER--->S3

S3,
  #R2:ADD D--->S4
S4,
  0.1":RANDI Y = Z; SET B(1)=Y;SHOW 2, TRIALS, D; IF B(1)=4 [@FOUR,@OTHER]

     @FOUR:SET B(2)=5, B(3)=6, B(4)=7, B(5)=8--->S5

     @OTHER:IF B(1)=5 [@FIVE,@OTHER]

          @FIVE:SET B(2)=4,B(3)=6,B(4)=7,B(5)=8--->S5

          @OTHER:IF B(1)=6 [@SIX,@OTHER]

               @SIX:SET B(2)=4,B(3)=5,B(4)=7,B(5)=8--->S5

               @OTHER:IF B(1)=7 [@SEVEN,@EIGHT]

                    @SEVEN:SET B(2)=4,B(3)=5,B(4)=6,B(5)=8--->S5

                    @EIGHT:SET B(2)=4,B(3)=5,B(4)=6,B(5)=7--->S5
```

```
S5,
  5":ON Y--->S6 \PREMATURE RESPONSE GOES HERE
S6,
  #RB(1):ADD E;ON ^PELLET;OFF Y;SHOW 6,CORRECT,E--->S7 \SET G=E+F;SHOW 6,
%CORRECT, (E/G*100);SHOW 7,TOT_RESP,G
  #RB(2)!#RB(3)!#RB(4)!#RB(5):OFF Y; ADD F--->S8 \INCORRECT,TIMEOUT ;SET
G=E+FSHOW 6, %CORRECT, (E/G*100);SHOW 7,TOT_RESP,G
  5":OFF Y;ADD C;SHOW 3, OMISSIONS, C--->S8
S7,
  0.1":OFF ^PELLET--->S3
S8, \TIMEOUT1
  0.1":OFF ^HOUSELIGHT--->S9
S9, \TIMEOUT2
  5":ON ^HOUSELIGHT--->S3



\-------------------------------------------------------------------------------

S.S.2,

S1,

  #START:--->S2

S2,

  1":ADD T;Show 1, Timer, T; IF D>100 [@END,@CONT]

        @END:SET D=100;IF G=0 [@NORESPONSE,@RESPONSE]
            @NORESPONSE:SET H = 999;SHOW 1, TIMER, T; SHOW 2, TRIALS, D;
SHOW 3, OMISSIONS, C;SHOW 4, PREMATURE, A; SHOW 6, CORRECT, E;SHOW
7,TOT_RESP,G--->STOPABORTFLUSH

            @RESPONSE:SHOW 1, TIMEr, T; SHOW 2, TRIALS, D; SHOW 3, OMISSIONS,
C;SHOW 4, PREMATURE, A; SHOW 6, CORRECT, e--->STOPABORTFLUSH
        @CONT:  IF T>=1800 [@END,@CONT]

            @END:SHOW 1, TIMER, T; SHOW 2, TRIALS, D; SHOW 3, OMISSIONS,
C;SHOW 4, PREMATURE, A; SHOW 6, CORRECT, e--->STOPABORTFLUSH

            @CONT:--->S2
```

APPENDIX H
Phase 4 of Training Programming

```
\Five choice serial reaction time task

\Written by Adam Prus and Mike Berquist

\January 18 2012

\0.5 sec light pulse in recepticle.  Nosepoke leads to food pellet.

\Session last 80 trials or 30 minutes
\incorrect or omission leads to 5 sec time out

\ Training steps = 1.  FT60 2.  Nosepoke = food pellet delivery (all receptacles lighted)  3.  Only light hole triggers pellet (stays lit until response - incorrect leads to timeout)

\4.  Lever press triggers light (light stays on) 5.  Lever press triggers light (light stays on for maybe 5 sec  6.  Same but reduce light duration until 0.5" responding occurs
\STEP 2.  Training Here



\Alias


^Poke1 = 4
^Poke2 = 5
^Poke3 = 6
^Poke4 = 7
^Poke5 = 8
^Houselight = 15
^CenterLever = 2
^Pellet = 9
^Fan = 16



\A=PREMATURE RESPONSE
\B=CORRECT RESPONSE
\C=OMISSIONS
\D=TRIAL
\E=CORRECT RESPONSE
\F=INCORRECT RESPONSE
\G=TOTAL RESPONSES
```

```
\H=FOR NO RESPONSE CALCULATION
\Y=CHOOSES LIGHT
\Z=LIST FOR RANDOM LIGHT

LIST Z = 4,5,6,7,8
DIM B = 6



\SHOW 1 = TIME
\SHOW 2 = NUMBER OF TRIALS
\SHOW 3 = OMISSIONS
\SHOW 4 = PREMATURE
\SHOW 5 =
\SHOW 6 = %CORRECT
\SHOW 7 = TOTAL RESPONSES

\-------------------------------------------------------------------------------

S.S.1,

S1,

  1":  ON ^FAN--->S2

S2,

  #START: ON ^HOUSELIGHT,^CENTERLEVER--->S3

S3,
  #R2:ADD D--->S4
S4,
  0.1":RANDI Y = Z; SET B(1)=Y;SHOW 2, TRIALS, D; IF B(1)=4 [@FOUR,@OTHER]

     @FOUR:SET B(2)=5, B(3)=6, B(4)=7, B(5)=8--->S5

     @OTHER:IF B(1)=5 [@FIVE,@OTHER]

          @FIVE:SET B(2)=4,B(3)=6,B(4)=7,B(5)=8--->S5

          @OTHER:IF B(1)=6 [@SIX,@OTHER]

               @SIX:SET B(2)=4,B(3)=5,B(4)=7,B(5)=8--->S5

               @OTHER:IF B(1)=7 [@SEVEN,@EIGHT]

                    @SEVEN:SET B(2)=4,B(3)=5,B(4)=6,B(5)=8--->S5

                    @EIGHT:SET B(2)=4,B(3)=5,B(4)=6,B(5)=7--->S5
```

```
S5,
  5":ON Y--->S6 \PREMATURE RESPONSE GOES HERE
  #RB(1)!#RB(2)!#RB(3)!#RB(4)!#RB(5):ADD A;SHOW 4, PREMATURE, A--->S8
S6,
  #RB(1):ADD E;ON ^PELLET;OFF Y;SHOW 6,CORRECT,E--->S7 \SET G=E+F;SHOW 6,
CORRECT, E;SHOW 7,TOT_RESP,G
  #RB(2)!#RB(3)!#RB(4)!#RB(5):OFF Y; ADD F--->S8 \INCORRECT,TIMEOUT ;SET
G=E+F;SHOW 6, CORRECT, E;SHOW 7,TOT_RESP,G
  5":OFF Y;ADD C;SHOW 3, OMISSIONS, C--->S8
S7,
  0.1":OFF ^PELLET--->S3
S8, \TIMEOUT1
  0.1":OFF ^HOUSELIGHT--->S9
S9, \TIMEOUT2
  5":ON ^HOUSELIGHT--->S3



\-------------------------------------------------------------------------------

S.S.2,

S1,

  #START:--->S2

S2,

  1":ADD T;Show 1, Timer, T; IF D>100 [@END,@CONT]

        @END:SET D=100;IF G=0 [@NORESPONSE,@RESPONSE]
            @NORESPONSE:SET H = 999;SHOW 1, TIMER, T; SHOW 2, TRIALS, D;
SHOW 3, OMISSIONS, C;SHOW 4, PREMATURE, A; SHOW 6, CORRECT, E;SHOW
7,TOT_RESP,G--->STOPABORTFLUSH

            @RESPONSE:SHOW 1, TIMEr, T; SHOW 2, TRIALS, D; SHOW 3, OMISSIONS,
C;SHOW 4, PREMATURE, A; SHOW 6, CORRECT, E--->STOPABORTFLUSH
        @CONT:  IF T>=1800 [@END,@CONT]

            @END:SHOW 1, TIMER, T; SHOW 2, TRIALS, D; SHOW 3, OMISSIONS,
C;SHOW 4, PREMATURE, A; SHOW 6, CORRECT, E--->STOPABORTFLUSH

            @CONT:--->S2
```

# APPENDIX I
## Phase 5_Final Phase of 5-CSRTT Training Programming

```
\Five choice serial reaction time task

\Written by Adam Prus and Mike Berquist

\April 4 2012

\0.5 sec light pulse in recepticle.  Nosepoke leads to food pellet.

\Session last 80 trials or 30 minutes
\incorrect or omission leads to 5 sec time out

\ Training steps = 1.  FT60 2.  Nosepoke = food pellet delivery (all receptacles lighted)  3.  Only light hole triggers pellet (stays lit until response - incorrect leads to timeout)

\4.  Lever press triggers light (light stays on) 5.  Lever press triggers light (light stays on for maybe 5 sec  6.  Same but reduce light duration until 0.5" responding occurs
\STEP 2.  Training Here



\Alias


^Poke1 = 4
^Poke2 = 5
^Poke3 = 6
^Poke4 = 7
^Poke5 = 8
^Houselight = 15
^CenterLever = 2
^Pellet = 9
^Fan = 16



\A=PREMATURE RESPONSE
\B=CORRECT RESPONSE
\C=OMISSIONS
\D=TRIAL
\E=CORRECT RESPONSE
\F=INCORRECT RESPONSE
\G=TOTAL RESPONSES
\H=FOR NO RESPONSE CALCULATION
```

```
\I=SIGNAL
\J=OMISSION
\K=RESPONSE
\L=open?
\M=OPEN?
\N=OPEN?
\Y=CHOOSES LIGHT
\Z=LIST FOR RANDOM LIGHT

\Z PULSES
\Z1=START SS3
\Z2=OMISSION

LIST Z = 4,5,6,7,8
DIM B = 6
DIM I = 100
DIM J = 100
DIM K = 100
DIM L = 100
DIM M = 100
DIM N = 100



\SHOW 1 = TIME
\SHOW 2 = NUMBER OF TRIALS
\SHOW 3 = OMISSIONS
\SHOW 4 = PREMATURE
\SHOW 5 = INCORRECT
\SHOW 6 = CORRECT
\SHOW 7 = TOTAL RESPONSES

\---------------------------------------------------------------------------

S.S.1,

S1,

  1":  ON ^FAN--->S2

S2,

  #START: ON ^HOUSELIGHT,^CENTERLEVER;SET D=0--->S3

S3,
  #R2:ADD D--->S4
S4,
  0.1":RANDI Y = Z; SET B(1)=Y;SHOW 2, TRIALS, D;Z1; IF B(1)=4 [@FOUR,@OTHER]
     @FOUR:SET B(2)=5, B(3)=6, B(4)=7, B(5)=8, L(D)=1--->S5 \tag
```

```
    @OTHER:IF B(1)=5 [@FIVE,@OTHER]
        @FIVE:SET B(2)=4,B(3)=6,B(4)=7,B(5)=8, L(D)=2--->S5 \tag
        @OTHER:IF B(1)=6 [@SIX,@OTHER]
            @SIX:SET B(2)=4,B(3)=5,B(4)=7,B(5)=8, L(D)=3--->S5 \tag
            @OTHER:IF B(1)=7 [@SEVEN,@EIGHT]
                @SEVEN:SET B(2)=4,B(3)=5,B(4)=6,B(5)=8, L(D)=4--->S5 \tag
                @EIGHT:SET B(2)=4,B(3)=5,B(4)=6,B(5)=7, L(D)=5--->S5 \tag
S5,
  5":ON Y--->S6 \PREMATURE RESPONSE GOES HERE
  #RB(1)!#RB(2)!#RB(3)!#RB(4)!#RB(5):ADD A;SHOW 4, PREMATURE, A--->S9
S6,
  #RB(1):ADD E;ON ^PELLET;OFF Y;SHOW 6,CORRECT,E;Z2--->S8 \SET G=E+F;SHOW
6, CORRECT, E;SHOW 7,TOT_RESP,G
  #RB(2)!#RB(3)!#RB(4)!#RB(5):OFF Y; ADD F;SHOW 5,INCORRECT,F;Z2--->S9
\INCORRECT,TIMEOUT ;SET G=E+F;SHOW 6, CORRECT, E;SHOW 7,TOT_RESP,G
  0.5":OFF Y;Z2--->S7
S7,
  #RB(1):ADD E;ON ^PELLET;OFF Y;SHOW 6,CORRECT,E--->S8 \SET G=E+F;SHOW 6,
CORRECT, E;SHOW 7,TOT_RESP,G
  #RB(2)!#RB(3)!#RB(4)!#RB(5):OFF Y; ADD F;SHOW 5,INCORRECT,F--->S9
\INCORRECT,TIMEOUT ;SET G=E+F;SHOW 6, CORRECT, E;SHOW 7,TOT_RESP,G
  5":OFF Y;ADD C;SHOW 3, OMISSIONS, C--->S9
S8,
  0.1":OFF ^PELLET;Z3--->S3
S9, \TIMEOUT1
  0.1":OFF ^HOUSELIGHT;Z3--->S10
S10, \TIMEOUT2
  5":ON ^HOUSELIGHT--->S3



\-------------------------------------------------------------------------------

S.S.2, \ENDS AFTER 30 MIN OR 100 TRIALS

S1,

  #START:--->S2

S2,

  1":ADD T;Show 1, Timer, T; IF D>100 [@END,@CONT]
        @END:SET D=100;IF G=0 [@NORESPONSE,@RESPONSE]
            @NORESPONSE:SET H = 999;SHOW 1, TIMER, T; SHOW 2, TRIALS, D;
SHOW 3, OMISSIONS, C;SHOW 4, PREMATURE, A;SHOW 5, INCORRECT, F;SHOW 6,
CORRECT, E;SHOW 7,TOT_RESP,G--->STOPABORTFLUSH
            @RESPONSE:SHOW 1, TIMER, T; SHOW 2, TRIALS, D; SHOW 3, OMISSIONS,
C;SHOW 4, PREMATURE, A;SHOW 5,INCORRECT,F; SHOW 6, CORRECT, E---
>STOPABORTFLUSH
```

```
        @CONT:  IF T>=1800 [@END,@CONT]
            @END:SHOW 1, TIMER, T; SHOW 2, TRIALS, D; SHOW 3, OMISSIONS,
C;SHOW 4, PREMATURE, A; SHOW 6, CORRECT, E--->STOPABORTFLUSH
            @CONT:--->S2
\-------------------------------------------------------------------------------
S.S.3, \DV PER TRIAL
S1,
  #Z1:SET I(D)=Y--->S2 \SIGNAL POSITION
S2,
  #Z2:SET J(D)=1--->S3 \ONLY GATHERS PREMATURES
  #R4:SET K(D)=4--->S1
  #R5:SET K(D)=5--->S1
  #R6:SET K(D)=6--->S1
  #R7:SET K(D)=7--->S1
  #R8:SET K(D)=8--->S1
S3,
  #Z3:SET M(D)=1--->S1 \ONLY GATHERS RESPONSES AFTER SIGNAL
  #R4:SET N(D)=4--->S1
  #R5:SET N(D)=5--->S1
  #R6:SET N(D)=6--->S1
  #R7:SET N(D)=7--->S1
  #R8:SET N(D)=8--->S1
```