An Exploration of the Aversive Properties of 2-deoxy-D-glucose in the Context of Metabolic Dysfunction and Mood Disorders

By

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A Thesis
Presented to
The University of Guelph

In partial fulfillment of requirements
For the degree of
Master of Science
In
Psychology and Neuroscience

Guelph, Ontario, Canada

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ABSTRACT

An Exploration of the Aversive Properties of 2-deoxy-D-glucose in the Context of Metabolic Dysfunction and Mood Disorders

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There is evidence that hypoglycemia alters mood and induces depressive-like behaviours. One method to precipitate a state of acute hypoglycemia is to administer the glucose antimetabolite 2-deoxy-D-glucose (2-DG). This thesis explored whether 2-DG could produce a conditioned place avoidance (CPA) in rats and examined whether pharmacological manipulation of monoamines could alter this effect. Pre-fed rats avoided a compartment paired with 2-DG (300 or 500 mg/kg). 2-DG also increased blood glucose and corticosterone (CORT), and suppressed locomotor activity. Interestingly food deprivation eliminated 2-DG-induced CPA, despite enhancing blood glucose and CORT levels. Clonidine (10 and 40 ug/kg) and bupropion (10 and 30 mg/kg) attenuated 2-DG-induced CPA and reduced increases in blood CORT caused by 2-DG, while bupropion reversed locomotor deficits. Overall, these results suggest that acute impairment of glucose metabolism can elicit a stress response which is aversive to rats, and which involves neurochemical mechanism of stress, arousal, and mood.
Acknowledgements

I would like to thank my advisor, Dr. Francesco Leri for guiding me through my degree. Thank you to Dr. Boyer Winters as my second committee member who has provided valuable advice along the way as well. Thank you to Dr. Maria Fernandez, who was involved in the conception and early progress of this project. I would also like to thank everyone in the Leri lab for training me on various protocols. In particular, Stephen Daniels trained me on place conditioning and Anne-Marie Levy who taught me how to run self-administration. There are also several students who have contributed to my work along the way that I would like to thank: Ben Fuller, Karson Theriault, Melissa Tigert, and Mike Wolter. Overall, this has been a very positive experience that I will look back on fondly as I continue my career in academia.
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<tr>
<td>2-DG</td>
<td>2-deoxy-D-glucose</td>
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<tr>
<td>5-TG</td>
<td>5-thio-glucose</td>
</tr>
<tr>
<td>ACTH</td>
<td>Adrenocorticotropic hormone</td>
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<tr>
<td>AGRP</td>
<td>Agouti-related peptide</td>
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<tr>
<td>AN</td>
<td>Arcuate Nucleus</td>
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<tr>
<td>CORT</td>
<td>Corticosterone</td>
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<tr>
<td>CPA</td>
<td>Conditioned Place Avoidance</td>
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<td>CPP</td>
<td>Conditioned Place Preference</td>
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<td>CRH</td>
<td>Corticotropin-releasing hormone</td>
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<tr>
<td>DA</td>
<td>Dopamine</td>
</tr>
<tr>
<td>DSAP</td>
<td>anti-dβh-saporin</td>
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<tr>
<td>FD</td>
<td>Food Deprived</td>
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<tr>
<td>FST</td>
<td>Forced Swim Test</td>
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<tr>
<td>GCR</td>
<td>Glucose Counterregulatory Response</td>
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<tr>
<td>HPA</td>
<td>Hypothalamic-Pituitary-Adrenal</td>
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<tr>
<td>IGM</td>
<td>Impaired Glucose Metabolism</td>
</tr>
<tr>
<td>LC</td>
<td>Locus Coeruleus</td>
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<tr>
<td>MetS</td>
<td>Metabolic Syndrome</td>
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<tr>
<td>NAc</td>
<td>Nucleus Accumbens</td>
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<tr>
<td>NE</td>
<td>Norepinephrine</td>
</tr>
<tr>
<td>NPY</td>
<td>Neuropeptide Y</td>
</tr>
<tr>
<td>NTS</td>
<td>Nucleus Tractus Solitarius</td>
</tr>
<tr>
<td>PF</td>
<td>Pre-fed</td>
</tr>
<tr>
<td>PVN</td>
<td>Paraventricular Nucleus</td>
</tr>
<tr>
<td>VTA</td>
<td>Ventral Tegmental Area</td>
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General Introduction

Depression is a leading cause of global disability (Mathers & Loncar, 2015). There is evidence suggesting that metabolic syndrome (MetS) may predispose vulnerable individuals to developing depression or depressive-like symptoms (Gheshlagh, Parizad, & Sayehmiri, 2016). MetS is a multifaceted condition with several components; however, dyslipidemia and impaired glucose metabolism (IGM) are implicated as important links between MetS and depression (Farooqui & Farooqui, 2012; Liaw et al., 2015; Van Reedt Dortland, Giltay, Van Veen, Zitman, & Penninx, 2010). One possible explanation for this association is that systems responsible for mediating energy homeostasis, motivation, and mood, including the hypothalamus and mesolimbic dopamine (DA) system are closely linked (Ferrario et al., 2016).

The current study focused primarily on mood disturbances associated with IGM. Glucose is an essential nutrient for brain metabolism (Siesjo, 1978). Thus, it is not surprising that the body has developed mechanisms responsible for preventing and correcting decreases in blood glucose, collectively referred to as the glucose counterregulatory response (GCR; Cryer, 1993). Recent evidence suggests that IGM associated with maladaptive GCR function activates a stress response that can alter mood state in humans and produce depressive-like behaviour in laboratory animals via norepinephrine (NE) activity in the hypothalamus (Gold, MacLeod, Frier, & Deary, 1995; Park, Yoo, Choe, Dantzer, & Freund, 2012). Exploring this effect could help elucidate the neurobiology involved in the link between depression and MetS while providing targets for future research investigating treatment.

It is conceivable that the mood disturbances experienced by individuals with metabolic dysfunction are caused by an aversive state associated with impaired glucose metabolism. Thus,
it was hypothesized that an acute impairment in glucose metabolism could produce an aversive state. To test this hypothesis, the current study investigated whether the glucose antimetabolite, 2-deoxy-D-glucose (2-DG) could serve as an unconditioned stimulus to produce a conditioned place avoidance (CPA) in rats. This hypothesis makes three predictions: the effects of 2-DG should be dependent on the feeding state of the animal. Therefore, Experiment 1 investigated 2-DG-induced CPA and measured changes in blood glucose concentrations after acute injection of 2-DG in pre-fed (PF) and food deprived (FD) rats. Second, the monoamines DA and NE should play a role in 2-DG-CPA. To study this, Experiment 2 administered clonidine or bupropion during conditioning to alter CPA expression. Finally, the hypothesis predicts that the Hypothalamic-Pituitary-Adrenal (HPA) stress axis should be involved in 2-DG-induced CPA. Thus, Experiment 3 investigated blood corticosterone (CORT) levels in response to a single injection of 2-DG and whether this effect could be altered by clonidine or bupropion. In addition, Experiment 4 investigated whether clonidine or bupropion could produce a conditioned place preference (CPP) when administered alone.

**Metabolic dysfunction and depressed mood**

According to the Global Burden of Disease project, depression is among the leading causes of disability (Mathers & Loncar, 2015), contributing to increased mortality rates and shorter life expectancy in North America (Pratt, Druss, Manderscheid, & Walker, 2016). Despite global prevalence, treatment is limited to approximately a 50% success rate (Nestler & Carlezon, 2006). Current knowledge contributing to treatment options comes from research investigating the pharmacological mechanism of drugs that have demonstrated some efficacy (Nestler & Carlezon, 2006). As a result, the neurobiology involved in the development of depression and other mood disorders is limited.
Depression is linked to MetS and often cooccurs with several disorders that involve metabolic dysfunction (Gheshlagh et al., 2016). MetS is a multifaceted condition characterized by several components including dyslipidemia, insulin resistance, IGM, hypertension and central obesity (Farooqui & Farooqui, 2012). These symptoms have been implicated in comorbid disorders such as diabetes, obesity, and depression (Heiskanen et al., 2006). Interestingly, obesity is characterized by several components of MetS and is a recognized marker of depression (Carey et al., 2014; Roberts, Kaplan, Shema, & Strawbridge, 2000). For example, using a population of 3000 participants, Carey et al. (2014) found that 23% of obese individuals, compared to only 12% of aged-matched normal-weight participants, displayed depressive symptoms (Carey et al., 2014). Notable characteristics of obesity that have been linked to depression are dyslipidemia and insulin resistance (Farooqui & Farooqui, 2012). A cohort study in the Netherlands examining MetS symptomology in 1217 depressed or anxious subjects cited dyslipidemia as a primary driver in the association between MetS and depression (Van Reedt Dortland, Giltay, Van Veen, Zitman, & Penninx, 2010). Moreover, evidence from Liaw et al. (2015) suggests that insulin resistance is an important mediating factor as well. Their results demonstrated that individuals expressing more MetS symptoms exhibited higher depression scores when insulin resistance was one of the symptoms.

The public health agency of Canada cites depression as a major side effect of diabetes mellitus (Pelletier et al., 2012). The defining characteristic of diabetes mellitus is an inability to regulate blood glucose levels (Pelletier et al., 2012), possibly contributing to mood disturbances seen in these populations. For example, a nation-wide survey across the United States found that individuals with type 2 diabetes, and with at least 1 reported episode of hypoglycemia, had lower overall mental health compared to healthy individuals (Green, Fox, & Grandy, 2012). In
addition, Lawrence et al. (2006), examined the rate of depressed mood in in 2672 youth aged 10-21 years old with type 1 or 2 diabetes. Their results demonstrated that 14% and 8.6% had mildly or severely depressed mood, respectively. Researchers cited poor glycemic control as a key contributor to these results, which was consistent with previous research (Lustman et al., 2000). Overall, these studies support a correlation between MetS and depression; however, a significant knowledge gap exists regarding their causal relationship.

The prevalence of comorbid depression and MetS could be explained, in part, by common neurobiology shared in both conditions. A growing body of evidence indicates that the systems responsible for mediating energy homeostasis, motivation and mood are closely linked (Ferrario et al., 2016). The mediobasal hypothalamus is central in mediating energy homeostasis in the brain (Morton & Schwartz, 2006). Thus, it has been theorized that the hypothalamus maintains appropriate energy balance by responding to circulating hormone signals such as leptin, insulin and ghrelin, and adjusts behaviour accordingly (Morton, Meek, & Schwartz, 2014). However, it has become increasingly clear that several of these signals influence behaviour via direct action on limbic structures including the hippocampus, ventral tegmental area (VTA), and nucleus accumbens (NAc), regions involved in regulating motivation, emotion, and mood (Nestler & Carlezon, 2006).

Several signals that mediate energy homeostasis may also play an important role in regulating motivation and mood. For example, ghrelin promotes seeking of palatable food through increased DA activity in the VTA (Skibicka, Hansson, Alvarez-Crespo, Friberg, & Dickson, 2011) and has been implicated in mediating stress-induced depressive symptoms (Lutter et al., 2008). Agouti-related peptide (AGRP) neurons are activated by ghrelin, stimulate feeding behaviour, and increase effortful food seeking behaviour (Krashes et al., 2011).
Interestingly, AGRP neuron activation also produces a CPA via optogenetic stimulation (Betley et al., 2015). Therefore, Ferrario et al. (2016) suggest that feeding behaviour elicited by AGRP is motivated by aversive states associated with hunger. The aversive states may reinforce behaviours in response to conditioned cues that reduce AGRP activity.

Leptin and insulin both act to suppress AGRP activity and are established in mediating VTA DA activity. Leptin released from the adipose tissue acts to reduce food intake and body weight (Halaas et al., 1997). Recent evidence indicates that leptin inhibits DA activity in the VTA (Hommel et al., 2006), inhibits food seeking by modulating the reward value of food (Domingos et al., 2011) and plays a role in emotion (Lu, Kim, Frazer, & Zhang, 2006). In fact, rats that are subjected to chronic stress show depleted leptin levels, elevated CORT levels, and express depressive-like behaviours, while administration of Leptin prior to stress can reverse these effects (Lu et al., 2006). Insulin is a pancreatic hormone that regulates glucose levels via peripheral mechanisms (Levin & Sherwin, 2011) and in the brain, reduces feeding behaviour (Woods, Lotter, McKay, & Porte, 1979). Figlewicz, Evans, Murphy, Hoen, & Baskin (2003) discovered that the VTA contains a rich population of insulin receptors. Increased insulin activity in this region can cause long-term depression of DA activity, attenuate CPP in rats, and reduce anticipatory behaviour mice (Labouebe et al., 2013), thereby revealing that metabolic systems regulate reward related reactivity to incentive stimuli. Taken together, the substantial overlap between systems that regulate energy homeostasis, motivation, and mood supports the idea that chronic dysfunction caused by MetS could produce mood disturbances.

Another important consideration is the role of stress in the regulation of glycemic control and depression. Dysregulation of the HPA stress axis is commonly asserted in depression etiology (Vreeburg, Hoogendijk, van Pelt, & DeRijk, 2013). Under normal circumstances, the HPA stress
response allows an organism to respond appropriately to external threats ("fight or flight") by increasing plasma glucocorticoids (cortisol in humans and CORT in rats; Fuchs & Flugge, 2011). Several studies have indicated that depression is associated with elevated daytime levels of HPA hormones including cortisol (Pfohl, Sherman, Schlechte, & Winokur, 1985; Vreeburg et al., 2013), corticotropin releasing hormone (CRH; Gold, Licinio, Wong, & Chrousos, 1995), and adrenocorticotropic hormone (ACTH; Pfohl et al., 1985) compared to controls. Moreover, there is emerging evidence that IGM can alter mood state in humans and produce depressive like behaviours through mechanisms associated with HPA activation.

Indeed, to maintain appropriate physiological glucose levels, the body has developed mechanisms responsible for preventing and correcting decreases in blood glucose, collectively referred to as the glucose counterregulatory response (GCR; Cryer, 1993). In order to examine how IGM may affect systems involved in energy homeostasis, motivation, and mood it is important to understand GCR organization. Watts & Donovan (2010) suggest that the GCR exhibits a functional hierarchy, beginning with the activation of reflex loops in the hind brain and hepatic portal system when blood glucose concentrations are just below normal physiological levels. These reflex loops mobilize neuroendocrine hormones that reduce the uptake of glucose into peripheral cells and promote hepatic glucose production via glycogenolysis and gluconeogenesis (Beall, Ashford, & McCrimmon, 2012). It is important to note that glucose counterregulation is responsible for correcting and preventing glucose deficits. Therefore, GCR activation begins prior to the presentation of physical symptoms or cognitive deficits seen in a severe glucose deficit (Cryer, 1993).

Further up the hierarchy, forebrain input can influence the neuroendocrine response via noradrenergic projections from the dorsal-medial hindbrain to the hypothalamus, stimulating
HPA stress axis and inducing feeding behaviour (Ritter, Li, Wang, & Dinh, 2011). The neurocircuitry associated with this feeding response is referred to as the emergency feeding circuit and has been the subject of extensive research beginning in the early 1950s (for a more comprehensive review see Morton et al., (2014) and Ritter et al., (2011)). This body of work has employed several glucose antimetabolites, such as 2-DG, which are transported into the cell and block glucose metabolism via competitive inhibition of phosphoglucoisomerase (Edmonds & Edwards, 1998). These compounds are particularly effective for examining GCR activity because glucose sensing in the brain is mediated primarily by glycolytic processing of glucose rather than blood glucose concentrations (Lamy et al., 2014).

Experimental evidence indicates that IGM alters mood state in humans (Gold et al., 1995) and produces depressive like behaviours in laboratory animals (Park et al., 2008, 2012). Gold et al. (1995) induced hypoglycemia in humans with a hyperinsulinemic glucose clamp, which measures insulin sensitivity, to examine mood changes in healthy participants. Their results demonstrate that induced hypoglycemia produced tense arousal, along with reduced hedonic tone and energetic arousal, collectively termed ‘tense tiredness’ that remained for more than 30 minutes after the restoration of euglycemia.

In animals, Park and colleagues investigated how mice respond to IGM. In their first study, insulin-induced hypoglycemia caused an almost complete social withdrawal in 12-week-old male mice that persisted for four hours, beyond return to euglycemia. Critically, in a separate group, co-administration of a β2 noradrenergic receptor antagonist blocked this effect, revealing a role for NE in this effect (Park et al., 2008). In a follow up study, Park et al. (2012) examined how mice would perform in a forced swim test (FST) and saccharin preference test, 24 hours after an insulin challenge to examine depressive-like behaviour. Their results were consistent with the
previous study; mice displayed decreased mobility in the FST, as well as reduced saccharin preference. Importantly, other cognitive functions and locomotor responses were not affected. Once again, these effects were enhanced by systemic administration of NE, and blocked by noradrenergic antagonists (Park et al., 2012). It is possible that the mood disturbances and depressive-like behaviour described in these studies is the result of aversive states associated with IGM. However, the affective response to IGM has not been studied.

The current study aimed to characterize the affective response to IGM using 2-DG. It was hypothesized that an acute decrease in glucose metabolism could produce an aversive state. To test this hypothesis, this study investigated whether 2-DG could serve as an unconditioned stimulus to produce a CPA. Place conditioning is commonly used to examine the affective properties of various substances including drugs of abuse (Cummins Jacklin et al., 2015; Daniels, Marshall, Leri, 2016, & Tan et al., 2012). CPA provides a useful measure of 2-DG-induced conditioned aversion by exploiting the animals’ innate tendency to avoid a compartment previously associated with a noxious or aversive stimulus.

Given the data presented above, the current hypothesis makes several predictions. First, the hypothesis predicts that the effect of 2-DG will depend on the feeding condition of the animal. It is known that hormones involved in energy balance respond to the feeding state of an organism (Ferrario et al., 2016). For example, during hunger ghrelin and AGRP are more active, while sated individuals exhibit elevated levels of insulin and leptin (Morton et al., 2014). This is especially relevant because AGRP is involved in producing the negative affective state associated with hunger (Ferrario et al., 2016). In addition, there is some evidence that the effect of 2-DG is influenced by feeding state at the time of injection. Booth and colleagues found that the effects of 2-DG on feeding behaviour were disrupted when animals were fasted prior to
administration (Booth, 1972). To account for these effects, Experiment 1 investigated 2-DG-induced CPA and measured changes in blood glucose concentrations after acute injection of 2-DG in pre-fed (PF) and food deprived (FD) rats.

Second, the hypothesis predicts that NE should be involved in the response to 2-DG. Noradrenergic cell groups (A1-A7) are located primarily in the brainstem, including the locus coeruleus (LC) and nucleus of the solitary tract (NTS; Goddard et al., 2010; Ritter et al., 1998). Despite this relatively confined localization, NE neurons project to several brain regions including: prefrontal cortex, hypothalamus, thalamus, hippocampus, amygdala, and mesolimbic systems (Sawchenko & Swanson, 1982). NE activity is central to many psychological functions, including attention and arousal, while LC neurons projecting to the mesolimbic DA system and other cortical regions can influence mood state (Goddard et al., 2010).

Smith & Epstein (1969) and Miselis & Epstein (1975) were among the first to explore the idea of glucose regulation in the central nervous system using 2-DG. They demonstrated that IGM in the brain mobilized hepatic glucose stores and produced marked hyperphagia in both rhesus monkeys and rats. Several subsequent studies have taken advantage of this glucoprivic feeding response to explore the neural pathways associated with glucose regulation in the brain (King et al., 2011; Luquet, Phillips, & Palmiter, 2007; Sindelar et al., 2004). These studies located glucose receptive sites associated with glucoprivic feeding in hind brain regions. In one key study, Ritter, Dinh, & Zhang, (2000) infused 142 hindbrain regions and 61 hypothalamic regions with another glucose antimetabolite, 5-thio-d-glucose (5-TG), and concluded that infusion sites that induced hyperphagia must contain these glucose receptive sites. Their results found 66 such sites in the ventrolateral and dorsomedial medulla.
It is known that glucose receptive cells stimulate hindbrain NE neurons projecting to the hypothalamus during IGM (Ritter, Dinh, & Li, 2006). Ritter, Dinh, & Li, (2006) demonstrated that blocking glucose metabolism with 2-DG in these regions mobilize NE, while lesioning NE projections to the paraventricular (PVN) and arcuate nucleus (AN) of the hypothalamus eliminates glucoprivic hyperphagia for standard laboratory chow in rats (Hudson & Ritter, 2004; Ritter, Bugarith, & Dinh, 2001).

Third, the hypothesis predicts that DA should be involved in the response to 2-DG. DA mediates motivation, associative learning, mood state (Bromberg-Martin, Matsumoto, & Hikosaka, 2010; Nestler & Carlezon, 2006) and the ability to experience or anticipate pleasure (Berridge & Robinson, 1998). The dopaminergic response to salient stimuli is complex. Bromberg-Martin et al. (2010), suggest that DA encodes the adaptive response to salient external stimuli in the following ways: 1) motivational value promoting seeking or avoidance behaviour; 2) motivational salience providing information regarding immediate relevance of appetitive and aversive stimuli; and 3) an alerting response that allows for a timely reaction to any salient stimuli. Dysfunction in these DA pathways has been implicated as a characteristic quality of depressed mood (Dunlop & Nemeroff, 2007)

There is evidence that NE projections in DA systems are involved in the behavioural response to impaired glucose metabolism. Luquet et al. (2007) demonstrated that glucoprivic hyperphagia for palatable food was not affected in animals with hypothalamic lesions in cell groups that mediate the regulation of food intake including neuropeptide Y (NPY) or AGRP neurons. This study fits well with evidence suggesting that impaired glucose metabolism induces carbohydrate specific hyperphagia (Kanarek, Marks-Kaufman, Ruthazer, & Gualtieri, 1983), and
supports a role for higher-order systems involved in motivation and emotion in the behavioural response to IGM, such as the mesolimbic DA system.

To investigate the role of NE and DA, Experiment 2 used systemic administration of clonidine and bupropion during 2-DG conditioning sessions to alter CPA expression. In addition, Experiment 4 investigated whether clonidine or bupropion alone could produce a CPP. Clonidine is a noradrenergic α2 receptor agonist. α2 receptors are primarily presynaptic, and when activated act to reduce the release of NE into the synapse resulting in an overall suppression of noradrenergic tone (Engberg & Eriksson, 1991). As a result, clonidine has been used as effective treatment for hypertension and other anxiety disorders (Kamibayashi & Maze, 2000). Given the prominent role for NE in GCR, it is predicted that administration of clonidine with 2-DG during conditioning will suppress 2-DG-CPA. Bupropion is a catecholamine (DA and NE) reuptake inhibitor that is used as an antidepressant medication and smoking cessation agent (Dean, Horndasch, Giannopoulos, & McCabe, 2016). Specifically, bupropion is thought to be effective at improving motivational deficits associated with depression (Dean et al., 2016). Thus, it is predicted that administration bupropion with 2-DG during conditioning will also attenuate 2-DG-CPA.

Finally, the hypothesis predicts that the HPA axis should be involved in 2-DG-induced CPA. As discussed above, dysregulation in HPA axis activity is a common factor in depressed mood as affected individuals exhibit elevated resting levels of hormones associated with HPA activity. Previous research has demonstrated that 2-DG can elevate serum levels of HPA axis hormones including CORT and ACTH, and that that this effect could be the result of NE activity in the hypothalamus (Ritter et al., 2011; Weidenfeld, Corcos, Wohlman, & Feldman, 1994). Thus, to examine the role of HPA activity in 2-DG-induced CPA, Experiment 3 investigated blood CORT
levels in response to a single injection of 2-DG in PF and FD rats and whether this effect could be altered by clonidine or bupropion.

Methods

Subjects

A total of 296 male Sprague-Dawley rats (Charles River, QC) weighting between 200-250 g at the beginning of the experiments, were individually housed in standard rat cages (polycarbonate; 50.5×48.5×20 cm), with bedding and environmental enrichment. Upon arrival, rats were given 1 week of acclimatization to the facility. They were maintained on a 12-h reverse light/dark schedule (lights off 7:00 AM, on 7:00 PM), and all behavioral testing was conducted during the dark period. Water was available ad libitum throughout the experiments and feeding schedules of chow (Teckblad Global 18% Rodent diet, Envigo RMS Inc, Indianapolis, IN, USA) are specified for each experiment. All experiments were approved by The Animal Care Committee of the University of Guelph, and were carried out in accordance with recommendations of the Canadian Council on Animal Care.

Apparatus

Place conditioning

Six semitransparent plexiglass chambers (University of Guelph, ON) were used for place conditioning. Each chamber included two distinct compartments of equal size (30×40×26 cm) separated by a removable insert (dark gray PVC). A small square opening (10×10 cm) at the back of the insert allowed access to both compartments during pre-conditioning and test sessions, and an identical insert without the opening was used for conditioning. Distinct visual (marbled white and black pattern on the wall of one compartment and vertical white and black stripes on
the wall of the other; objects external to the boxes including cabinets, tables, and computer) and tactile (one compartment in each chamber contained a black ceramic floor tile) cues were maintained constant throughout the experiment. Black wire mesh covered the front of each compartment allowing for automatic video tracking (EthoVision v11.5, Noldus, The Netherlands). The software was also employed to create a virtual transition zone (approximately the size of a 400-g rat) creating a third, middle compartment. Time spent in this virtual compartment was not included in data analysis.

**Procedures**

**Experiment 1**

This dose-finding experiment aimed to investigate CPA and changes in blood glucose concentrations after acute administration of 2-DG. All animals were maintained on a 3-hr daily feeding schedule. In the PF condition, the feeding period occurred immediately prior to behavioural testing. In the FD condition, the feeding period occurred immediately after behavioural testing. Blood glucose levels were measured in PF and FD rats 0, 15, 30, 45, 60, 90, and 120 min after injecting 0, 300, or 500 mg/kg 2-DG (n=6-10 each). Blood was collected from a small incision at the tip of the tail and blood glucose concentration was measured using Accu-Check glucose meter (Roche Diabetes Care, Inc, Indianapolis, IN, USA).

In separate groups, 2-DG-induced CPA was examined in PF and FD rats. On the preconditioning test, the inserts with openings were used, and rats had free access to both compartments for 20 min. The compartment in which each rat spent the most time was designated as “preferred” and was subsequently paired with 2-DG. The following day, the solid inserts were used to fully separate the compartments, and rats were injected with vehicle or 0, 300 or 500 mg/kg 2-DG (n=8 each), and were immediately confined to the vehicle- or 2-DG-
paired compartment for 30 min. Over 6 days, rats received a total of three vehicle and three 2-DG compartment pairings, counterbalanced, each 24 h apart. Following the last day of conditioning, animals were tested for CPA using the inserts with openings for 20 min.

Experiment 2

Experiment 2 investigated whether clonidine or bupropion could alter 2-DG-induced CPA in PF rats only. The PF feeding condition was selected based on results from Experiment 1 indicating that FD rats did not display CPA. The same conditioning protocol of Experiment 1 was followed, with the exception that animals were conditioned with 500 mg/kg 2-DG alone, or 500 mg/kg 2-DG in combination with 10 and 40 μg/kg clonidine or 10 and 30 mg/kg bupropion (n=8 each). Each group was conditioned with only 500 mg/kg 2-DG because this dose produced robust CPA in Experiment 1.

Experiment 3

Experiment 3 investigated blood CORT levels in response to a single injection of 2-DG in PF and FD rats. Animals were injected with 0, 300 or 500 mg/kg 2-DG (n=8 each). Thirty min after the injection, trunk blood was collected and analyzed for plasma CORT concentrations via radioimmunoassay. A separate group was used to determine whether 2-DG-induced increases in blood CORT levels could be altered by clonidine or bupropion in PF rats only. The same protocol was followed, with the exception that rats were injected with 0 mg/kg 2-DG plus vehicle, 500 mg/kg 2-DG plus vehicle, 0 mg/kg 2-DG plus 40 μg/kg clonidine, 500 mg/kg 2-DG plus 40 μg/kg clonidine, 0 mg/kg 2-DG plus 30 mg/kg bupropion, or 500 mg/kg 2-DG plus 30 mg/kg bupropion (n = 8 each). The dose and feeding condition were selected to match the feeding protocol and results from Experiment 2.
Experiment 4

The final experiment investigated whether clonidine or bupropion could produce a CPP in PF rats when administered on their own. The same conditioning protocol of Experiment 2 was followed, with the exception that on the pre-conditioning test the compartment in which each rat spent less time was designated as “less preferred” and was subsequently paired with the drug. Animals were conditioned with vehicle or 10 and 40 ug/kg clonidine, or 10 and 30 mg/kg bupropion (n=8 each). The dose and feeding condition were selected to match the protocol from Experiment 2.

Radioimmunoassay

At the time of decapitation, trunk blood from each rat was collected in tubes, placed on ice, and was spun in a refrigerated centrifuge. Plasma was separated and stored at −80°C for hormonal measurements by radioimmunoassay. Levels of corticosterone immunoreactivity were assayed from unextracted plasma by using kits from DiaSorin Inc. (Stillwater, MN). All values were determined in duplicate in a single assay.

Drugs

2-deoxy-D-glucose (2-DG; Sigma-Aldrich, St. Louis, MO), clonidine hydrochloride (Sigma-Aldrich, St. Louis, MO), and bupropion hydrochloride (Toronto Research Chemicals, Toronto, ON) were dissolved in deionized water and injected subcutaneously (SC) at a volume of 1 ml/kg. The doses of 2-DG were selected based on previous behavioural results demonstrating that 50-500 mg/kg effectively induced glucoprivic feeding (Ritter et al., 2011), while higher doses induce prolonged locomotor depression and retching (Smith & Epstein, 1969). Doses of clonidine were selected based on previous research demonstrating effective reduction of NE
neuronal firing and release (Engberg & Eriksson, 1991). Finally, doses of bupropion selected have been shown to interfere with nicotine priming, conditioned taste avoidance (CTA), and conditioned place preference (CPP; Budzynska & Biaa, 2011; Rauhut, Hawrylak, & Mardekian, 2008).

Data Analysis

One, two, or three-way mixed design analysis of variance (ANOVA) were used as appropriate. Significant interactions were followed by multiple comparisons using the Student-Newman-Keuls or Bonferroni methods. The threshold for significance was at $\alpha \leq 0.05$. The exact values of non-significant analyses are not reported

Results

Experiment 1

Figure 1 represents mean blood glucose levels in PF and FD rats injected with 0, 300 or 500 mg/kg 2-DG. The ANOVA identified a significant interaction between Feeding Condition, Dose, and Time [$F(12, 252) = 6.034, p < 0.0001$], a significant interaction between Dose and Feeding Condition [$F(2, 252) = 10.633, p < 0.001$], a significant interaction between Feeding Condition and Time [$F(6, 252) = 4.629, p < 0.001$], a significant interaction between Dose and Time [$F(12, 252) = 66.308, p < 0.0001$], a significant main effect of Dose [$F(2, 252) = 130.241, p < 0.0001$], and a significant main effect of Time [$F(6, 252) = 226.637, p < 0.0001$]. Multiple comparisons revealed that 2-DG elevated blood glucose levels 30 min after the injection in both feeding conditions indicated by significant differences found between rats injected with 300 or 500 mg/kg 2-DG and rats injected with 0 mg/kg 2-DG. This effect was dose dependent in the PF but not FD feeding condition as indicated by the significant differences between PF rats injected
with 300 mg/kg 2-DG and 500 mg/kg 2-DG. In addition, food deprivation enhanced the blood glucose level response to 2-DG as indicated by the significant differences between PF and FD rats injected with 300 mg/kg 2-DG.

Figure 2A represents mean time spent in the 2-DG-paired compartment during the CPA test in PF and FD rats that were conditioned with 0, 300 and 500 mg/kg 2-DG. The ANOVA identified a significant interaction between Dose and Feeding Condition [F(2, 42) = 5.421, p < 0.01], a significant main effect of Dose [F(2, 42) = 4.776, p < 0.05], and a significant main effect of Feeding Condition [F(1, 42) = 14.139, p < 0.001]. In PF animals, multiple comparisons revealed that rats injected with 300 or 500 mg/kg 2-DG spent significantly less time in the 2-DG-paired compartment than rats injected with 0 mg/kg 2-DG. These groups differences were not observed in FD rats who displayed no CPA.

Figure 2B represents mean total distance moved during the drug conditioning sessions in PF and FD rats conditioned with 0, 300 and 500 mg/kg 2-DG. The ANOVA identified a significant interaction between Dose and Feeding Condition [F(2, 42) = 8.569, p < 0.001], a significant main effect of Dose [F(2, 42) = 64.424, p < 0.001], and a significant main effect of Feeding Condition [F(1, 42) = 24.660, p < 0.001]. In both feeding conditions, multiple comparisons revealed that rats injected with 300 or 500 mg/kg 2-DG moved significantly less than rats injected with 0 mg/kg 2-DG and this effect was significantly more pronounced in FD rats.

**Experiment 2**

Figure 3A represents mean time spent in the drug-paired compartment during the CPA test in PF rats that were conditioned with 500 mg/kg 2-DG alone, or in combination with 10 (low dose) and 40 ug/kg (high dose) clonidine or 10 (low dose) and 30 mg/kg (high dose) bupropion. The
ANOVA identified a significant effect of Group \([F(4, 35) = 5.439, p < 0.01]\). Multiple comparisons revealed significant differences between rats injected with the combination of 2-DG and either the low dose of bupropion or the high doses of bupropion and clonidine compared to rats injected with 2-DG alone.

Figure 3B represents mean total distance moved during drug conditioning sessions in PF rats conditioned with 500 mg/kg 2-DG alone, or in combination with 10 (low dose) and 40 ug/kg (high dose) clonidine or 10 (low dose) and 30 mg/kg (high dose) bupropion. The ANOVA identified a significant effect of Group \([F(4, 35) = 18.994, p < 0.001]\). Multiple comparisons revealed that rats injected with both the high and low dose of bupropion but not clonidine moved significantly more than rats injected with 2-DG alone.

**Experiment 3**

Figure 4A represents mean blood levels of CORT in PF or FD rats injected with 0, 300 and 500 mg/kg 2-DG. The ANOVA identified a significant main effect of Dose \([F(2, 42) = 5.922, p < 0.01]\) and a significant main effect of Feeding Condition \([F(1, 42) = 5.574, p < 0.05]\). That is, over all, animals that were injected with 2-DG had elevated blood levels of CORT and this effect was further increased in FD rats.

Figure 4B represents mean blood levels of CORT in PF rats injected with 0 mg/kg 2-DG plus vehicle, 500 mg/kg 2-DG plus vehicle, 0 mg/kg 2-DG plus 40 ug/kg clonidine, 500 mg/kg 2-DG plus 40 ug/kg clonidine, 0 mg/kg 2-DG plus 30 mg/kg bupropion, or 500 mg/kg 2-DG plus 30 mg/kg bupropion. The ANOVA identified a significant main effect of 2-DG \([F(1, 57) = 19.019, p < 0.001]\). That is, overall, animals that were injected with 2-DG had elevated blood levels of CORT. However, it appears that this effect is attenuated by clonidine and bupropion, which was
confirmed by multiple comparison corrected with Bonferroni adjustment within drug groups. That is, rats injected with 2-DG alone had significantly higher blood CORT levels than rats injected with 0 mg/kg 2-DG \( [t(30) = 4.309, p < 0.001] \). These differences were not observed in rats injected with clonidine or bupropion.

**Experiment 4**

Mean (SEM) time spent in the drug-paired compartment during the CPP test was analyzed in PF animals injected with vehicle [589.7 (36.5)], or 10 [508.0 (39.5)] and 40 ug/kg [528.1 (26.7)] clonidine, or 10 [711.6 (43.9)] and 30 mg/kg [719.9 (37.0)] bupropion. The ANOVA identified no significant effects, indicating that animals did not spend significantly more time in the drug-paired compartment compared to controls.

**Discussion**

Components of metabolic dysfunction have been linked to mood disturbances that may leave people vulnerable to developing depression or depressive like behaviours (Gheshlagh et al., 2016). Evidence suggests that IGM can alter mood state in humans and induce depressive like behaviours in animals (Gold, MacLeod, Frier, & Deary, 1995; Park, Yoo, Choe, Dantzer, & Freund, 2012). It is conceivable that these mood disturbances are the result of aversive states associated with IGM. To test this hypothesis, the current study explored whether an acute decrease in glucose metabolism caused by 2-DG could produce a CPA.

Experiment 1 measured changes in blood glucose concentrations after an acute injection of 0, 300 or 500 mg/kg 2-DG in PF and FD rats. In a separate group, 0, 300, or 500 mg/kg 2-DG was paired with an initially preferred compartment during conditioning in PF and FD rats; avoidance was measured by comparing the time spent in the 2-DG paired compartment during a test phase.
after conditioning. Based on results from Experiment 1, Experiment 2 examined whether 0, 10, or 40 ug/kg clonidine or 0, 10, or 30 mg/kg bupropion during conditioning could alter 2-DG-CPA in PF rats. Experiment 3 measured changes in blood CORT concentrations after an acute injection of 0, 300, or 500 mg/kg 2-DG in PF and FD rats and investigated this effect could be altered by 0, 10, or 40 ug/kg clonidine, or 0, 10 or 30 mg/kg bupropion in PF rats. Finally, Experiment 4 determined if 0, 10, or 40 ug/kg clonidine or 0, 10, or 30 mg/kg bupropion could be used to produce a CPP when injected alone.

Results from the Experiment 1 demonstrated that 2-DG caused an increase in blood glucose levels and that FD enhanced this effect. In addition, rats avoided a compartment that was paired with 2-DG and locomotor suppression was observed during 2-DG conditioning sessions. Interestingly, FD animals did not express CPA despite exhibiting augmented locomotor suppression compared to PF animals. Experiment 2 found that clonidine and bupropion attenuated avoidance behaviour caused by 2-DG. In addition, bupropion reversed locomotor deficits caused by 2-DG. Experiment 3 demonstrated that 2-DG caused an increase in blood levels of CORT and that FD enhanced this effect. Clonidine and bupropion attenuated 2-DG-induced increases in blood levels of CORT. Finally, Results from Experiment 4 indicated that neither clonidine or bupropion administered alone produced a CPP. Overall these results suggest that an acute decrease in glucose metabolism elicits a stress response that can cause an aversive state by engaging systems that mediate stress, arousal, and mood.

In the experiments reported above, PF animals generally spent significantly less time in an environment paired with the effects of 2-DG at doses ranging between 300 and 500 mg/kg. These results are consistent with previous research demonstrating that 500 mg/kg 2-DG is capable of producing a CTA to a sweet saccharine solution (Stephan, Smith, & Fisher, 1999).
Research has shown that both humans and other animals have an innate tendency to avoid environments paired with aversive stimuli (O’Doherty, 2004). Investigators have used this phenomenon to examine the affective response to various stimuli including the aversive consequences of opioid withdrawal (Daniels, Marshall, & Leri., 2016). Therefore, while the dose of 2-DG did not significantly mediate the avoidance behaviour observed in this study, the results support our hypothesis that an acute decrease in glucose metabolism causes an aversive state.

Interestingly, several physiological and behavioural effects caused by 2-DG were modulated by the feeding condition of the animal at the time of injection. First, 300 and 500 mg/kg 2-DG caused an elevation in blood glucose levels that was enhanced by FD in animals that received 300 mg/kg 2-DG. This is consistent with previous research indicating that 2-DG elevates blood glucose as the body attempts to compensate for the decrease in glucose metabolism (Iguchi et al., 1990; Marin-Spiotta, Levin, & Tkacs, 2004). However, it is possible that our results revealed a ceiling effect. More specifically, there was no differences observed between FD or PF animals that received 500mg/kg 2-DG or FD animals that received 300 mg/kg 2-DG. This suggests that FD-induced elevations in blood glucose levels could not be further augmented by a higher dose of 2-DG.

Second, FD enhanced 2-DG-induced elevations in blood CORT levels. CORT is a biomarker commonly used to measure HPA stress axis activation (Herman, 2011). Thus, these results suggest that food deprivation may have enhanced the stress response associated with 2-DG. In line with these findings, Booth (1972) demonstrated that fasting prior to 2-DG administration disrupted 2-DG induced feeding behaviour, suggesting that the behavioural effects of 2-DG are due to the inhibition of nutrient processing rather than a separate hunger indicator. In addition, Delprete & Scharrer (1992) demonstrated that the 2-DG induced feeding behaviour could also be
modified by manipulating the composition of carbohydrates and fats in the diet. Therefore, it is not surprising that our feeding manipulation modulated the physiological effects of 2-DG.

Surprisingly, despite enhancing the effect of 2-DG on blood glucose and CORT levels, FD animals did not express 2-DG-induced CPA. That is, FD animals conditioned with 300 or 500 mg/kg 2-DG did not spend significantly less time in the 2-DG-paired compartment during the test phase compared to FD animals that received 0 mg/kg 2-DG. It is possible that the absence of 2-DG-induced CPA in FD rats could be the result of memory impairments associated with 2-DG.

As discussed above, glucose is an essential nutrient for brain metabolism (Siesjo, 1978), and the effect of glucose on memory modulation has seen extensive attention in recent decades (for a more extensive review see Messier, 2004). Messier (2004) proposed the hypothesis that some memory impairments are the direct result of impaired glucose tolerance, which could contribute to some cognitive deficits associated with diabetes. For example, Messier, Desrochers, & Gagnon (1999) demonstrated that students with impaired glucose regulation showed deficits in free recall, paragraph recall and word order recall compared to healthy controls. In addition, an acute injection of glucose restored memory performance in these individuals but did not enhance performance in healthy individuals, indicating that memory performance is sensitive to glucose deficits (Messier et al., 1999). It is possible that an acute decrease in glucose metabolism by 2-DG could produce memory impairments, while combining FD with 2-DG may intensify this effect and interfere with the animal’s ability to form stable associations during 2-DG conditioning. Therefore, future research should consider the effect of 2-DG on learning and memory.

Alternatively, the absence of 2-DG-induced CPA in FD animals could the result of an inability to distinguish the aversive effects of 2-DG and the aversive effects of hunger. Indeed,
CPA relies on the animal’s ability to effectively discriminate the affective state associated with each compartment. There is evidence that the hunger state alone is aversive, which contributes to the drive to seek out consume food (Ferrario et al., 2016). In fact, previous research has demonstrated that AGRP activity is elevated during hunger and can produce CPA that is independent of glucose activity (Betley et al., 2015). If this is true, it may indicate that FD animals did not form sufficient contrasting contextual associations between compartments to express 2-DG-induced CPA.

Our results also demonstrated that 2-DG caused locomotor deficits during conditioning sessions. Specifically, animals injected with 300 and 500 mg/kg 2-DG traveled significantly shorter distances during conditioning compared to controls. It is possible that this effect could be the result of energy deficits associated with an acute reduction in glucose metabolism. This interpretation is supported by our findings that FD augmented locomotor deficits observed during conditioning with 300 and 500 mg/kg 2-DG compared to PF animals at the same doses. That is, greater energy deficits could result in greater locomotor suppression. However, another interpretation to consider is that 2-DG-induced locomotor deficits are the result of lassitude associated with an acute decrease in glucose metabolism. Locomotor activity is often used in animal research to examine the central nervous system effects of various substances including drugs of abuse (Clarke & Kumar, 1983; Montgomery & Grottick, 1999). It is possible that locomotor deficits observed here could be associated with a negative motivational state or mental fatigue exhibited during 2-DG conditioning sessions. In addition, lassitude is a major consequence of depression and is linked to alterations in neurotransmitter systems involved in arousal and mood such as NE and mesolimbic DA systems (Buyukdura, Mc Clintock, &
Croarkin, 2011). Therefore, if this interpretation is correct, it would be expected that manipulating these systems should alter the behavioural response to 2-DG.

Indeed, clonidine and bupropion administration during 2-DG conditioning sessions attenuated 2-DG-induced CPA. That is, animals that were conditioned with 500 mg/kg 2-DG in combination with either 40 ug/kg clonidine, 10 or 30 mg/kg bupropion spent more time in the drug-paired compartment compared to animals that received 500 mg/kg 2-DG alone. It is important to note that neither clonidine or bupropion administered alone produced a CPP. Or more specifically, animals that were conditioned with 10 or 40 ug/kg clonidine, or 10 or 30 mg/kg bupropion alone did not show a preference for either the drug-paired or vehicle-paired compartment. Clonidine has been used in the past to alleviate symptoms of opiate withdrawal (Delfs, Zhu, & Druhan, 2000; Kosten, 1994). Kosten (1994) demonstrated that clonidine also attenuated naloxone-induced CPA in morphine treated rats, but had no effect when animals were not pretreated with morphine. Thus, this evidence suggests that clonidine and 2-DG have synergistic effects. Budzynska & Biaa (2011) demonstrated that bupropion can attenuate nicotine priming in the reinstatement of CPP. In addition, Rauhut, Hawrylak, & Mardekian (2008) demonstrated that bupropion can attenuate nicotine CTA and nicotine CPP, while producing a CPP when administered alone. This is inconsistent with the current results; however, differences in the timing of bupropion administration prior to conditioning could explain these discrepancies. Thus, the mechanism involved in the attenuation of 2-DG-induced CPA by bupropion is unclear.

Overall, these results support a role for NE and DA in the response to 2-DG. Clonidine is a noradrenergic α2 auto-receptor agonist, which effectively reduces NE release from the nerve terminal and suppresses NE activity at the current doses (Engberg & Eriksson, 1991). Thus, it is possible that NE hyperactivity contributes to the development of 2-DG-CPA. Bupropion is
characterized as a monoamine reuptake inhibitor, which effectively increases DA and NE activity in the synapse (Dean et al., 2016). It is possible that the effects of bupropion involve DA activity primarily if clonidine’s effects on 2-DG-induced CPA are the result of blunted NE activity. This would be consistent with previous research demonstrating that the mesolimbic DA system is an important system required for CPA (Tan et al., 2012), and should be explored further.

In addition, bupropion reversed the locomotor deficits observed during 2-DG conditioning. Animals that were conditioned with 500 mg/kg 2-DG in combination with 10 or 30 mg/kg bupropion travelled a significantly greater distance than animals that were conditioned with 500 mg/kg 2-DG alone. This supports the idea that 2-DG induced locomotor deficits may be the result of lassitude rather than energy deficits. Or more specifically, it demonstrates that if 2-DG does produce energy deficits, this is not a limiting factor. However, future studies should employ specific DA agonists and antagonists to elucidate the mechanisms involved.

Finally, clonidine and bupropion attenuated 2-DG-induced elevation of blood CORT. That is, animals injected with 500 mg/kg 2-DG alone demonstrated a significant increase in blood concentration of CORT and this effect was not seen in animals that were injected with 500 mg/kg 2-DG in combination with 40 ug/kg clonidine or 30 mg/kg bupropion. This result is consistent with previous research demonstrating that 2-DG induces the adrenocortical response via HPA axis activation (Weidenfeld et al., 1994) and suggests that elevated CORT driven by HPA stress axis activation could be involved in 2-DG-induced CPA.

Various feedback loops required for HPA regulation have been investigated which highlight the importance of considering peripheral glucocorticoid activity in brain regions that regulate
motivation and mood (Gold, Drevets, & Charney, 2002). Corticosteroids have been shown to affect dopaminergic (Cyr et al., 2001), and noradrenergic systems (Gannon and McEwen, 1990; Stone, Lin, & Quartermain, 2008), which play a central role in CPA (Tan et al., 2012). Wang et al. (2009) demonstrated that removing the adrenal gland of Long-Evans rats blocked the expression of formalin induced CPA, but that CORT supplementation restored this effect. Conversely, there is also evidence that NE-induced enhancement of CPA by the α2 receptor antagonist yohimbine does not require HPA axis activation (Banihashemi & Rinaman, 2006), suggesting that the effect of NE on CPA could be a centrally mediated effect. Therefore, the role of peripheral corticosterone on 2-DG-induced CPA should be explored further.

There are a few limitations in the current study that should be addressed by future research. First, only male Sprague-Dawley rats were used. Thus, it is unclear if the same effects of 2-DG would occur in females or other species of rat, and the conclusions cannot be applied to humans. Previous research has demonstrated that female Long Evans rats exhibit greater CORT elevation than males in response to an acute stressor (Wilson & Biscardi, 1994). In addition, the current study examined CORT levels at a single time point (30 min after 2-DG administration) while the time course of this effect remains unclear. Second, there are other methods for investigating IGM. For example, previous research has employed insulin-induced hypoglycemia to examine depressive-like behaviour in mice (Park et al., 2012), and this can be appropriate because it is possible that mood disturbances experienced by individuals who suffer from diabetes result from insulin treatment (Lawrence, 2006). As an acute manipulation, insulin primarily promotes peripheral uptake and processing of glucose (Morton et al., 2014), and has differential effects by acting on insulin receptors located in brain regions involved in motivation and mood (Stouffer et al., 2015). Regardless, it could be useful to investigate motivational differences between insulin...
and 2-DG in place conditioning. Finally, the CPP protocol used in each experiment was a biased design, meaning that the drug-paired compartment was designated in the initially preferred compartment. This was appropriate for 2-DG based on previous work examining negative mood states associated with IGM. However, the predicted outcomes for clonidine and bupropion CPP were less clear, suggesting an unbiased design could be more appropriate.

Despite these limitations, results from the current study provide direction for future research. Several studies have employed localized manipulations, including intracranial infusions of 2-DG to investigate the regions involved in glucoprivic feeding behaviour (Ritter et al., 2011). Park et al. (2008, 2012) have infused specific NE receptor agonists and antagonists intracranially to investigate neural pathways involved in the behavioural effects of insulin-induced hypoglycemia. In addition, lesioning studies have exposed NE pathways that are required for 2-DG induced feeding behaviour (Ritter et al., 2001) using the toxin-antibody complex antidβh-saporin (DSAP) that destroys NE neurons in a retrograde fashion. Region-specific intracranial manipulations are the next logical step for future studies investigating the behavioural and physiological effects of 2-DG. In addition, employing receptor-specific drugs will help identify systems that might be involved in 2-DG-induced CPA.

Specifically, the current results implicate two notable brain pathways that warrant further investigation. It is possible that NE projections to mesolimbic DA systems are involved in 2-DG-CPA. NE mediates stress, attention, and arousal and includes projections from hind-brain regions, including the LC to mesolimbic DA systems (Goddard et al., 2010; Robertson, Plummer, de Marchena, & Jensen, 2013). DA’s role in aversive learning and the behavioural response to stressful stimuli has been investigated (Bromberg-Martin et al., 2010; Tan et al., 2012) and evidence suggests that NE can mediate these effects. For example, Masana,
Bortolozzi, & Artigas (2011) demonstrated that selective noradrenergic agonists and antagonists can affect mesolimbic DA activity including enhancing DA output in the prefrontal cortex and nucleus accumbens. Moreover, Ungless (2004) found that aversive stimuli can strongly inhibit DA neurons in the VTA, while resilience to behavioural and neurochemical adaptations associated with chronic stress are mediated by NE activity in the VTA (Isingrini et al., 2016). In fact, a recent study by Solecki and colleagues found that inhibiting NE-α1 receptors in the VTA can block behavioural response to stress and the acquisition of fear memories (Solecki, Szklarczyk, Pradel, Dobrznaski, & Przewlocki, 2017). These studies indicate that, NE-DA interactions in the VTA is a relevant target for future research investigating the aversive effects of 2-DG.

NE projections to the hypothalamus may also play a significant role in 2-DG-induced CPA. Ritter, Bugarith, & Dinh (2001) demonstrated that lesioning NE projections to the PVN and AN of the hypothalamus disrupt 2-DG-induced feeding behaviour. Moreover, as discussed above, homeostatic signals from the hypothalamus can act in mesolimbic systems to mediate motivation (Ferrario et al., 2016). This suggests that the relationship between the hypothalamus and the VTA is more complex than simply regulating energy balance. However, these signals have not been explored in the context of 2-DG. Conversely, NE activity in the hypothalamus also activates the HPA stress axis (Goddard et al., 2010). Deactivating the dorsal medial hypothalamus (DMH; Evans et al., 2004) and lesioning the lateral hypothalamus (LH; Weidenfeld et al., 1984) blocks HPA activation in response to insulin-induced hypoglycemia and 2-DG induced glucoprivation, respectively. Elevated CORT during HPA activation is important for central nervous system feedback responsible for regulating HPA activity (Fuchs & Flugge, 2011). Thus, it is possible that stress-induced corticosterone activity in mesolimbic DA systems
produces the aversion associated 2-DG. However, based on the current results, the role of the hypothalamus in 2-DG-CPA is unclear and further research is required.

Overall, the results from this study support four main conclusions: First, 2-DG did produce a CPA which supports our hypothesis that an acute decrease in glucose metabolism is associated with an aversive state; second, clonidine and bupropion attenuated 2-DG-CPA which suggests that the DA and NE neurotransmitter systems may be involved in this effect; Third, locomotor deficits observed during 2-DG conditioning could result from lassitude associated with acute decrease in glucose metabolism; and, finally, the feeding condition during 2-DG conditioning sessions modulated the physiological and behavioural effects of 2-DG, demonstrating that food intake is an important variable to control while investigating the effects of 2-DG.

The implications for the results of the current study are threefold. First, for the first time, we have demonstrated that an acute decrease in glucose metabolism by 2-DG can produce conditioned avoidance behaviour. Previous studies have already determined that IGM can cause mood disturbances in humans (Gold et al., 1995), and produce depressive like behaviour in laboratory animals (Park et al., 2012). Our results support the idea that these mood disturbances could be the result of aversive states associated with IGM. Second, these results support the possibility that there is a causal relationship between MetS and affective disorders. Previous research has primarily investigated correlations between the prevalence of MetS and depression (Gheshlagh et al., 2016). Our results support the idea that IGM is a stressor that can cause mood disturbances that may lead to the development of depression or depressive like symptoms.

Finally, the current study provides support for the idea that a common neuropathology may underlie affective disorders. Over time cumulative effects of repeated exposure to acute stressors
can cause neuroadaptations in NE and DA systems similar to those seen after chronic exposure to drugs of abuse (El Mansari et al., 2010; Nestler & Carlezon, 2006). These adaptations manifest as impairments in reward sensitivity that ultimately lead to a loss of interest in previously enjoyable activities along with diminished motivation or negative affect. This is a common symptom exhibited by individuals suffering from depression referred to as anhedonia (Stone et al., 2008). Stone et al. (2008) suggest that this should direct future research investigating treatment for depression. Our results contribute to this by demonstrating that metabolic dysfunction is a relevant stressor that also engages systems implicated in the neuroadaptations associated with anhedonia. Therefore, this effect should be explored further as a model to investigating depressive-like behaviour in animals, and to understand how IGM associated with diabetes or obesity may predispose vulnerable individuals to developing depressive symptoms.
References


Masana, M., Bortolozzi, A., & Artigas, F. (2011). Selective enhancement of mesocortical dopaminergic transmission by noradrenergic drugs: therapeutic opportunities in...


Figure Legends

Figure 1.

Mean (SEM) blood glucose concentrations in PF and FD rats injected with 0, 300 and 500 mg/kg 2-DG. The * indicates significant differences to the 300 and 500 mg/kg groups within a given feeding condition. The ** indicates a significant difference between the 500 mg/kg and the 300 mg/kg groups within a given feeding condition. The # indicates a significant difference between feeding conditions within the same dose.

Figure 2.

A) Mean (SEM) time spent in the 2-DG-paired compartment in PF and FD rats conditioned with 0, 300 and 500 mg/kg 2-DG. B) Mean (SEM) total distance moved during drug conditioning sessions in pre-fed and food deprived rats conditioned with 0, 300 and 500 mg/kg 2-DG. The ** indicates a significant difference to the 0 mg/kg group within a feeding condition. The % indicates a significant difference between PF and FD animals in the same dose group.

Figure 3.

A) Mean (SEM) time spent in the drug-paired compartment during test in PF rats that were conditioned with 500 mg/kg 2-DG alone, or in combination with 10 (low dose) and 40 ug/kg (high dose) clonidine or 10 (low dose) and 30 mg/kg (high dose) bupropion. B) Mean (SEM) total distance moved during drug conditioning sessions in PF rats conditioned with 500 mg/kg 2-DG alone, or in combination with 10 (low dose) and 40 ug/kg (high dose) clonidine or 10 (low dose) and 30 mg/kg (high dose) bupropion. The ** indicates a significant difference to the 2-DG group.
Figure 4.

A) Mean (SEM) blood concentration of corticosterone in PF or FD rats injected with 0, 300 and 500 mg/kg 2-DG. B) Mean (SEM) blood concentration of corticosterone in PF rats injected with 0 mg/kg 2-DG plus vehicle, 500 mg/kg 2-DG plus vehicle, 0 mg/kg 2-DG plus 40 ug/kg clonidine, 500 mg/kg 2-DG plus 40 ug/kg clonidine, 0 mg/kg 2-DG plus 30 mg/kg bupropion, and 500 mg/kg 2-DG plus 30 mg/kg bupropion. The ** indicates significant difference with respect to animals that were injected with 0 mg/kg 2-DG within Drug.
Tables and Figures

Figure 1.
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Figure 3.
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