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Effects of Galanin on Energizing and Hedonic Aspects of Consumption

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Ilan Michael McNamara

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Ilan Michael McNamara

We, the dissertation committee for the above candidate for the Doctor of Philosophy

degree, hereby recommend acceptance of this dissertation.

John K. Robinson, Ph.D.-Dissertation Advisor
Associate Professor, Psychology

Brenda J. Anderson, Ph.D.-Chairperson of Defense
Associate Professor, Psychology

Arthur G. Samuel, Ph.D.
Professor, Psychology

Samuel John Gatley, Ph.D.
Professor, Pharmacological Sciences, Northeastern University

This Dissertation is accepted by the Graduate School

Lawrence Martin
Dean of the Graduate School

Abstract of the Dissertation

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Prior research has shown that the neuropeptide galanin strongly stimulates food intake in sated rats when food is made freely available. However, when access to food is made contingent upon lever pressing on a progressive ratio schedule, no such stimulation occurs. This dissociation is consistent with the theorized "behavioral energizing" function of the ascending mesolimbic dopamine system, which purports that this ascending dopamine system is involved in only the goal directed effort maintaining (appetitive) and not the hedonic (consummatory) aspects of reward. Further, these results suggest that galanin may play an inhibitory role therein. The current dissertation tests a prediction of this theory by determining if a similar dissociation also applies to non-food rewards. Prior galanin research has only utilized caloric foods and therefore the non-caloric-but-hedonic reward of saccharin was chosen. Indeed, galanin significantly increased free consumption of a non-nutritive 0.2% saccharin solution but not when operant responding was required for access to saccharin. Recent reports suggest that galanin increases hedonic responses to an alcohol solution. Interestingly, the current study did not replicate a galanin-induced increase in *ad libitum* consumption of caloric-but-not-palatable 7% alcohol solution, suggesting that the taste qualities may be a specific hedonic factor involved in galanin stimulation of free consumption. As a positive control, the current work replicated the galanin-induced free consumption of a high-fat milk/cream liquid. Furthermore, the current research extended this finding by showing that a novel GalR2 receptor agonist, M1145, had no effect on free milk/cream consumption, suggesting that the GalR2 receptor is not involved in galanin-induced free food consumption. Taken together, the current results indicate that galanin may be acting as a mesolimbic dopamine inhibitor or itself may be inhibited by mesolimbic dopamine release. Additionally, these results offer further indirect support for the theory that mesolimbic dopamine regulates energetic "work" behavior more than hedonic "liking" behavior.

Table of Contents

List of Tables

List of Figures

1.0 Introduction

Galanin is a 29-residue neuropeptide in rodents and a 30-residue neuropeptide in humans (Wynick, et al., 1998). The positions of the first 15 N-terminal amino acids are identical between rodents, humans, and other species and likely contain the active receptor-binding properties of galanin (Gundlach, 2002). Since the initial discovery of galanin in the small intestine of pigs (Tatemoto et al., 1983), a multidisciplinary approach has been utilized to explore its effects on the nervous system. In the peripheral nervous system, galanin has been implicated in smooth muscle contraction (Ekblad et al., 1985; Bek et al., 1988), hyperglycemia (McDonald et al., 1985), gastrointestinal inflammation following injury (Simpson et al., 2008), cutaneous blood flow (Santha et al., 1998), changes in nociception after injury to the dorsal root ganglion or spinal cord (Flatters et al., 2002; Liu et al., 2001; Hao et al., 1999), as well as various other functions (see Lang et al., 2007 for a comprehensive review). However, its expansive presence in the central nervous system and correlative behavioral modifications has encouraged an exploration into the physiological and behavioral properties of this neuropeptide. To date, galanin has been implicated in several diverse and diffuse processes in the central nervous system including learning (Malin et al., 1992; Sundstrom et al., 1988; Ukai et al., 1995), memory (Crawley, 1993; Mastropaolo et al., 2002), anxiety (Holmes & Picciotto, 2006; Holmes et al., 2002; Karlsson & Holmes, 2006), depression (Kuteeva et al., 2007; Weiss et al., 1998), sexual behavior (Fraley et al., 2004; Poggioli et al., 1992), and ingestion of food (reviewed by Crawley, 1999), water (Brewer et al., 2005), and alcohol (Lewis et al., 2004; Schneider et al., 2007).

Although an impressive amount of research over the last 25 years has focused on galanin, the precise effects of galanin on the underpinnings of behaviors are incomplete and have often yielded seemingly ambiguous findings—particularly in the domains of food and water consumption. For example, galanin is known to increase food consumption in food-sated rats, but not when effort is required to obtain the same food (Brewer & Robinson, 2008). This raises important questions regarding the effect of galanin on *instrumental (i.e. appetitive) behavior,* defined as behavior that reliably leads to the opportunity to engage in a *consummatory behavior*. Consummatory behavior is defined as actions that end an appetitive behavior such as, but not limited to, consuming a biologically relevant reward (e.g. food or water). The current dissertation explores galanin-induced changes in consummatory and instrumental behavior by determining if a similar dissociation also applies to the non-caloric-but-hedonic reward of saccharin. The influence of galanin on alcohol consumption in rats trained to freely consume alcohol will also be examined. As a positive control, data replicating previous reports that galanin increases free consumption of a high-fat milk/cream liquid will be presented. Finally, the current research extends this replication by showing that a novel GalR2 receptor agonist, M1145, has no effect on free milk/cream consumption.

This work begins by providing a brief overview of the anatomical distribution of galanin in the central nervous system followed by its proposed function in the brain. The prolific and widespread location of galanin-producing neurons and receptors situates galanin in an ideal position to influence consumption of food and water which are

detailed in Chapters two and three. Finally, I will discuss galanin-dopamine interactions and the modern theory that mesolimbic dopamine serves as an energizing system, which is an important undertone to the research findings I will describe.

1.1 Anatomical Distribution

In the central nervous system, Galanin was first mapped using an immunohistochemical analysis by Skofitsch & Jacobowitz (1985). Results indicated prolific and widespread galanin-*ir*, where galanin-like immunoreactive cell bodies were found throughout the telencephalon diencephalon, mesencephalon, pons, medulla oblongata, pituitary, and sensory ganglia (See Table 1 for a listing of structures most applicable to the current study). Of interest to the current study, a highly dense accumulation of galanin has been located in the preoptic, paraventricular (PVN), dorsomedial, ventromedial, supraoptic (SON), and lateral nuclei of the hypothalamus (Gundlach et al., 2001). This robust density of galanin-immunoreactivity in the hypothalamus has been suggested to modulate survival behaviors such as reproductive behavior (Bloch et al., 1996), feeding (Crawley, 1999), and drinking (Brewer et al., 2005). Yet since galanin is widely dispersed throughout the central nervous system, its effects are diverse and likely affect various neural circuitries that produce a variety of behaviors. For example, the presence of galanin within the prefrontal cortex, bed nucleus of the stria terminalis (BNST), rostral cingulate, medial forebrain bundle, amygdala, ventral hippocampus, medial nucleus accumbens, septum, raphe nuclei, and locus coeruleus have the potential to modulate a plethora of systems and resulting behaviors (Ch'ng et al., 1985; Skofitsch & Jacobowitz, 1985; Melander et al., 1986). Because motivation and reward processes are governed by numerous expansive and interconnecting brain circuits, the prolific locations galanin-containing neurons have potential to modulate instrumental and consummatory behaviors.

1.2 Receptors and Signal Transduction

The diverse behavioral effects reported in the early studies exploring galanin led to the suggestion of multiple receptor subtypes (Bartfai et al., 1993; Wynick et al., 1993; Gundlach, 2002). To date, three galanin receptors have been identified and studied—GalR1, GalR2, and GalR3. Whereas all galanin receptor subtypes are membrane-bound and G-protein-coupled, they differ in amino acid sequence, distribution, pharmacology, G-protein-coupling, and cellular signaling.

GalR1 and GalR3 are strongly coupled to $G_{i/o}$ proteins and result in cascading effect of inhibition of adenylate cyclase activity, decreases in cAMP (Chen et al., 1992; Karleson & Langel, 1998) and reduction in protein kinase A activity in the cell. Ultimately this cascade results in a hyperpolarization of the neuron due to opening of K^+ channels. GalR2 is coupled to $G_{i/o}$ and $G_{q/11}$ trans-membrane proteins to stimulate phospholipase C and inositol phosphate production; interestingly, the GalR2 is also weakly coupled to adenylate cyclase inhibition. Activation of phospholipase C-β increases inositol triphosphate production (Wang et al., 1998), which results in the

opening of Ca^{2+} channels in the endoplasmic reticulum and release of Ca^{2+} from its intracellular storage locations. Therefore, stimulation of GalR1 and GalR3 typically leads to hyperpolarization and cell inhibition and stimulation of GalR2 (releasing Ca^{2+} into the cytoplasm) is believed to produce depolarization and cell excitation (see Lang et al., 2007 for a review).

Northern blot and in situ hybridization studies have suggested that GalR1 and GalR2 are widely distributed in the central nervous system. For example, galanin receptor mRNA for GalR1 and GalR2 have been located in areas ranging from the spinal cord, cerebellum, pons, medulla, midbrain areas, hypothalamus, thalamus, hippocampus, limbic system, cortex, and olfactory system (O'Donnell et al., 1999). Of the three subtypes of receptors, GalR1 receptor mRNA is overwhelmingly expressed in areas including the piriform cortex, lateral septum, various nuclei within the hypothalamus and thalamus, and autonomic nuclei in the midbrain and brainstem. Given that these locations correspond with the location of [^{125}I]galanin binding sites, it has been suggested that GalR1 receptor may be the predominant galanin receptor (Gundlach, 2002). As shown in Table 2, GalR2 is predominantly found in the dorsal hippocampus and the mammillary nuclei and is most often associated with learning and depression-like behaviors in rodents (O'Donnell et al. 1999; Kuteeva et al., 2008). GalR3 has a more restricted distribution and in the CNS is found primarily in the preoptic area, PVN, dorsomedial, and ventromedial nuclei (Mennicken et al. 2002).

2.0 Galanin and Food Intake

With a high concentration of receptors in the paraventricular (PVN), dorsomedial, lateral, arcuate, and ventromedial nucleus of the hypothalamus, it is no surprise that galanin has been reliably implicated in food intake. Only three years after the initial discovery of galanin, Kyrkouli et al. (1986) first reported that administration of galanin into the lateral ventricle increased food consumption in sated rats. Microinjection of galanin into the PVN also increased food consumption in sated rats (Kyrkouli et al., 1990). Galanin microinjected into other hypothalamic nuclei including the lateral hypothalamus, ventromedial nucleus of the hypothalamus and nucleus of the solitary tract increased food intake in sated rats, yet injections into the PVN produced the largest increase in food intake (Schick et al., 1993). It's important to emphasize that the animals in the aforementioned studies were food-sated, meaning that galanin caused them to consume an excess of caloric food. This galanin-induced feeding behavior in food-sated rats can be reversed with the galanin antagonists M40 and C7. For example, Corwin et al. (1993) found that injection of M40 and C7 five minutes prior to galanin injection blocked the effects of galanin on food consumption. Although injection of M40 or C7 alone had no significant effect on food consumption, M40 had been previously reported to decrease spontaneous ingestion of a fat diet in freely feeding animals (Leibowitz & Kim, 1992).

Studies examining the effect of endogenous (natural) galanin have shown that galanin preferentially increased consumption of fat and carbohydrate as opposed to other nutrients (Smith et al., 1997; Tempel et al., 1988). The preference for fat or carbohydrate

ingestion may contain a circadian pattern of preference, with fat preference occurring in the middle of the feeding cycle (Leibowitz & Wortley, 2004). Fat ingestion, circulating glucose, and body fat—but not carbohydrates—increase galanin production, release, and gene expression in the PVN and external zone of the median eminence (Akabayashi et al., 1994; Leibowitz et al., 1998). Blocking endogenous binding of galanin receptors in the PVN with antisense oligodeoxynucleotides, results in decreased fat and calorie intake for a 24-hour period (Akabayashi et al., 1994). Furthermore, obesity-prone rats with a natural preference for fat intake show increased galanin mRNA and production in the PVN (Dourmashkin et al., 2005), and pharmacological blockade of fat oxidation reduces galanin expression in the PVN (Wang et al., 1998). As such, galanin is considered a non-homeostatic, forward or positive feedback system, where higher levels of galanin increase the intake of food (with preferential for fat and carbohydrates over other micronutrients) and fat ingestion/oxidation increases endogenous galanin.

Although most studies have focused on the effects of galanin in sated animals, administration of galanin has been shown to increase food intake in 24-hour food deprived animals (Schick et al., 1993). This increased consumption in acute food-deprived animals was of a considerably smaller percent increase compared to sated animals and thus galanin appears relatively insensitive to signals of food deprivation (Leibowitz, 2007). A recent study in our lab found that galanin caused a significant increase in food consumption in non-food restricted rats with free access to food (Brewer & Robinson, 2008). However, when adding a work requirement that forced rats to lever press for food, there was no significant galanin-induced increase in food consumption. Thus it appears that galanin increases food consumption regardless of deprivation state, but increases consumption if food is readily available (*viz.* consummatory behavior) and not if metabolic work (*viz.* instrumental behavior) is required.

3.0 Galanin and Water Intake

Water balance is maintained by numerous galanin-rich brain regions including the preoptic area, median eminence, and superoptic nucleus (SON) (McCann et al., 1997). Galanin is colocalized in neurons that contain the primary antidiuretic hormone, vasopressin, and may interact in the paraventricular nucleus (PVN) and SON during physiological states of dehydration (Melander et al., 1986; Landry et al., 2003). Microinjections of galanin in dehydrated rats reversed the typical dehydration-induced increase in vasopressin mRNA in the PVN and SON (Landry et al., 1995). The same study found that microinjections of galanin followed by the galanin antagonist M15 increased vasopression mRNA to the level of dehydrated-control rats. Moreover, galanin appears to be sensitive to cues of dehydration; osmotic manipulations, which produce hyperosmosis and hypervolemia, upregulate galanin and GalR1 mRNA in the PVN (Burazin et al., 2001; Koenig et al., 1989; Landry et al., 1998).

Intracerebroventricular (i.c.v.) injection of high doses of galanin (10-20µg i.c.v.) has been shown to reduce water intake in twenty-three hour water restricted rats (Brewer et al., 2005). This reduction in water was found in conditions where water restricted rats were given free access to water and where the same rats could access water on a fixed-

time schedule (FT 20). Interestingly, the same study found that lower doses of galanin failed to reduce water intake and other studies have shown no changes in water consumption when galanin was microinjected in the nucleus accumbens, lateral nucleus of the hypothalamus, or PVN (Schneider et al., 2007; Lewis et al., 2004). Further, galanin has no effect or even a stimulatory effect on water intake in water-sated animals (Malendowicz et al., 1994; Mazzocchi et al., 1992). Taken together, the aforementioned studies examining galanin and water intake suggest that either: 1) osmotic imbalance due to dehydration is necessary for any galanin-induced decreases in water intake; unlike its role in feeding, galanin requires a deprivation status to have a significant effect, *or* 2) As detailed below, the decrease in water intake in dehydrated rats required to lever-press for water may also be a repercussion of an increased work requirement, which may be inhibited by galanin.

3.1 Galanin and Instrumental Behavior: Insights from Studies of Cognition

Several studies have implicated galanin in basal forebrain cholinergic activity (Beal et al., 1990; Bowser et al., 1997; Chan-Palay, 1988). As such, it is logical that that galanin may function in forebrain circuits to influence cogntion. Galanin has been demonstrated to impair performance in the delayed non-match to position (DNMTP, described below) task using water as a reward (McDonald & Crawley, 1996; Robinson & Crawley, 1993*)*. Yet a closer evaluation of the effects of galanin on the reward (water) may change the implications of the results from cognition-based to those related to behavioral motivation.

Of the paradigms used to assess learning and memory, most utilize a food or water reward as motivational incentive to perform the task. Typically in the delayed non-match to position task (DNMTP), subjects must attend to a sample stimulus (such as a light presented in one of two locations of the operant chamber), hold that location "on-line" during a variable retention delay period, and then choose the stimulus that was not presented during the sample phase. Whereas most studies utilize food as a reward, the food interactions with galanin confounded the use of this reward and thus a water-reward was chosen instead (prior to the galanin-water interactions reported by Brewer et al., 2004 and Brewer et al., 2005, described below).

Although galanin had been suggested to worsen performance on the DNMTP, a closer examination of the data by Brewer et al (2004) suggest that galanin-microinjected rats had a persistent reduction in the number of trials the animals completed per session, meaning that these "thirsty" rats were not willing to complete the task to obtain a water reward. In addition, reviewing previous data suggests that galanin impairs the choice accuracy independent of retention delay period length. Together, these reevaluations of the data suggest that galanin, which decreases the water intake in dehydrated- rats, may also decrease the *incentive value* of the water as a reward. Further analyses of the DNMTP data suggest that galanin only reduced the total number of bar presses for the water reward and not reaction time or motor responses. The implications from this Galanin-DNMTP phenomenon combined with the increased free-food consumption but decreased instrumental behavior for a food reward reported by Brewer et al. (2005),

suggest that that galanin reduces "work" for reward when the reward is contingent upon a larger metabolic cost.

4.0 Galanin and Dopamine

Dopamine is a monoamine synthesized from the precursor, 3,4-dihydroxyphenylalanine (L-DOPA). Three primary dopamine pathways are found in the brain: the tuberoinfundibular-, nigrostriatal-, and mesolimbic- pathways. Whereas galanin is found in numerous locations throughout the brain and these pathways, it is the mesolimbic "reward" pathway (described in the next section) that has been overwhelmingly implicated in rewarded behaviors and is most pertinent to the current proposed study. The three mesolimbic structures that receive the most attention in research on reward are the dopamine rich substantia nigra (SN) ventral tegmental area (VTA) and the nucleus accumbens (NAc), although the pathway also includes the frontal cortex, hypothalamus and amygdala. The first research to establish the presence of galanin in the mesolimbic pathway utilized autoradiography to show galanin-like binding in the core and shell of the nucleus accumbens (NAc) and ventral tegmental area (VTA; Skofitsch et al. 1986). Since this initial report, GalR1-, GalR2-, and GalR3 -*ir* has been found in the VTA, SN and NAc (Hawes & Picciotto, 2004). Of the three receptors, the same study found that GalR1 was the most abundant receptor found in the VTA, SN, and NAc. Direct evidence to establish that galanin is colocalized in dopaminergic neurons of the mesolimbic pathway is not currently available, however, a recent study has indicated that the 60-amino acid related neuropeptide, galanin-like peptide (GALP) is colocalized with dopamine-containing neurons of the tuberoinfundibular pathway (Kageyama et al., 2008).

Although largely indirect, *in vitro* and *in vivo* evidence indicates that galanin may decrease dopamine expression. For example, decreased dopamine synthesis was reported in the VTA following galanin treatment (Ericson & Arenius, 1999) and striatal tissue pretreated with galanin attenuated the typical dopamine release *in vitro* (Tsuda et al., 1998). A potential mechanism that may explain the diminution of dopamine by galanin has recently been described; galanin reduced levels of tyrosine hydroxylase of ventral mesencephalic embryonic cultures and inhibited dibutyryl cAMP-induced tyrosine hydroxylase immunoreactivity (Counts et al., 2002). Moreover, whereas GalR1, GalR2, and GalR3 were found in the ventral mesencephalic tissue, pretreatment with dibutyryl cAMP resulted in a 200% increase in GalR1 receptor mRNA. As such, it appears that much of the role of galanin in the mesolimbic pathway may be due to GalR1 activation. Yet not all studies indicate a diminution of dopamine as a result of galanin; *in vivo* microdialysis of galanin microinjected into the PVN resulted in an increase in dopamine in the NAc (Rada et al., 1998). Together, the aforementioned studies provide evidence of a modulatory effect of galanin on dopamine that may involve several pathways and produce varying effects.

4.1 Theorized Roles for Dopamine in Reward Systems

As a monoamine neurotransmitter with diffuse modulatory effects, dopamine has been implicated in a multitude of diseases including Parkinson's disease (Greer & Williams, 1963; Cotzias et al., 1969), schizophrenia (Carlsson, 1974; Meltzer & Stahl, 1976; Snyder, 1972), depression (Willner, 1983; Dailly et al., 2004), attention deficit hyperactivity disorder (Levy, 1991; Oades, 2002), and addiction (Wise 1987; Wise & Bozarth, 1987; Koob & Weiss, 1992). Of these, perhaps it is the proposed role of dopamine in addiction that has received the most attention; originally proposed by Roy Wise , dopamine has been theorized to mediate "reward" processes of the subjective and pleasurable aspects of consumption (See Alcaro et al., 2007 for a comprehensive review). It was presumed that blocking dopamine, originally with atypical antipsychotic medication, would impair pleasure and thus this theory was dubbed "The General Anhedonia Model". This theory has received a substantial amount of attention in the scientific literature (Bozarth & Wise, 1981; Wise, 1982; Wise, 1987; Dackis & O'Brien, 2001) and popular press (Newsweek, 12 February 2001).

This General Anhedonia Model (GAM) presupposes that mesolimbic dopamine innervates the dopamine-rich nucleus accumbens where it serves as the primary mediator of the motivational- and rewarding- properties of food, water, sex, and drugs of abuse. Although mesolimbic DA has often been equivocally paired with reward, several problems exist with the omnibus dopamine-reward hypothesis. Depletion of endogenous dopamine from Parkinson's disease does not affect perceived pleasantness of taste stimuli as the GAM would suggest (Sienkiewicz-Jarosz et al. 2005). Moreover, dopamine antagonists failed to attenuate the euphoric effects of the dopamine antagonists cocaine (Gawin, 1986; Brauer & De Wit, 1997) and methamphetamine (Wachtel et al., 2002) as one would expect based on the hypothesis. Indirect evidence from fMRI gambling studies using human participants has revealed that the dopamine-rich nucleus accumbens is more active during a prediction of reward rather than presentation of reward (Knutson et al., 2001). The GAM would suggest the opposite—that the nucleus accumbens would be most active during the hedonic (reward) phase of a gambling task.

Animal research has allowed for a more direct examination of the dopamine-reward hypothesis. Dopamine release can occur in response to aversive stimuli including footshock, tailshock, tailpinch, restraint stress, instrumental avoidance, and conditioned aversive stimuli, (Salamone, 1994; Salamone, 1996; McCullough et al. 1993a; McCullough et al., 1993b; Tidey & Miczek 1996; Salamone et al. 1997; Datla et al. 2002; Young 2004). Taken together, these findings suggest that dopamine can be released as a response to both hedonic and aversive stimuli, which adds profound ambiguity to the specificity of dopamine in mediating hedonic reward. Pertaining most to the current study, *in vivo* measures of dopamine release and metabolism in the dopamine-rich NAc were found to be related to the instrumental behavioral response of lever pressing more than the consummatory consumption of large quantities of food (reviewed by Salamone, 1994). Numerous studies show that dopamine depletions using 6-OHDA reduce lever pressing (*viz.* "work") for food reward while increasing free food consumption (Cousins et al., 1999;; Cousins et al., 1993; Salamone et al., 1991). As

shown in Table 3 several classes dopamine antagonists, administered both intra-accumbens and systemic, reduce lever presses for food rewards. It is interesting that pharmacological blockage of both D1 and D2 dopamine receptors reduce instrumental lever pressing, suggesting that both are involved in instrumental behavior (Cousins et al., 1994). Finally, in a rodent high- and low- effort choice task, the D2 receptor DA antagonist pimozide has been shown to switch the preference from effortful acquisition of sucrose-water to non-effortful consumption of water (Hsiao & Chen, 1995).

It should be noted that only dopamine depletions to the ventrolateral striatum produce severe motor impairments (Cousins et al., 1993); depletions to the nucleus accumbens or other mesolimbic regions do not impair lick rate or motor responding (Salamone, 1994). In light of this recent evidence, the functions of mesolimbic dopamine may not reflect an all-encompassing reward system. Rather, as suggested by Salamone et al., (2006; 2005; 2002; 1997), mesolimbic dopamine may serve as a *behavioral energizing* system, designed to maintain effortful acquisition of rewarding commodities.

5.0 Present Study: Galanin and Energizing and Hedonic Aspects of Consumption

The parsimonious nature of the studies examining the effects of galanin on food- and water- consumption, described in Chapters 2 and 3, is that they clearly separate instrumental behavior from consummatory behavior. Instrumental behavior, defined as behavior that reliably leads to an outcome, could thus be viewed as lever presses in an operant box that are required to obtain food; the lever presses reliably lead to a desirable outcome (food) and therefore can be referred to as instrumental in nature. With this definition, instrumental behavior corresponds to "precurrent" reactions described by Sherrington (1906) or "appetitive" behavior used by Craig (1917), and, can be extended to include both approach behaviors and non-overt actions such as remaining inactive for a period of time. Consummatory behavior, sometimes described as "hedonic" behavior, is defined in the current study as engaging in an act of consuming a commodity or preferred activity. Galanin stimulates food intake in sated rats with free access to food (non-response-contingent) showing that it plays a role in consummatory or "hedonic" behavior. Yet on a progressive ratio 1, where the number of responses required per reinforcer increase by one for each subsequent reinforcer, galanin inhibits bar presses (*viz.* instrumental behavior). A similar effect occurs with a water reward—galanin inhibits lever presses for a water reward, particularly when the lever press requirement is high.

The dissociation above is consistent with the theorized behavioral energizing function of the ascending mesolimbic dopamine system, which purports that this ascending dopamine system is involved in only the goal directed effort maintaining (appetitive) and not the hedonic (consummatory) aspects of reward. The current dissertation tests a prediction of this theory by determining if a similar dissociation also applies to the non-caloric-but-hedonic reward of saccharin in experiment one. Recent reports suggest that galanin increases hedonic responses to a caloric but not-palatable alcohol solution. Experiment two served as an attempt to replicate these findings using a different methodology and offers a direct contrast between the non-caloric-but-hedonic

saccharin reward in experiment one and a caloric-but-not-hedonic reward of alcohol. As a positive control, experiment three replicated the galanin-induced free consumption of a high-fat milk/cream liquid and examined the effects of a novel GalR2 receptor agonist, M1145, on the same high-fat milk/cream liquid. Taken together, these experiments have the ability to refine the theory on which galanin acts on consumption of commodities, and also has the ability to add indirect convergent validity to the "behavioral energizing" theory proposed in Section 4.1.

5.1 General Methods

Subjects

A total of 19 Sprague-Dawley rats (Taconic Farms, Germantown, NY) were used for experiments. A subset of rats was used for each of the three experiments in the current work. Rats were housed individually in a temperature (20-22^{o}C) controlled room under a 0700 on and 1900 off 12-hour light-dark cycle with *ad libitum* access to food and water. All procedures were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and received prior approval from the SUNY Institutional Animal Care and Use Committee.

Surgical Procedures

Each animal received a cannula made of stainless-steel hypodermic tubing (24 gauge and 1.7cm long) into the right lateral ventricle under 100mg/kg I.P. ketamine and 10 mg/kg I.P. Xylazine anesthesia (coordinates from Bregma are: +0.5 anterior/posterior, -3.5 dorsal/ventral, and -1.0 laterally). Rats received an additional bilateral cannulation in the paraventricular nucleus of the hypothalamus (n = 10; coordinates from Bregma are: -2.0 anterior/posterior, -6.0 dorsal/ventral, and +/-0.5 laterally), ventral tegmental area (n = 10, coordinates from Bregma are: -5.0 anterior/posterior, -7.0 dorsal/ventral, and +/-1.0 laterally), or nucleus accumbens (n = 10; Coordinates from Bregma are: +1.2 anterior/posterior, +5.3 dorsal/ventral, and +/-2.0 laterally); these site-specific cannulae were used to gather pilot data (not presented in the current dissertation). Four stainless screws and epoxy were used to secure the small plastic tubing (which protects the cannula) in place. Post-operative analgesic was administered two-times daily (buprenorphine, 0.1mg/Kg I.P.) until animals recovered from surgery. At the completion of the experiments, the rats were sacrificed by decapitation under deep anesthesia (sodium pentobarbital) and the correct placement of the cannula in the lateral ventricle was verified.

Drug Preparation & Injection

Galanin (Bachem Bioscience, King of Prussia, PA) was dissolved in 0.9% physiological saline and administered into the right lateral ventricle (I.C.V.) using a Hamilton syringe connected by plastic tubing to a 31 gauge, 1.9 cm stainless steel injector. Each injection delivered a total of 5 µl of solution for I.C.V. over a span of 30 seconds. The injectors remained in place for an additional 60 seconds following the injections to assure complete drug delivery.

Behavioral Testing: Free access

Free consumption testing took place in four identical free access chambers measuring 45 cm X 24 cm with plastic ventilated lids. The liquid commodity was placed in removable plastic cups measuring 3.5 cm in diameter that were affixed to the floors of the chambers during testing. Thus the change in weight of the cup could be measured to provide an accurate amount of free consumption. Because 1.0g is equivalent to 1.0ml of liquid, results are expressed in ml consumption. For experiments one and two, rats were habituated to the testing environment followed by at least three baseline sessions on consecutive days. Animals were injected with galanin (or M1145 in experiment three) and placed in the free access chamber after a 10-minute post-injection lag. After the completion of the free-access testing, the change in weight of the cup was recorded and the chambers were cleaned with alcohol and water.

6.0 Experiment One: Galanin and Saccharin Consumption

As detailed in chapter 2, galanin has been shown to robustly increase free consumption of food and caloric high fat milk in food- and water- sated animals. On the contrary, galanin appears to reduce water intake at a high 20µg dose but not at a low 5µg dose in water deprived rats (Brewer et al., 2005). This dissociation in galanin's stimulation of reward consumption could be a result of the relative caloric value of the reward or may reflect the intrinsic "hedonic" taste associated with the reward itself—high fatty foods having a preferred "hedonic" taste compared to the neutral or "tasteless" water. Further, galanin increases food intake only when testing for consummatory behavior—when food is made freely available. Adding a work requirement does not increase food intake (Brewer & Robinson, 2008). As such, the current experiment sought to use a food-like aqueous solution with the properties of water but hedonic taste of a food.

Saccharin is a first generation non-caloric artificial sweetener with sweetness that is 300x the relative potency of natural sucrose. Rats freely ingest saccharin and it has been used in a plethora of studies investigating mechanisms that regulate "hedonic" taste (Inui-Yamamoto et al., 2009), taste aversion (Stephens & Riley, 2009), food intake (Tordoff, 1988), and drug abuse (Carroll & Lac, 1998). Because saccharin is prepared as an aqueous solution and is a non-caloric-but-hedonic reward, it provides the unique opportunity to dissociate the effects of galanin on a "hedonic" liquid with the caloric properties of water but the taste of a food-like caloric substance.

The current experiment explored the effects of galanin on free consumption of a saccharin solution using a design that is similar to that of Brewer and Robinson (2008). Whereas galanin increases free consumption of food, galanin does not increase instrumental responding for a food reward. Therefore, the second portion of the current experiment also examined the effects of galanin on instrumental "work" for a saccharin reward.

Methods

Experiment 1A: Consummatory Behavior Testing

Cannulated animals (N=19; surgery described in General Methods) were used to examine galanin's influence on consummatory behavior for saccharin. Briefly, animals were acclimated to the free access chamber for twenty minutes followed by a three day 20-minute free consumption baseline measurement of 0.2% sodium saccharin (Weight/Volume; Sigma Aldrich, St Louis, MO). On the second and third days of baseline measurement, all animals were injected with 0.9% physiological saline to assure proper functioning of the cannulae and reduce stress associated with the injection procedure. Testing days used a Latin Square design and animals were injected I.C.V. with 5µg or 10µg of galanin, or 0.9% saline and placed in the chamber for 20 minutes. Two animals were removed from analyses due to methodological problems in measuring saccharin consumption and thus the total number of animals analyzed was 17.

Experiment 1B: Instrumental Behavior Testing

Three identical standard operant conditioning chambers (MED Associates, Hatfield, VT) were used for instrumental behavioral testing. Liquid drop dispensers delivered the reinforcer (0.2% sodium saccharin) to a cup located in a recess between the response levers mounted on the front wall of the chamber. Rats (N = 15) were initially autoshaped to press the left lever on a fixed ratio of 1 (FR 1) schedule of reinforcement, where one drop of alcohol was administered following a left lever press. Sessions ended when rats reached a performance criterion of 50 lever-presses or 30 minutes elapsed. If the rat had successfully mastered three days of testing on an FR 1 schedule, progressive ratio (PR) scheduled testing commenced. During PR testing, subjects had to increase the total number of responses by one for each subsequent trial. For example, trial one required the animal to press the lever once for a reward, trial two required two level-presses, trial three required three lever-presses, etc. Pharmacological testing began after establishing a reliable baseline (approximately ten days of PR testing). Utilizing a Latin square design, subjects were injected with galanin at a dose of 5 or 10 µg or 0.9% saline and placed in an operant conditioning chamber for the PR testing. Two animals were dropped from analyses due to clogged cannulae and thus 13 animals were used for data analyses. For statistical analyses, the number of saccharin reward drops administered was compared between conditions.

Results

Experiment 1a

Free consumption of saccharin (Experiment 1a) was converted to ml consumed/body weight (kg). A one-way repeated measures analysis of variance was conducted to determine if galanin influenced free consumption of saccharin. Galanin administration into the lateral ventricle produced a significant increase in saccharin consumption under free access conditions [$F(2,32) = 7.90$, $p < 0.05$; Figure 1]. Bonferroni post-hoc tests showed both the 5 and 10 µg groups were significantly different from saline (p=0.01 and p=0.001, respectively).

Experiment 1b

Instrumental responding for a saccharin reward (Experiment 1b) was measured using two factors: the total number of rewards awarded and the total number of lever presses. A one-way repeated measures analysis of variance was conducted to determine if galanin changed the number of lever presses attempted in the 30 minute session. Galanin administration into the lateral ventricle produced a significant decrease in the number of lever presses [$F(2,24) = 12.87$, $p < 0.05$)]. Bonferroni post-hoc tests showed both the 5 and 10µg groups were significantly different from saline (p=0.001 and p=0.008, respectively; see Figure 2). A one-way repeated measures analysis of variance was completed to determine if galanin altered the number of rewards awarded in the 30 minute session. Galanin administration into the lateral ventricle significantly decreased the number of rewards awarded [$F(2,24) = 7.38$, $p < 0.05$)]. Bonferroni post-hoc tests revealed that the 5 and 10µg groups differed significantly from saline (p=.03, p=.008, respectively; see Figure 3).

Discussion

As shown in Figures 1, 2, and 3, galanin increased free consumption of 0.2% saccharin but decreased the amount of instrumental "work" rats performed in a PR task. Although prior reports of intracerebroventricular (i.c.v.) administration of the neuropeptides orexin, melanin-concentrating hormone, and neuropeptide Y have demonstrated increased saccharin consumption (Furudono et al., 2006), the current study is the first to report galanin-induced free consumption of a non-caloric food-like substance. This suggests that either saccharin is treated as a caloric substance or galanin is sensitive to the hedonic taste properties of this commodity. It is interesting that galanin increased free consumption of saccharin but decreased the breaking point for "work" for a saccharin reward. A similar dissociation has been found with a liquid food reward, where galanin increased free consumption of high-fat milk/cream liquid but did not increase instrumental "work" for the same reward (Brewer et al. (2008). The differences between the results from Brewer et al. (2008) which did not find a significant decrease in "work" for a food-like reward and the current study which found a significant diminution in work for a saccharin reward could be due to the distinct demand and response output for each of the two commodities. Low fixed ratios are associated with relatively equal lever responding for non-caloric saccharin and caloric food rewards in the Rhesus Monkey (Hursh, 1991). However, as the fixed ratio of responding increases, the number of responses for saccharin dramatically drops off while the number of responses for food slightly increases. As such, increases in "work" required to obtain a reward at higher fixed ratios (or perhaps the progressive ratio in the current study) result in the demand for food to increase relative to demand for saccharin. The sharp drop-off in responding for saccharin rewards at a higher progressive ratio in the current study using a saccharin reward would be described in behavioral economic terms as having more "elastic" demand compared to a caloric commodity.

As reviewed in Chapter 4, galanin may act as a dopamine antagonist. Mesolimbic dopamine is associated with goal directed behavior and galanin's inhibition of

mesolimbic dopamine could explain the decrease in lever pressing in the PR task. This is similar to dopamine antagonists and dopamine depletions that decrease lever pressing for rewards (Salamone, 1994; See Table 3) and inhibit effortful acquisition of a sucrose reward (Hsiao & Chen, 1995). Therefore, the current experiment adds indirect evidence that supports the behavioral energizing theory of mesolimbic dopamine.

It should be noted that because of a lack of studies directly testing galanin-dopamine interactions, these results could also suggest that *dopamine inhibits the stimulatory effects of galanin* on saccharin consumption. If dopamine regulates goal directed behavior, as suggested by the behavioral energizing theory, then dopamine should increase when rats are required to lever press for a saccharin reward in the progressive ratio task. Galanin decreased lever pressing in the progressive ratio task when we would expect dopamine transmission to be elevated, and, galanin increased saccharin consumption under free access conditions when we would *not* expect dopamine transmission to be elevated. Whereas circumstantial evidence is not available to implicate dopamine as a galanin antagonist, the stimulatory effect on saccharin consumption could have been blocked by an increase in dopamine transmission associated with acquiring the reward in the progressive ratio tasks.

With either of the aforementioned theories, if dopamine is the neurotransmitter mediating the diminution of "work" for a saccharin reward, it is likely that the GalR1 receptor subtype is interacting with dopamine; pretreatment with dibutyryl cAMP resulted in a 200% increase in GalR1 receptor mRNA in dopamine-rich ventral mesencephalic tissue with no significant increase in other receptor subtypes (Counts et al., 2002). Further, the GalR1 receptor has been implicated in food intake (Zorrilla et al., 2007; Krasnow et al., 2004). If saccharin is serving as a food-like substance, it is likely that the same receptor subtype as caloric food mediates its intake; however, at the time of this publication, no direct evidence exists to show this relationship. In addition, because galanin and food intake form a positive loop where increased food intake raises endogenous galanin levels which results in further food intake, there is a potential for a similar mechanism to occur with saccharin. Evidence suggests that in both rats and humans, non-caloric sweeteners such as saccharin increase free feeding of other caloric nutrients (Tordoff & Friedman, 1989; Rogers & Blundell, 1989) and drinking of saccharin has been shown to elevate the mRNA levels of orexin and neuropeptide Y (Furudono et al., 2006).

The results from experiment one suggest that galanin increases free consumption of a hedonic-but-not-caloric saccharine solution. In experiment two, I investigated the effect of galanin on a non-hedonic-but caloric solution of alcohol. In combination, these experiments have the potential to answer if galanin is sensitive to a broader "hedonic tone" of a commodity.

7.0 Experiment Two: Galanin and Alcohol Consumption

Alcohol is a widely used drug of abuse with caloric properties. Although galanin preferentially increases the intake of fat over other caloric sources, circumstantial

evidence provides an indirect link between galanin and alcohol intake. For example, alcohol ingestion increases circulating lipids and lipid storage (Lieber, 1988) and fat ingestion/oxidation upregulates galanin. This may explain why alcohol has been known to increase food intake, although research has not directly tested an alcohol-galanin-lipid positive-feedback relationship. Moreover, food deprivation has been shown to increase both food intake and alcohol intake (Meisch & Henningfield 1977).

More recently, preliminary research has indicated that chronic administration of alcohol increased the expression of galanin in the PVN of the hypothalamus—an area previously found to upregulate galanin with increased fat intake (Leibowitz et al., 2003; Rada et al., 2005). The first direct evidence that tested the relationship between galanin and alcohol was completed by Lewis et al. (2004). Rats repeatedly exposed to alcohol were given microinjections of galanin (1nmol or 3nmol), the galanin antagonist M40 (1nmol), or a combination of M40 and galanin into the third ventricle. When given a choice of water or alcohol, those rats previously injected with only galanin increased alcohol consumption in both the light and dark period. Microinjections of M40 and galanin failed to increase alcohol intake and microinjections of only M40 slightly decreased alcohol consumption. A similar study performed by the same group of researchers confirmed the previous findings that galanin injected into the third ventricle increased alcohol consumption and also found that a similar effect was observed when microinjecting galanin into the PVN (Rada et al., 2004). Importantly, studies that have confirmed the stimulatory effects of galanin on alcohol consumption (Lewis et al., 2004, Rada et al., 2004, and Schneider et al., 2007) tested rats with free access to food and water and reported no increased intake of food. This finding may suggest that availability and consumption of alcohol diminishes the stimulatory effect of galanin on food or that the pharmacological properties of alcohol result in a preferred commodity.

Similar to its feed-forward effects on food intake, preliminary studies suggest that galanin can stimulate *ad libitum* alcohol intake and is upregulated as a result of alcohol intake. Although the precise mechanism underlying this system has not been fully delineated, Lewis (1996) suggested that alcohol may interact with brain systems responsible for motivation that also mediate appetite and food intake. The unpalatable taste of alcohol is typically aversive to alcohol-naïve Sprague-Dawley rats (used in the aforementioned studies) and thus it has been suggested that the increase in alcohol intake after galanin injections is due to the pharmacological properties of alcohol rather than taste. Although alcohol has been shown to influence serotonin, GABA, and the opioid system, it is the dopamine system that has served as the reason for galanin-induced increases in ethanol consumption. Injections of galanin in the PVN have been shown to increase dopamine release in the NAc (Rada et al., 1998), however, this finding has not been replicated.

Ethanol alcohol is not a preferred commodity for Sprague Dawley rats. Although caloric, it is relatively unpalatable in a pure form and Sprague Dawley rats will not freely consume ethanol. These properties provide a unique contrast to the calorie-free-but-palatable saccharin commodity used in Experiment One. The current experiment examined the effects of galanin microinjection into the lateral ventricle on alcohol consumption when alcohol is provided *ad libitum*. This provides a necessary replication

from a separate laboratory of the galanin-alcohol interaction detailed above, and, also tests whether a relatively low calorie, non-hedonic liquid can be influenced by galanin. As detailed below, the current experiment will use a different alcohol training procedure to replicate the work of Lewis et al. (2004) and test the effects of galanin on free consumption of alcohol in food-sated rats.

Methods

Behavioral Testing: Free access

Prior to free access testing, rats (N = 18) were habituated to 7% ethanol using a sucrose-replacement protocol based on that of Samson et al. (1999). Briefly tubs in the consumption chambers were initially filled with 10% sucrose and 0% ethanol (W/V from a 190 proof stock) and rats were exposed to this liquid for 20 minutes for two days. Sucrose was faded and ethanol was increased using the following progression: 10% sucrose, 2% ethanol; 10% sucrose, 5% ethanol; 10% sucrose, 7% ethanol; 5% sucrose, 7% ethanol; 2% sucrose, 7% ethanol; 7% ethanol. Rats were exposed to solutions in consumption chambers for twenty minutes per day and moved to the next concentration only after verification of two consecutive days of consumption.

After an average of 14 days of consumption of 7% unsweetened ethanol, rats were injected in the right lateral ventricle with 0.9% saline and allowed to freely consume 7% unsweetened ethanol for twenty minutes. An identical procedure was followed the next day with an extended 60-minute testing procedure. Using a Latin square design, each rat was injected with galanin at a dose of 5 or 10 µg or 0.9% saline and placed in the cage for 60 minutes at which time the volume of remaining ethanol will be measured.

Results

Free consumption of 7% ethanol was converted to ml consumed/body weight (kg). A one-way repeated measures analysis of variance was conducted to determine if galanin influenced free consumption of ethanol. Galanin administration into the lateral ventricle did not produce any significant difference in ethanol consumption under free access conditions [$F(2,34) = 0.54$, $p > 0.05$; Figure 4].

Discussion

The current experiment sought to replicate initial studies that demonstrated that galanin increases free consumption of an ethanol reward in rats shaped to consume alcohol. As shown in Figure 4, galanin did not significantly increase alcohol intake at a 5µg or 10µg dose compared to saline control, although a non significant trend for increased alcohol intake was demonstrated at the 5µg dose and a non significant trend for decreased alcohol intake was demonstrated at the 10µg dose. Whereas not reported in earlier papers from the same laboratory, Schneider et al. (2007) found that galanin increased alcohol consumption only in high-ethanol consuming animals and not in low-consuming animals. As shown in Figure 5, I performed a median split to differentiate high and low alcohol consumers based on average baseline alcohol consumption. In doing so, the inverted U-shaped function was amplified, yet doses still did not significantly change free consumption of alcohol in high ($p = 0.11$) or low ($p = 0.81$)

drinkers, although the high drinkers approached marginal significance. Due to a lack of significant alcohol consumption under infusion of galanin, I did not examine the effect of galanin on instrumental responding for an alcohol reward; however, a prior report by Czachowski et al. (2001) found that inter-accumbens microinjections of dopamine D2 antagonist raclopride reduced instrumental responding (lever presses) for 10% ethanol without influencing consummatory behavior in rats trained to consume ethanol in a similar fashion as the current study.

Whereas the results from the current experiment do not replicate those of Lewis et al. (2004), Rada et al., (2004) or Schneider et al. (2007), methodological differences could (at least partially) explain differences in replication. The ethanol training protocol and testing chambers were slightly different in the current experiment compared to the prior galanin-alcohol studies; previous studies utilized a two-bottle choice paradigm where rats were exposed to a bottle of water and a bottle of alcohol for 12 hours per day. Because the bottles of water and alcohol are vulnerable to leaks and only allow measurement increments of 1ml (reported during the dark period) and 0.1ml (reported during the light period), I used an alternate method of inducing ethanol consumption where rats were exposed to tubs of alcohol for twenty minutes per day. Measuring consumption to the nearest 0.01ml allowed the current study to more precisely detect galanin-induced differences, especially because of the low consumption amounts. It is possible that the difference in exposure time and delivery method alters the experience of consuming alcohol; whereas the hour baseline consumption during the light period was extremely low in prior reports (less than 0.25ml with no reported standard error from the mean in Lewis et al., 2004), the current experiment reports a baseline of 0.56ml +/- 0.12. This suggests that the shorter training for ethanol and/or non-homecage environment in the current study may change the experience of drinking ethanol. The higher baseline in the current study could have contributed to the non-significant trend between groups as well.

Even though differences exist between my results and those from prior studies, the trend for increased ethanol intake at 5µg suggests that galanin may have a small effect on ethanol intake in at least some animals. However, this phenomenon may not be unique to the pharmacological properties of alcohol. Low doses of galanin slightly increase water intake in a similar fashion (Brewer et al., 2005; Schneider et al., 2007). Antithetically, high doses of galanin have been shown to reduce water intake in water-deprived rats (Brewer et al., 2005) and may account for the diminished alcohol consumption at 10µg. Overall, the pattern of galanin-induced changes in alcohol consumption and water consumption are strikingly similar and both contain a non significant inverted-U-shaped function.

Prior studies examining galanin-induced increases in alcohol consumption have suggested that caloric intake may mediate elevated ethanol consumption. Although ethanol is caloric, perhaps the 7% ethanol does not contain enough calories to be truly viewed as a caloric substance. In the current experiment, rats consumed approximately 0.56ml of 7% ethanol in the hour baseline session and not much more after 5µg galanin injection. Assuming that 1ml of 95% ethanol contains 7 Kcal, 1ml of 7% ethanol should contain approximately 0.52 Kcal and half of that (approximating the mean baseline

measurement) is 0.26 Kcal. The relatively low amount of calories in this amount of alcohol may not be high enough for galanin to have an effect. In contrast, Brewer and Robinson (2008) show that galanin robustly increases free consumption of a high-fat milk/cream liquid, which contains 1.33Kcal per ml. Perhaps galanin, which has demonstrated particular sensitivity to fat and high carbohydrate foods, did not influence the intake of ethanol due to the low calorie count in 7% unsweetened alcohol.

It can be concluded that the taste of unflavored alcohol is not palatable to rats. In the current experiment, free consumption of unflavored alcohol was induced by weeks of training using a sucrose fading technique. This is contrasted with results from experiment one, which found that a non-caloric aqueous saccharin solution with high hedonic value is increased by galanin injection. Therefore, taken together, experiments one and two suggest that galanin may influence the "hedonic" experience of consumption.

8.0 Experiment Three

Experiment three served as a control to verify the potency of our galanin and functionality of the lateral ventricle cannulae. The most robust and replicated effect of galanin is an increase in free feeding. Prior research from our laboratory has suggested that galanin increases free consumption of a high-fat milk/cream liquid. As such, the first portion of the current experiment sought to replicate these results using a similar method and dose of galanin.

Although the GalR1 receptor subtype has been most associated with increased feeding, due to the inavailability of other receptor subtype agonists or antagonists, few studies have addressed the specific contribution of GalR2 or GalR3 to feeding. Therefore, the second part of this experiment evaluated M1145, a novel GalR2 agonist created by Johan Runesson and described in Runesson et al. (2009).

The first GalR2 receptor specific agent, AR-M1896, was created by truncating the amino acid sequence (2-11) to reduce the affinity for GalR1 (Liu et al., 2001). Years later, the chimeric peptide M871 was created and reported by Sollenberg et al. (2006). M871 was reported to have a 30-fold higher affinity for the GalR2 over GalR1 receptor and offered post synaptic inhibition. Recently, M1145 was designed to improve the receptor subtype selectivity of the chimeric ligand M871. M1145 has been shown to have more than a 90-fold higher affinity for the GalR2 over GalR1 and a 76-fold higher affinity for GalR2 over GalR3; therefore it has been classified as a GalR2 agonist with the highest specificity for the GalR2 receptor of all GalR2 agonists.

Part two of the current experiment was designed to test the effects of M1145 on free consumption of a high-fat milk/cream liquid. Because GalR1 has been most implicated with consumption of food and GalR2 locations are found in areas not associated with caloric ingestion, it was predicted that M1145 would not have any detectable alteration of consummatory behavior.

Methods

Behavioral Testing: Free access

Behavioral testing used free consumption chambers (described above). Briefly, rats were acclimated to consumption chambers and high-fat milk/cream liquid for one day to reduce neophobia. On the following day, rats freely consumed the high-fat milk/cream liquid from the tubs affixed to the bottom of the cage.

Experiment 3a

Rats (N=15) were randomly assigned to a saline (n=8) control group or galanin (n=7) group and injected in the lateral ventricle with 0.9% saline or 10µg of galanin. Rats were allowed to consume the high-fat milk/cream liquid commodity for 20 minutes.

Experiment 3b

Using a Latin Square design, animals (N=18) were injected in the lateral ventricle with 1µg, 5µg or 10µg of M1145, or 0.9% saline and placed in the Free access chamber with high-fat milk/cream liquid for 20 minutes.

Results

Experiment 3a

Free consumption of high-fat milk/cream liquid was converted to ml consumed/body weight (kg). A one-way analysis of variance was conducted to determine if galanin influenced free consumption of high-fat milk/cream liquid. Galanin administration into the lateral ventricle produced a significant increase in high-fat milk/cream liquid consumption under free access conditions [$F(1,13) = 4.47$, $p = 0.05$; Figure 6].

Experiment 3b

Free consumption of high-fat milk/cream liquid was converted to ml consumed/body weight (kg). A one-way repeated measures analysis of variance was conducted to determine if M1145 influenced free consumption of high-fat milk/cream liquid. M1145 administration into the lateral ventricle produced no significant change in high-fat milk/cream liquid consumption under free access conditions [$F(3,51) = 0.49$, $p > 0.05$); Figure 7].

Discussion

As detailed in Chapter 2, the most documented and robust effect of galanin is increased free feeding, particularly of high fat foods (Tempel et al., 1988). Experiment 3a served a dual purpose: to replicate data from Brewer and Robinson (2008) showing that galanin increases free consumption of a high-fat milk/cream liquid and to serve as a positive control for experiments 1 and 2 of current work. Injection of galanin robustly increased free consumption of high-fat milk/cream liquid in a similar fashion as presented by Brewer and Robinson (2008), showing that food intake in both solid and liquid forms are increased under galanin. Since the galanin used in the current experiment was from

the same manufactured batch as that in experiment 2, it can be concluded that the galanin was pharmacologically active.

As shown in Table 1, the distribution of galanin receptors varies throughout the brain. Galanergic receptive sites have been located in the paraventricular nucleus (PVN), dorsomedial nucleus (DMN) and ventromedial nucleus (VMN) and have been shown to increase food intake under galanin (Leibowitz, 2005; Crawley, 1999). As shown in Table 2, these three sites crucial for feeding regulation contain high amounts of GalR1 receptors. The GalR2 receptors are found particularly in the mammillary nucleus and dorsal dentate gyrus—areas not typically associated with feeding homeostasis. Therefore is no surprise that M1145 had no effect on consumption of a high-fat milk/cream liquid commodity. One prior study utilizing early GalR2 and GalR3 receptor agonist found that neither stimulated food consumption (Wang et al., 1998). It follows that GalR1 is likely to be the galanin receptor most implicated in feeding, however this has not been directly tested.

9.0 General Discussion

The current dissertation found that galanin (5µg, 10µg) increases free consumption of 0.2% saccharin (experiment 1a) while not significantly affecting the free consumption of 7% ethanol alcohol (experiment 2). The differences in the type of commodity between experiments 1a and 2 are critically relevant for interpreting the results. Although non-caloric, saccharin is highly palatable and rats readily consume saccharin within the first two exposures. Ethanol is relatively unpalatable and requires weeks of sucrose fading as a way to initiate the consumption of the initially unpalatable solution. Prior galanin studies had not addressed the palatability of the commodity, but have found that caloric (and arguably palatable) fatty foods are most influenced by galanin (Tempel et al., 1988; Leibowitz, 2005). The current study expands this and suggests that galanin may alter the "hedonic value" of a commodity. It would follow that the saccharin and the high-fat milk/cream liquid solutions (experiments 1a and 3a) contain high "hedonic value" and are consumed more after galanin injection. The "non-hedonic" unpalatable ethanol solution (experiment 2) was not influenced by galanin injection. Whereas the caloric composition and pharmacological properties of alcohol may be a distinct difference between alcohol and saccharin, the factor that may control galanin-induced ingestion of these two different commodities seems to be palatability.

Other neuropeptides that control food intake have been shown to influence both alcohol and saccharin intake in a similar fashion as shown by the current work. For example, neuropeptide Y stimulates carbohydrate and saccharin ingestion (Leibowitz, 2005; Furudono et al., 2006) but decreases ethanol intake in rats trained to consume alcohol (Rimondini et al., 2005). The neuropeptides orexin, melanin-concentrating hormone, and neuropeptide Y have demonstrated increased food intake (reviewed by Beck, 2000) and saccharin consumption (Furudono et al., 2006).

If saccharin is treated as a caloric food, it is likely that galanin modulates its consumption through the brain's opiate system. Similar to galanin, opioids are heavily involved in regulating the ingestion of palatable food with high caloric value, particularly

under conditions of food-satiety (Kelley et al., 2005). Administration of opioid agonists that stimulate the μ receptor also stimulate food consumption (Zhang et al., 1998) and the opiate antagonist naltrexone reduces food consumption in control animals as well as in animals injected with galanin (Barton et al., 1996; Dube et al., 1994). High doses of naltrexone have also decreased free saccharin consumption (Biggs & Myers, 1998). As such, the opiate system may at least partially account for the increases in free consumption of the palatable high-fat milk/cream liquid and the palatable saccharin solution. Interestingly, alcohol, which also stimulates opiate receptors, is attenuated by the opiate antagonist naltrexone in alcohol-preferring rats (Davidson & Amit, 1997; Altshuler et al., 1980). Perhaps this further implicates that galanin is specific to the palatability of the commodity and that alcohol preference is a necessary precondition for any galanin-induced changes to free consumption. Alternately, attenuated release of vasopressin by alcohol could interact with galanin and explain the lack of galanin-induced stimulation independently from galanin-opiate interactions. Alcohol naïve rats that prefer alcohol and those that do not prefer alcohol differ in μ -opioid binding sites (McBride et al., 1998) and therefore it is also possible that alcohol-preference is a necessary precondition for changes to the opiate system (which would alter alcohol ingestion) to take place. This would suggest that the methodology chosen to initiate alcohol consumption may modify the opiate system and any effects of galanin on the system.

Experiments 1a and 1b add to the growing data suggesting that galanin influences both consummatory and instrumental behaviors independently (See Figure 8). Attenuated lever responses for a saccharin reward due to galanin injection are antithetical to the robust increase in free saccharin consumption. However, these results make sense when compared with those used to support the behavioral energizing theory (Salamone, 1994). Numerous studies (reported in Table 3) have shown that dopamine antagonists have the ability to reduce "work" for food reward while not influencing or even stimulating food intake. The results from experiments 1a and 1b, along with the indirect evidence that galanin serves as a dopamine antagonist, add indirect support for the behavioral energizing theory of mesolimbic dopamine. The mesolimbic dopamine pathway as an all-encompassing reward pathway purported by the GAM is useful in that it makes testable some predictions but may be an oversimplification that ignores the expansive conditions that are inherent to reward. As such, it is easy to specify a more precise role of dopamine in reward seeking behavior in instrumental- versus consummatory- behavior which has the additional advantage of not implying some degree of introspection or subjective evaluation of the pleasurable properties of the reward as a mediator in the proposed process. Although not reported in the literature, it is also possible that dopamine release from instrumental "work" acts as a galanin antagonist thereby reducing galanin's stimulation of feeding. This alternate theory is depicted in Figure 9.

Having raised these issues, it is worth noting that the *reduction* (rather than just a neutralization) in "work" for a saccharin reward observed presently is potentially partially due to some galaninergic-opiate system interactions. Naltrexone has been shown to attenuate progressive ratio "work" for a saccharin reward in rhesus monkeys

(Rodefer et al., 1999). This is a question for future evaluation, exploring further galanin-opiate interactions, which is another poorly studied topic.

9.1 Conclusions

The current dissertation demonstrates the novel finding that galanin significantly increases free consumption of a non-nutritive 0.2% palatable saccharin solution, but not when operant responding was required for saccharin reward. Using a different methodology to initiate alcohol consumption, the current work was unable to replicate prior reports that galanin increased ethanol free consumption. Together, these findings suggest that galanin may be particularly sensitive to the hedonic taste of a reward. As a positive control, the current work replicated the galanin-induced free consumption of a high-fat milk/cream liquid, on which which the novel GalR2 receptor agonist, M1145, had no effect. This suggests that the GalR2 receptor is not involved in galanin-induced free food consumption. Taken together, the current results indicate that galanin may be acting as a mesolimbic dopamine inhibitor or itself may be inhibited by mesolimbic dopamine release. These results offer further indirect support for the theory that mesolimbic dopamine regulates energetic "work" behavior more than hedonic "liking" behavior thereby supporting the behavioral energizing theory of dopamine and discounting the GAM.

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Table 1: Immunohistochemical Mapping of Galanin-Like Neurons in the Telencephalon, Diencephalon, and Mesencephalon from Skofitsch & Jacobowitz (1985).

General Region	Specific Brain Region	Amount of Staining Reported
Telencephalon	Parietal cortex	Few
	Central amygdala	Few
	Basal amygdala	Few
	Posterior piriform cortex	Few-to-moderate
	Hippocampus	Few-to-moderate
	Medial amygdala	Few-to-moderate
	Rostral cingulate	Moderate
	Medial prefrontal cortex	Moderate
	Caudate nucleus	Moderate
Diencephalon	Septal area	Few
	Bed nucleus of the stria terminalis	Few
	Medial forebrain bundle	Few
	Dorsal Premamillary	Few
	Arcuate nucleus	Few
	Posterior hypothalamic nucleus	Few-to-moderate
	Ventral premamillary nucleus	Few-to-moderate
	Nucleus of the diagonal tract	Moderate
	Preoptic nucleus	Moderate
	Suprachiasmatic nucleus	Moderate
	Periventricular nuclei	Moderate
	Supraoptic nucleus	Dense
	Interstital nucleus of the stria terminals	Dense
	Periventricular nucleus	Dense
	Arcuate nucleus	Dense
	Paraventricular nucleus	Dense
	Aona incerta	Dense
	Mamillary body	Dense
	Thalamic area	Dense
	Dorsomedial nucleus	Very Dense
	Perifornical nucleus	Very Dense
	Hypothalamic medial forebrain bundle	Very Dense
Mesencephalon	Central gray	Few
	Reticular formation	Few
	Cuneiform nucleus	Few
	Substantia nigra	Few-to-moderate
	Lateral lemniscus	Few-to-moderate
	Interpeduncular nucleus	Few-to-moderate
	Parabrachian nucleus	Few-to-moderate

Table 2: Location of galaninergic receptor sites in the brain. Brain regions in **bold** contain the highest reported levels of galanin receptors.

Reference	Galanin Receptor	Identified Brain Region
Parker et al. (1995)	GalR1	BNST Lateral septum Diagonal band (nuc vertical and horizontal) **Amygdaloid nuc** CA2/**CA3** **Ventral Subiculum** Preoptic area Periventricular nuc Suprachiasmatic nuc Anterior hypo area **Supraoptic nuc** **PVN** VM DH
O'Donnell et al. (1999)	GalR2	BNST Diagonal band (nuc vertical) Medial septum Amygdaloid nuc DG (**dorsal**/ventral) DH LH VM Arc nuc Periventricular nuc **Mammillary nuc**
Mennicken et al. (2002)	GalR3	BNST Diagonal band (nuc vertical and horizontal) MPOA Ventromedian POA DM LH Posterior hypothalamic area Ventromedial hypothalamic area Premammillary body

Table 3: Evidence suggesting that dopamine antagonists reduce instrumental responding.

Reference	DA antagonist	Summary of finding
Salamone et al. (1991)	6-hydroxy-dopamine (non specific) Haloperidol (D2)	Peripheral and intra-accumbens administration of 6-hydroxy-dopamine and haloperidol significantly reduced lever pressing for preferred food reward but not for a standard rat chow reward
Cousins et al. (1994)	Haloperidol (D2) SCH 23390 (D1) Cis-flupentixol (non-specific) Sulpiride(D2)	Peripheral injections of haloperidol, SCH23390, and Cis-flupentixol reduced lever pressing for food but robustly increased free consumption of food. Peripheral injection of sulpiride reduced lever pressing for food and slightly increased free consumption of food
Salamone et al. (1996)	Haloperidol (D2) Clozaphine (D2) Thioridazine (D2)	Peripheral injections of haloperidol and thioridazine significantly reduced lever pressing and increased chow intake. Peripheral injection of clozapine suppressed lever pressing but did not increase chow intake
Nowend et al. (2001)	SCH23390 (D1) Raclopride (D2)	Microinjection of SCH23390 and Raclopride in the NAc core suppressed lever pressing for a preferred food but increased free consumption of the same food
Salamone et al. (2002)	SKF83566 (D1) Raclopride (D2)	Peripheral injections of SKF83566 and raclopride decrease lever pressing for food but increase free food consumption

Figure 1. Experiment 1: Effect of galanin injection on free 0.20% saccharin consumption in food and water sated animals

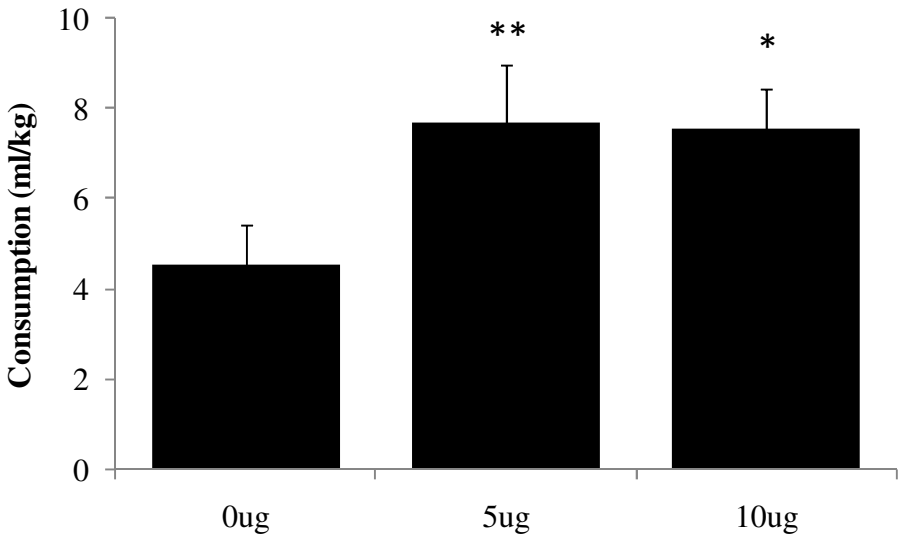

Figure 2. Experiment 1b: Effect of galanin injection on lever presses for a 0.20% saccharin reward

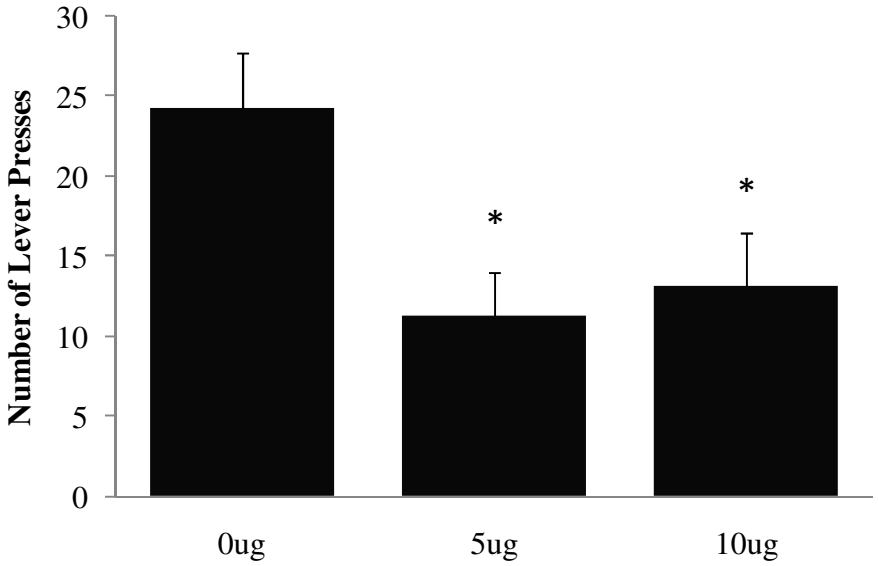

Figure 3. Experiment 1b: Effect of galanin injection on total number of 0.20% saccharin rewards

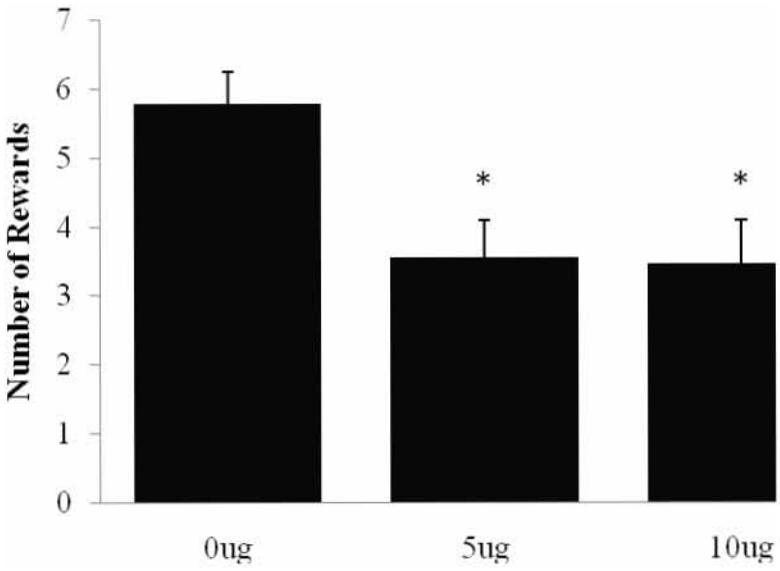

Figure 4. Experiment 2: Effect of galanin injection on free 7% ethanol consumption in food and water sated animals

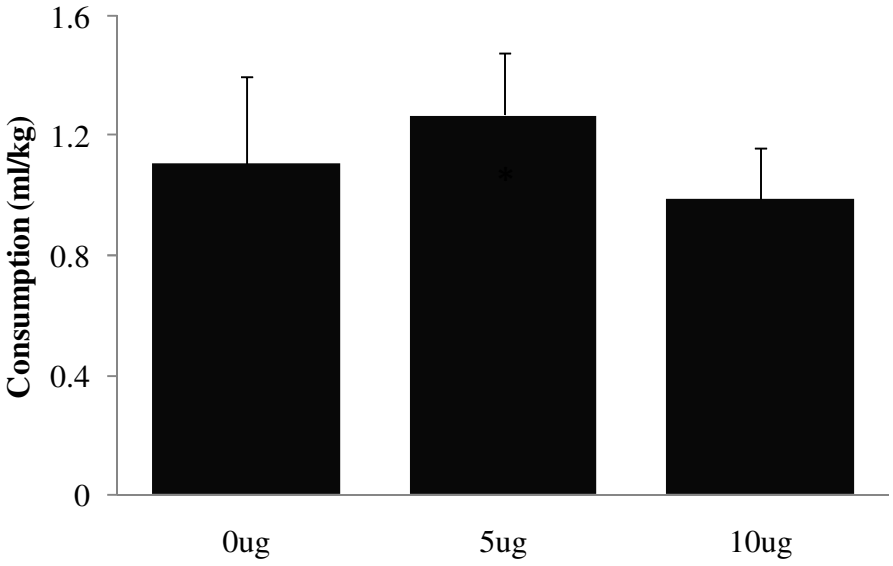

Figure 5. Experiment 2: Effect of galanin injection on free 7% ethanol consumption in low and high alcohol consuming animals

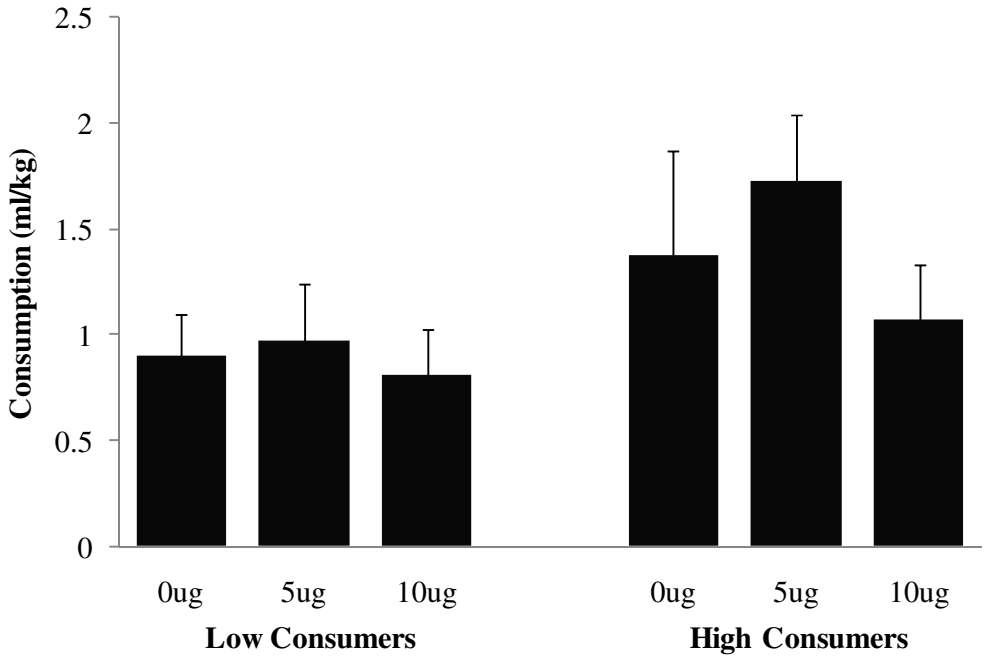

Figure 6. Experiment 3: Effect of galanin injection on free high-fat milk/cream liquid consumption in food and water sated animals

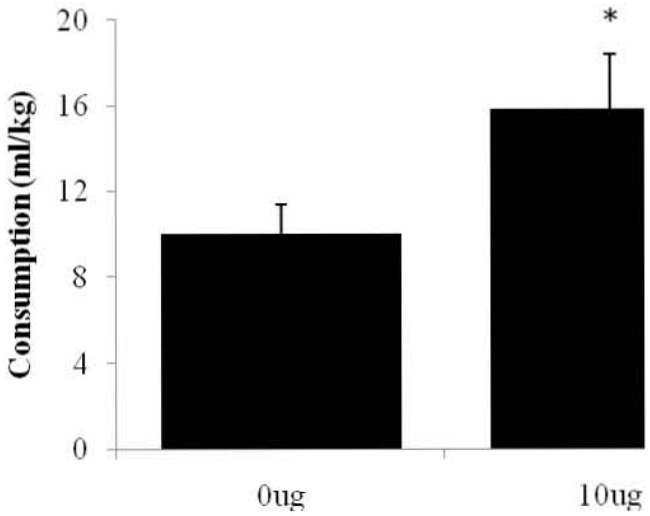

Figure 7. Experiment 3: Effect of M1145 injection on free high-fat milk/cream liquid consumption in food and water sated animals

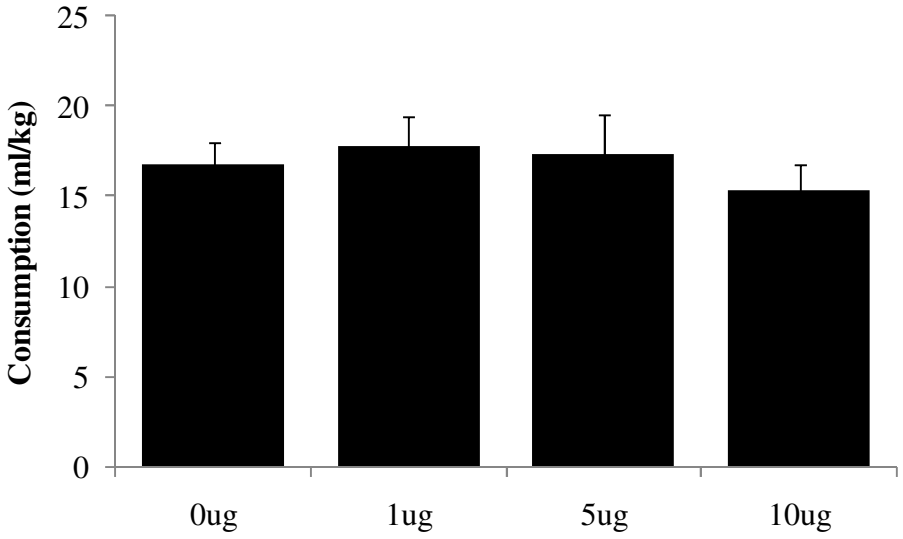

Figure 8: Differential effects of galanin on consummatory and instrumental behavior assuming galanin acts as a dopamine antagonist. It is suggested that galanin stimulates consummatory behavior, but inhibits mesolimbic dopamine, which is necessary for instrumental responding.

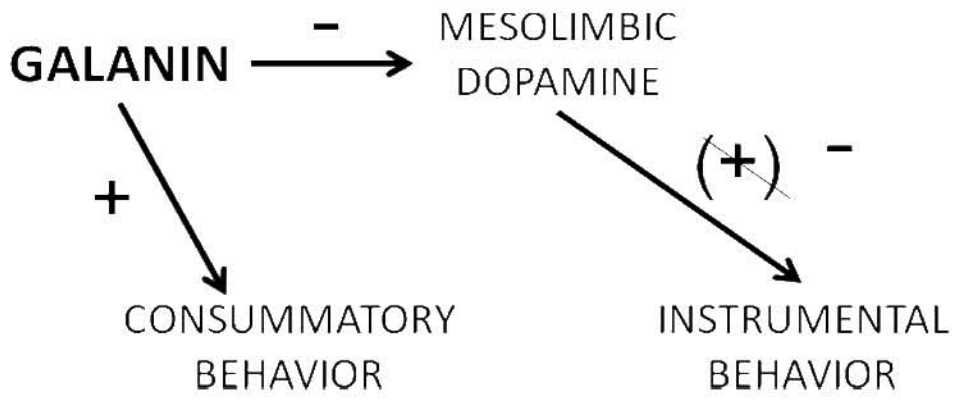

Figure 9: Differential effects of galanin on consummatory and instrumental behavior assuming dopamine acts as a galanin antagonist. In this scenario, instrumental behavior increases mesolimbic dopamine, which inhibits galanin's stimulatory effects on consummatory behavior.