A Proteomic Analysis of the Behavioural Effects of Sugar Cross-Sensitisation with Methamphetamine in the Nucleus Accumbens

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Abstract

The ease in accessibility to sugar-rich products has contributed to a dramatic increase in the consumption of sugar among the adolescent population. Likewise, due to its reinforcing properties, methamphetamine (METH) abuse and its associated mental health problems are another global burden. Both sugar and METH elicit their effects on similar neuronal systems, primarily the mesolimbic dopaminergic system which regulates the nucleus accumbens (NAc) of the brain. This study aimed to investigate the effects of chronic sugar consumption during adolescence on METH-induced locomotor activity in adulthood and associated changes to protein levels in the NAc of rats. Following 4 weeks of chronic sugar or water exposure, and a 6 week treatment-free period, rats were challenged with METH or vehicle and their locomotor behaviour measured. Following 24 hours, changes in protein levels in the NAc were identified using a proteomic approach. In comparison to the controls treated with water and challenged with saline (Water/Saline), the Water/METH group demonstrated significantly greater locomotor activation ($p < 0.05$). Providing support for behavioural cross-sensitisation, the Sugar/METH condition significantly demonstrated greater locomotor activity compared to the Water/METH condition ($p < 0.05$). Proteomic analyses revealed that a total of 93 differentially expressed proteins were identified in the NAc of the Water/METH rats compared to controls. These proteins were mainly involved in coordinating mitochondrial functioning and neuronal morphology. In comparison to the Water/METH animals, the Sugar/METH animals demonstrated significant changes to a total of 102 proteins that were mainly involved in mitochondrial dysfunction and synaptic plasticity. Proteomic analyses identified a total of 74 differentially expressed proteins in the Sugar/METH condition compared to controls which were mainly critical in regulating cellular processes and molecular transport.

Statement of Authentication and Ethical Accordance

The work presented in this thesis is, to the best of my knowledge and belief, original except as acknowledged in the text. I hereby declare that I have not submitted this material, in full or in part, for a degree at this or any institution.

All animal research carried out in this thesis was approved by the Macquarie University Animal Ethics Committee in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, 8th Edition (National Health and Medical Medical Research Council, 2013).

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Signature: CAOckert…….. Date: 10.10.2014

In recent times, there has been a dramatic increase in the excessive consumption of sugar among adolescents. Today, a majority of children and adolescents aged between 10 and 16 are consuming sugar-rich foods and drinks in excess of the recommended level (AIHW Australia's Food and Nutrition, 2012). An alarming aspect of this excess in sugar intake is that the long-term behavioural and neurochemical consequences are largely unknown. Another burden plaguing society at a global scale is the high rates of methamphetamine (METH) abuse and associated mental health disorders. Recently, a global report identified METH as one of the most commonly abused drugs around the world (UNODC Global Synthetic Drugs Assessment, 2014). Evidently, excessive sugar intake and METH abuse are major threats to public health in today's society.

Excessive sugar intake and METH use may not be directly related; however, the effects of these two health problems do share similar fundamental neural and behavioural characteristics (Kelley et al., 2002; Vanderschuren & Kalivas, 2000). The neural mechanisms within the nucleus accumbens (NAc) brain region is critical in regulating food motivation and consumption, as well as mediating feeding reward processes (Alsio et al., 2010; Kelley et al., 2005). Similar neuronal networks in the NAc are also stimulated by METH use, which trigger rewarding effects (Koob, 1992) and the formation of drug-seeking behaviours (Parsegian & See, 2014). Since both sugar and METH administration engage similar neural pathways in the NAc, it is possible that pre-exposure to excessive sugar may prime the neurons within the NAc making them more sensitive to the effects of METH (Avena & Hoebel, 2003). Such a relationship between chronic sugar intake and METH exposure has been little studied and there has been limited research investigating the molecular processes underlying this cross-sensitisation relationship between sugar and METH. A greater understanding of the molecular mechanisms regulating these health concerns will allow for the development and implementation of effective treatment and preventative strategies to combat against the development of reward-related behaviours.

1.1 Brain Circuitry Involved in Reward and Motivation

The behavioural experience of reward and motivation is mainly dictated by the mesocorticolimbic dopamine system (Koob, 1992). Theoretically, the behavioural states of reward and motivation in humans are difficult to conclusively define in a neurobiological manner because it is hard to determine a holistic process of the brain in inducing such behaviours from isolated neurobehavioural mechanisms (Robbins & Everitt, 1996; Salamone et al., 2007). However, as an operational definition, reward-related and reward-motivated behaviours can be well-explained through the neurobehavioural processes of the mesocorticolimbic dopamine system (Koob, 1992). This system originates from the ventral tegmental area (VTA) and sends dopaminergic projections to forebrain regions, including the prefrontal cortex (PFC) and the NAc (Koob, 1992). When the dopamine cells of the VTA are stimulated by powerful external stimuli such as sugar or drugs of abuse, dopamine is released in an altered manner that elicits differential reward-related responses in the PFC and NAc (Koob, Sanna, & Bloom, 1998). In the PFC, the excessive release of dopamine initiates reward-dependent behavioural processes underlying addiction and dependence (Robbins & Everitt, 1996), including habit-formation, poor decision-making and compulsive behaviours (Lyvers, 2000; Hester & Garavan, 2004). In contrast, altered dopamine release in the NAc triggers the development of early reward-motivated learning processes that facilitate the development of reward-related behaviours, such as locomotor approach behaviours towards stimuli producing positive affect and heightened energy in response to rewarding stimuli (Salamone & Correa, 2002; Robbins & Everitt, 1996).

1.1.1 The functional-anatomical characteristics of the nucleus accumbens

The nucleus accumbens (NAc), forming part of the striatum, is located in the basal forebrain and belongs to various connecting anatomical and neuronal pathways, such as the mesocorticolimbic reward pathway (David, 2008, p.2–4). Additionally, the NAc can be separated into three anatomically and functionally distinct domains, including the core, the shell and the rostral pole (Hanlon, Baldo, Sadeghian, & Kelley, 2004). The core and the shell

of the NAc have been thoroughly examined in locomotor and reward processes due to their neurochemical connections to critical brain regions, such as the PFC and VTA (Zhang, Balmadrid, & Kelley, 2003).

The core receives afferent projections directly from the VTA, PFC, hippocampus and amygdala (Robbins & Everitt, 1996). Consequently, the neuronal signals from these brain regions mediate the formation of reward-related learning and memories, and facilitate motivational responding to novel stimuli and contexts (Kelley et al., 2005). Furthermore, the core is an integral component in controlling motor functions through its neural connections to the basal ganglia (Kelley et al., 2005). In contrast, the shell subregion of the NAc receives afferent connections from the prelimbic and infralimbic cortices of the PFC and the amygdala which conveys information about the functions of visceral mechanisms and flavour (David, 2008, p.16; Kelley et al., 2005). Additionally, internal homeostatic information is relayed from the lateral hypothalamus to the NAc shell in order to modulate feeding-related behaviours (Kelley et al., 2005). From the shell, efferent projections are sent to the hypothalamic circuitry to initiate motor functions and autonomic arousal (Kelley et al., 2005). In conjunction with the lateral hypothalamus, the efferent projections from the shell to the ventral pallidum (VP) integrate and associate reward-directed motor behaviours (Smith & Berridge, 2007). Although it seems that the NAc shell and core are anatomically and functionally distinct, both subregions send and receive neuronal signals to overlapping brain regions in order to regulate behaviours related to reward, motor functions, reinforcement and motivation (Kelley et al., 2005). Such overlapping brain regions include the amygdala, VTA, hippocampus, lateral hypothalamus and VP (David, 2008, p.16-18).

The NAc consists of various distinct neuronal networks that can be differentiated by the functions they subserve and their connective projections. These neuronal systems include the γ-aminobutyric acid (GABA), glutamatergic, dopaminergic and endogenous opioid systems (Blumenthal & Gold, 2010). The NAc mainly consists of medium-spiny neurons (MSNs), which primarily contain GABA, glutamate and dopamine receptors (David, 2008,

p.4–6). The GABAergic system mediates fast inhibitory synaptic transmission (Greengard, 2001). This system contains the GABA A and GABA B receptors which coordinate motor approach behaviours by regulating synaptic transmission efficiency of the dopaminergic and glutamatergic systems (Georgetti et al., 2002).

In contrast, the glutamatergic system is responsible for regulating fast excitatory synaptic transmission through the functions of its receptors, including the *N*-methyl-D-aspartate (NMDA) receptors (Greengard, 2001). The activation of the NMDA receptors in the corticostriatal pathway modulates the formation of durable reward-related behaviours and motivational drive through its governing function in neuronal development and synaptic plasticity (Miyazaki et al., 2013; Parsegian & See, 2014). Additionally, since the NMDA receptors target the GABA receptors, direct interactions between these neural systems may trigger the development of long-term depression (LTD) of inhibitory synaptic transmission (Luscher, Fuchs, & Kilpatrick, 2011). As a result, the glutamate receptors have been identified as one of the key substrates involved in synaptic transmission and plasticity (Reith, 2002, p.281).

Entangled in the functions of the glutamatergic system is the dopaminergic system. This system contains the dopamine D1 and D2-like families of receptors which are involved in modulating the levels of extracellular dopamine through the dopamine transporter (DAT), the primary structure for dopamine reuptake (Grimm, Shaham, & Hope, 2002). The dopamine D1 and D2 receptors of the NAc MSNs are fundamental in modulating various neuronal mechanisms, including glutamatergic signalling (Wise, 2002). Consequently, dopamine facilitates the synaptic strength and transmission of the glutamatergic neurons (Gerdjikov et al., 2004; Valjent et al., 2005). This integrative signalling activity of dopamine and glutamate is controlled by the DA- and cAMP-regulated phosphoprotein (Mr 32 kDa, DARPP-32) (Rauggi et al., 2005). These interactive properties of dopamine in the NAc highlights that it is an important neuronal system underlying motivation and reinforcement, reward-related learning and goal-directed behaviours (Avena, Rada, & Hoebel, 2008).

In addition to glutamate, the dopaminergic system also plays a neuromodulatory function over the endogenous opioid system (Spanagal, Herz, & Shippenberg, 1992). The endogenous opioid system consists of the opioid peptides, dynorphin and enkephalin, which are synthesised by the preproenkephalin and preprodynorphin genes (Spangler et al., 2004). These peptides are highly co-expressed with dopamine in the NAc. Due to this co-expression, the dopamine and opioid receptors (mu, delta and kappa) functionally interact with each other in the NAc to produce psychological states of reward and pleasure, which in turn influences hedonic-driven behaviours (Avena, Rada, & Hoebel, 2008).

1.2 Rewarding and Motivational Aspects of Sugar

In today's western society, sugar-rich foods and drinks are easily and readily available at a very low cost. As a result, this ease in accessibility has contributed to the alarming finding that children and adolescents are the highest groups to consume sugar at excessive amounts (AIHW Australia's Food & Nutrition, 2012). Although the long-term physical implications have been greatly examined, the long-term neural and behavioural implications are yet to be discovered. Despite sugar being a natural reward, consuming sugar in excess can alter the neuronal mechanisms in the brain that liken the rewarding effects of drugs of abuse (Avena, Rada, & Hoebel, 2008). These neural alterations may contribute to the development of maladaptive behaviours.

Initially, much research concluded that the neurotransmitter dopamine may be the driving force in regulating the motivational and rewarding aspects associated with sugar intake (Salamone & Correa, 2002). Release of dopamine to act at dopamine receptors in the NAc is triggered by the consumption of sugar (Muscat & Willner, 1989). Consequently, this produces a psychological state of reward and pleasure, which may be responsible in developing preferences for sweet tastes. This preference for sweets, in turn, stimulates consummatory and approach behaviours for the sugar reward (Salamone & Correa, 2002). For instance, Rada and colleagues (2005) demonstrated that, in food-restricted rats, the daily intermittent exposure to sugar significantly heightened extracellular dopamine levels in the

NAc. Hence, these accounts suggested that the dopamine system may be involved in triggering motivational behaviours, such as locomotor approach behaviours, in response to sweet preferences (Ikemoto & Panksepp, 1996). Accordingly, the pleasurable qualities associated with the sweet taste may activate the excessive consumption of sugar (Berridge & Robinson, 1998).

An equally significant neurotransmitter involved in the motivational and rewarding properties associated with sugar consumption is the opioid system. The endogenous opioid system has been linked with producing pleasurable sensations associated with the consumption of sugar-rich substances (Salamone & Correa, 2002). Recent research has demonstrated that blockade of the endogenous opioid receptors in the NAc reduced the consumption of the sugar substance while leaving motivational behaviours for the sugar reward intact (Barbano & Cador, 2006). These findings highlight that, in combination with the dopamine system, the endogenous opioid system may be responsible for encoding preferences for sweet tastes (Barbano & Cador, 2006). Thus, once opioid receptors are activated in response to a sweet taste, dopamine receptors may be triggered in order to initiate reward-directed locomotor behaviours that are motivated by pleasure and palatability (Zhang & Kelley, 2002).

The excessive consumption of sugar can also induce enduring alterations in other neural mechanisms to contribute to the development of more persistent and durable behaviours, such as heightened reward-seeking behaviours (Tukey et al., 2013). One prominent neurotransmitter involved in the activation of enduring synaptic changes is glutamate, which is co-activated with dopamine receptors (Berridge & Robinson, 1998; Baldwin et al., 2002). For instance, the repeated administration of sugar has been recently shown to elevate stable levels of extrasynaptic glutamate (Tukey et al., 2013) and together with the activation of dopamine, induces enduring changes in the synaptic and neuronal morphology of the glutamate system (Bello et al., 2003; Baldwin et al., 2002). Accordingly, these neuroadaptative alterations in both the dopamine and glutamate systems contribute to

persistent behavioural responses, such as reward-seeking behaviours and excessive consumption of sugar, that have been previously encoded by the combined functions of the opioid and dopamine receptors (Kelley, 2004; Baldo & Kelley, 2007).

1.2.1 Functions of the nucleus accumbens: consequences of sugar consumption

The functional role of the NAc dopaminergic system in regulating the rewarding and motivational properties of sugar intake has ignited much controversy. Initially, general conclusions from earlier work suggested that dopamine in the NAc is involved in activating pleasurable appraisal for sweet rewards (Salamone & Correa, 2002). However, further analysis has demonstrated that dopamine in the NAc may be important in activating "incentive motivation" for sweet rewards (Ikemoto & Panksepp, 1999). In other words, the NAc dopaminergic system may play a fundamental role in engaging primary reward-related learning processes and facilitating motor approach behaviours (Salamone et al., 2005). For instance, the presentation of a novel sugar reward can elevate dopamine release in the NAc (Gambarana et al., 2003); and blockade of this heightened dopamine activity with dopamine D1 receptor antagonists in the NAc can subsequently reduce the acquisition of learning sugar flavour preferences (Touzani, Bodnar, & Sclafani, 2008). In contrast, inhibiting the activity of the dopamine receptors in the NAc does not impair sucrose consumption, but instead it impairs locomotor approach behaviours for sucrose (Ikemoto & Panksepp, 1996). Based on such findings, the current consensus is that the NAc dopamine system is critical in initiating the development of reward-related learning processes in response to novel sugar rewards (Baldo & Kelley, 2007) to drive motivational activation of locomotor behaviours in order to acquire the sugar reward (Baldo et al., 2002).

In contrast to the functions of the dopaminergic system, the endogenous opioid system in the NAc is reported to regulate the pleasurable sensations associated with the consumption of palatable foods (Salamone & Correa, 2002). The inhibition of opioid, but not dopamine, receptors in the NAc reduced the consumption of food while leaving anticipatory behaviours for the palatable substance intact (Barbano & Cador, 2006). This distinct function of the NAc

endogenous opioid system is regulated by the μ-opioid receptors whereby the stimulation of these receptors in the NAc activates feeding behaviours motivated by pleasure and palatability (Zhang & Kelley, 2002). These findings suggest that there may be discrete neural systems in the NAc involved in modulating specific reward-related feeding behaviours. Specifically, the NAc dopaminergic system may be involved in assigning positive motivational value to sweet rewards to regulate food-seeking behaviours, while the endogenous opioid system may be responsible in activating the hedonic properties associated with palatable stimuli (Hanlon et al., 2004).

However, there is evidence to suggest that both neuronal systems may be functioning in an interactive manner to control behaviours associated with the consumption of sugar as it is difficult to discern at which point the release of dopamine terminates and the release of the opioids begin (Baldo & Kelley, 2007). For example, Spanagel and colleagues (1992) demonstrated that the stimulation of the μ-opioid receptors in the VTA elevated the release of dopamine in the NAc. Correspondingly, investigations into the behavioural implications of consuming sugar-rich substances demonstrated that the stimulation of opioid neurons within the NAc-VP circuit generated a behavioural state of pleasure in response to the sugar reward, which in turn activated sugar-seeking motor behaviours (Smith & Berridge, 2007). In addition, in response to novel experiences with sugar, the NAc dopaminergic and endogenous opioid systems may both be recruited to encode a stable link between the sweet substance and the behavioural states of reward and pleasure (Baldo & Kelley, 2007). This may stimulate enduring locomotor behaviours to consume the rewarding substance every time it is presented. Therefore, the excessive consumption of sugar may induce long-term alterations in the functioning of the interacting neuronal systems within the NAc. As a result, this may stimulate enduring reward-directed behaviours (Salamone & Correa, 2002).

Given, the extensive focus directed toward the dopamine and opioid systems, the involvement of other neuronal mechanisms within the NAc associated with sugar reward has been less studied. For example, it is possible that the serotonergic and endocannabinoid

systems within the NAc may be functioning in conjunction with the dopaminergic and opioid systems to regulate reward-motivated behaviours for sugar (Salamone et al., 2005) as both systems have been implicated in sugar feeding behaviours (Kirkham et al., 2002; Pratt et al., 2009). If excessive exposure to sugar consumption impairs the functioning of even one of the neuronal systems in the NAc, this can subsequently have knock-on effects for other neurotransmitters. This, in turn, can potentially trigger long-term maladaptive reward-seeking behaviours in order to achieve behavioural states of reward and pleasure.

1.2.2 Functions of the nucleus accumbens: the development of reward-related dependence

A primary element that initiates the development of reward-related dependence is "bingeing". The behavioural hallmarks of sugar bingeing include the escalation of sugar intake combined with a high proportion of consumption during the presentation of sugar. These characteristic bingeing behaviours are accompanied by distinct alterations in neuronal functioning within the NAc (Avena et al., 2008). For instance, in food-restricted rats, the daily intermittent exposure to sugar can significantly heighten extracellular dopamine and delayed the release of acetylcholine in the NAc (Avena et al., 2008; Rada et al., 2005). Specifically, sugar consumption primarily targets the dopamine D1 and D2 receptors by upregulating the dopamine D1 receptors and reducing the density of the dopamine D2 receptors (Colantuoni et al., 2001; Bello et al., 2002). These changes in dopamine signalling may be due to alterations in DAT binding in the NAc (Bello et al., 2003) altering dopaminergic tone to augment the motivational value and rewarding properties attached to sugar, which may trigger persistent reward-seeking behaviours (Hajnal & Norgren, 2002; Bello et al., 2002).

An enduring implication of sugar bingeing is the development of craving. Craving emerges when motivation for the sugar reward is heightened due to a period of abstinence (Avena et al., 2008). The "deprivation effect" paradigm is used to investigate behaviours associated with craving. This involves training rats to self-administer sucrose and subsequently depriving them of the sucrose solution for a substantial period of time (i.e.

abstinence period). After the abstinence period, rats are allowed to self-administer the sucrose solution, which indicates the subjects' motivation for the sugar reward (Avena, Long, & Hoebel, 2005). Interestingly, findings using this paradigm demonstrate that long periods of daily sugar exposure (6 or 12 hours) heighten responding for the sugar reward after a period of abstinence (Avena et al., 2005; Grimm et al., 2005). These findings suggest that, due to the persistent neuronal imbalances firstly induced by bingeing and withdrawal, the rewarding and motivational value of sugar has become excessively magnified along with the cues and contexts linked with the presentation and consumption of sugar (Grimm, Fyall, & Osincup, 2005). Consequently, when the sugar substance is removed, heightened reward-seeking behaviours may be activated in order to produce feelings associated with reward and pleasure (Avena et al., 2008).

Dopamine regulation of the NAc has been postulated to play a prominent function in triggering craving-related behaviours, such as activating motivational reward-seeking behaviours when sugar or cues associated with its consumption are presented (Volkow et al., 2008). This is due to its involvement in initiating the development of early motor learning behaviours in response to the rewarding properties connected with sugar consumption (Baldo & Kelley, 2007). However, Grimm and colleagues (2002) demonstrated that behaviours evident during the abstinence period, such as heightened motivation and sugar-seeking responses, were not accompanied by alterations in tyrosine hydroxylase (rate-limiting enzyme in dopamine production) functioning and DAT expression in the NAc. Instead, due to the interactive functions of the dopaminergic and glutamatergic neuronal systems within the NAc, glutamate receptors may be recruited to encode more durable behavioural adaptations associated with sugar reward and motivation (Kelley, 2004). For instance, the repeated consumption of sugar altered the expression of glutamate in the NAc, which could be later stimulated by re-exposure to sugar (Tukey et al., 2013). This long-term potentiation of glutamate was accompanied by heightened locomotor activity in response to sugar consumption (Tukey et al., 2013). These findings suggest that prolonged sugar bingeing may

reconfigure the signalling responses and temporal coordination of the NAc dopaminergic and glutamatergic systems (Kelley, 2004). As a result, this synaptic plasticity within the neuronal mechanisms of the NAc contributes to the expression of augmented reward-motivated behaviours (Tukey et al., 2013). These synaptic alterations may encode an exaggerated value attached to sugar in order to produce coordinated motor behaviours to procure the reward (Kelley, 2004).

However, there is much controversy surrounding the plausibility for sugar craving because studies using human participants demonstrate that individuals most commonly indicate cravings for savoury foods, such as pizza, and for sweet/fatty products, including chocolate (Weingarten & Elston, 1991). It is very rare for individuals to actually indicate specific cravings for pure sugar. But it is possible that the reported cravings for such foods may be mistaken for craving for tryptophan, which functions as an amino acid precursor for serotonin (Fortuna, 2010). Since tryptophan is scarcely found in food and must also compete with several other amino acids in order to enter the brain, foods containing high amounts of tryptophan will have greater priority in accessing the brain (Fortuna, 2010). Interestingly, foods containing tryptophan, such as wheat products and chocolate, are commonly reported as the food items mainly craved (Benton, 2010). However, human research fails to take into account that the metabolic implications associated with food consumption also interact with the neural processes underlying the rewarding and motivational effects of palatable food consumption. As a result, it is difficult to determine if craving-related behaviours are triggered solely due to neuronal mechanisms or due to the combined functioning of the metabolic effects and neural processes involved in palatable food consumption. Thus, although animal research is able to isolate the neural functions involved in triggering craving-related behaviours, this research may not validly reflect behaviours specific to sugar craving.

1.3 The Neural and Behavioural Implications of Methamphetamine Use

Psychostimulant abuse, such as METH, is a significant societal problem that is affecting physical and psychological health globally. The use of psychostimulants can alter the function of the neuronal networks within the mesocorticolimbic system. As a result, the continuous stimulation of these neuronal networks can induce enduring neuroadaptations in the brain regions forming the mesocorticolimbic reward system (Wakida et al., 2014). These synaptic changes, in turn, contribute to dysfunctional and maladaptive behavioural responses, such as anxiety and psychosis (Toda, McGinty, & Kalivas, 2002; Krasnova et al., 2010). Methamphetamine, a powerful psychostimulant derivative of amphetamine, elicits its effects on monoaminergic neurotransmitters (including dopamine, serotonin and epinephrine) and glutamate receptors within the mesocorticolimbic reward pathway (Narita et al., 2004).

Methamphetamine is a synthetic psychostimulant drug that is characterised as a neurotoxin, which is capable of altering levels of dopamine release (Krasnova et al., 2010; Vandershuren & Kalivas, 2000). Specifically, METH enhances the release of extracellular dopamine by binding and reversing the dopamine transporter (Ritz et al., 1987; Vanderschuren & Kalivas, 2000). This effect of METH elevates the amount of dopamine available within the synaptic cleft affecting brain regions forming the reward pathway, including the NAc and PFC (Toriumi et al., 2014). The acute administration of METH immediately induces altered psychological states, such as euphoria and heightened energy, due to this heightened availability of dopamine (Robinson & Berridge, 2001; Martin et al., 2012). These immediate responses may reinforce the repeated use of the drug in order to achieve a state of intense reward and euphoria (Hyman, Malenka, & Nestler, 2006). The excessive and long-term use of METH recruits other neuronal systems, such as glutamate, to produce more durable dysfunctional behavioural responses such as drug dependence and drug-seeking behaviours (Parsegian & See, 2014).

In a similar manner to dopamine, METH heightens the amount of glutamate in the brain regions involved in inducing reward and sensitisation, including the VTA and the NAc

(Vandershuren & Kalivas, 2000). With the repeated and excessive use of METH, the continuous stimulation of the glutamate and dopamine receptors induces neuroplastic changes within the mesocorticolimbic system (Berridge & Robinson, 1998; Kalivas et al., 2005). As a consequence of these maladaptive neurological responses, long-lasting dysfunctional behavioural patterns are reinforced and maintained, such as habitual drug-seeking behaviours and behavioural sensitisation (Goldstein et al., 2009). Therefore, the initial state of reward and pleasure induced by dopamine reinforces the use of METH and may lead to the gradual and persistent use of the drug due to the neuroadaptations of the glutamate and dopamine receptors in the mesocorticolimbic reward system.

1.3.1 The neural and behavioural implications of methamphetamine use in the nucleus accumbens

Methamphetamine administration hijacks the neural systems involved in processing natural rewards, which subsequently facilitates motivation for these rewards. As a result, the synthetic effects of METH inhibit these neural pathways from adaptive functioning by forcing their activation in response to the drug (Blumenthal & Gold, 2010). These neural pathways involved in generating the acute effects of METH, as well as natural rewards, are mediated by the activation of the dopaminergic system in the VTA-NAc pathway (Nestler, 2001). More specifically, acute METH administration enhances synaptic levels of dopamine in the NAc by inhibiting the activity of the DAT and stimulating the release of dopamine into the synapse (Panenka et al., 2013). Consequently, this produces powerful behavioural effects including a heightened state of arousal, increased energy and euphoria (Steinkellner et al., 2011). The rapid uptake of METH combined with a slow rate of clearance from the striatum contributes to its reinforcing properties (Davidson et al., 2005; Fowler et al., 2008).

Additionally, the reinforcing effects of METH are mediated by the availability of the DAT (Fowler et al., 2008). For instance, Fukushima and others (2007) demonstrated that the acute administration of METH failed to induce heightened locomotor activity in mice lacking the DAT. This suggests that acute METH administration alters the functions of DAT

trafficking in the synapse, which subsequently changes dopaminergic tone by enhancing extracellular dopamine levels (Schmitt & Reith, 2010). Evidently, the distinct functions of the DAT in regulating the reinforcing properties of acute METH administration parallel the effects of sugar. Similar to METH, sugar consumption alters NAc dopaminergic tone by enhancing the activation of striatal DAT binding (Bello et al., 2003). Hence, it is possible that sugar may share a common neural pathway as METH to generate dysfunctional behavioural responses for the substances.

The continued use of METH can alter synaptic plasticity of neuronal pathways within the NAc. This, in turn, promotes enduring neuronal changes that contribute to persistent drug-seeking behaviours (Hyman, Malenka, & Nestler, 2006). Since METH induces powerful effects in the NAc dopaminergic system, the chronic use of METH primarily activates durable synaptic changes within this system (Fowler et al., 2008). For example, a recent study modelling METH addiction demonstrated that extended METH self-administration induced tolerance to the extracellular increases of dopamine in the NAc (Le Cozannet, Markou, & Kuczenski, 2013). That is, long-term access to METH intake promotes the development of neurochemical adaptations within the NAc, which may contribute to heightened drug-seeking and drug-taking behaviours (Le Cozannet et al., 2013). A surprising effect of these neuronal adaptations is that after 21 days of withdrawal, the NAc dopaminergic system is still sensitive to the synthetic effects of METH (Lominac et al., 2012). Hence, the long-term intake of METH produces neurochemical adaptations within the NAc dopaminergic system by altering the synaptic plasticity. This, in turn, dictates the neuronal reorganisation of the dopaminergic system to produce persistent reward-directed behaviours (Hyman, Malenka, & Nestler, 2006).

Furthermore, due to the interactive relationship between the NAc dopaminergic and glutamatergic systems, the combined activation of the glutamatergic and dopaminergic neuronal mechanisms is critical in developing enduring synaptic plasticity (Baldwin et al., 2002). Consequently, the neurochemical adaptations associated with METH administration can activate the formation of reward-related learning and reward-motivated behaviours

(Lominac et al., 2012). In particular, the long-term administration of METH can alter the homeostasis of the NAc glutamatergic and dopaminergic systems by decreasing basal glutamate levels (Parsegian & See, 2014). As a result, this generates the formation of enduring glutamate and dopamine system sensitisation such that when cues associated with METH intake are presented, reward-seeking and drug-taking behaviours become activated (Parsegian & See, 2014; Lominac et al., 2012). Evidently, these findings suggest that chronic METH intake can change the morphology of the NAc dopaminergic and glutamatergic neuronal systems through altering the dendritic profile of the neuronal networks and structural reorganisation of the synaptic connections (Robinson & Kolb, 1999). Accordingly, changes in synaptic morphology promote the development of reward-related learning, which subsequently activates drug-taking and drug-seeking behaviours (Kelley, 2004). Interestingly, the co-activation of the NAc dopaminergic and glutamatergic systems parallel sugar-induced reward-related learning which suggests that the behaviours associated with the excessive consumption of sugar may be functioning in the same neuronal networks as METH (Kelley, 2004).

1.4 The Neural and Behavioural Effects of Sugar Cross-Sensitisation with Psychostimulants

With chronic consumption of sugar, it is possible that individuals can become sensitised to the rewarding effects of subsequent exposure to psychostimulants (Avena et al., 2009). This is defined as cross-sensitisation, and the presence of this phenomenon suggests that the neurobehavioural consequences associated with sugar consumption may be operating in a similar manner as psychostimulant drugs (Alsio et al., 2012). For instance, Avena and Hoebel (2003) demonstrated that rats pre-exposed to intermittent sugar access were more likely to exhibit heightened motor activity (cross-sensitisation) in response to amphetamine administration (Avena & Hoebel, 2003). These findings highlight that psychostimulants, such as amphetamine, may be functioning in overlapping neuronal systems as sugar (Avena & Hoebel, 2003). In particular, both sugar and amphetamine may be eliciting their effects on the

NAc dopaminergic and glutamatergic systems (Kelley, 2004; Avena & Hoebel, 2003). Both substances may induce structural changes in the synaptic morphology such that the neuronal receptors have become sensitive to the effects of other substances targeting those specific receptors (Robinson & Kolb, 1999; Avena et al., 2008). As a result, after the conclusion of excessive sugar consumption, the NAc dopaminergic and glutamatergic neurons may become sensitive such that the immediate rewarding effects associated with the acute intake of psychostimulants may be exaggerated (Avena et al., 2008; Kelley, 2004). These immediate behavioural effects may be extremely intense, which can subsequently reinforce persistent drug use (Steinkellner et al., 2011).

However, in comparison to the underlying mechanisms involved in drug abuse, the neural and molecular processes involved in the development of sugar dependence are much less understood. This is primarily due to the discrepancies in the methodologies used to model sugar consumption and the presence of greater heterogeneity in sugar preferences (Nestler, 2005). As a result, it is difficult to conclusively identify the specific neural and molecular pathways functioning to induce the rewarding and motivational effects of sugar consumption. Based on these drawbacks, there is much debate concerning the credibility of the cross-sensitisation between sugar and psychostimulants. Recently, Cameron and Carelli (2012) indicated that the expression of neural phasic activity in the NAc for cocaine intake was distinctly different to the pattern of cell activation coding for sucrose intake. These findings emphasise that psychostimulants may not elicit the same activational pattern as sugar to code the rewarding value for the substance (Cameron & Carelli, 2012). For example, sugar and cocaine may stimulate the release of dopamine in the NAc, yet the underlying processes involved in the development of neuronal adaptations may be functionally distinct (Benton, 2010; Di Chiara, 2002; Vendruscolo et al., 2010).

Although the neuronal adaptations associated with cocaine and sugar intake may be functionally distinct within the NAc, other psychostimulants, including METH, may activate parallel neuroadaptive processes within the NAc. In contrast to cocaine, the uptake of METH

is rapid, and this is followed by a slow rate of clearance from cortical grey matter (Fowler et al., 2008). This pattern of neuronal activation may be more receptive to the enduring changes induced firstly by excessive sugar consumption, which may in turn produce more pronounced behavioural states of reward after subsequent METH exposure (Alsio et al., 2012). Individuals with disordered eating behaviours, such as bulimia and obesity, show a selective preference for sugar-rich foods, and will often engage in reward-seeking and consummatory behaviours for such foods (Fortuna 2010). Neuroimaging techniques have demonstrated that these individuals exhibit a biological marker, called the A1 allele, which is strongly associated with attenuated dopamine D2 receptor binding and heightened sensitivity for reward (Davis et al., 2008). Interestingly, this genetic marker has also been implicated in METH dependence, suggesting that the neuronal processes of sugar consumption and METH intake may be primarily targeting the dopaminergic structures (Fortuna, 2010). Hence, the neuronal adaptations involved in sugar consumption may be functioning through a similar mechanism involved in METH intake. This may subsequently promote cross-sensitisation (Avena et al., 2008).

1.5 Molecular and Proteomic Research Examining the Effects of Sugar consumption or Methamphetamine use

1.5.1 Molecular and proteomic analysis of the effects of sugar consumption

Chronic sugar consumption can induce long-term molecular adaptations within the NAc through activating various neuronal mechanisms. This, in turn, may induce stable reward-seeking behaviours due to a heightened sensitivity for rewarding stimuli (Nestler & Aghajanian, 1997). The NAc dopaminergic system is a prominent neural target influencing these molecular adaptations due to its role in activating various second messengers, including cAMP (Kelley, 2004). Due to alterations in dopaminergic tone in response to excessive sugar intake, the expression of dopamine-activated cAMP may be upregulated in the NAc (Chao & Nestler, 2004; Bello et al., 2003). Consequently, this altered expression of cAMP triggers protein kinase A (PKA) signalling in a way that alters the functioning of further downstream

molecular targets, such as DARPP-32 and the cAMP-response-element-binding (CREB) protein (Heyser et al., 2013).

DARPP-32 is a major protein that is controlled by the cAMP-dependent-PKA pathway (Rauggi et al., 2005). The regulatory functions of DARPP-32 are determined by the phosphorylation of the threonine residue (Valjent et al., 2005). Through the dopamine-activated PKA pathway, the residue threonine-34 (Thr34) is phosphorylated on to DARPP-32 which converts it into an inhibitor for protein phosphatase-1 (PP1) (Chen & Chen, 2005). Interestingly, Rauggi and others (2005) demonstrated that consumption of a novel sugar reward heightened the expression of phosphorylated-Thr34 DARPP-32 (DARPP-32/Thr34) in the NAc. These findings suggest that sugar can modify the activation of DARPP-32 to function as a phosphatase inhibitor (Chen & Chen, 2005). Consequently, this activation may modify the homeostatic functions performed by PP-1, such as the regulation of membrane receptors, cell progression and neuronal activities (Heyser et al., 2013; Chiang & Chen, 2007). As a result, these altered molecular functions may encode an exaggerated rewarding and motivational value for sugar, which may foster the development of reward-seeking behaviours (Rauggi et al., 2005).

Additionally, since PKA activates CREB, the excessive release of dopamine in response to chronic sugar consumption may alter the transcription and synthesis of CREB (Baldwin et al., 2002). As a result, molecular changes in the physiological activity of CREB may encourage persistent approach and appetitive behaviours due to the heightened reward value of sugar (Kelley, 2004). For example, the overexpression of CREB in the NAc reduced motor responding, whereas the presence of the mutated CREB variant, which prevents the activation of CREB, heightened locomotor activity for the sugar reward (Barrot et al., 2002). These findings suggest that CREB is a key contributor in the formation of motor learning behaviours for rewarding substances (Kelley, 2004). Hence, alterations originating from the NAc dopaminergic system in response to sugar consumption can induce enduring molecular

adaptations through the functioning of PKA and associated processes (Rauggi et al., 2005; Baldwin et al., 2002).

Another significant molecular substrate implicated in the behavioural processes associated with excessive sugar consumption is ΔFosB. The transcription factor, ΔFosB is rapidly stimulated in response to the presentation of stimuli; however, the expression of ΔFosB also persists long after other molecular substrates have returned to regular levels, which makes it an important factor in triggering enduring modifications to the functions of various molecular substrates and in producing long-term behavioural changes (Chao & Nestler, 2004; Nestler & Aghajanian, 1997). It has been shown that the excessive consumption of sugar heightens the expression of ΔFosB levels in the NAc and that elevated expression of ΔFosB levels in the NAc triggers an increase in sugar consumption (Wallace et al., 2008). These findings suggest that ΔFosB activation may regulate the synaptic morphology and functions within the NAc, which thereby contributes to the development of reward-directed behaviours (Neslter, Barrot, & Self, 2001). An alarming aspect of these findings is that since ΔFosB is a transcription factor, the long-term activation of altered ΔFosB levels may induce persistent modifications to the physiological activity of downstream genetic factors (Nestler et al., 2001). Overall, these biological and molecular adaptations can trigger behavioural plasticity to promote persistent reward-directed behaviours (McClung et al., 2004).

Previous research has thoroughly examined the neurobehavioural effects of chronic sugar exposure, yet there has been limited research examining the protein profile of key brain areas associated with long-term sugar consumption. Proteomic analysis is an effective tool for examining the protein properties of the whole cell within a given brain region (van den Oever et al., 2006). Studies investigating the effects of chronic sugar consumption using proteomic analysis have mainly focused on the hippocampus and medial prefrontal cortex (mPFC). For example, the chronic consumption of a high fat and refined sugar diet altered the expression of hippocampal proteins involved in energy metabolism, synaptic assembly, and cellular

maintenance and functioning (Francis et al., 2013). Correspondingly, within the mPFC, the self-administration of sugar induced changes in the expression of proteins responsible for regulating cytoskeletal organisation, oxidative stress, neurotransmission, energy metabolism and neuronal growth (van den Oever et al., 2006). These findings suggest that the chronic consumption of sugar as part of a high-fat diet can produce enduring molecular adaptations in the structural morphology, synthesis and functional activities of cells, neuronal processes and synaptic plasticity within the brain (Francis et al., 2013; van den Oever et al., 2006). Enduring changes in these proteins may consequently contribute to persistent negative behavioural responses, such as poor cognitive functioning, lack of inhibitory control, reward-seeking behaviours and symptoms of anxiety (Francis et al., 2013; van den Oever et al., 2006).

1.5.2 Molecular and proteomic analysis of the effects of methamphetamine use

The acute exposure to METH elevates the levels of dopamine in the NAc through the combined stimulation of dopamine release and reversal of the DAT (Wallace, Gudelsky, & Vorhees, 1999). Accordingly, the increase in dopamine signalling at dopamine D1 receptors triggers activated PKA and downstream molecular targets, including DARPP-32 and CREB (Baldwin et al., 2002; Cadet et al., 2014; Miyazaki et al., 2013). In particular, PKA activation of DARPP-32/Thr34 has been recognised as a critical element in regulating learning and plasticity associated with chronic METH administration (Chen & Chen, 2005). For example, Valjent and colleagues (2005) demonstrated that amphetamine administration activated extracellular-signal-regulated kinases (ERK) within the NAc; but this ERK activation was prevented in mice lacking the phosphoprotein DARPP-32/Thr34. The ERK pathway has been implicated in the formation of reward-related learning by facilitating long-term potentiation and synaptic plasticity within the NAc dopaminergic and glutamatergic systems (Gerdjikov et al., 2004; Mazzucchelli et al., 2002). This suggests that DARPP-32/Thr34 is a key component in regulating the activation of ERK (Valjent et al., 2006). As a result, triggering the ERK pathway via DARPP-32/Thr34 may be fundamental in establishing behavioural sensitisation

to psychostimulants, such as amphetamine and METH, by inducing long-term synaptic adaptations within the NAc neuronal systems (Valjent et al., 2005).

The activation of the ERK pathway can trigger the phosphorylation of multiple transcription factors to directly or indirectly activate the expression of immediate early genes, such as the Fos family (Valjent et al., 2006), including ΔFosB, which has been implicated in both the acute and chronic administration of METH (Chao & Nestler, 2004). Following the acute administration of METH, the expression of ΔFosB is rapidly elevated within the NAc for a period of 2 hours and subsequently returns to basal levels (Martin et al., 2012). This increase in ΔFosB heightens sensitivity to the rewarding and locomotor-activating properties of METH (Nestler, Barrot, & Self, 2001) and may reinforce the chronic use of METH in the short term. In contrast, the chronic use of METH induces the long-term expression of heightened ΔFosB that persists for as long as 14 days without the use of the drug (McDaid, Graham, & Napier, 2006). Since ΔFosB is a transcription factor, the persistent expression of heightened ΔFosB within the NAc may mediate the enduring neural and behavioural adaptations associated with METH dependence by altering the expression of its target genes to promote reward-seeking behaviours (Chao & Nestler, 2004; Nestler et al., 2001). Hence, due to the stability of ΔFosB expression, this transcription factor has been postulated as a molecular switch that facilitates the progression from acute drug use to chronic drug use by inducing neural and behavioural plasticity (Chao & Nestler, 2004).

An interesting aspect of ΔFosB is its regulatory ability to activate the transcription of cyclin-dependent kinase 5 (Cdk5), which is an essential kinase involved in the regulation of neurodevelopment, neurodegeneration, neuronal cytoarchitecture, synaptic functioning and dopamine signalling in the NAc (Chao & Nestler, 2004; Dhavan & Tsai, 2001). The activation of Cdk5 initiates the phosphorylation of the residue threonine 75 on to DARPP-32 (DARPP-32/Thr75). For example, Chen and Chen (2005) demonstrated that Cdk5 activity in the NAc was transiently increased in response to acute METH treatment. This was accompanied by a delayed activation of DARPP-32/Thr75 signalling within the NAc after the

activation of DARPP-32/Thr34 had subsided (Chen & Chen, 2005). Consequently, the phosphorylation of threonine 75 transforms DARPP-32 into a kinase inhibitor, which thereby antagonises the DARPP-32/Thr34 and PP-1 cascade by inhibiting the activity of the PKA pathway (Rauggi et al., 2005). These alterations in the molecular cascades of Cdk5, PKA and DARPP-32 may indirectly impair the activity of CREB. To illustrate, repeated METH administration decreased the expression of phosphorylated CREB within the NAc (McDaid, Graham, & Napier, 2006). Interestingly, these findings suggest that METH administration did not directly affect the activity of CREB, but instead METH altered the functioning of the kinases and phosphatases targeting CREB, such as Cdk5, PKA and DARPP-32 (McDaid et al., 2006). Accordingly, the changes in CREB functioning may initiate the formation of augmented behavioural responding for METH by modulating long-term synaptic adaptations within the NAc (Baldwin et al., 2002; Barrot et al., 2002).

Therefore, METH treatment induces changes in the activities of multiple molecular substrates within the NAc, likely through the initial increase in synaptic levels of dopamine. Dopamine neurotransmission activates multiple downstream molecular cascades and transcription factors, including the cAMP-dependent-PKA pathway, Cdk5, DARPP-32, CREB and ΔFosB (Chao & Neslter, 2004). As a result of chronic METH use, persistent alterations in the expression of these kinases, phosphatases and transcription factors induce long-term neuronal and behavioural adaptations. An intriguing aspect of these molecular modifications is that many of the alterations are similar to the effects of chronic sugar consumption, such as changes in the functioning and expression of DARPP-32/Thr34, ΔFosB and CREB (Kelley, 2004). Hence, these molecular similarities emphasise that the behaviours associated with chronic sugar consumption, such as learning reward-directed behaviours, may contribute to heightened responsiveness to METH administration and subsequent METH abuse.

Proteomic analysis provides for a greater understanding of the interactive molecular mechanisms within a complex biological system (Iwazaki et al., 2006). In light of this,

proteomics allows for a global perspective of the long-lasting changes of METH use. For instance, Iwazaki and colleagues (2006) demonstrated that a single administration of METH altered the expression of proteins within the striatum involved in regulating mitochondrial functioning, protein degradation and protection, oxidative metabolism and cytoskeletal organisation. These findings emphasise that a single exposure to METH can cause enduring modifications to neuronal signalling and structure (Lubec et al., 2003; Iwazaki et al., 2006). These immediate modifications to protein functioning may subsequently influence the development of neural adaptations that contribute to altered behavioural responses, such as heightened incentive value for METH and reward-seeking behaviours (Iwazaki et al., 2007).

The chronic intake of METH extends the development of enduring molecular modifications evident after a single METH treatment. This, in turn, may strengthen the formation of long-term dysfunctional behavioural responses. As an example, Iwazaki and colleagues (2007) demonstrated that chronic METH administration not only induced behavioural sensitisation, but also modified the expression of proteins implicated in cytoskeletal regulation and synaptic functioning in the striatum. In particular, chronic METH administration altered the expression of the synaptosomal-associated protein 25 (SNAP-25) which is directly involved in regulating the release of neurotransmitters and the trafficking of presynaptic vesicles (Iwazaki et al., 2007). The expression of SNAP-25 in the striatum was also altered in response to a single treatment of METH, and changes to this protein were inhibited after the administration of a dopamine D1 receptor antagonist (Isao & Akiyama, 2004). These findings highlight that a single exposure to METH can promote the development of long-term modifications to molecular functioning. This, in turn, promotes enduring neuronal adaptations that encourage augmented behavioural responding to METH (Iwazaki et al., 2007).

However, the advantages of proteomic research have not been applied to its full potential since only limited research has used this tool to investigate the effects of METH exposure within the key brain areas. Further research using proteomic analysis in combination

with methods assessing behaviour is essential in order to determine the molecular adaptations associated with the development of dysfunctional behavioural responses; especially in the NAc and in response to chronic sugar administration and subsequent METH cross-sensitisation.

1.6 Modelling Sugar Cross-sensitisation with Methamphetamine

The majority of the current literature has investigated the neurobiological and behavioural effects of chronic sugar consumption or acute METH administration in isolation. It is surprising that there has been very little focus on the molecular implications associated with chronic sugar consumption and subsequent METH administration as both substances produce similar behavioural responses, and their neurobiological effects operate in overlapping systems. However, in light of these neurobiological similarities, some research has been undertaken to model the neural and behavioural effects associated with sugar consumption and subsequent drug use, highlighting that the cross-sensitisation paradigm is an effective neural and behavioural paradigm.

1.6.1 Sugar exposure and subsequent cross-sensitisation with methamphetamine

Using adolescent rats is effective in modelling the chronic effects of excessive sugar consumption because their neural and behavioural developmental processes are similar to those of human adolescents (Spear, 2000). However, limited research has examined the neural and behavioural implications of chronic sugar consumption during adolescence. Chronically exposing rats to sugar treatment allows for a greater understanding of the long-term consequences of excessive sugar consumption in humans because it validly models the duration and behaviours associated with sugar consumption (Vendruscolo et al., 2010).

Following sugar overconsumption during adolescence, subsequent METH challenge during adulthood provides an insight of the impact long-term sugar consumption have on underlying neurobiology through measures of behavioural sensitisation. Behavioural sensitisation is characterised as augmented locomotor activity as a consequence of neuroadaptive changes within the neuronal systems due to repeated exposure to the substance

(Robinson & Berridge, 1993). Correspondingly, cross-sensitisation aims to model these behavioural and neuronal effects, but repeated exposure to one substance is followed by treatment with a substance of a different class (Avena & Hoebel, 2003). Evidence of behavioural cross-sensitisation emerges when a single administration of a substance (e.g. METH challenge) heightens locomotor activity subsequently after the termination of chronic substance administration (e.g. sugar consumption) (Hoebel et al., 2009). This heightened locomotor responding suggests that the different substances are activating similar neuronal pathways (Avena et al., 2008), such that one substance produces neuroadaptations to enhance the locomotor effect of the second substance. Locomotor activity is a reliable and valid measure of reward-motivated behaviour because it provides an indication that the neuronal responses in the NAc are triggered in order to produce activational motivation in response to the administered substance (Salamone & Correa, 2002).

1.6.2 Proteomics: protein structure and function

Proteomic analysis is a large-scale methodology that aims to examine the functional and structural properties of proteins (Palzkill, 2002, p.1). Additionally, proteomics primarily focuses on the interaction of various proteins within a complex biological network (Liebler, 2002, p.4). As a result, this method is an effective tool in examining the global changes in the protein expression profile in the NAc in response to chronic sugar consumption and subsequent METH exposure (Iwazaki et al., 2007; Liebler, 2002, p.6). Although there is no direct examination of this relationship to date, there has been some proteomic research investigating the protein expression profile in response to sugar overconsumption and acute METH treatment in isolation. Of particular interest, previous studies using a proteomic approach on specific brain areas have demonstrated significant changes in the expression of proteins involved in regulating cellular processes, synaptic plasticity and mitochondrial functioning in chronic sugar consumption as part of a high fat diet or acute METH treatment (Francis et al., 2013; Iwazaki et al., 2006). These findings highlight that proteomics is an effective tool to examine the consequences of environmental modifications on the regulatory

activity of proteins within a whole biological context and in a given brain region (Liebler, 2002, p.6).

1.7 The Present Research

1.7.1 Rationale, research aims and design

Excessive sugar consumption is at its highest level during adolescence (AIHW Australia's Food and Nutrition, 2012). In combination with this, adolescence is a period of major developmental transformations in behaviour and in the neuronal reward system (Spear, 2000). These neural and behavioural shifts during adolescence may contribute to long-term adaptations that influence neural and behavioural processing in adulthood. Hence, it is important to model the neural and behavioural effects of chronic sugar consumption during this age period in order to gain a greater understanding of the long-term behavioural implications. The subsequent administration of METH provides an insight of whether the behavioural and neural modifications produced by chronic sugar consumption during adolescence would alter behavioural and neuronal activity during adulthood. METH is used to examine these neural and behavioural adaptations because it is one of the most commonly abused drugs, and its pharmacological properties can induce powerful neuronal and molecular modifications that contribute to long-term changes in behaviour and mental health (United Nations Office on Drugs & Crime, 2011).

To date, there have been no reports of research using a proteomic approach to investigate sugar cross-sensitisation with METH in the NAc. The present study will use a rodent model of behavioural cross-sensitisation in order to explore the neuroadaptations associated with sugar overconsumption and METH cross-sensitisation. From these behavioural measures, the protein expression profile of the NAc will be analysed with label-free quantitative shotgun proteomics. Determining the molecular adaptations occurring within the NAc in response to sugar cross-sensitisation with METH will provide a clear picture of the biological changes, and in turn determine the factors contributing to the development of

reward-dependent behaviours and will provide targeted areas for the development of preventative strategies to reduce the risk of forming maladaptive behaviours.

1.7.2 Hypotheses

Five hypotheses have been deduced from the present review of the literature:

Hypothesis 1: An acute METH challenge will significantly increase locomotor activity in comparison to rats given an acute saline challenge.

Hypothesis 2: Acute METH challenge will increase locomotor behaviour in rats pre-exposed to chronic sugar treatment (Sugar/METH; i.e. cross-sensitisation) in comparison to rats pre-exposed to chronic water treatment (Water/METH) and those given a saline challenge (Water/Saline).

Hypothesis 3: In comparison to the control treatment group (Water/Saline), rats in the water-METH treatment group (Water/METH) will demonstrate alterations in the expression of proteins involved in regulating neuronal modifications, such as cytoskeletal proteins and synaptic vesicle-associated proteins, and proteins involved in regulating cell functioning and structure, such as metabolism enzymes and mitochondrial proteins.

Hypothesis 4: In comparison to the combined water-METH treatment group (Water/METH), the combined sugar-METH treatment group (Sugar/METH) will show differentially expressed proteins in the NAc involved in the regulation of neuronal modifications, cell functioning and structure and mitochondrial dynamics following an acute METH challenge.

Hypothesis 5: Compared to the controls (Water/Saline), the proteomic analyses will identify proteins in the NAc of the combined sugar-METH-treated rats involved in coordinating cellular interactions and transporting molecular substrates to and from the NAc.

2. Materials and Methods

2.1 Subjects and Exposure to Sucrose in Adolescence

In the present study, 36 adolescent (postnatal day 30; P30) male Sprague Dawley rats were used from the Animal Resource Centre (Canningvale, WA, Australia). Upon arrival, the rats weighed between 110-115g and were housed in groups of four in standard high top cages [64 cm (L) × 40 cm (W) × 20 cm (H)] enriched with shredded paper, wood shavings and blocks of wood. Inside the animal holding room, the temperature was constantly maintained at 21 ± 1°C on a 12-hour light-dark cycle (lights on at 2400 h and off at 1200 h). The rats had *ad libitum* access to food and water inside their home cages, but not during the experimental procedures. The experimental procedures were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council, 2004) and ethical approval was provided by the Macquarie University Animal Ethics Committee (ARA reference number 2012_063).

Prior to commencing the experimental procedures, the rats were allowed 5 days of acclimation and an additional 5 days of handling (2 min per day) in order to adjust to their new environment. Animals were designated into one of three treatment conditions, which included water/saline (Water/Saline; i.e. control) (n = 12), water/methamphetamine (Water/METH) (n = 12) and sugar/methamphetamine (Sugar/METH) (n = 12). The subjects were tagged by texta marks on the tail and were regularly weighed throughout the experiment. After habituation, the rats commenced sugar treatment for 26 days. Twenty-six days of treatment was determined because it is the developmental period of rats progressing from adolescence to young adulthood. Animals in the Water/Saline and Water/METH treatment groups received *ad libitum* tap water (Water), and rats in the Sugar/METH treatment group received *ad libitum* access to a sugar solution (Sugar; 100g/L sugar in tap water; 17 kJ/g). Additionally, the subjects had continuous access to standard rat chow (13 kJ/g; fat ≈ 6% energy, sugar ≈ 1% energy, carbohydrate ≈ 37.2% energy) throughout the experiment. Total

body weight, food consumption and treatment intake were measured every three days throughout the experiment.

2.2 Behavioural Testing

2.2.1 Apparatus

Sixteen identical locomotor chambers [40.5 cm (L) × 30.5 cm (W) × 30 cm (H)] were used to record locomotor activity. The walls and ceiling of the locomotor chambers were made up of aluminium and Plexiglas, and each chamber was sound-attenuated and contained an exhaust fan for ventilation. The floor of the locomotor chambers consisted of a stainless grate (bars 3.2 mm wide, 1.1 cm apart) elevated above an aluminium tray. In order to monitor locomotor activity, the walls of the chambers were lined with four infrared photo beam sensors located 2.9 cm above the floor of each chamber. The computer software MED-PC (MED Associates, St. Albans, VT, USA) recorded counts of locomotor behaviour from the detectors.

2.2.2 Procedure

Prior to the commencement of the 26-day sugar treatment schedule, adolescent rats were permitted 12 hours of acclimation in the locomotor chambers. During this acclimation period, the animals had *ad libitum* access to only water and standard rat chow.

On the first day (D1) of sugar treatment, adolescent rats were placed in the locomotor chambers for 24 hours to record locomotor activity. The rats were permitted *ad libitum* access to food and either Water or Sugar. Subsequently, after locomotor testing, the animals returned back to their home cages and remained there for a further 24 days. At their home cages, the subjects were given continuous access to standard rat chow and either Water or Sugar. On the last day of the 26-day sugar treatment regimen (D26), locomotor activity was measured for 24 hours in the locomotor chambers. The animals were provided with *ad libitum* access to food and either Water or Sugar during this measure of locomotor activity.

Once rats concluded their 26 day treatment schedule, and entered into adulthood, they had a six-week washout from treatment. The six-week washout period ensures that the rats are

full adults before METH challenge testing. During this time, all animals endured other behavioural testing procedures unrelated to the primary objective of this study which is to measure locomotor cross-sensitisation. After six weeks, locomotor activity was measured in response to the acute treatment of METH. In order to habituate to the experimental protocol, the animals were provided 15 minutes of acclimation to explore the locomotor chamber. Subsequently, each rat was intraperitoneally injected with saline (1 ml/kg) and then placed in the locomotor chamber for 2 hours to measure locomotor activity. The next day, the Water/Saline treatment group was challenged with saline (1 ml/kg, i.p.), and the Water/METH and Sugar/METH treatment groups were challenged with METH (1 mg/kg, i.p.). Following 15 minutes of acclimation in the locomotor chambers, locomotor responding was measured for 2 hours after receiving a challenge dose of saline (1 ml/kg, i.p.) or METH (1 mg/kg, i.p.). The low dose of METH was chosen to avoid inducing stereotypies and to heighten locomotor responding to METH treatment.

2.2.3 Euthanasia

Precisely twenty-four hours after the last measure of locomotor activity, the rats were killed for proteomic analysis of brain tissue. An interval of twenty-four hours after measuring locomotor activity ensures that protein manufacturing has occurred to detect significant changes to the abundance of protein. The rats were firstly anaesthetised with an intraperitoneal injection of pentobarbitone sodium 325 mg/ml (1:1 ml dilution of Lethobarb; Virbac, Milperra, Australia) and shortly after decapitated by guillotine after no response to tail pinch. To determine protein expression, the brains were removed rapidly and snap frozen in liquid nitrogen, and then stored in a -80°C freezer. On dry ice and by using the rat brain matrix, the frozen nucleus accumbens (NAc) was dissected into 1 mm thick coronal sections (relative to bregma; AP 1.2 – 2.2; ML 0 – 1, bilateral; DV 6.5 – 7.5) and stored at -80°C in preparation for proteomic analysis. Additionally, the orbitofrontal cortex, the prelimbic cortex and infra-limbic cortex were dissected and stored at -80°C for future experimentation.

2.2.4 Drugs

Methamphetamine hydrochloride (METH) was purchased from the Australian Government Analytical Laboratories (Pymble, NSW, Australia). METH was dissolved in isotonic saline (0.9%) and was injected intraperitoneally (i.p.) at a volume of 1 ml/kg each administration.

2.2.5 Statistical analyses for behavioural data

Total energy intake was calculated for each rat prior to statistical analyses. Repeated measures ANOVA were carried out separately with SPSS version 21 to analyse total energy intake, body weight, and locomotor behaviour measured during the 26-day treatment regimen (Day 1 and Day 26) and during METH or saline challenge at six weeks post treatment. Results were considered significant if $p < 0.05$ and between group comparisons were corrected with Bonferroni multiple comparisons adjustments.

2.3 Proteomic Analysis

2.3.1 Sample homogenisation and protein fractionation using SDS-PAGE

After weighing the prepared nucleus accumbens brain slices, they were homogenised in buffer containing 0.32mM sucrose, 2mM EDTA and 1% SDS at a ratio of 1:10. The homogenised solution was then centrifuged at 134 000 rpm for 40 minutes and stored at -20°C in preparation for protein extraction. Prior to protein extraction, a BCA protein assay was performed in order to determine the protein concentration for each aliquot. After running the BCA protein assay, each aliquot containing 20µl was combined with 2µl DTT, 5µl of H_2O and 2µl of SDS. This was then loaded into a well of a Bio-Rad 10% Tris-HCl precast gel and run at 70 V for 5 minutes and at 150 V for a further 1 hour. The gel was stained with Coomassie Brilliant Blue G-250 (Bio-Rad) for 1 hour and destained with H_2O overnight in preparation for in-gel digestion.

2.3.2 Trypsin in-gel digestion

A scalpel was used to slice each individual gel lane into approximately 16 equal pieces. The gel pieces were further chopped and placed into a well of a 96-well plate.

Subsequently, the gel pieces were repeatedly washed for 10 minutes each time with 150µl of 50% Acetonitrile (ACN)/50% 100mM NH_4HCO_3 in order to completely destain the gel pieces. The gel pieces were dehydrated for 5 minutes with 100% ACN and then, after the removal of ACN, the samples were air dried and reduced with 50µl of 10mM DTT/50mM NH_4HCO_3 at 37°C for 1 hour. At room temperature, the samples were then subjected to alkylation with 50µl of 50mM iodoacetamide/NH_4HCO_3 (50%) for 45 minutes in the dark. The samples were briefly washed with 100mM NH_4HCO_3 and then briefly washed twice with 200µl of 50% ACN/100mM 50% NH_4HCO_3. In preparation for trypsin digestion, the gel pieces were dehydrated with ACN (100%) and this was then removed to air dry the samples. Finally, trypsin digestion involved firstly digesting the sample with 30µl of trypsin (12.5ng/ml 50mM NH_4HCO_3) on ice for 30 minutes and then at 37°C overnight.

2.3.3 Peptide extraction

Following overnight trypsin digestion of proteins, peptides from each sample were extracted by adding 60µl of 50% ACN/2% formic acid, then incubated for 30 minutes at 37°C. This process was repeated in order to produce a peptide extraction volume of approximately 60µl for each sample. Subsequently, a vacuum centrifuge dried the peptide extractions of each sample until it was reduced to 10µl with 2% formic acid. The dried samples were then subjected to analysis with the mass spectrometer.

2.3.4 Nanoflow liquid chromatography/tandem mass spectrometry

In order to control for false positive results and therefore identify statistically significant differences between control and treatment groups, the proteomic method recognises that a specific protein is consistently expressed as an identified change across all three biological replicates. Based on the proteomic methods adopted from Gammulla and colleagues (2010) and Mirzaei et al. (2012), nanoflow liquid chromatography/tandem mass spectrometry (nanoLC-MS/MS) using LTQ-XL linear ion trap mass spectrometer (Thermo, San Jose, CA) was used to analyse each of the 16 reconstituted fractions and determine the differences in protein expression between the sample groups. The reversed-phase columns

were loaded in-house approximately 7 cm (100 µm id) using 100 Å, 5 µm Zorbax C18 column (Agilent Technologies, CA, USA). These reverse-phase columns were placed inside a fused silica capillary that includes an integrated electro-spray tip. Through a liquid junction located upstream of the C18 column, an electrospray voltage of 1.8 kV was administered. The surveyor autosampler injected the samples onto the C18 column. Subsequently, the samples inside the column were initially washed for 10min at 1 mL/min with buffer A (5% v/v ACN, 0.1% v/v formic acid), which was then followed by an elution process for 58 min at 500 nL/min with buffer B (95% v/v ACN, 0.1% v/v formic acid). Consequently, the eluate produced inside the column was positioned into a nanospray ionization source of the mass spectrometer. Spectra ranging between 400–1500amu were scanned. Finally, MS/MS of the six strongest precursor ions at 35% normalization collision energy, dynamic exclusion and automated peak recognition were executed with the use of Xcalibur software (Version 2.06, Thermo).

2.3.5 Database search for protein and peptide identification

The global proteome machine software (version 2.1.1) and the X!Tandem algorithm was used to convert the raw files of nanoLC-MS/MS data into a mzXML format. At the same time, the raw files were also searched against the National Center for Biotechnology information (NCBI) *Rattus Norvegius* Reference Sequence (RefSeq) database. For each sample, the 16 fractions were processed in a sequential order with output files created for the fractions. This process generated a merged, non-redundant output file for protein identifications with log(*e*) values less than -1. A 0.4 Da fragment mass error determined the identification of peptides. Carbamidomethyl was recognised as a complete modification, and oxidation of methanionine and tryptophan were considered as potential modifications. Finally, MS/MS spectra were searched against the NCBI *Rattus Norvegius* RefSeq database, and reverse sequence database searching was performed to estimate the false discovery rates (FDR).

2.3.6 Data processing and quantitation

For each experimental condition (control, sugar and METH), the samples were run in triplicates. Proteins were only retained if they were reproducibly present in all three replicates and if they demonstrated a total spectral count (SpC) greater than six (i.e. SpC > 6). After this filtering process, protein and peptide FDRs were calculated. Consequently, the protein FDR was calculated based on (total number of reversed protein hits in the list / total number of proteins in the list) × 100. Additionally, the peptide FDR was equal to (total number of peptides representing reversed protein hits in the list / total number of peptides representing all reversed proteins in the list) × 100. The normalised spectral abundance factor (NSAF) recognises that the number of spectral counts (SpC) is determined by the length of the protein. As a result, a NSAF value was calculated for each protein, described by Zybailov et al. (2006) and Neilson et al. (2011). This involves dividing the number of SpC identifying the protein by its length (SpC/L). Based on this calculation, the product is then divided by the sum of SpC/L for all proteins in the experiment to generate the total NSAF value. To account for null values, a spectral fraction value of 0.5 was added to all spectral counts.

2.3.7 Statistical analyses for proteomics data

Three separate Ingenuity Pathway Analyses (IPA) were conducted to preform two-way comparisons between the three treatment groups. These comparisons included Water/Saline and Water/METH, Sugar/METH and Water/METH, and Water/Saline and Sugar/METH. Results were considered significant if $p < 0.05$. Only two-way analyses were performed because IPA is unable to run three-way analyses. Based on this limitation, three treatment groups were used to interpret the effects of sucrose treatment on proteins expressed by METH administration. Additionally, a one-way ANOVA was conducted between the three treatment groups in order to determine the uniquely expressed proteins for each treatment group from the identified differentially expressed proteins. Results were considered significant if $p < 0.05$.

After protein identification and data processing was performed, the output files produced a list of proteins identified by their ensemble number. Each protein ensemble number was searched using the online protein search engines UniProt (http://www.uniprot.org/) and NCBI (http://www.ncbi.nlm.nih.gov/protein) in order to identify the names of the up-regulated and down-regulated proteins for each two-way comparison.

2.3.8 Statement of tasks performed

Since the current study was part of a larger research project, all behavioural testing procedures were conducted by the PhD candidate, Jane Franklin. For proteomic analysis, the researcher of this current study performed a majority of the procedures, including sample homogenisation and protein fractionation using SDS-PAGE, trypsin in-gel digestion, peptide extraction, data processing and quantitation, and searching for protein ensemble numbers. Nanoflow liquid chromatography/tandem mass spectrometry, database search for protein and peptide identification, and Ingenuity Pathway Analyses were conducted by experts in the proteomics field.

3. Results

3.1 Energy Intake and Body Weight during Treatment in Adolescence

During the acclimation period, there were no significant differences found between the Water/Saline, Water/METH and Sugar/METH treatment groups in total energy intake measured in kilojoules (F (2, 28) = 1.549, p = 0.230). On the first day of the 26-day sugar-treatment regimen, total energy intake significantly differed between the treatment groups (F (2, 28) = 9.311, p = 0.001; Figure 3.1). The Sugar/METH treatment group significantly consumed more kilojoules of food and sugar-treatment on average (M = 374.10, SEM = 9.63; Figure 1 in Appendix E) in comparison to the Water/Saline treatment group (M = 301.60, SEM = 10.93; F (1, 28) = 16.376, p = 0.000) and the Water/METH treatment group (M = 316.33, SEM = 19.01; F (1, 28) = 9.802, p = 0.004). There were no significant differences in

energy intake between the Water/Saline and Water/METH treatment groups (F (1, 28) = 0.587, p = 0.450). There were significant differences in energy intake on the fifth day of the sugar-treatment period between the treatment groups (F (2, 28) = 29.654, p = 0.000; Figure 3.1). On average, the Sugar/METH treatment group consumed more kilojoules of energy from food and sugar-treatment (M = 438.06, SEM = 12.00) compared to the Water/Saline treatment group (M = 347.10, SEM = 7.26; F (1, 29=8) = 47.739, p = 0.000) and Water/METH treatment group (M = 355.33, SEM = 6.13; F (1, 28) = 37.231, p = 0.000). The Water/Saline and the Water/METH treatment groups did not significantly differ in total energy intake (F (1, 28) = 0.340, p = 0.565). Total energy intake significantly differed between the treatment groups on the fifteenth day of the sugar-treatment period (F (2, 28) = 20.195, p = 0.000; Figure 3.1). The Sugar/METH treatment group consumed on average significantly more kilojoules of food and sugar-treatment (M = 472.68, SEM = 7.45) in comparison to the Water/Saline treatment group (M = 365.30, SEM = 20.37; F (1, 28) = 35.177, p = 0.000) and the Water/METH treatment group (M = 385.67, SEM = 8.67; F (1, 28) = 21.778, p = 0.000). There were no significant differences in total energy intake between the Water/Saline and Water/METH treatment groups (F (1, 28) = 1.099, p = 0.303). On the last day of the sugar-treatment period, there were no significant differences evident between the Water/Saline, Water/METH and Sugar/METH treatment groups in total energy intake (F (2, 28) = 1.799, p = 0.184).

Body weight measured in grams significantly differed between the treatment groups during the acclimation period (F (2, 28) = 5.120, p = 0.013; Figure 2 in Appendix E). The Water/METH treatment groups recorded on average a significantly higher body weight (M = 123.83, SEM = 4.11) in comparison to the Water/Saline treatment group (M = 112.87, SEM = 3.77; F (1, 28) = 5.487, p = 0.026) and the Sugar/METH treatment group (M = 109.88, SEM = 1.76; F (1, 28) = 9.657, p = 0.004). There were no significant differences in body weight between the Water/Saline and Sugar/METH treatment groups (F (1, 28) = 0.472, p = 0.498). During the 26-day sugar-treatment regimen, there were significant differences in body weight

between the treatment groups on the first day of treatment (F (2, 28) = 4.830, p = 0.016; Figure 2 in Appendix E). The Water/METH treatment group weighed significantly greater (M = 144.27, SEM = 3.95) in comparison to the Water/Saline treatment group (M = 132.46, SEM = 4.21; F (1, 28) = 5.571, p = 0.025) and the Sugar/METH treatment group (M = 129.93, SEM = 2.13; F (1, 28) = 8.914, p = 0.006). The Water/Saline and Sugar/METH treatment groups did not significantly differ in body weight (F (1, 28) = 0.294, p = 0.592). There were no significant differences in body weight evident between the Water/Saline, Water/METH and Sugar/METH treatment groups on the fifth day of sugar-treatment (F (2, 28) = 2.382, p = 0.111), the fifteenth day of sugar-treatment (F (2, 28) = 1.877, p = 0.172) and the last day of sugar-treatment (F (2, 28) = 2.518, p = 0.099).

3.2 Energy Intake and Body Weight during Adulthood

During the METH challenge period, there were significant differences in total energy intake measured in kilojoules between the treatment groups (F (2, 28) = 6.084, p = 0.006; Figure 3.1). The Sugar/METH treatment group consumed significantly more kilojoules on average (M = 413.83, SEM = 5.86) compared to the Water/Saline treatment group (M = 370.58, SEM = 12.13; F (1, 28) = 10.958, p = 0.003) and the Water/METH treatment group (M = 380.89, SEM = 10.73; F (1, 28) = 5.994, p = 0.021). The Water/Saline and Water/METH treatment groups did not significantly differ in total energy intake (F (1, 28) = 0.540, p = 0.468). There were no significant differences in body weight measured in grams between the Water/Saline, Water/METH and Sugar/METH treatment groups during the METH challenge period (F (2, 28) = 0.289, p = 0.751), indicating that body weight had stabilised.

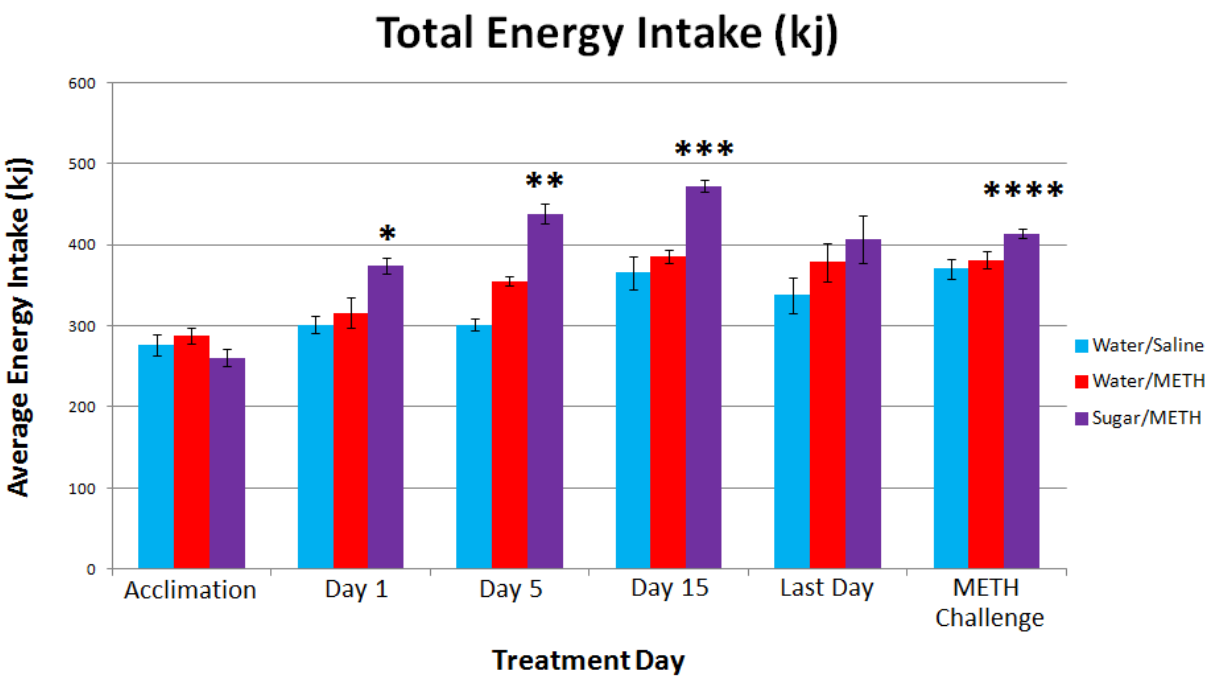

Figure 3.1. Total energy intake represents the average amount of energy (measured in kilojoules) consumed from food and fluid treatment throughout the duration of the experiment (± standard error of the mean, SEM). During the 26-day treatment period, the rats in the Sugar/METH condition consumed significantly more energy on average compared to the rats in the Water/Saline and Water/METH conditions on the first day of treatment (*$p < 0.05$), the fifth day of treatment (**$p < 0.05$) and the last day of treatment (***$p < 0.05$). The Sugar/METH group consumed significantly more energy on average compared to the Water/Saline and Water/METH conditions during the METH challenge period (****$p < 0.05$).

3.3 Behavioural Tests

3.3.1 Locomotor activity during adolescence

On the first day of the 26-day sugar-treatment regimen, there were no significant differences found between the Water/Saline, Water/METH and Sugar/METH treatment groups in total 24 hours of locomotor activity (F (2, 28) = 0.183, p = 0.834; Figure 3 in Appendix E). There were also no significant differences in the total 24 hours of locomotor activity between the three treatment groups on the last day of the sugar-treatment period (F (2, 28) = 1.842, p = 0.178; Figure 3 in Appendix E).

3.3.2 Locomotor cross-sensitisation during adulthood

During the METH challenge period, there were no significant differences in locomotor activity during the 15 minutes acclimation period between the Water/Saline, Water/METH and Sugar/METH treatment groups (F (2, 28) = 0.222, p = 0.802).

There were significant differences in locomotor activity between the treatment groups during the first hour after the administration of the challenge injection (F (2, 28) = 22.411, p = 0.000; Figure 3.2 and Figure 4 in Appendix E). There were significant differences in locomotor activity between the saline-treated rats (Water/Saline) and the METH-treated rats (Water/METH and Sugar/METH) (F (1, 28) = 33.858, p = 0.000; Figure 3.2). Specifically, the Water/METH treatment group recorded significantly higher bouts on average of locomotor activity (M = 801. 33, SEM = 161.844) compared to the Water/Saline treatment group (M = 223.80, SEM = 39.788; F (1, 28) = 12.437, p = 0.001). The Sugar/METH treatment group also recorded on average significantly greater bouts of locomotor activity (M = 1245.25, SEM = 107.61) in comparison to the Water/Saline group (M = 223.80, SEM = 39.788; F (1, 28) = 44.800, p = 0.000). Additionally, there was evidence of locomotor cross-sensitisation during the first hour after the challenge injection. The Sugar/METH treatment group significantly recorded higher bouts on average of locomotor activity (M = 1245.25, SEM = 107.61) compared to the Water/METH treatment group (M = 801. 33, SEM = 161.844; F (1, 28) = 7.978, p = 0.009; Figure 3.2).

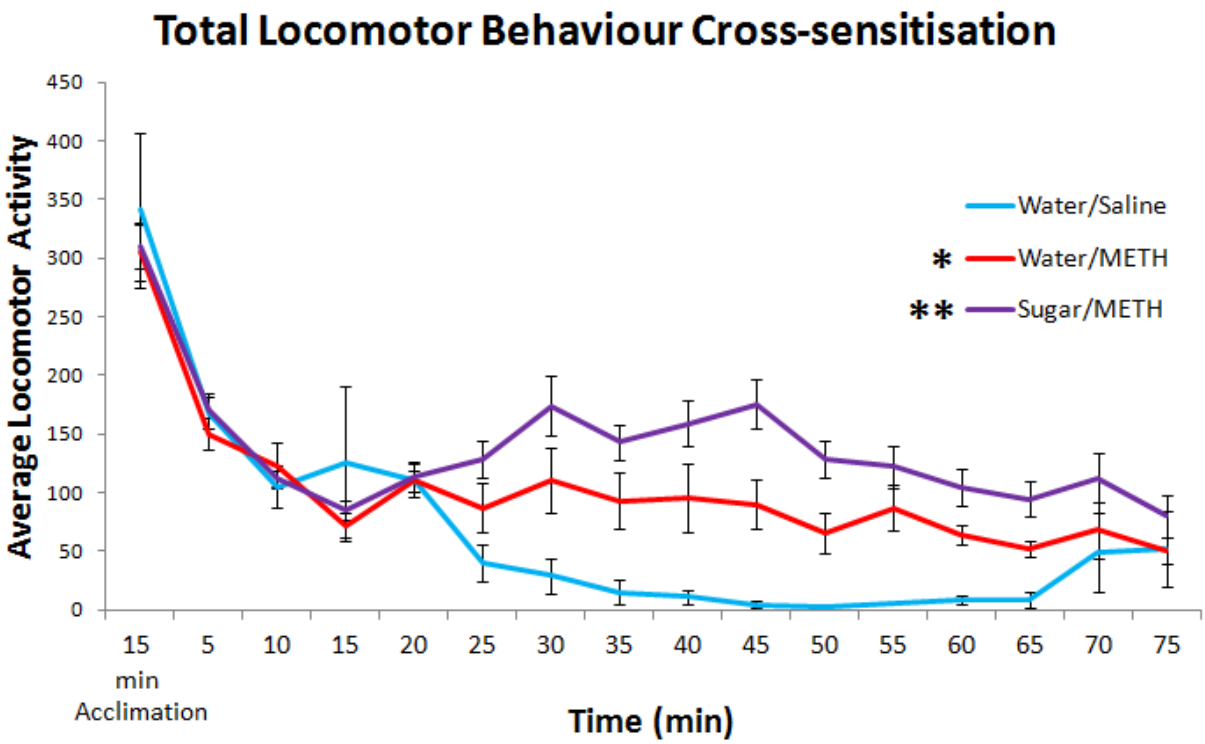

Figure 3.2. Total locomotor behaviour cross-sensitisation represents average locomotor activity for 60 minutes (± SEM) after given 15 minutes acclimation and administration of the challenge injection. The Water/METH group demonstrated significantly more locomotor activity on average in comparison to the Water/Saline group (*$p < 0.05$). As an indication of behavioural cross-sensitisation, the Sugar/METH condition exhibited significantly greater bouts of locomotor activity on average compared to the Water/METH group (**$p < 0.05$).

3.4 Proteomic Analysis of the Nucleus Accumbens

The results from the proteomics analyses were significantly reliable and reproducible. From the proteomic analyses of the three biological replicates, the proteomic datasets consistently detected a total of 1519 proteins in the Water/Saline and Water/METH comparison, a total of 1472 proteins in the Sugar/METH and Water/METH comparison, and a total of 1509 proteins in the Water/Saline and Sugar/METH comparison. The protein and peptide FDRs for each comparison were calculated based on the proteins identified in the biological replicates (Table 3.1). In the Water/Saline and Water/METH comparison, the protein FDR ≈ 0.39% and the peptide FDR ≈ 0.03%. The protein FDR ≈ 0.27% and the peptide FDR ≈ 0.03% for the Sugar/METH and Water/METH comparison, and the protein FDR ≈ 0.27% and the peptide FDR ≈ 0.03% for the Water/Saline and Sugar/METH comparison (Table 3.1). All of the protein and peptide FDRs were below 1% and thus the proteomic results did not require subsequent filtering or analyses.

Three separate Student's *t*-tests were used to analyse significant differences between the treatment groups based on the natural log NSAF values. Differences in the abundance of identified proteins between the three treatment groups was considered statistically significant if $p < 0.05$. The *t*-test analyses identified 33 proteins up-regulated (Table 3.2) and 60 proteins down-regulated (Table 3.3) in the Water/Saline and Water/METH comparison, 68 proteins up-regulated (Table 3.4) and 34 proteins down-regulated (Table 3.5) in the Sugar/METH and Water/METH comparison, and 31 proteins up-regulated (Table 3.6) and 43 proteins down-regulated (Table 3.7) in the Water/Saline and Sugar/METH comparison.

The one-way ANOVA between the three treatment groups identified a total of 36 proteins uniquely differentially expressed in the NAc of rats in the Water/Saline, Water/METH and Sugar/METH groups (Table 3.8). A total of 11 uniquely expressed proteins were found in the Water/Saline treatment group, 13 proteins in the Water/METH treatment group, and 12 proteins in the Sugar/METH treatment group (Table 3.8).

Table 3.1. Summary of proteomic analysis of the nucleus accumbens for Water/Saline, Water/METH and Sugar/METH rats

No. Reproducibly identified proteins	No. of Peptides						Protein FDR (%)	Peptide FDR (%)
Summary of proteomic analysis for Water/Saline and Water/METH comparison								
	Water/METH treatment group			Water/Saline treatment group				
	1	2	3	1	2	3		
1519	31027	37429	30359	30341	32005	34984	0.39%	0.03%
Summary of proteomic analysis for Sugar/METH and Water/METH comparison								
	Sugar/METH treatment group			Water/METH treatment group				
	1	2	3	1	2	3		
1472	31712	28825	30913	30623	37090	30337	0.27%	0.03%
Summary of proteomic analysis for Water/Saline and Sugar/METH comparison								
	Sugar/METH treatment group			Water/Saline treatment group				
	1	2	3	1	2	3		
1509	31916	29012	31287	30399	32140	35033	0.27%	0.3%

Table 3.2. Proteins identified as up-regulated in the nucleus accumbens of Water/METH rats in comparison to Water/Saline rats

Identifier: ENSRNOP00'	Symbol	Protein Description	Fold Change	*p*-value
000039289	HIST3H2A	Histone cluster 3, H2a	86.899	1.04E-06
000044007	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	104.056	3.44E-06
000039671	RTN4	Reticulon 4	42.0906	8.15E-06
000061921		Uncharacterised protein	9.246	5.21E-05
000021512	PECR	Peroxisomal trans-2-enoyl-CoA reductase	4.770	5.38E-05
000016749	CPNE4	Copine IV	6.161	0.002279
000025385	MAP1S	Microtubule-associated protein 1S	5.287	0.002785
000008477	VDAC1	Voltage-dependent anion channel 1	1.381	0.004408
000062198	DOCK10	Dedicator of cytokinesis 10	4.913	0.009249
000032320	ALDOA	Aldolase A, fructose-bisphosphate	1.128	0.010233
000010545	PDHB	Pyruvate dehydrogenase (lipoamide) beta	1.566	0.010256
000021171	SFXN3	Sideroflexin 3	1.414	0.01357
000007100	YWHAE	Tyrosine 3-monooxygenase/ tryptophan 5-monooxygenase activation protein, epsilon	1.408	0.013732
000026928	PSMA5	Proteasome (prosome, macropain) subunit, alpha type, 5	1.965	0.014221
000012796		Uncharacterised protein	3.315	0.015635
000018453	FAM213B	Family with sequence similarity 213, member B	4.206	0.016626
000012425	NDUFS3	NADH dehydrogenase (ubiquinone) Fe-S protein 3, 30kDa (NADH-coenzyme Q reductase)	1.233	0.019767
000015344	DNAJC13	DnaJ (Hsp40) homolog, subfamily C, member 13	2.971	0.021868
000031940	ALDOA	Fructose-bisphosphate aldolase A	1.146	0.022229
000001954	YWHAG	Tyrosine 3-monooxygenase/ tryptophan 5-monooxygenase activation protein, gamma	1.461	0.025148
000013354	CA2	Carbonic anhydrase II	1.228	0.028662
000052868	CAPZA1	Capping protein (actin filament) muscle Z-line, alpha 1	1.368	0.029515
000058202		Uncharacterised protein	10.191	0.032271
000056469	H2AFX	H2A histone family, member X	1.528	0.037893
000014062	XPO1	Exportin 1	1.476	0.039062
000024707	SFXN1	Sideroflexin 1	1.324	0.039314
000011501	YWHAQ	Tyrosine 3-monooxygenase/ tryptophan 5-monooxygenase activation protein, theta	1.117	0.041946
000038375		Uncharacterised protein	4.983	0.041988
000007403		Guanine nucleotide-binding protein subunit gamma	2.576	0.04408
000062107		Histone H3.1	3.580	0.044327
000014658	HADH	Hydroxyacyl-CoA dehydrogenase	4.125	0.046587
000002938	IBSP	Integrin-binding sialoprotein	2.334	0.047218
000063484	EIF3A	Eukaryotic translation initiation factor 3, subunit A	1.725	0.049251

Table 3.3. Proteins identified as down-regulated in the nucleus accumbens of Water/METH rats in comparison to Water/Saline rats

Identifier: ENSRNOP00'	Symbol	Protein Description	Fold Change	*p*-value
000029604	MYO5A	Unconventional myosin-Va	-103.913	1.24E-06
000028785	RTN3	Reticulon 3	-64.225	4.01E-06
000022626	RAB13	Ras-related protein Rab-13	-27.263	1.58E-05
000026920	HSP90AB1	heat shock protein 90kDa alpha (cytosolic), class B member 1	-1.131	1.94E-05
000058953	VTI1A	Vesicle transport through interaction with t-SNAREs 1A	-5.241	4.22E-05
000040338	METTL7A	Methyltransferase like 7A	-6.511	0.000363
000016842	INPP1	Inositol polyphosphate-1-phosphatase	-2.294	0.000397
000028188	DDB1	Damage-specific DNA binding protein 1, 127kDa	-13.865	0.000456
000015828	CBX3	Chromobox homolog 3	-9.196	0.000835
000004970	GABRB2	Gamma-aminobutyric acid (GABA) A receptor, beta 2	-9.408	0.001256
000029044	MLLT4	Myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 4	-5.168	0.002412
000018796	PGRMC2	Progesterone receptor membrane component 2	-1.747	0.002842
000020174	EXOG	Endo/exonuclease (5'-3'), endonuclease G-like	-1.747	0.002842

000004613	CADM3	Cell adhesion molecule 3	-1.808	0.004139
000056213	HSDL2	Hydroxysteroid dehydrogenase like 2	-5.288	0.0042
000022621	ISOC2	Isochorismatase domain-containing protein 2, mitochondrial	-8.257	0.005671
000020785	USP5	Ubiquitin specific peptidase 5 (isopeptidase T)	-1.443	0.008777
000001518	RPLP0	Ribosomal protein, large, P0	-1.507	0.008809
000031201	GSTA2	Glutathione S-transferase alpha 2	-6.610	0.00953
000008948	LANCL2	LanC lantibiotic synthetase component C-like 2 (bacterial)	-2.409	0.010081
000034775	ANK3	Ankyrin 3, node of Ranvier (ankyrin G)	-1.339	0.010455
000019214	DLGAP3	Discs, large (Drosophila) homolog-associated protein 3	-1.708	0.010538
000055092		Uncharacterised protein	-5.065	0.010827
000016851	GSTO1	Glutathione S-transferase omega 1	-1.608	0.011316
000021921	SLC12A2	Solute carrier family 12 (sodium/potassium/chloride transporter), member 2	-2.054	0.012696
000004942	TMED7	Transmembrane emp24 protein transport domain containing 7	-4.756	0.013497
000024711	PARK7	Parkinson protein 7	-1.433	0.014632
000022485	NGEF	Neuronal guanine nucleotide exchange factor	-1.438	0.017177
000027768	NCAN	Neurocan	-1.992	0.017806
000062102	UBE2V2	Ubiquitin-conjugating enzyme E2 variant 2	-1.346	0.018446
000012847	CCT4	Chaperonin containing TCP1, subunit 4 (delta)	-1.791	0.019745
000045650	ATP1A1	ATPase, Na+/K+ transporting, alpha 1 polypeptide	-1.403	0.022128
000018860	PTPN5	Protein tyrosine phosphatase, non-receptor type 5 (striatum-enriched)	-5.111	0.022329
000017573	BIN1	Bridging integrator 1	-1.312	0.023563
000018049	SFPQ	Splicing factor proline/glutamine-rich	-2.013	0.024042
000060478		Uncharacterised protein	-2.229	0.025156
000045959	HAPLN1	Hyaluronan and proteoglycan link protein 1	-1.919	0.027766
000016130	PSIP1	PC4 and SFRS1 interacting protein 1	-6.160	0.029806
000016813	RAB5A	RAB5A, member RAS oncogene family	-1.726	0.033447
000011784	ECI1	Enoyl-CoA delta isomerase 1	-2.318	0.034511
000006370	SRRM2	Srrm2 protein	-2.588	0.034654
000006407	CRK	V-crk avian sarcoma virus CT10 oncogene homolog	-2.503	0.035448
000021419	AP1G1	Adaptor-related protein complex 1, gamma 1 subunit, isoform CRA_b	-4.496	0.037488
000021387	HINT2	Histidine triad nucleotide binding protein 2	-2.070	0.039731
000027869	C11orf68	Chromosome 11 open reading frame 68	-3.365	0.040166
000023326	CSPG4	Chondroitin sulfate proteoglycan 4	-3.365	0.040166
000028881	PRNP	Prion protein	-1.778	0.042205
000025279	MOBP	Myelin-associated oligodendrocyte basic protein	-1.393	0.042425
000027076	ETHE1	Ethylmalonic encephalopathy 1	-3.822	0.042487
000035517	WDFY3	WD repeat and FYVE domain containing 3	-4.991	0.042495
000002738	ALCAM	Activated leukocyte cell adhesion molecule	-1.422	0.043523
000025217	RPL17	Ribosomal protein L17	-1.745	0.043937
000036487	CDC42BPB	CDC42 binding protein kinase beta (DMPK-like)	-2.251	0.044133
000005125	BLMH	Bleomycin hydrolase	-2.545	0.04421
000004269	MAT2B	Methionine adenosyltransferase II, beta	-1.915	0.044872
000007558	CSNK2A1	Casein kinase 2, alpha 1 polypeptide	-2.148	0.046785
000001247	RAN	RAN, member RAS oncogene family	-1.484	0.04783
000004725	GABRA1	Gamma-aminobutyric acid (GABA) A receptor, alpha 1	-4.155	0.048395
000001013	PPP1R11	Protein phosphatase 1, regulatory (inhibitor) subunit 11	-3.501	0.049076
000001160	VARS	Valyl-tRNA synthetase	-1.810	0.049647

Table 3.4. Proteins identified as up-regulated in the nucleus accumbens of Sugar/METH rats in comparison to Water/METH rats

Identifier: ENSRNOP00'	Symbol	Protein Description	Fold Change	*p*-value
000044593		Uncharacterised protein	76.922	5.78E-07
000002479	DNM1L	Dynamin1-like protein	84.025	1.57E-06
000022626	RAB13	Ras-related protein Rab-13	18.582	7.42E-06
000013515	ACOT2	Acyl-CoA thioesterase 2	5.466	1.88E-05
000007540	IL36A	Interleukin 36, alpha	5.466	1.88E-05

000054496	GMPS	GMP synthase (glutamine-hydrolyzing)	5.466	1.88E-05
000029604	MYO5A	Unconventional myosin-Va	85.821	2.58E-05
000025279	MOBP	Myelin-associated oligodendrocyte basic protein	1.692	2.72E-05
000015828	CBX3	Chromobox homolog 3	6.91	0.000103
000003083	ENOPH1	Enolase-phosphatase 1	6.209	0.00021
000012301	FBXO6	F-box only protein 6	7.687	0.000523
000040969	ATP5L	ATP synthase, H+ transporting, mitochondrial Fo complex, subunit G	2.061	0.001223
000040162	PAK2	Serine/threonine-protein kinase PAK 2	9.772	0.001292
000062949	IgG-2a	Gamma-2a immunoglobulin heavy chain	5.433	0.002544
000023934	ATP2B3	ATPase, Ca++ transporting, plasma membrane 3	1.413	0.003288
000040338	METTL7A	Methyltransferase like 7A	5.5	0.003676
000040592	KIAA1211L	KIAA1211-like	8.131	0.005379
000022375	PIP4K2A	Phosphatidylinositol-5-phosphate 4-kinase, type II, alpha	7.724	0.007771
000004613	CADM3	Cell adhesion molecule 3	1.658	0.008046
000043221	ITIH3	Inter-alpha-trypsin inhibitor heavy chain 3	3.067	0.009188
000025217	RPL17	Ribosomal protein L17	1.687	0.009571
000024258		Uncharacterised protein	2.569	0.009856
000053123	RPL10	Ribosomal protein L10	2.139	0.0101
000021419	AP1G1	Adaptor-related protein complex 1, gamma 1 subunit, isoform CRA_b	4.908	0.010993
000017227	STX12	Syntaxin 12	1.951	0.011097
000012747	WIPF3	WAS/WASL interacting protein family, member 3	9.202	0.011359
000001247	RAN	RAN, member RAS oncogene family	1.551	0.011641
000004942	TMED7	Transmembrane emp24 protein transport domain containing 7	4.938	0.011671
000057517	CYLD	Ubiquitin carboxyl-terminal hydrolase CYLD	6.696	0.011932
000062205		Uncharacterised protein	1.879	0.014694
000022849	EPB41L3	Erythrocyte membrane protein band 4.1-like 3	1.472	0.015291
000015179	VCL	Vinculin	1.891	0.017366
000049512		Uncharacterised protein	1.504	0.01742
000014359	CLDN11	Claudin 11	1.121	0.018019
000001461	HIP1R	Hip1r protein	2.993	0.018212
000049552	DCLK1	Serine/threonine-protein kinase DCLK1	1.516	0.018484
000060948		Uncharacterised protein	1.297	0.018853
000020066	HSPE1	Heat shock 10kDa protein 1	1.719	0.021158
000016104	ACSBG1	Acyl-CoA synthetase bubblegum family member 1	1.906	0.023073
000063893	SEPT8	Septin 8	1.509	0.024514
000018049	SFPQ	Splicing factor proline/glutamine-rich	1.895	0.025197
000038889	ABR	Active BCR-related	2.888	0.025316
000015993	VAT1L	Vesicle amine transport 1-like	2.163	0.026496
000062871	IL3	Interleukin enhancer-binding factor 3	3.912	0.026692
000040096	PITPNM2	Phosphatidylinositol transfer protein, membrane-associated 2 (Predicted), isoform CRA_b	3.229	0.027401
000019531	TCP1	T-complex 1	1.262	0.027669
000027869	BLES03	UPF0696 protein C11orf68 homolog	3.441	0.027993
000006370	Srrm2	Srrm2 protein	3.709	0.028996
000036391	Rpl23a	Ribosomal protein L23A	2.165	0.030442
000060198	RABEP1	Rab GTPase-binding effector protein 1	2.995	0.030909
000059624	CFL1	Cofilin 1 (non-muscle)	1.362	0.031633
000058649	SMARCC2	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily c, member 2	4.04	0.032673
000061342	DYNLL1	Dynein, light chain, LC8-type 1	6.663	0.033483
000006407	CRK	V-crk avian sarcoma virus CT10 oncogene homolog	2.189	0.033987
000033271	KPNA1	Karyopherin alpha 1 (importin alpha 5)	3.952	0.037638
000008813	HK2	Hexokinase 2	4.973	0.03764
000012670	FBXO2	F-box protein 2	2.381	0.037888
000023303		Uncharacterised protein	3.907	0.03875
000030866	TLN2	Talin 2	1.29	0.040855
000013184	DYNC1I1	Dynein, cytoplasmic 1, intermediate chain 1	2.102	0.04225
000012415	PRKAR2B	Protein kinase, cAMP-dependent, regulatory, type II, beta	1.51	0.043146

000012279	DYNLL2	Dynein, light chain, LC8-type 2	3.205	0.04428
000010017		Uncharacterised protein	7.036	0.045212
000058540	PREPL	Prolyl endopeptidase-like	1.872	0.045323
000049421	ZBS559	Protein LOC100363716	4.137	0.046721
000002091	GAP43	Growth associated protein 43 or Neuromodulin	1.588	0.048076
000058924		Uncharacterised protein	1.838	0.048926
000045959	HAPLN1	Hyaluronan and proteoglycan link protein 1	1.551	0.049267

Table 3.5. Proteins identified as down-regulated in the nucleus accumbens of Sugar/METH rats in comparison to Water/METH rats

Identifier: ENSRNOP00'	Symbol	Protein Description	Fold Change	*p*-value
000038546		Uncharacterised protein	-84.483	3.56E-08
000000064	GAK	Cyclin G associated kinase	-1.752	1.42E-05
000000222		Uncharacterised protein	-10.558	1.48E-05
000008355	SF3A1	Splicing factor 3a, subunit 1, 120kDa	-4.573	2.98E-05
000010545	PDHB	Pyruvate dehydrogenase (lipoamide) beta	-1.565	0.001422
000016749	CPNE4	Copine IV	-5.906	0.002338
000045180	H2AFV	H2A histone family, member V	-1.331	0.003113
000008736	ATP6V0C	V-type proton ATPase 16 kDa proteolipid subunit	-8.403	0.003832
000063484	EIF3A	Eukaryotic translation initiation factor 3, subunit A	-3.166	0.003867
000017967	PGAP1	Post-GPI attachment to proteins 1	-4.606	0.005158
000020192	HAGH	Hydroxyacylglutathione hydrolase	-1.532	0.005801
000022406	GLRX3	Glutaredoxin 3	-11.472	0.006056
000002358	PSMD2	Proteasome (prosome, macropain) 26S subunit, non-ATPase, 2	-1.137	0.006237
000007528	FAM49B	Family with sequence similarity 49, member B	-1.533	0.012524
000018462	VDAC2	Voltage-dependent anion channel 2	-1.28	0.014942
000002366	BDH1	3-hydroxybutyrate dehydrogenase, type 1	-3.931	0.021785
000024707	SFXN1	Sideroflexin 1	-1.334	0.022129
000019552	ANXA5	Annexin A5	-1.67	0.02252
000006567	SAR1B	Secretion associated, Ras related GTPase 1B	-3.423	0.022944
000011166	ABCB1	ATP-binding cassette, sub-family B (MDR/TAP), member 1	-3.102	0.023249
000059617		Uncharacterised protein	-3.571	0.025172
000020727		Uncharacterised protein	-4.766	0.025856
000003697	PRPSAP2	Phosphoribosyl pyrophosphate synthetase-associated protein 2	-1.837	0.026367
000007403		Guanine nucleotide-binding protein subunit gamma	-3.361	0.027085
000049647		Uncharacterised protein	-6.24	0.030701
000026792	ACTR1A	ARP1 actin-related protein 1 homolog A, centractin alpha (yeast)	-1.374	0.033378
000058202		Uncharacterised protein	-9.779	0.034398
000006605	PSMD14	Proteasome (prosome, macropain) 26S subunit, non-ATPase, 14	-3.041	0.035629
000045257	SH3BGRL	SH3 domain binding glutamate-rich protein like	-3.429	0.036016
000015475	ATPB13	ATPase, Na+/K+ transporting, beta 3 polypeptide	-1.83	0.038716
000015344	DNAJC13	DnaJ (Hsp40) homolog, subfamily C, member 13	-2.723	0.039718
000014167	MTOR	Mechanistic target of rapamycin (serine/threonine kinase)	-3.973	0.043884
000035846	FBXL16	F-box and leucine-rich repeat protein 16	-2.122	0.045426
000041042	HIST1H4J	Histone cluster 1, H4j	-1.429	0.047835

Table 3.6. Proteins identified as up-regulated in the nucleus accumbens of Sugar/METH rats in comparison to Water/Saline rats

Identifier: ENSRNOP00'	Symbol	Protein Description	Fold Change	*p*-value
000061921		Uncharacterised protein	8.682	2.17E-05
000027226		Uncharacterised protein	11.5	4.79E-05
000014871	GNB4	Guanine nucleotide binding protein (G protein), beta polypeptide 4	44.273	6.73E-05
000003083	ENOPH1	Enolase-phosphatase 1	5.919	0.000171
000053012		Uncharacterised protein	14.173	0.000239

000057517	CYLD	Ubiquitin carboxyl-terminal hydrolase CYLD	10.096	0.002343
000022983	SERPINH1	Serpin peptidase inhibitor, clade H (heat shock protein 47), member 1, (collagen binding protein 1)	1.736	0.004163
000022375	PIP4K2A	Phosphatidylinositol-5-phosphate 4-kinase, type II, alpha	7.359	0.008132
000009666	PSMA6	Proteasome (prosome, macropain) subunit, alpha type, 6	2.126	0.01232
000013515	ACOT2	Acyl-CoA thioesterase 2	3.253	0.015529
000048390	FAM65B	Family with sequence similarity 65, member B	2.206	0.01598
000008813	HK2	Hexokinase 2	1.416	0.018793
000052868	CAPZA1	Capping protein (actin filament) muscle Z-line, alpha 1	1.333	0.020061
000059909	ACTR3	Actin-related protein 3	1.385	0.021814
000000772	HINT1	Histidine triad nucleotide binding protein 1	1.917	0.022809
000060229	SUCLA2	Succinate-CoA ligase, ADP-forming, beta subunit	1.377	0.023309
000012301	FBXO6	F-box only protein 6	4.257	0.023562
000038375		Uncharacterised protein	6.849	0.026541
000007540	IL36A	Interleukin 36, alpha	3.113	0.027002
000022735	Hist1h1t	Histone H1t	2.294	0.028313
000012279	DYNLL2	Dynein, light chain, LC8-type 2	2.895	0.028801
000019574	TXNDC17	Thioredoxin domain containing 17	2.23	0.033161
000059480		Uncharacterised protein	1.838	0.033177
000008980	DLD	Dihydrolipoamide dehydrogenase	1.292	0.035046
000009198	RNPEP	Arginyl aminopeptidase (aminopeptidase B)	2.591	0.037946
000024258		Uncharacterised protein	1.621	0.039401
000023078	PPP5C	Protein phosphatase 5, catalytic subunit	1.932	0.040247
000020860	HSDL1	Hydroxysteroid dehydrogenase like 1	2.442	0.040739
000035734	CLIP2	CAP-GLY domain containing linker protein 2	1.512	0.042922
000019059	IDH2	Isocitrate dehydrogenase 2 (NADP+), mitochondrial	1.378	0.047277
000012255		Uncharacterised protein	1.433	0.049941

Table 3.7. Proteins identified as down-regulated in the nucleus accumbens of Sugar/METH rats in comparison to Water/Saline rats

Identifier: ENSRNOP00'	Symbol	Protein Description	Fold Change	*p*-value
000022184	RPS12	Ribosomal protein S12	-16.879	4.89E-08
000028888	CDS2	CDP-diacylglycerol synthase (phosphatidate cytidylyltransferase) 2	-4.794	8.74E-06
000030297		Uncharacterised protein	-5.372	1.58E-05
000032971		Uncharacterised protein	-34.619	4.38E-05
000026297	NUDT21	Nudix (nucleoside diphosphate linked moiety X)-type motif 21	-5.951	0.000294
000028188	DDB1	Damage-specific DNA binding protein 1, 127kDa	-12.694	0.000468
000024459	SV2C	Synaptic vesicle glycoprotein 2C	-1.726	0.000568
000024063	AKR7A2	Aldo-keto reductase family 7, member A2 (aflatoxin aldehyde reductase)	-1.791	0.001035
000005374	GIPC1	GIPC PDZ domain containing family, member 1	-5.327	0.002664
000025446	ECHS1	Enoyl CoA hydratase, short chain, 1, mitochondrial	-1.541	0.002844
000021170	CDH2	Cadherin 2, type 1, N-cadherin (neuronal)	-2.053	0.004547
000016965	NDUFV2	NADH dehydrogenase (ubiquinone) flavoprotein 2, 24kDa	-1.375	0.005344
000024711	PARK7	Parkinson protein 7	-1.369	0.005518
000020192	HAGH	Hydroxyacylglutathione hydrolase	-2.129	0.006011
000027150	LAMTOR1	Late endosomal/lysosomal adaptor, MAPK & MTOR activator 1	-4.863	0.006238
000008522	RAB2A	RAB2A, member RAS oncogene family	-1.471	0.007062
000008736	ATP6V0C	V-type proton ATPase 16 kDa proteolipid subunit	-6.365	0.00818
000003252	EPRS	Eprs protein	-1.53	0.009222
000054347	SFXN5	Sideroflexin 5	-1.125	0.011861
000039466	SEPT5	Septin 5	-1.299	0.012129
000010054	ARFGEF2	ADP-ribosylation factor guanine nucleotide-exchange factor 2 (brefeldin A-inhibited)	-5.187	0.012604
000007104	CNRIP1	Cannabinoid receptor interacting protein 1	-1.377	0.013203
000026482		Uncharacterised protein	-4.747	0.014328

000028484	PSMB4	Proteasome (prosome, macropain) subunit, beta type, 4	-1.885	0.015103
000009086	ACYP1	Acylphosphatase 1, erythrocyte (common) type	-4.451	0.016827
000055092		Uncharacterised protein	-4.438	0.019172
000061017	INPP4A	Type I inositol 3,4-bisphosphate 4-phosphatase	-4.395	0.022742
000019047	HINT3	Histidine triad nucleotide binding protein 3	-4.321	0.023052
000045257	SH3BGRL	SH3 domain binding glutamate-rich protein like	-3.384	0.024411
000019350	AP1M1	Adaptor-related protein complex 1, mu 1 subunit	-4.954	0.025546
000006355	ARF6	ADP-ribosylation factor 6	-1.825	0.027279
000041046	DIRAS2	DIRAS family, GTP-binding RAS-like 2	-1.652	0.028806
000026528	RPS5	Ribosomal protein S5	-1.867	0.029797
000000500	AGPAT1	1-acylglycerol-3-phosphate O-acyltransferase 1	-1.686	0.031265
000020443	Anp32a	Acidic (leucine-rich) nuclear phosphoprotein 32 family, member A	-1.291	0.034208
000021921	SLC12A2	Solute carrier family 12 (sodium/potassium/chloride transporter), member 2	-1.623	0.037274
000022836	IKBKAP	Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein	-1.175	0.03793
000008193	CPSF6	Cleavage and polyadenylation specific factor 6, 68kDa	-1.65	0.040671
000025159	ARFIP2	ADP-ribosylation factor interacting protein 2	-3.457	0.04331
000002478	DNM1L	Dynamin 1-like	-1.237	0.045644
000022626	RAB13	RAB13, member RAS oncogene family	-1.466	0.046333
000023806		Uncharacterised protein	-1.152	0.048682
000027088	TOMM20	Translocase of outer mitochondrial membrane 20 homolog (yeast)	-2.834	0.049844

Table 3.8. Proteins identified as uniquely expressed in the nucleus accumbens of rats in the Water/Saline, Water/METH and Sugar/METH treatment groups

Water/Saline Condition: 11 proteins identified as uniquely differentially expressed			
Identifier	Symbol	Protein Description	*p*-value
ENSRNOP00000024711	PARK7	Parkinson protein 7	0.007662
ENSRNOP00000021921	SLC12A2	Solute carrier family 12 (sodium/potassium/chloride transporter), member 2	0.008688
ENSRNOP00000045650	ATP1A1	ATPase, Na+/K+ transporting, alpha 1 polypeptide	0.012518
ENSRNOP00000021170	CDH2	Cadherin 2, type 1, N-cadherin (neuronal)	0.013865
ENSRNOP00000024459	SV2C	Synaptic vesicle glycoprotein 2C	0.014863
ENSRNOP00000008948	LANCL2	LanC lantibiotic synthetase component C-like 2 (bacterial)	0.015184
ENSRNOP00000016842	INPP1	Inositol polyphosphate-1-phosphatase	0.016027
ENSRNOP00000052868	CAPZA1	Capping protein (actin filament) muscle Z-line, alpha 1	0.017281
ENSRNOP00000012847	CCT4	Chaperonin containing TCP1, subunit 4 (delta)	0.045557
ENSRNOP00000062102	UBE2V2	Ubiquitin-conjugating enzyme E2 variant 2	0.049657
ENSRNOP00000008522	RAB2A	RAB2A, member RAS oncogene family	0.049963
Water/METH Condition: 13 proteins identified as uniquely differentially expressed			
Identifier	Symbol	Protein Description	*p*-value
ENSRNOP00000004613	CADM3	Cell adhesion molecule 3	0.001253
ENSRNOP00000025279	MOBP	Myelin-associated oligodendrocyte basic protein	0.003173
ENSRNOP00000010545	PDHB	Pyruvate dehydrogenase (lipoamide) beta	0.004328
ENSRNOP00000063484	EIF3A	Eukaryotic translation initiation factor 3, subunit A	0.005496
ENSRNOP00000018049	SFPQ	Splicing factor proline/glutamine-rich	0.014351
ENSRNOP00000045959	HAPLN1	Hyaluronan and proteoglycan link protein 1	0.017339
ENSRNOP00000001247	RAN	RAN, member RAS oncogene family	0.022269
ENSRNOP00000024707	SFXN1	Sideroflexin 1	0.023856
ENSRNOP00000025217	RPL17	Ribosomal protein L17	0.028192
ENSRNOP00000007100	YWHAE	Tyrosine 3-monooxygenase/ tryptophan 5-monooxygenase activation protein, epsilon	0.028682
ENSRNOP00000006407	CRK	V-crk avian sarcoma virus CT10 oncogene homolog	0.032033
ENSRNOP00000007528	FAM49B	Family with sequence similarity 49, member B	0.03555
ENSRNOP00000008477	VDAC1	Voltage-dependent anion channel 1	0.035864
Sugar/METH Condition: 12 proteins identified as uniquely differentially expressed			
Identifier	Symbol	Protein Description	*p*-value
ENSRNOP00000020192	HAGH	Hydroxyacylglutathione hydrolase	0.002427

ENSRNOP00000040969	ATP5L	ATP synthase, H+ transporting, mitochondrial Fo complex, subunit G	0.01145
ENSRNOP00000024258		Uncharacterised protein	0.01244
ENSRNOP00000023934	ATP2B3	ATPase, Ca++ transporting, plasma membrane 3	0.018957
ENSRNOP00000022735	Hist1h1t	Histone H1t	0.029335
ENSRNOP00000017227	STX12	Syntaxin 12	0.033272
ENSRNOP00000020066	HSPE1	Heat shock 10kDa protein 1	0.036344
ENSRNOP00000012415	PRKAR2B	Protein kinase, cAMP-dependent, regulatory, type II, beta	0.038703
ENSRNOP00000059480		Uncharacterised Protein	0.039262
ENSRNOP00000002091	GAP43	Growth associated protein 43 or Neuromodulin	0.041135
ENSRNOP00000060948		Uncharacterised Protein	0.047887
ENSRNOP00000022849	EPB41L3	Erythrocyte membrane protein band 4.1-like 3	0.04955

3.4.1 Top biological functions and signalling pathways

The Ingenuity Pathway Analysis software (IPA, Ingenuity Systems, www.ingenuity.com) was used to determine the top molecular and cellular functions and the top canonical pathways from the up-regulated and down-regulated proteins in each comparison. The IPA compares the protein abundance levels in each comparison between the treatment groups to allow for a greater understanding of the interactive functions of the significantly identified proteins. The top molecular and cellular functions for the Water/Saline and Water/METH, the Sugar/METH and Water/METH, and the Water/Saline and Sugar/METH comparisons are shown in Table 3.9 and Figures 3.3-3.8. The proteins involved in the top canonical pathways for the three comparisons between the three treatment groups are displayed in Table 3.10.

Table 3.9. Top molecular and cellular functions involved in the effect of methamphetamine cross-sensitisation in the rat nucleus accumbens

Top molecular and cellular functions between Water/Saline and Water/METH rats			
	p-value	No. of Proteins	Proteins
Cell Death and Survival	5.10E-06 - 2.83E-02	40	ALCAM, ALDOA, ANK3, ATP1A1, BIN1, CA2, CCT4, CRK, CSNK2A1, CSPG4, DDB1, EIF3A, ETHE1, EXOG, GABRA1, GABRB2, GSTA2, H2AFX, HSP90AB1, IBSP, INPP1, LANCL2, MAP1S, MLLT4, PARK7, PPP1R11, PRNP, PSIP1, PTPN5, RAN, RPLP0, SFPQ, SLC12A2, UBE2V2, VDAC1, VTI1A, XPO1, YWHAE, YWHAG, YWHAQ
Cellular Assembly and Organisation	5.39E-06 - 2.85E-02	23	ANK3, BIN1, CBX3, CRK, CSPG4, DLGAP3, NCAN, NGEF, SLC12A2, UBE2V2, YWHAG, MLLT4, PRNP, PARK7, RAB13, RTN3, EIF3A, RAN, H2AFX, VDAC1, VTI1A, ALDOA,

			CDC42BPB
Molecular Transport	4.05E-05 - 2.71E-02	17	RAN, XPO1, ANK3, ATP1A1, CA2, GSTO1, GABRA1, GABRB2, SFXN1, SLC12A2, VDAC1, YWHAE, EXOG, H2AFX, PARK7, PRNP, RAB13
RNA Trafficking	4.05E-05 - 1.47E-02	3	RAN, XPO1, EIF3A
Protein Trafficking	6.77E-05 - 2.71E-02	5	RAN, XPO1, YWHAE, YWHAG, YWHAQ
Top molecular and cellular functions between Sugar/METH and Water/METH rats			
	p-value	No. of Proteins	Proteins
Cell-to-Cell Signalling and Interaction	2.36E-04 - 4.74E-02	15	CLDN11, RAB13, VCL, DNM1L, SF3A1, TLN2, GAP43, HAPLN1, MTOR, ANXA5, CRK, ATP6V0C, EIF3A, PRKAR2B, CFL1
Cellular Assembly and Organisation	2.36E-04 - 4.81E-02	21	CLDN11, RAB13, CFL1, CRK, ANXA5, GAK, ATP6V0C, DYNLL1, DNM1L, MTOR, VCL, GAP43, TLN2, KPNA1, RAN, EIF3A, TCP1, CBX3, ABR, HK2, STX12
Cellular Function and Maintenance	2.36E-04 - 4.44E-02	17	CLDN11, RAB13, CFL1, CRK, DNM1L, MTOR, VCL, TLN2, ABR, DYNLL1, GAP43, KPNA1, RAN, ANXA5, ATP6V0C, GAK, SFPQ
Cell Morphology	2.60E-04 - 4.41E-02	19	CFL1, DNM1L, MTOR, VCL, CRK, GAP43, ABCB1, ABR, ACSBG1, ATP6V0C, CLDN11, EPB41L3, FBXO2, HK2, PRKAR2B, RAB13, RAN, WIPF3, ANXA5
Protein Degradation	5.16E-04 - 2.62E-02	6	FBXO2, FBXO6, MTOR, PSMD14, PSMD2, STX12
Top molecular and cellular functions between Water/Saline and Sugar/METH rats			
	p-value	No. of Proteins	Proteins
Cellular Assembly and Organisation	6.18E-04 - 4.86E-02	14	Anp32a, ARF6, ARFGEF2, ARFIP2, ATP6V0C, CDH2, DNM1L, HK2, LAMTOR1, RAB2A, RAB13, SEPT5, SERPINH1, SLC12A2
Cell-To-Cell Signalling and Interaction	7.15E-04 - 4.64E-02	11	ARF6, ATP6V0C, CDH2, DNM1L, FAM65B, GIPC1, PARK7, RAB13, SEPT5, SLC12A2, SV2C
Drug Metabolism	7.15E-04 - 3.94E-02	4	ATP6V0C, HAGH, PARK7, SEPT5
Molecular Transport	7.15E-04 - 4.24E-02	16	Anp32a, AP1M1, ARF6, ARFGEF2, ATP6V0C, DNM1L, GIPC1, HAGH, HK2, PARK7, PIP4K2A, RAB13, RAB2A, SEPT5, SFXN5, SLC12A2
Small Molecule Biochemistry	7.15E-04 - 4.77E-02	14	ACOT2, AGPAT1, ARF6, ARFGEF2, ATP6V0C, DLD, DNM1L, ENOPH1, HK2, PARK7, PIP4K2A, RAB2A, SEPT5, SUCLA2

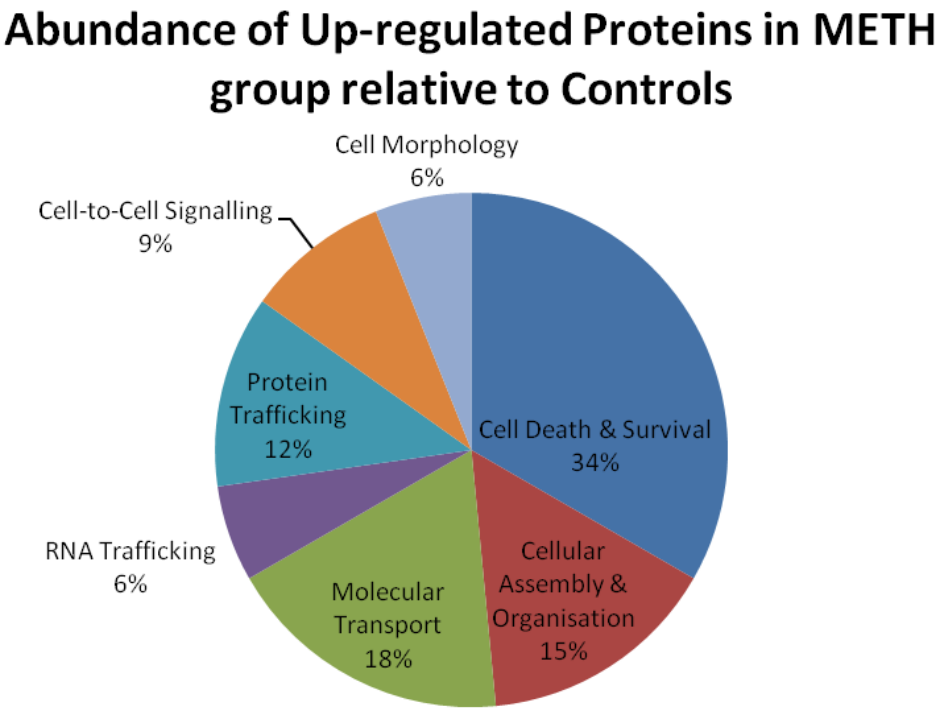

Figure 3.3. Abundance of up-regulated proteins involved in regulating the top molecular and cellular functions in the NAc of METH-treated rats (Water/METH) compared to controls (Water/Saline) after challenge injection

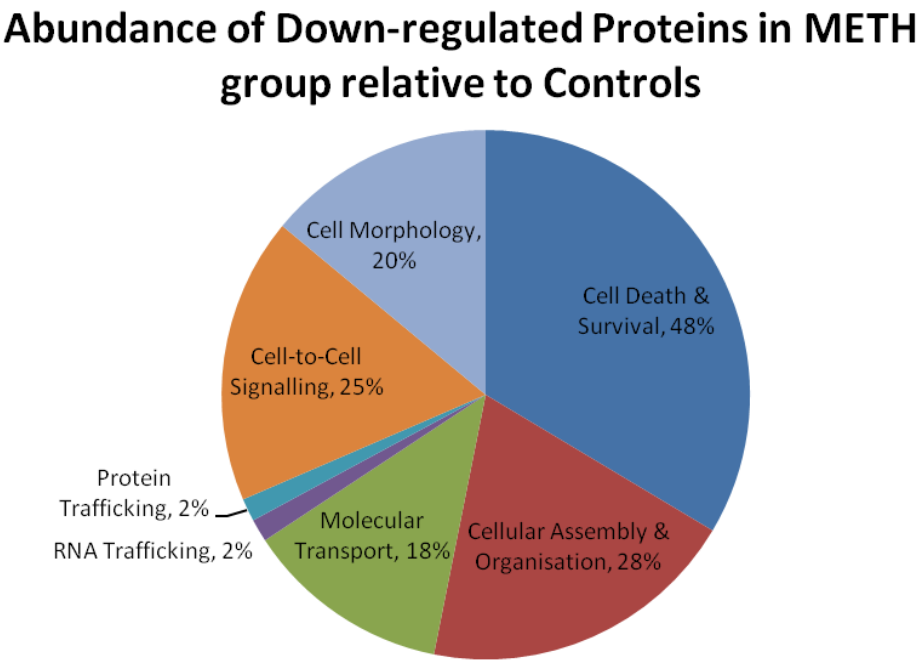

Figure 3.4. Abundance of down-regulated proteins involved in regulating the top molecular and cellular functions in the NAc of METH-treated rats (Water/METH) compared to controls (Water/Saline) after challenge injection

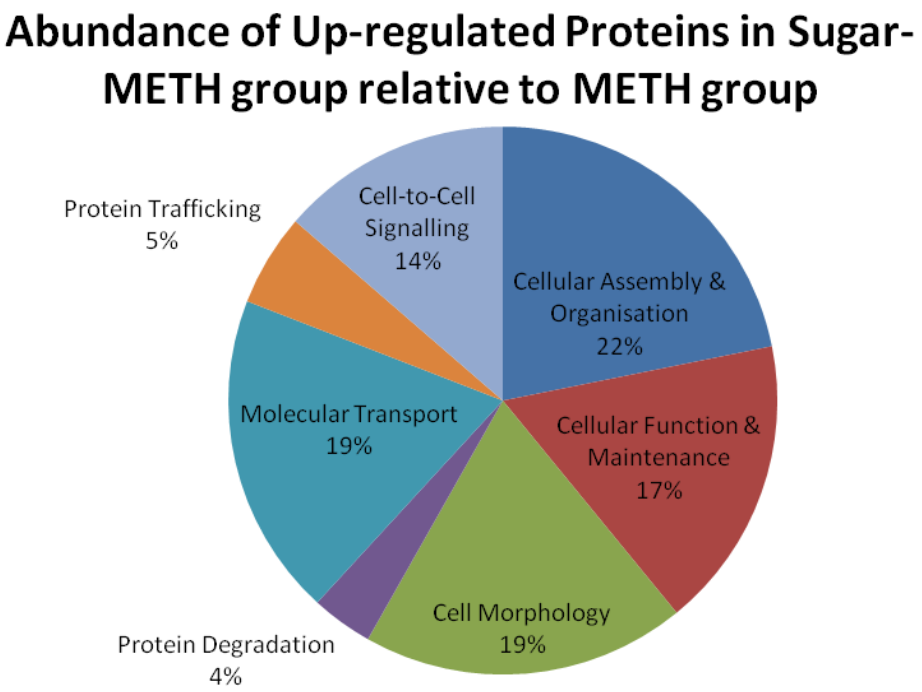

Figure 3.5. Abundance of up-regulated proteins involved in regulating the top molecular and cellular functions in the NAc of sugar-METH-treated rats (Sugar/METH) compared to METH-treated rats (Water/METH) after challenge injection

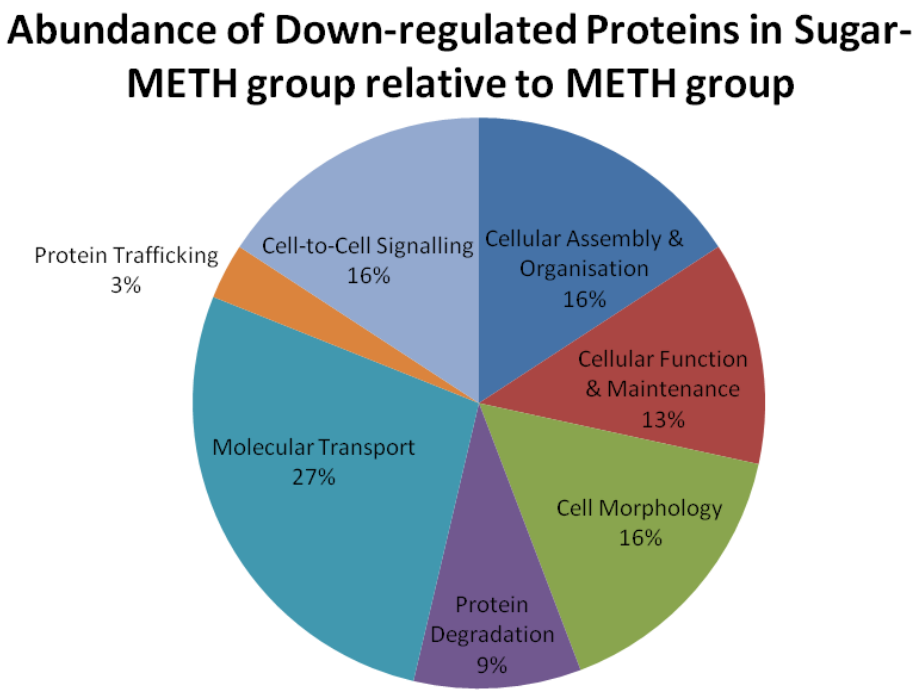

Figure 3.6. Abundance of down-regulated proteins involved in regulating the top molecular and cellular functions in the NAc of sugar-METH-treated rats (Sugar/METH) compared to METH-treated rats (Water/METH) after challenge injection

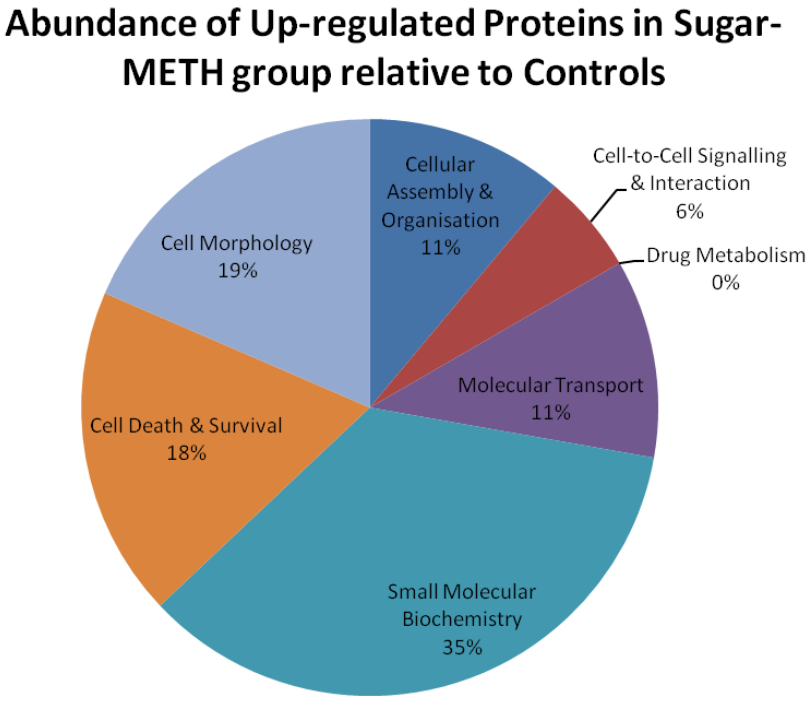

Figure 3.7. Abundance of up-regulated proteins involved in regulating the top molecular and cellular functions in the NAc of sugar-METH-treated rats (Sugar/METH) compared to controls (Water/Saline) after challenge injection

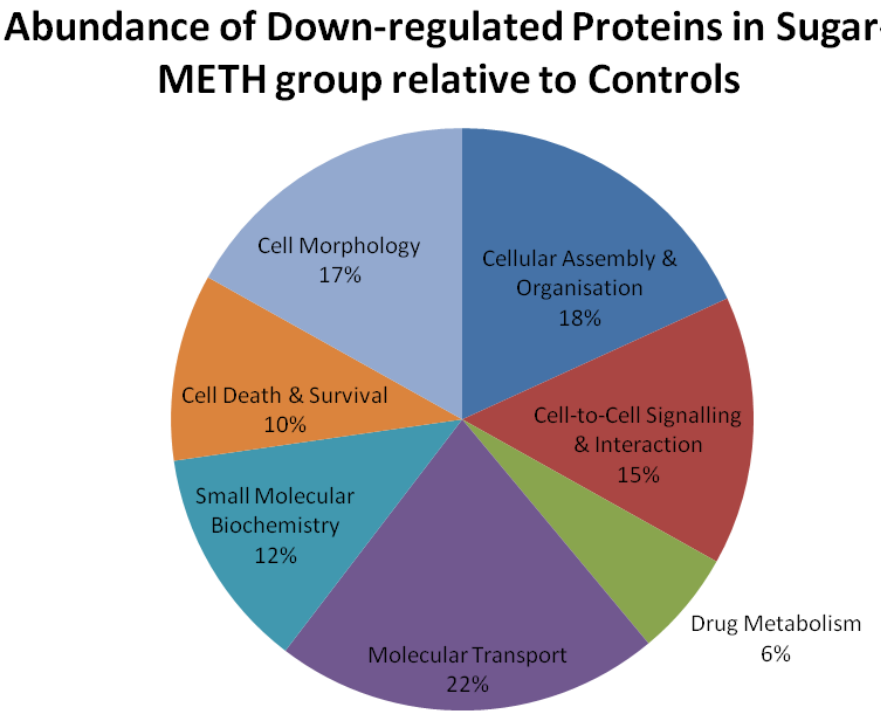

Figure 3.8. Abundance of down-regulated proteins involved in regulating the top molecular and cellular functions in the NAc of sugar-METH-treated rats (Sugar/METH) compared to controls (Water/Saline) after challenge injection

Table 3.10. Top canonical pathways involved in the effect of methamphetamine cross-sensitisation in the rat nucleus accumbens

Top canonical pathways between Water/Saline and Water/METH rats			
	p-value	Ratio	Molecules
IGF-1 Signalling	4.62E-04	4/97 (0.041)	YWHAQ, YWHAG, YWHAE, CSNK2A1
Cell Cycle: G2/M DNA Damage Checkpoint Regulation	7.94E-04	3/49 (0.061)	YWHAQ, YWHAG, YWHAE
PI3K/AKT Signalling	1.13E-03	4/123 (0.033)	YWHAQ, YWHAG, YWHAE, HSP90AB1
Myc Mediated Apoptosis Signalling	1.3E-03	3/58 (0.052)	YWHAQ, YWHAG, YWHAE
ERK5 Signalling	1.65E-03	3/63 (0.048)	YWHAQ, YWHAG, YWHAE
Top canonical pathways between Sugar/METH and Water/METH rats			
	p-value	Ratio	Molecules
Actin Cytoskeleton Signalling	9.06E-04	5/217 (0.023)	TLN2, CFL1, CRK, VCL, PIP4K2A
RAN Signalling	1.54E-03	2/17 (0.118)	RAN, KPNA1
Tight Junction Signalling	2.63E-03	4/167 (0.024)	CLDN11, PRKAR2B, RAB13, VCL
FAK Signalling	3.36E-03	3/87 (0.034)	TLN2, CRK, VCL
Paxillin Signalling	5.25E-03	3/102 (0.029)	TLN2, CRK, VCL
Top canonical pathways between Water/Saline and Sugar/METH rats			
	p-value	Ratio	Molecules
Cleavage and Polyadenylation of Pre-mRNA	5.24E-04	2/12 (0.167)	CPSF6, NUDT21
Signalling by Rho Family GTPases	5.5E-04	5/234 (0.021)	GNB4, CDH2, SEPT5, ARFIP2, PIP4K2A
Isoleucine Degradation I	7.2E-04	2/14 (0.143)	ECHS1, DLD
CDP-diacylglycerol Biosynthesis I	8.29E-04	2/15 (0.133)	AGPAT1, CDS2
Parkinson's Signalling	9.45E-04	2/16 (0.125)	SEPT5, PARK7

4. Discussion

The present study sought to determine the effect of chronic sucrose exposure during adolescence on locomotor activity and protein expression in the NAc following acute METH challenge in adulthood. During the 26-day treatment regimen, there were time-dependent changes in total energy intake between the water-treated and sucrose-treated groups, where excessive sucrose consumption resulted in compensatory reductions in chow consumption in sucrose-treated rats by the end of treatment. The behavioural results demonstrated that the acute METH challenge significantly heightened locomotor activity in the water-treated animals (i.e., Water/METH) compared to rats given the acute saline challenge (i.e. Water/Saline controls), providing support for hypothesis one. The findings of the current study support hypothesis two, providing evidence for an effect of chronic sucrose exposure during adolescence to produce behavioural cross-sensitisation to METH administration in adulthood.

The findings from the proteomic analyses of the NAc supported hypothesis three, indicating that in comparison to the controls, the rats in the water-METH condition exhibited differential changes in the expression of a total of 93 proteins within the NAc. These proteins were mainly involved in the regulation of neuronal and synaptic modifications, and cellular structure and functioning. This is the first known study to analyse the expression of protein changes in the NAc in response to METH after chronic sugar consumption. Providing support for hypothesis four, the findings from the proteomic analyses significantly identified a total of 102 proteins in the NAc as differentially expressed in the sugar-METH condition compared to the water-METH group. A large proportion of these detected proteins were involved in coordinating cell signalling and interaction, cell structure and function, and cell morphology. The proteomic analyses also supported hypothesis five, indicating that the combination of sugar pre-exposure and METH challenge significantly changed the expression of a total of 74 proteins compared to control treated rats. These differentially expressed proteins were involved in regulating cellular interactions, maintaining cellular structure and morphology, and transporting molecular substrates. Thus, the present study highlights that the effects of chronic sugar consumption can induce distinct changes in the NAc neuronal networks and synapses that may contribute to the augmented behavioural responding to METH.

4.1 Energy (Kilojoule) Intake across Treatment

During the 26-day treatment regimen, in comparison to the controls (Water/Saline) and water-METH treated rats, the rats in the combined sugar-METH treatment group consumed significantly more energy due to sucrose intake, yet, there were no significant differences in food intake between the three treatment groups on the first and fifth days of the sugar-treatment regimen. However, on the last day of the sugar-treatment regimen, there were no significant differences in total energy intake between the treatment groups. These findings highlight that, at the beginning of the sugar-treatment period, the rats in the sugar-METH treatment group did not adjust their food consumption in order to compensate for the excessive amount of energy consumed from the sugar treatment (Vendruscolo et al., 2010).

Such consummatory behaviours parallel that seen in human adolescents whereby a large proportion of adolescents fail to balance their food intake in order to compensate for indirect sugar consumption (Lien et al., 2006; Vendruscolo et al., 2010). However, by the last day of the treatment period, rats had learned to compensate for the amount of energy consumed as the sugar-METH treatment group consumed less food, similar to that shown in older rats on a high fat refined sugar diet (Francis et al., 2013).

A surprising finding is that during adulthood, on the METH challenge day, the combined sugar-METH animals consumed significantly more energy from food and water compared to controls and METH-treated animals. Although this may influence locomotor behaviour in response to METH, the heightened amount of energy intake in adulthood suggests that the consummatory behaviours acquired during adolescence may subsequently influence feeding behaviours during adulthood (Lien et al., 2006). Thus, exposure to excessive sugar during adolescence may encourage the development of augmented appetitive behaviours in adulthood. These developmental shifts in consummatory behaviour in the sugar-METH group may be indicative of the processes involved in METH behavioural cross-sensitisation.

4.2 Locomotor Behaviour in Response to Acute Methamphetamine Challenge

The present study demonstrated that a single challenge injection of METH induced heightened locomotor activity in comparison to saline-treated animals (Koob, 1992; Iwazaki et al., 2006). These findings are in accordance with the literature and indicate that due to the pharmacological properties of the drug, the acute administration of METH can significantly alter dopaminergic activity to augment locomotor behaviour (Blumenthal & Gold, 2010). In particular, the administration of METH immediately elevates synaptic dopamine expression within the NAc, by reversing the function of the DAT to produce greater availability of activating dopamine D1 receptors (Nairn et al., 2004; Koob, 1992). These effects, in turn, have been well-described to heighten locomotor and activational motivation (Le Moal & Simon, 1991; Vanderschuren & Kalivas, 2000; Fukushima et al., 2007; Panenka et al., 2013)

Although the locomotor behavioural effects of acute METH treatment are temporary, the immediate dopaminergic activation in response to a single METH injection is powerful enough to induce long-term changes in the functional expression of the proteins within the NAc (Iwazaki et al., 2006). These modifications to protein functioning can stimulate morphological alterations to synaptic plasticity and trigger a cascade of neuroadaptations (Nestler, 2001). Synaptic and neuronal adaptations within the NAc set the stage for the development of reward-motivated behaviours that later progress to reward-dependent behaviours with continued strengthening to these neuroadaptations (Koob, 1992; Koob et al., 1998).

4.3 Locomotor Cross-sensitisation of Sucrose and Methamphetamine

In comparison to rats exposed to water during adolescence, rats pre-exposed to sucrose during adolescence demonstrated significantly greater locomotor activation in response to the METH challenge injection. These findings are consistent with previous research demonstrating behavioural cross-sensitisation between prior sucrose exposure and amphetamine administration (Avena & Hoebel, 2003; De Sousa et al., 2000). Together, this research suggests that sugar may be operating in corresponding neuronal networks within the NAc similar to METH and amphetamine to exaggerate the behavioural effects produced by the administration of these psychostimulants (Hoebel et al., 2009).

The behavioural cross-sensitised response is likely due to the functions of the dopaminergic system within the NAc (Avena et al., 2008). Specifically, chronic exposure to sugar consumption can induce enduring changes in the expression of the NAc dopaminergic system by enhancing the expression of the D1 dopamine receptors and reducing the expression of the D2 dopamine receptors (Bello et al., 2002; Colantuoni et al., 2001). These long-term modifications to the dopamine receptors within the NAc may in turn alter the structural and functional morphology of the DAT (Robinson & Kolb, 1999). Since the reinforcing behavioural effects of METH are regulated by DAT availability within the NAc, the synaptic modifications in the DAT induced firstly by sucrose exposure may augment the

behavioural effects of METH (Bello et al., 2003; Avena et al., 2008). This stimulates excessive levels of dopamine within the synapse to amplify the subjective and behavioural effects of METH (Steinkellner et al., 2011).

In the current study, the sugar-treated rats demonstrated a gradual increase in the consumption of sucrose (Figure 1 in Appendix E). This highlights that the continuous access to sugar may heighten sensitivity for rewarding substances by inducing changes to neuronal morphology. In light of this, the subsequent administration of METH may strengthen these neuroadaptations to reinforce the development of the primary stages underlying reward-motivated behaviours.

Additionally, the distinct neuroadaptations between the sugar-METH and water-METH treatment groups may determine different reward-related learning processes. Since the consumption of sugar can produce intense feelings of reward and pleasure, the learning processes forming these connections between the sugar substance and reward become established (Alsio et al., 2012; Ikemoto & Panksepp, 1999). The link between reward and the initial stimuli can be applied to other substances that produce intense feelings of reward, such as METH and amphetamine. This may reinforce reward-motivated behaviours and prolong the use of the substance (Kelley, 2004). For instance, De Sousa and colleagues (2000) demonstrated that subjects identified as having a greater preference for sugar consumption were more likely to show augmented responding for amphetamine. These findings suggest that preferences for rewarding substances, such as sugar, may encourage synaptic plasticity and induce structural changes in the axons and dendrites that underlie the building blocks of reward-motivated learning to strongly reinforce continued use of psychostimulants (Berridge & Robinson, 1998; Robinson & Kolb, 1999; Blumenthal & Gold, 2010).

From the proteomic analyses, a majority of the differentially expressed proteins in the sugar-METH group, compared to the METH-treated group, were critical to the development of synaptic plasticity and neuroadaptations. This is supportive of the potential role the NAc glutamatergic system may play in encoding reward-related learning processes for both sugar

and METH through its interaction with the dopaminergic system (Kelley, 2004). For example, the prolonged consumption of sugar strengthened the long-term potentiation of the glutamate receptors within the NAc (Tukey et al., 2013). Correspondingly, prolonged METH treatment altered the expression of the glutamate receptors within the NAc (Parsegian & See, 2014). In light of these findings, the enduring modifications in the expression of both dopamine and glutamate during sugar pre-exposure may modify the structural and functional morphology of the synapse (Tukey et al., 2013; Kelley, 2004). This, consequently, may change the strength of the synapse to subsequently facilitate learning and encoding of reward-relevant information (Lominac et al., 2012). As a result, since METH targets the NAc dopaminergic and glutamatergic systems, these alterations in synaptic strength may aid the acquisition of learning associations between METH and reward, and promote the formation of reward-dependent behaviours (Parsegian & See, 2014).

Although the cross-sensitisation effect is evident in response to amphetamine and METH, it is not generalizable to all psychostimulants that activate the functions of the NAc dopaminergic system (De Sousa et al., 2000). Unlike amphetamine, there has been limited evidence demonstrating sugar cross-sensitisation in response to cocaine administration. For example, Vendruscolo and others (2010) demonstrated that continuous pre-exposure to excessive sugar consumption during adolescence failed to significantly affect the self-administration of cocaine during adulthood. This may be due to differences in the temporal activation of the NAc neuronal networks between sugar and cocaine (Cameron & Carelli, 2012). Both sugar and cocaine stimulate the release of dopamine, but the different phasic neuronal activation may trigger divergent neuroadaptations to encode reward-related behavioural plasticity (Di Chiara, 2002; Vendruscolo et al., 2010).

Additionally, the temporal activity of the NAc dopaminergic system between METH and cocaine is distinctly different (De Sousa et al., 2000). Specifically, in comparison to cocaine, METH induces more rapid stimulation of dopamine release which is followed by the delayed clearance of dopamine within the NAc (Fowler et al., 2008). Accordingly, the

immediate neuronal pharmokinetic activation of METH may be more sensitive and responsive to the synaptic modifications induced by the pre-exposure of sugar (Avena et al., 2008; Fowler et al., 2008). Thus, the subsequent administration of METH may augment the subjective behavioural effects and reinforce the rewarding and motivational value of the drug because the modifications in the NAc dopaminergic system induced firstly by sugar may correspond to the neuronal effects of METH (Avena et al., 2003; Kelley, 2004).

4.4 Proteomic Analysis

The molecular findings from the present proteomic analyses contribute to and expand on the current neurobiological understanding of locomotor cross-sensitisation. This is the first known study to detail and compare the protein expression profile of the NAc associated with the effects of acute METH administration and METH behavioural cross-sensitisation to adolescent exposure to sucrose. A total of 93 proteins were significantly identified as differentially expressed in the METH treatment group compared to the controls. A large proportion of these proteins were involved in regulating cell death and survival, and cellular assembly and organisation, which may influence the activational structure of the NAc dopaminergic and glutamatergic systems. Accordingly, changes in the proteins governing such functions suggest neurotoxic changes in the functioning of mitochondria to induce oxidative stress and neuroadaptations within the NAc, such as alterations in synaptic strength and dendritic branching.

A total of 102 proteins were significantly identified as differentially expressed in the NAc of the combined sugar-METH treatment group, in comparison to the METH group. The molecular functions of a majority of these proteins were involved in the regulation of cell-to-cell signalling, cellular assembly and organisation, and cellular function and maintenance. Interestingly, proteins involved in cell death were less indicated following METH treatment in sucrose-treated animals, with alterations to protein levels predominately highlighting structural and functional modifications to the morphology of the synapses and neuronal networks within the NAc.

Compared to the controls, the proteomic analyses significantly identified a total of 74 differentially expressed proteins in the NAc of the sugar-METH group. Changes to the proteins in this comparison were involved in the regulation of cell structure, cell signalling and molecular transport, which also suggest alterations to the structure and activity of cellular and neuronal morphology. Differences in protein expression found between the controls and the sugar-METH group was used to confirm that the differential expression of proteins between the sugar-METH and METH treatment groups were specific to that comparison.

Within the proteomic data, a number of proteins were identified as greatly differentially expressed, including histone cluster 3 H2a (HIST3H2A), reticulon 4 (RTN4), reticulon 3 (RTN3) and unconventional myosin-Va (MYO5A). Firstly, through its structural functioning in the nucleosome, HIST3H2A plays a critical maintenance function in regulating DNA transcription and repair (Rando & Ahmed, 2007). The up-regulation of HIST3H2A in the METH-treated animals, compared to the controls, suggest that HIST3H2A may be excessively activated in order to repair and restore the structural morphology and functioning of the cellular processes that were damaged in response to acute METH treatment (Martin et al., 2012).

Additionally, RTN4, which is involved in cellular apoptotic death and membrane trafficking (Watari & Yutsudo, 2003) was identified as up-regulated in the METH-treated group compared to controls. This heightened expression may indicate that the pharmacological properties of METH stimulated neuronal cell death within the NAc mediated by the activity of RTN4 (Murayama et al., 2006). However, RTN3 is also involved in coordinating membrane trafficking and apoptosis (Wakana et al., 2005; Murayama et al., 2006), and this protein was identified as down-regulated in the METH-treated condition compared to controls. Although both proteins are involved in similar functions, RTN3 is highly enriched in neurons and RTN4 expression is more abundant in the oligodendrocytes (He et al., 2004). These findings suggest that the administration of METH may stimulate RTN3 activity to promote functional membrane trafficking in order to stabilise excessive

apoptosis in the NAc neuronal cells; whereas the pharmokinetic effects of METH may exhaust the activity of RTN4 to induce axonal and dendritic structure degradation within the NAc neuronal networks (He et al., 2004; Wakana et al., 2005).

MYO5A was identified as down-regulated in the METH-treated rats compared to the controls. MYO5A participates in coordinating the functioning of synaptic and neuronal morphology through the mechanisms of the actin cytoskeletal pathway (Tilelle et al., 2003; Lise et al., 2009). This reduction in the expressional activity of MYO5A may be directly linked to the reduced functioning of the actin cytoskeletal pathway induced by METH treatment (Shen et al., 2009; Lise et al., 2009). In response, this may reduce dendritic spine formation and subsequently reduce the activational potential of synaptic transmission within the NAc neuronal networks (Lise et al., 2009; Braithwaite et al., 2006).

Conversely, in comparison to the METH-treated rats, MYO5A was identified as up-regulated in the sugar-METH treated rats. The preserved functioning of MYO5A in the sugar-METH group compared to the METH-treated group suggests that the pre-exposure to sugar may induce adaptive changes in the actin cytoskeleton pathway that may have protected the phosphorylation of MYO5A in response to METH treatment. This protective mechanism may induce morphological changes within the NAc neuronal systems by stimulating the excessive growth of axonal and dendritic spines (Yue et al., 1999; Lai & Ip, 2009; Lise et al., 2009). These morphological changes may strengthen synaptic and neuronal transmission to promote efficient encoding of reward-related learning (Braithwaite et al., 2006). Better known proteins aligned with the effects of METH will be discussed.

4.4.1 Mitochondrial dysfunction: distinct differences between the treatment groups

A profound effect of acute METH treatment is its ability to alter the functioning of the mitochondria, which is the power house of all cells to regulate their functioning and cell death (Detmer & Chan, 2007; Ren et al., 2011). In light of the important functions of the mitochondria, dysfunctions to the mitochondria can consequently alter the axonal and dendritic morphology of neurons, which ultimately lead to alterations in synaptic transmission

(Detmer & Chan, 2007). In the current study, the proteomic analyses identified significant changes to mitochondrial functioning in the METH-treated rats compared to controls. Mitochondrial dysfunction was associated with the heightened expression of voltage-dependent anion channel 1 (VDAC1). The VDAC protein group consists of three isoforms, including VDAC1, VDAC2 and VDAC3, and the pores generated by the VDAC1 and VDAC2 components are located on the outer membrane of the mitochondria (Sampson et al., 1996; Colombini et al., 1996). The VDAC proteins are critical in sustaining mitochondrial structure and function, regulating cellular growth and survival, and maintaining synaptic morphology through the mitochondria (Reddy, 2012; Raghaven et al., 2012).

Previous studies using a proteomic approach have demonstrated that the acute administration of METH upregulated the expression of VDAC1 in the striatum (Iwazaki, McGregor, & Matsumoto, 2006). This suggests that the pharmacological effects of a single treatment of METH can exert powerful modifications and disruptions to mitochondrial functioning, and consequently influence synaptic plasticity (Brown & Yamamoto, 2003). As a consequence of the combined alterations in VMAT-2 functioning and dopamine availability produced by acute METH administration, the structure and activational properties of the mitochondria become impaired which triggers oxidative stress and the formation of free radicals, such as reactive oxygen species (ROS) (Brown & Yamamoto, 2003; Yamamoto et al., 2010) (see Figure 4.1). For instance, Fumagalli and colleagues (1999) demonstrated that impairments in the genetic expression of VMAT-2 enhanced METH-induced dopaminergic neurotoxicity within the striatum. This dopaminergic neurotoxicity was accompanied by the formation of free radicals. These findings highlight that the METH-induced alterations in VMAT-2 activity and dopaminergic homeostasis reduces mitochondrial functioning to promote oxidative stress (Miyazaki & Asamima, 2008; Yamamoto et al., 2010). The disruptions in mitochondrial activity promote modifications to the structure of the axonal terminals and dendritic spines, which thereby alters synaptic plasticity, neurotransmission and downstream molecular regulation (Detmer & Chan, 2007).

In conjunction with the down-regulated expression of VDAC1, the expression glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was heightened in response to acute METH treatment in the METH-treated animals compared to controls. This protein is involved in converting glucose into ATP, and regulates apoptotic cell death under conditions of oxidative stress (Nakajima et al., 2009). Previous studies have demonstrated that acute exposure to METH heightened the expression of GAPDH, and this was associated with elevated synaptic dopamine and glutamate levels (Mark et al., 2007; Nakajima et al., 2009). Interestingly, this suggests that the energy regulation system of GAPDH may mediate restoring the functioning of the glutamatergic and dopaminergic synaptic vesicles (Ikemoto, Bole, & Ueda, 2003). Since GAPDH was up-regulated in the current findings, the excessive stimulation of GAPDH activity in response to METH treatment may be activated in order to swiftly restore and refill the glutamate and dopamine vesicles within the NAc (Ikemoto et al., 2003; Nakajima et al., 2009). However, the over-production of energy generated by GAPDH in order to compensate for depleted glutamate and dopamine levels may consequently produce excessive levels of oxidative stress (Mark et al., 2007). As a result, this may induce apoptotic cell death primarily in the glutamatergic and dopaminergic neuronal receptors, and thereby reduce efficient synaptic and neural transmission (Nakajima et al., 2009; Mark et al., 2007). The continued strengthening to these morphological alterations may contribute to the potential development of reward-motivated behaviours acquired through learning associations between the drug and reward (Koob et al., 1998; Robinson & Kolb, 1999).

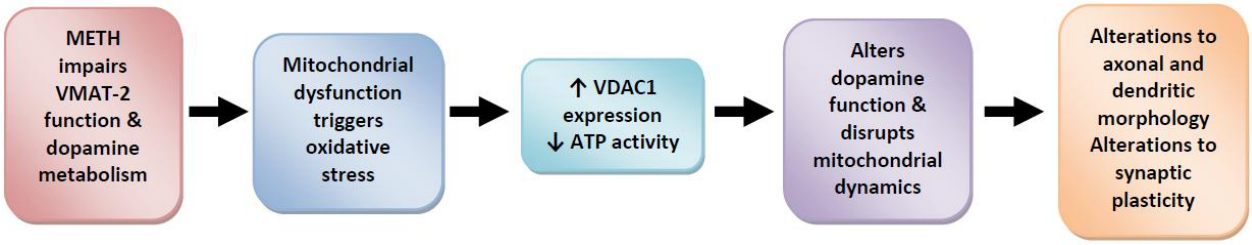

Figure 4.1. Alterations to mitochondrial dynamics in response to METH treatment in the METH-treated rats. Methamphetamine reduces VMAT-2 and dopamine uptake into vesicles, which subsequently influences VMAT-2 functioning and dopamine metabolism to trigger mitochondrial dysfunction & the production of oxidative stress. This increases VDAC1 activity and reduces ATP/ADP transmission into the mitochondria to alter the functional morphology of the NAc neuronal networks.

Alterations in mitochondrial dysfunction were also accompanied by the down-regulated expression of Parkinson protein 7 (PARK7) in the METH-treated animals compared to the controls. The presence of PARK7 in the mitochondria serves a protective role by maintaining the internal homeostasis of the mitochondria and regulating oxidative stress response signals (Taira et al., 2004; Hayashi et al., 2009; Irrcher et al., 2010). However, the loss of PARK7 functioning promotes alterations in mitochondrial morphology and functioning (Silvestri et al., 2005; Irrcher et al., 2010). As a result, this triggers the excessive production of ROS which is a critical molecular substrate that stimulates neuronal cell destruction (Irrcher et al., 2010). The disruptions in mitochondrial morphology and functioning, and the production of ROS heightens vulnerability to the development and pathogenesis of neurological diseases, such as Parkinson's disease (PD) (Irrcher et al., 2010). For instance, recent studies have demonstrated that the binding of PARK7 onto the mitochondrial complex I isoform was critical in maintaining internal homeostasis within the mitochondria (Hayashi et al., 2009). However, the administration of METH antagonises the functioning of the mitochondrial complex I, triggering mitochondrial dysfunction (Klongpanichapak et al., 2006; Yamamoto et al., 2010). As such, the combined impairments in mitochondrial activity and ROS production may induce neuronal cell death that heightens PD pathogenesis following METH administration (Fornai et al., 2005; Yamamoto et al., 2010; Irrcher et al., 2010).

However, the expression of PARK7 was identified as down-regulated in the sugar-METH rats in comparison to controls, indicating that the expression of PARK7 was unique to the control group. This suggests that the protective functions of PARK7, such as mitochondrial homeostasis, were intact in the controls (Hayashi et al., 2009), and the treatment of METH and the combined treatment of sugar and METH reduced the adaptive functioning of PARK7. The lack of significant differences in the expression of PARK7 between the METH-treated and sugar-METH-treated rats highlight that the reduced activity of PARK7 was not unique to METH treatment nor the combined treatment of sugar and METH.

Mitochondrial dysfunction was also identified in the sugar-METH-treated group compared to the METH-treated animals. The alterations in mitochondrial dysfunction were associated with the heightened expression of ATP synthase subunit G (ATP5L). ATP5L is located along the mitochondrial membrane and it is involved in converting adenosine diphosphate (ADP) into ATP (del Castillo et al., 2009). Interestingly, using a proteomic method, long-term exposure to a high-fat and refined sugar diet reduced the expression of ATP5L within the hippocampus (Francis et al., 2013). Furthermore, del Castillo and others (2009) indicated that the expression of ATP synthase subunit A in the NAc was down-regulated in cocaine-treated rats. However, in the present study and in comparison to the METH-treated animals, ATP5L levels in the sugar-METH-treated group are increased and may allow for more efficient regulation of ATP/ADP into the mitochondria (Brown & Yamamoto, 2003). This highlights that sugar pre-treatment may potentially function as a protective mechanism against the toxic effects of METH treatment that allows for preserved energy production and transmission into the mitochondria (Tsujimoto & Shimizu, 2002; Brown & Yamamoto, 2003).

The relatively preserved energy functioning of the mitochondria may encourage the adaptive functioning of the mitochondrial protein, dynamin 1-like protein (DNM1L). In the current study, DNM1L was greatly up-regulated in the sugar-METH-treated animals compared to the METH-treated animals (Chen et al., 2000). This protein facilitates synaptic

development and morphology through the governing mechanisms of mitochondrial fission (Chen et al., 2000; Yoon et al., 2003; Li et al., 2008). The excessive stimulation of DNM1L activity in the sugar-METH group compared to METH-treated group suggests that the pre-treatment of sugar may induce changes to mitochondrial dynamics that promote efficient mitochondrial division, even after the treatment of METH (Li et al., 2008). This combined efficiency of ATP5L and DNM1L, in turn, may heighten the synaptic strength of the dopamine terminals and preserve dopamine functioning (Yamamoto & Raudensky, 2008) after METH treatment. These changes in synaptic plasticity and dopamine transmission may promote the development of reward-motivated behaviours that are distinctly augmented in the sugar-METH-treated group compared to the METH-treated animals (Chan et al., 1994; Yamamoto & Raudensky, 2008).

4.4.2 Neuronal and cellular modifications: distinct differences between the treatment groups

In support for hypothesis three, the pharmacological properties of a single administration of METH induces changes in the activity of proteins within the NAc involved in regulating neuronal and cellular structure and functioning. More specifically, ten differentially expressed proteins in the METH-treated rats in comparison to controls were involved in coordinating changes to synaptic plasticity and neuronal activity. These changes in synaptic and neuronal structure and functioning were directly related to changes in the actin cytoskeleton pathway, ephrin receptor signalling, the DARPP-32 signalling cascade, GABA receptor signalling, the ERK/MAPK pathway and mTOR signalling. As an extension to the effects of METH, the present proteomic analyses support hypothesis four indicating that the combined treatment of sugar and METH induces distinct changes in the proteomic profile of the NAc that is different to METH only administration. The findings indicated that six differentially expressed proteins involved in regulating synaptic and neuronal structure and functioning in the sugar-METH-treated group, compared to the METH-treated rats, were linked to the altered pathways identified in the METH-treated rats compared to the controls.

These proteomic findings suggest that the pre-treatment of sugar may alter the neuronal and synaptic morphology of the NAc that augments behavioural responding to METH treatment.

4.4.2.1 Modulation of Actin cytoskeletal pathway

The actin cytoskeleton is a dynamic network that is composed of various complex interactions involved in regulating cellular modifications and transport in response to extracellular stimuli (Dos Remedios et al., 2003). In light of these functions, powerful extracellular stimuli, such as drugs of abuse, can induce alterations in cellular morphology and assembly to in turn affect the functioning and signalling activity of the NAc neuronal networks (Dos Remedios et al., 2003). The present proteomic study demonstrated that the METH-treated condition demonstrated alterations in the signalling activity of the actin cytoskeleton pathway in comparison to the controls. These changes in actin cytoskeleton signalling were related to the down-regulated expression of V-crk avian sarcoma virus CT10 oncogene (CRK) protein within the NAc. CRK is an adaptor protein implicated in the activation of various signal transduction pathways, including the actin cytoskeleton pathway, through interacting with tyrosine-related proteins (Huang et al., 2002). Recent evidence has demonstrated that cocaine treatment degraded the structural and functional mechanisms of the actin cytoskeleton within the NAc (Shen et al., 2009). These impairments in the actin cytoskeleton may be due to the reduced activity of CRK in maintaining and regulating cytoskeletal dynamics (Huang et al., 2002). As a result, the combined reduction in the functional structure of CRK and actin cytoskeletal activity may promote modifications to dendritic spine morphology to alter synaptic strength and density of the neuronal receptors (Meng et al., 2003; Shen et al., 2009). These neuroadaptations may underlie the early stages in the development of reward-motivated behaviours.

Conversely, in conflict with the present findings, previous studies have also demonstrated that a single injection of METH heightened the expression of CRK (Jayanthi, McCoy, Ladenheim, & Cadet, 2002). Interestingly, these findings suggest that the heightened expression of CRK may contribute to the activation of the actin cytoskeleton pathway that

may enhance cellular processing and functioning (Dos Remedios et al., 2003). The increased cellular motility may subsequently recruit cellular mechanisms that stimulate dendritic branching and axonal expansion to increase synaptic strength and neuronal functioning (Meng et al., 2003; Huang et al., 2002). These discrepancies in the findings may be due to the measure of neurobiological activity within the NAc in response to METH at different time points. As a result, in the current analysis, the down-regulated expression of CRK in the METH-treated animals may be driven by interactions with other proteins that are affected by the reduced availability of dopamine and glutamate within the NAc at 24 hours post injection (Shen et al., 2009; Jayanthi et al., 2002).

The dysregulation of other proteins in response to METH treatment in the current study may imply an interaction with CRK to impair actin cytoskeletal dynamics. A putative protein is protein tyrosine phosphatase, non-receptor type 5 (PTPN5), which has been implicated in directly regulating the phosphorylation of the tyrosine side chain onto the functional domains of CRK (Dadke & Chernoff, 2002). Accordingly, since the expression of PTPN5 was down-regulated in the METH-treated animals compared to controls, this may trigger the dephosphorylation of CRK which may reduce its activity and impair cellular motility and functioning (Nguyen et al., 2002; Braithwaite et al., 2006). These impairments may reduce the efficiency of synaptic transmission and neuronal functioning not only through the actin cytoskeleton but also indirectly through the NMDA receptors (Liu et al., 1996; Braithwaite et al., 2006). In particular, the combined activation of CRK and PTPN5 has been shown to stimulate efficient synaptic signalling and strengthen neuronal receptor functioning by phosphorylating the NMDA receptors (Salter & Kalia, 2004). However, in the present analyses, the down-regulated expression of both CRK and PTPN5 may reduce the phosphorylation and alter the activity of the NMDA receptors to reduce synaptic signalling strength and neuronal receptor functioning following acute METH treatment (Braithwaite et al., 2006). As a result, these dramatic shifts in synaptic plasticity and the neuronal mechanisms in the METH-treated rats, compared to controls, may contribute to the initial

processes underlying the development of reward-related learning and reward-motivated behaviours.

Chronic exposure to sucrose consumption prior to METH challenge also produced alterations in the actin cytoskeleton signalling pathway when compared to the METH-treated rats. Sucrose-exposed animals had heightened expression of CRK, cofilin (CFL1) and talin 2 (TLN2). Both CFL1 and TLN2 are cytoskeletal proteins playing critical roles in the regulation of synaptic functioning and dendritic morphology (Meng et al., 2003; Zhou et al., 2004). The pre-exposure to sugar may have induced changes in the signalling responses from the actin cytoskeleton to preserve the intact functioning of CRK, CFL1 and TLN2 in response to METH treatment, compared to the water-METH-treated rats. In response to METH, the phosphorylation of CFL1 and TLN2 may strengthen its interaction with CRK to stimulate the activational properties of both CFL1 and TLN2 (Guan, 1997). These structural and functional changes within the NAc may preserve the intact functioning of the neural receptors to promote synaptic strengthening and efficient neurotransmission compared to the water-METH treated rats (Braithwaite et al., 2006; Meng et al., 2006).

Therefore, sugar pre-treatment may have served a protective function in order to moderate the reduced expression of PTPN5 and CRK in response to METH treatment (Meng et al., 2003; Francis et al., 2013). This long-term potentiation of the NAc proteins may promote the primary stages of reward-related learning developed during sugar pre-exposure to strengthen the development of reward-dependent behaviours to acute METH exposure. The differences in CRK expression between the sugar-METH and the METH-treated groups were not found in the comparison between the controls and the sugar-METH group, indicating that the differential alterations in CRK functioning were specific to the METH-treated rats in comparison to the controls.

4.4.2.2 Modulation of Ephrin receptor signalling

Ephrin receptor signalling is fundamental in cellular interactions, and the stimulation of the A-class and B-class ephrin receptors is critical in controlling neuronal morphology and

synapse formation and plasticity (Klein, 2009; Lai & Ip, 2009). Alterations in ephrin receptor signalling were identified in both the sugar-METH group and the METH condition. However, activational changes to ephrin A signalling were detected in the METH group compared to the controls, whereas alterations in the activation of ephrin B signalling were identified in the sugar-METH group compared to the METH condition. In support for the differential activation of ephrin receptor signalling, the sugar-METH condition demonstrated changes to the activation of ephrin B receptor signalling compared to the controls. This suggests that distinct forms of synaptic morphology and neuronal plasticity were developing in the sugar-METH and METH treatment groups in response to METH treatment. Specifically, changes to ephrin receptor signalling in the METH-treated rats compared to the controls was associated with the combined down-regulated expression of CRK and neuronal guanine nucleotide exchange factor (NGEF), which is a protein involved in regulating axon guidance and dendritic morphology (Shamah et al., 2001). This impaired functioning of CRK and NGEF may initiate reductions in axonal guidance and dendritic differentiation through their interactions with the signalling mechanisms of the ephrin A receptors (Klein, 2009; Shamah et al., 2001).

The activation of ephrin A receptor signalling modifies the synaptic profile and neuronal physiology by restricting the unlimited development of dendritic spines (Lai & Ip, 2009) and may influence the restructuring and reorganisation of the morphology of the NAc dopaminergic system (Cooper, Kobayashi, & Zhou, 2009). For example, Sieber and colleagues (2004) demonstrated that the pharmacological inhibition of the ephrin A receptors reduced the total volume of the dopaminergic neurons in the striatum that was associated with a reduced behavioural response to administered amphetamine, indicating that the upregulation of ephrin A may be involved in the locomotor response to acute METH treatment. Ephrin A receptor signalling also activates Cdk5 to induce changes to the phosphorylation of NGEF (Shamah et al., 2001). In the current study, the acute treatment of METH was associated with upregulation of Cdk5, which may alter the phosphorylation of NGEF to induce the DARPP-

32 signalling cascade, and transform the morphogenesis of the NAc synapse and dendritic spine profile (Shamah et al., 2001; Bibb et al., 1999). The present findings demonstrated that METH-induced enhancement of Cdk5 levels was associated with the down-regulation of protein phosphatase 1 (PPP1R11), an enzyme involved in learning and memory through its interaction with Cdk5 and the DARPP-32 signalling cascade (Fernandez et al., 2006; Nairn et al., 2004). The down-regulation of PPP1R11 in the METH-treated rats, compared to controls, was also involved in modifying the functions of dopamine receptor signalling, protein kinase A (PKA) signalling, and dopamine-DARPP-32 feedback in cAMP signalling. These neurochemical changes may play a significant role in creating the fundamental structures that underlie the development of behaviours directed for rewarding substances (Nairn et al., 2004; Sieber et al., 2004).

Changes to ephrin B, but not ephrin A, signalling were evident in the combined sugar-METH-treated rats compared to the METH-treated rats. Through its interaction with downstream molecular substrates, ephrin B receptor signalling modulates dendritic formation and synaptic morphology by promoting dendritic spine growth (Klein et al., 2009; Shi et al., 2009). An important downstream molecular target for the ephrin B receptors is CFL1 (Shi et al., 2009), which was associated with ephrin B receptor signalling in the present proteomic analyses. The pre-treatment with sucrose may have induced structural adaptations within the NAc to encourage adaptive alterations of the ephrin B receptors in response to METH treatment compared to the METH-treated rats. Changes in ephrin B receptor signalling may have in turn activated CFL1 to trigger the restructuring of the dendritic profile of the NAc, and alter the excitatory potential of the synapse (Shi et al., 2009).

Modifications to synaptic strength may also be influenced by alterations in the interaction between the ephrin B receptors, CFL1, and the dopaminergic and glutamatergic neuronal systems (Yue et al., 1999; Klein, 2009). For instance, previous studies have demonstrated that alterations in ephrin B receptor signalling was accompanied by the activational signalling pattern of dopamine (Yue et al., 1999), and directly influenced

interactions with the NMDA receptors (Dalva et al., 2000; Takasu et al., 2002). Enduring changes in the ephrin B-CFL1 signalling pathway may promote dendritic spine morphogenesis to enhance the structural elaboration of the dopaminergic receptors and the NMDA receptors (Yue et al., 1999; Lai & Ip, 2009), contributing to the development of long-lasting synaptic long-term potentiation (LTP) to strengthen and augment the formation of reward-related behaviours (Antion et al., 2010; Klein, 2009). This may reduce locomotor control to thereby encourage motivational drive for substances that trigger feelings of reward.

4.4.2.3 Modulation of DARPP-32

Long-term changes in synaptic plasticity and neurotransmission in response to METH treatment may be initiated by the DARPP-32 signalling cascade phosphorylated by the threonine 75 residue (Chen & Chen, 2005). In accordance with the down-regulated expression of PPP1R11, changes to Cdk5 signalling in the present findings may direct the phosphorylation of PPP1R11 at residue T320 to inhibit PPP1R11 activity (Hou et al., 2013). As a result, the changes in the functioning in Cdk5 in response to acute METH treatment may initiate the phosphorylation of DARPP-32/Thr75 (Bibb et al., 1999). Previous studies have demonstrated that the acute administration of METH was followed by the delayed activation of DARPP-32/Thr75 signalling in the NAc (Chen & Chen, 2005). The activation of DARPP-32/Thr75 may inhibit the activation of PKA signalling to influence the functions of the dopaminergic system (Dhavan & Tsai, 2001).

Alterations to PKA signalling was accompanied by the down-regulated expression of PPP1R11 and PTPN5, and the upregulation of the variants of tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation proteins, including theta (YWHAQ), gamma (YWHAG) and epsilon (YWHAE), in the METH-treated animals compared to the controls. The YWHAQ, YWHAG and YWHAE proteins are adaptor proteins involved in regulating cell survival and protein trafficking through their interaction with PKA (van Heusden, 2005). The impairments in PKA signalling activity may alter interactions with the YWHAQ, YWHAG and YWHAE proteins to influence the development of neurite and

axonal growth (Yang & Terman, 2012; Kent et al., 2010). In combination with this, the reduced expression of PPP1R11 and PTPN5 may consequently suppress synaptic strength to initiate the long-term depression (LTD) of the NAc glutamatergic and dopaminergic systems (Shi et al., 2009; Braithwaite et al., 2006). The changes in the NAc synaptic and neuronal organisation may promote a blueprint for reward-related and reward-motivated learning processes.

However, in contrast to the present findings, previous studies have demonstrated that acute treatment of amphetamine stimulated the phosphorylation of DARPP-32/Thr34 and reduced the activity of DARPP-32/Thr75 (Nishi et al., 2000; Svenningsson et al., 2004). The differential activation of DARPP-32 between the current study and previous studies may be primarily due to the different time points in measuring dopaminergic activity. The immediate pharmacological effects of METH and amphetamine instantly elevate the availability of local dopamine by activating the D1 receptors (Nairn et al., 2004). As a result, the greater activation of the D1 receptors enhances the phosphorylation of DARPP-32/Thr34 and reduces the phosphorylation of threonine 75 on to DARPP-32 (Nishi et al., 2000). Since the animals in the current study were sacrificed 24 hours after METH treatment, the activational availability of the dopamine D1 receptors may have become reduced, which thereby heightens activational dopamine D2 receptor levels in the NAc (Nairn et al., 2004). In conjunction with PPP1R11, the increased levels of the dopamine D2 receptors within the NAc may stimulate the enhanced phosphorylation of DARPP-32/Thr75 to inhibit protein kinase signalling (Nishi et al., 2000; Bibb et al., 1999). The present proteomic analyses demonstrated that numerous proteins characterised as protein kinases were identified as down-regulated in the METH-treated group in comparison to controls, including CDC42 binding protein kinase beta (CDC42BPB) and casein kinase 2, alpha 1 polypeptide (CSNK2A1). Hence, the acute administration of METH may initiate the DARPP-32/Thr75 signalling cascade to subsequently induce changes in the synaptic morphology, and facilitate the development of LTD in the synapse (Hou et al., 2013). This reduced strength and efficacy of synaptic

functioning may alter dopaminergic and glutamatergic neurotransmission to initiate reward-motivated behavioural plasticity (Bamford et al., 2008; Thomas et al., 2001).

The dopamine-DARPP-32-cAMP signalling cascade was activated in the combined sugar-METH-treated rats compared to the METH-treated rats indicated by increased expression of the PKA isoform, protein kinase cAMP-dependent regulatory type 2 beta (PRKAR2B) (Bibb et al., 1999), which is highly enriched in the medium spiny neurons of the striatum and has been implicated in synaptic LTP and axonal guidance (Parisiadou et al., 2014; Brandon et al., 1998). The elevated expression of PRKAR2B was also implicated in altering the functions of dopamine receptor signalling, cAMP-mediated signalling and synaptic LTP. The chronic pre-treatment of sugar may have induced enduring functional adaptations in the availability of local D1 and D2 dopamine receptors within the NAc (Bello et al., 2003). In particular, it is possible that sugar pre-exposure may have stimulated structural changes in the dendritic and axonal morphology of the dopamine receptors to facilitate the efficient restoration of the D1 dopamine receptors, and suppress D2 dopamine receptor levels (Bello et al., 2003; Robinson & Kolb, 1999). Accordingly, the acute administration of METH may not exhaust dopamine D1 levels because the neuroadaptations developed during chronic sugar consumption may promote the restoration of the dopaminergic system to encourage functional neuronal homeostasis within the NAc (Nestler, 2001), and to facilitate locomotor cross-sensitisation (Bello et al., 2003; Avena et al., 2008).

4.4.2.4 Modulation of GABA receptor signalling

The changes in the phosphorylation of DARPP-32 in the METH-treated animals compared to the controls likely contributed to increases in PKA signalling to promote changes in the functioning of downstream neuronal networks, such as GABA A regulation (Hu et al., 2008; Moss et al., 1992). The GABA A receptors are crucial in regulating fast inhibitory synaptic neurotransmission in order to maintain homeostasis in neuronal functioning and coordinate neuronal plasticity (Brickley et al., 2001; Luscher & Keller, 2004).

Modifications in GABA receptor signalling in response to acute METH treatment was identified due the down-regulation of gamma-aminobutyric acid A receptor, beta 2 (GABRB2) and gamma-aminobutyric acid A receptor, alpha 1 (GABRA1), which are the subunit classes of the GABA A receptors (Bedford et al., 2001). Although there has been limited research investigating the activity of GABA A receptor functioning in response to acute METH treatment within the rat NAc, previous studies examining the functioning of the GABA B receptors within the VTA have demonstrated that after 24 hours, a single treatment of METH significantly reduced the expression of the GABA B receptors, and this reduction persisted for seven days (Padgett et al., 2012). Liu and others (2005) indicated that repeated cocaine treatment reduced the inhibitory activation of the GABA A receptors within the VTA. In combination with previous studies investigating GABA receptor functioning, the present proteomic analyses suggest that acute METH treatment can induce powerful effects on GABA receptor functioning. As a result, the prolonged and short-term reduction of GABA receptor signalling may trigger the dephosphorylation of the GABA A receptor subunits, including GABRB2 and GABRA1 (Couve et al., 2002; Sakaba & Neher, 2003). The reduced expression of the GABA A receptor substrates may in turn alter the functioning and recruitment of downstream neuronal networks to affect dopaminergic signalling (Sakaba & Neher, 2003; Hu et al., 2008; Farrant & Nusser, 2005). This effect on GABA signalling was not present in sugar-METH treated animals when compared to water-METH treated rats and may suggest that a change to GABAergic function may promote the developmental stages of locomotor sensitisation for substances that trigger reward.

4.4.2.5 Modulation of ERK/MAPK signalling

Due to the differential stimulation of the DARPP-32 pathways between the METH-treated group and the sugar-METH-treated group, the distinct activation of DARPP-32 may subsequently influence the differential functioning of the ERK/MAPK signalling pathway. In particular, in comparison to the controls, the ERK/MAPK signalling pathway was identified as altered in the METH-treated rats. The altered functioning of ERK/MAPK signalling was

associated with the heightened expression of YWHAQ and YWHAG, and the reduced expression of PPP1R11 and CRK (see Figure 4.2). The alterations in these proteins were also linked with modifications to DARPP-32/Thr75 which may subsequently influence ERK/MAPK signalling. For example, Valjent and others (2005) demonstrated that the phosphorylation of DARPP-32/Thr75 by Cdk5 signalling failed to significantly alter the stimulation of the ERK/MAPK signalling pathway. This failure to activate ERK/MAPK signalling pathway may be primarily due to the over-expression of Cdk5 inhibiting the functions of PKA (McDaid, Graham, & Napier, 2006).

However, previous studies examining the relationship between the DARPP-32 signalling cascade and the ERK/MAPK signalling pathway only provide a broad explanation of this relationship. As a result, it is difficult to discern the specific molecular substrates contributing to the inactivity of the ERK/MAPK signalling cascade. In light of this, the length and intensity of the activation of the ERK/MAPK signalling pathway is largely dependent on various molecular substrates regulating this signalling cascade, including YWHAQ, YWHAG, PPP1R11 and CRK (Mitsuhashi et al., 2003). The interactive relationship between the YWHAQ and YWHAG proteins and the Rapidly Accelerated Fibrosarcoma (RAF) kinase coordinates the signalling transduction of the ERK/MAPK pathway (Xing et al., 2000). This relationship between the YWHAQ and YWHAG proteins and RAF is mediated by the activational properties of PPP1R11 (Mitsuhashi et al., 2003). As a result of this interactive relationship, the down-regulated expression of PPP1R11 evident in this study may reduce the activational strength of RAF (Mitsuhashi et al., 2003). RAF also functions as a molecular switch to unite CRK activation to ERK/MAPK signalling (Barberis et al., 2000). Since CRK was identified as down-regulated, the reduced efficiency of CRK functioning may consequently inhibit the phosphorylation of RAF to the ERK/MAPK substrate (Mitsuhashi et al., 2003). Under such circumstances, the reduced functional relationships between PPP1R11, RAF and CRK may reduce the magnitude of ERK/MAPK signalling and suppress the prolonged activation of the ERK/MAPK cascade (Kawahara et al., 2013). As a result, this

may initiate cell migration and facilitate the development of LTD within the synaptic and neural networks of the NAc (Kawahara et al., 2013; Valjent et al., 2005).

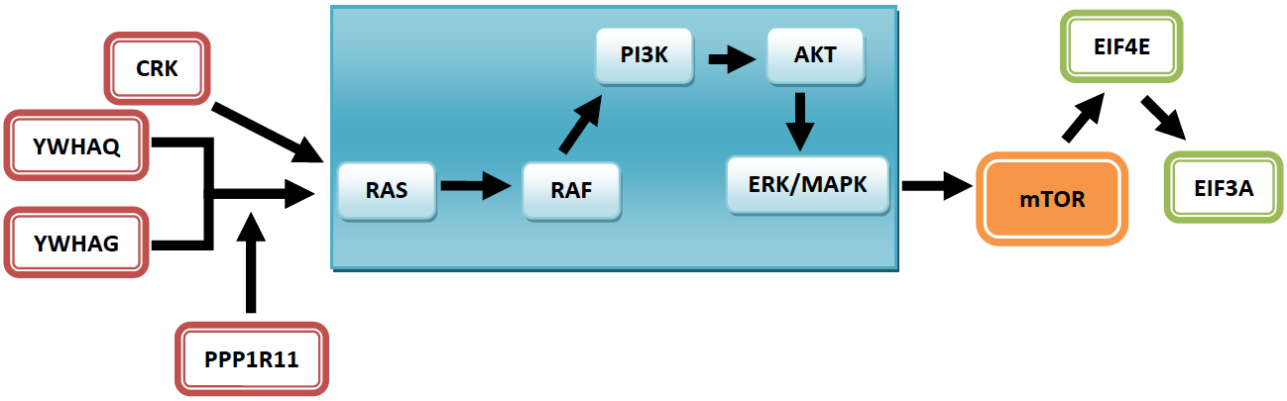

Figure 4.2. Interaction between the ERK/MAPK signalling cascade and mTOR signalling in the METH-treated animals. Reduced expression of PPP1R11 alters interaction between YWHAQ and YWHAG proteins and RAF through RAS. Reduced CRK expression also alters RAF activation through RAS. RAS activates PI3K through RAF to subsequently stimulate AKT. This stimulates ERK/MAPK signalling cascade to alter its interaction with mTOR signalling. Activation of mTOR pathway stimulates the phosphorylation of EIF4E and EIF3A.

Conversely, phosphorylation of DARPP-32/Thr34 may trigger modifications to ERK/MAPK signalling in the sugar-METH-treated rats compared to the METH-treated rats. Previous studies have demonstrated that the phosphorylation of DARPP-32/Thr34 in the striatum was necessary for the activation of the ERK/MAPK signalling cascade (Valjent et al., 2005). The stimulation of the ERK/MAPK pathway may strengthen the development of reward-related learning and memory (Mazzucchelli et al., 2002). For instance, Gerdjikov and colleagues (2004) demonstrated that the ERK/MAPK pathway regulated the learning processes involved in establishing the link between the substance and reward, and this associative learning response was abolished due to the inhibition of the ERK/MAPK pathway. This suggests that chronic sugar pre-treatment may trigger a cascade of adaptive molecular modifications in the synaptic and neuronal profile of the NAc to facilitate the development of reward-induced plasticity which underlies the building blocks of reward-motivated behaviours (Chao & Nestler, 2004). Subsequent METH treatment may intensify these neuroadaptations to reinforce enduring behaviours directed for rewarding substances.

Although the link between the phosphorylation of DARPP-32/Thr34 and the activation of the ERK/MAPK signalling pathway is well established, it is difficult to conclusively determine the specific molecular mechanisms involved in this relationship. In more detail, the present study demonstrated that alterations to the ERK/MAPK pathway was related to the up-regulated expression of TLN2, PRKAR2B and CRK in the combined sugar-METH-treated rats compared to the METH-treated rats (see Figure 4.3). The up-regulated expression of TLN2 may amplify its interaction with the cell-extracellular matrix to subsequently enhance the expression of CRK and influence the functional activation of the substrates within the rat sarcoma (RAS) family (Guan, 1997). In addition to the interaction between TLN2 and CRK, PRKAR2B and CRK also interact with each other to influence the activation of B-RAF within the RAS family (Stork & Schmitt, 2002). Stimulating the activation of B-RAF, in turn, triggers the ERK/MAPK signalling cascade (Vossler et al., 1997). Consequently, due to the heightened expression of PRKAR2B and CRK in the present findings, the interaction may enhance the intensity and prolong the sustained activation of the ERK/MAPK pathway (Kawahara et al., 2013). This prolonged activation is crucial in stimulating cell differentiation and cell migration and the activation of neuron-specific genes, such as CREB (York et al., 1998; Barberis et al., 2000).

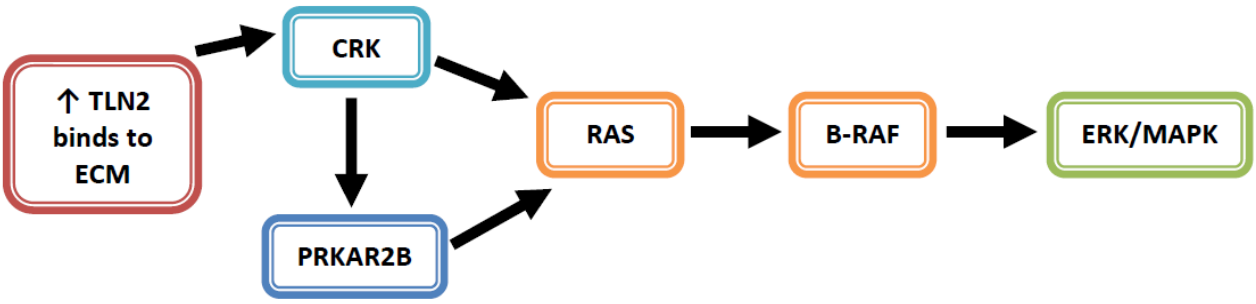

Figure 4.3. Activation of the ERK/MAPK pathway in the Sugar-METH group. Heightened TLN2 expression enhances its interaction with the cell-extracellular matrix (ECM) to increase the phosphorylation of CRK. CRK stimulates B-RAF activation through its interaction with RAS. Increased phosphorylation of B-RAF is also influenced by the interaction between PRKAR2B and CRK. Activation of B-RAF triggers the ERK/MAPK signalling cascade.

The activation of the ERK/MAPK signalling cascade through the functional interactions of the PKA isoform, PRKAR2B, stimulates the phosphorylation of CREB (Stork

& Schmitt, 2002). In the present study, the sugar-treated rats demonstrated significant modifications to CREB signalling in neurons compared to the METH-treated group. This was associated with the up-regulated expression of PRKAR2B. The activation of PKA signalling may induce the phosphorylation of CREB through the prolonged activation of the ERK/MAPK pathway (Stork & Schmitt, 2002; York et al., 1998). As a result, since CREB is a functionally crucial transcription factor, stimulation of CREB signalling can induce enduring changes in the NAc neuronal networks by altering gene expression (Svennigsson et al., 2004). This interactive relationship between PKA signalling, the ERK/MAPK pathway and CREB is recognised as a critical molecular modulator in regulating reward-related learning, behavioural plasticity and long-term adaptations (Baldwin et al., 2002). For instance, previous studies have demonstrated that the expression of the mutated CREB variant within the NAc heightened motivational responding for the sugar substance (Barrot et al., 2002). Hence, due to the modifications in PKA signalling and the ERK/MAPK pathway, long-term changes in the activational functions of CREB within the NAc neuronal system may regulate the value of rewarding stimuli to encourage and reinforce behavioural motivation for rewarding substances (Kelley, 2004; Barrot et al., 2002).

Although the heightened expression of ΔFosB is induced by the ERK/MAPK signalling pathway (Valjent et al., 2006) and in response to acute and chronic METH treatment (Martin et al., 2012; McDaid et al., 2006), the present study did not demonstrate significant changes to the differential expression of proteins involved in regulating ΔFosB expression between the treatment groups. This may be primarily due to the methodological procedure of measuring neurobiological activity in the NAc 24 hours after the METH challenge. The expression of ΔFosB is rapidly elevated for a period of 2 hours before returning to basal levels in response to acute METH treatment (Martin et al., 2012); and in response to chronic METH treatment ΔFosB expression can be sustained for up to 14 days (McDaid et al., 2006). Accordingly, ΔFosB may activate the initial developmental stages of

reward-induced neuronal plasticity within the sugar-METH-treated animals, and once ΔFosB levels have subsided, CREB signalling may be initiated to prolong these synaptic adaptations.

4.4.2.6 Modulation of mTOR pathway

As an extension of the alterations found within the ERK/MAPK signalling cascade, it is evident that the ERK/MAPK pathway can coordinate synaptic and neuronal morphology by interacting with other protein kinases, such as the mammalian target of rapamycin (mTOR) (Jaworski et al., 2005). The present proteomic analyses significantly identified that mTOR signalling was altered in the METH-treated animals in comparison to the controls. mTOR is a serine/threonine kinase that is involved in the regulation of cell growth and proliferation (Jaworski & Sheng, 2006). The interaction between the ERK/MAPK signalling cascade and the mTOR signalling pathway is mediated by the substrates within the RAS family (see Figure 4.2), which determines the magnitude and length of ERK/MAPK signalling (Kumar et al., 2005). In this complex relationship, RAS initiates the activation of phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) which stimulates protein kinase B (AKT). The activation of AKT, in turn, triggers mTOR signalling (Hay & Sonenberg, 2004; Tsokas et al., 2007). For instance, Tsokas and others (2007) demonstrated that stimulation of the midbrain synapses induced the activation of substrates regulated by mTOR signalling. The heightened expression of these mTOR substrates was subsequently inhibited by the administration of ERK/MAPK antagonists. These findings suggest that the ERK/MAPK pathway may coordinate the stimulation of mTOR signalling through the RAS-PI3K-mTOR pathway (Hay & Sonenberg, 2004; Tsokas et al., 2007). Accordingly, activation of mTOR signalling through the ERK/MAPK pathway may induce enduring changes to dendritic branching formation and synaptic strength (Kumar et al., 2005; Jaworski & Sheng, 2006).

Although mTOR signalling influences synaptic and neuronal plasticity, these modifications are initiated by the downstream molecular targets of mTOR signalling (Hay & Sonenberg, 2004). The activation of mTOR signalling directly or indirectly induces the phosphorylation and activation of components within the translation initiation network

(Hoeffer & Klann, 2009). In the present study, alterations in mTOR signalling were associated with the heightened expression of the eukaryotic translation initiation factor 3, subunit A (EIF3A) in the METH-treated animals compared to the controls. The acute treatment of METH may trigger the activation of mTOR signalling through the ERK/MAPK pathway, which stimulates the phosphorylation and activity of the translation initiation factor EIF3A (Hay & Sonenberg, 2004). Consequently, the activation of EIF3A may induce synaptic plasticity and alter the dendritic profile of the NAc by accelerating cellular growth and proliferation (Jaworski & Sheng, 2006; Hay & Sonenberg, 2004). For example, Narita and others (2005) demonstrated that METH sensitisation enhanced the phosphorylation of the upstream molecular regulators of mTOR signalling within the NAc. This activates mTOR signalling to enhance the phosphorylation of the translation initiation factor EIF4E, and subsequently activate the phosphorylation of EIF3A (Richter & Klann, 2009; Hay & Sonenberg, 2004). The heightened expression of the translation initiation factors, EIF4E and EIF3A, accelerates cellular growth and division to trigger long-term modifications to synaptic signalling strength and neuronal morphology (Jaworski & Sheng, 2006; Hay & Sonenberg, 2004). Despite the excessive focus on the chronic effects of psychostimulant use on mTOR signalling within the NAc (Narita et al., 2005; Wang et al., 2010); the present findings corroborate and extend previous studies demonstrating that acute METH treatment may trigger the coordinated activation of the ERK/MAPK and mTOR signalling pathways to induce long-term modifications in synaptic plasticity and neuronal physiology within the NAc through the activation of EIF3A.

mTOR signalling was also identified as differentially expressed in the combined sugar-METH-treated rats compared to the METH-treated rats. However, alterations in mTOR signalling were associated with the reduced expression of both EIF3A and the mechanistic target rapamycin (MTOR) in the sugar-METH-treated group. Distinct alterations in EIF3A expression between the sugar-METH and METH-treated rats were not found in the sugar-METH treatment group compared to the controls, highlighting that the differential changes to

EIF3A activity were specific to the METH-treated condition in comparison to the sugar-METH condition. These findings highlight that the pre-treatment of sugar may have induced modifications to the coordinated mechanisms of mTOR signalling that is distinct to the rats pre-treated with water. The distinct changes in mTOR signalling have contributed to divergent responses to subsequent METH treatment. As such, chronic pre-exposure to sugar may overstimulate the phosphorylation and activity of MTOR (Woods & Ramsay, 2011). Recently, Orr and colleagues (2014) demonstrated that long-term consumption of a high sucrose diet induced the hyper-phosphorylation and hyperactivity of MTOR. The overstimulation of mTOR signalling may be triggered to preserve energy homeostasis (Orr, Salinas, Buffenstein, & Oddo, 2014). Accordingly, the activation of mTOR signalling triggers the phosphorylation of the eukaryotic translation initiation factor 4E (EIF4E)-binding protein (4E-BP1), which subsequently deactivates the functions of 4E-BP1 (Patel et al., 2001; Fingar et al., 2004). This inactivation accelerates the activity of EIF4E and, the stimulation of EIF3A activity to promote enduring modifications to synaptic and neuronal functioning (Hay & Sonenberg, 2004).

Over time, the excessive stimulation of mTOR signalling may become inactive and unresponsive to powerful stimuli, such as METH (Orr et al., 2014). This reduction in mTOR signalling may be associated with the down-regulated expression of MTOR in the present findings. Reductions in the activity of mTOR signalling decrease the phosphorylation state of 4E-BP1, which subsequently inhibits the activity of EIF4E and its downstream molecular targets, including EIF3A (Fingar et al., 2004; Patel et al., 2001). The reduced activity of the substrates within the mTOR signalling pathway, including MTOR, 4E-BP1, EIF4E and EIF3A, may promote the development of an inhibitory mechanism within the mTOR signalling pathway (Jaworski & Sheng, 2006). As a consequence of this inhibitory response, the subsequent administration of METH may fail to trigger the stimulation of mTOR signalling to stabilise dendritic morphology and synaptic plasticity within the NAc (Jaworski et al., 2005). In parallel with this, the expression of the late endosomal/lysosomal adaptor

MAPK and MTOR activator 1 (LAMTOR1) was identified as down-regulated in the sugar-METH rats compared to the controls. LAMTOR1 regulates cell growth and the recycling of the neural receptors through the activation of the mTOR signalling pathway (de Araujo et al., 2013). Since the expression of LAMTOR1 was reduced, this suggests that the activation of the mTOR signalling pathway may be inhibited, which may restrain cellular growth in the NAc neuronal receptors (Nada et al., 2009). The present findings suggest that the pre-exposure to sugar may function as a "protective mechanism" such that the deactivation of the mTOR signalling cascade may maintain synaptic strength and preserve neuronal functioning (Jaworski & Sheng, 2006).

Therefore, in conjunction with previous studies, the present proteomic analyses suggest that the pre-treatment of chronic sugar induces distinct modifications to the mTOR signalling cascade. As a result, subsequent METH treatment may be prevented in contributing to the enduring changes in the synaptic and neuronal morphology within the NAc through the mTOR signalling cascade. Instead, other signalling pathways, such as the actin cytoskeleton pathway, the DARPP-32 signalling cascade, the ERK/MAPK pathway and CREB signalling, may be functioning in a coordinated manner to accelerate synaptic strength and preserve neurotransmission. In contrast, the acute treatment of METH in the absence of prior sugar consumption may decrease the efficiency of synaptic and neuronal transmission through the DARPP-32 signalling cascade and the coordinated interaction between the mTOR signalling pathway and the ERK/MAPK pathway.

4.5 Limitations and Methodological Considerations

The main behavioural finding from the current analysis is the first to demonstrate that pre-exposure to excessive sugar consumption during adolescence augments locomotor behaviour in response to acute METH administration in adulthood. Since this is the first to demonstrate METH behavioural cross-sensitisation to prior sucrose exposure, further research is needed in order to reliably validate that the present cross-sensitisation findings are reproducible.

Additionally, due to limited molecular research investigating the effects of cross-sensitisation within the NAc, many uncharacterised proteins were identified as differentially expressed in the current proteomic analysis. This hampered the strength of the analysis because it is difficult to specifically illustrate a comprehensive examination of the cumulative molecular effects of cross-sensitisation until these proteins have been identified.

A methodological limitation was that there were significant differences in energy intake between the sugar-METH animals and the control and METH treatment groups at the challenge day. This effect did not translate to changes in locomotor activity between treatment groups, however the interpretation of the effect of sucrose on METH locomotor responding and protein levels must therefore take into account that any neuroadaptations may not only be the result of sugar intake per se, but the amount of energy consumed.

The NAc is a highly innervated brain structure consisting of various neuronal networks, including the dopaminergic system and the glutamatergic system (Blumenthal & Gold, 2010). While several previous studies have reliably and validly demonstrated that excessive sugar consumption and METH treatment alter the functions of the NAc dopaminergic and glutamatergic neuronal systems (Avena et al., 2008; Tukey et al., 2013; Nestler, 2001; Parsegian & See, 2014), the present analysis did not directly measure the activity of these neural systems in response to excessive sugar administration and METH treatment and all interpretations are inferred from previous research. Accordingly, the results from the present analysis are unable to conclusively determine the underlying neuronal networks within the NAc that are triggering the development of METH behavioural cross-sensitisation to prior sucrose exposure.

The present proteomic findings are based on an exploratory “shotgun” approach. The amount of data populated by this technique resulted in the exclusion of a sugar-saline treatment group. Thus, it is difficult to specifically identify the specific protein modifications that occur in response to excessive sugar consumption, as a baseline contributor to METH-induced behaviour. Future refinement of the present research using a targeted proteomic

approach would benefit from the inclusion of a sugar-saline treatment group in order to determine the key proteins involved in behavioural cross-sensitisation of sucrose exposure with METH.

Another major methodological short-coming is that, after excessive sugar consumption, the animals were exposed to five weeks of behavioural testing prior to acute METH treatment. Although during the five week testing period the animals did not consume any foreign substances or were administered with any drugs, the behavioural measures may have induced alterations in the physiology and functional activity of the proteins. The exposure to novel and unfamiliar environments may have contributed to further protein changes (Hoeffer & Klann, 2009) that may be unrelated to the behavioural cross-sensitisation with METH. Consequently, it is difficult to determine if the expression of proteins were due to the environmental manipulations outlined in this study or a combination of the effects of the 26-day treatment regimen and the five weeks behavioural testing period. However, this methodological limitation was unavoidable in the current protocol because the study was part of a larger analysis.

The NAc consists of functionally and anatomically distinct sub-regions, the core and the shell. Since the core and the shell send and receive signals to and from various different brain regions, each sub-region is involved in regulating distinct behavioural responses. For instance, while the core regulates reward-related learning processes, the shell governs the initiation of reward-directed behaviours (Kelley et al., 2005). In the present analysis, the whole NAc brain region was examined. While this is one of the first studies to specifically investigate the NAc region as opposed to striatal samples using a proteomic approach, the examination of whole brain region will not provide a detailed perspective of the specific protein changes in subregions of the NAc that are involved in regulating locomotor activity in response to cross-sensitisation. Although there are currently methodological limitations to investigating small subregion samples, future examination of the sub-regions within the NAc

may provide a comprehensive insight of the critical proteins involved in regulating METH behavioural cross-sensitisation to prior sugar exposure during adolescence.

4.6 Future Research Directions

The current research project only examined locomotor activity in response to behavioural cross-sensitisation and did not explore other various reward-related behaviours, regulated by the functions of the NAc, which may underlie the development of METH behavioural cross-sensitisation. Techniques to investigate reward-directed motivation, reward-related learning and the developmental processes of addiction, such as self-administration paradigms could be used in future studies (Kelley, 2004). The self-administration paradigm is a well-validated measure used to investigate reward-motivated learning processes and reward-related dependence underlying the development of addiction (Berridge & Robinson, 1998). Use of the self-administration paradigm would allow a detailed examination of the transmission of reward-directed learning processes in METH dependence that may have developed from sugar pre-exposure.

The current analyses used protein-expression profiling to determine the functions of NAc proteins in response to METH behavioural cross-sensitisation. This method is effective in determining the expression levels of individual proteins, but it largely neglects to examine the manner in which the proteins interact to trigger the alterations in protein expression (Liebler, 2002, p.11). Due to these shortcomings, the critical molecular factors that contributed to the functional and physiological modifications to each protein are based on inferences from the literature. A combination of proteomic analysis techniques, such as protein-expression profiling combined with protein-network mapping, may allow for a more detailed perspective of the physiological effects of METH behavioural cross-sensitisation on the NAc proteins because both methods will be able to examine the interactive status between all proteins within a pathway and allow for a clear perspective of the molecular processes that dictate the functional activity of the NAc protein networks (Liebler, 2002, p.11-12).

Since the proteomics method involves homogenising the whole NAc, it is difficult to discern the presynaptic and postsynaptic transmission of the proteins involved in regulating neuronal and cellular activity. Future studies would benefit from using immunohistochemistry to validly determine the functions of the identified proteins. In immunohistochemistry, antigens including proteins and neuronal receptors are detected with the use of antibodies binding specifically to the antigen biomarker. This allows for a comprehensive understanding of the functional expression and structural distribution of the specifically targeted antigens in response to the methodological manipulations (Koylu et al., 1998).

Future confirmatory analyses should be considered to validate the present findings in order to reliably determine the underlying proteins and neuronal networks involved in regulating behavioural cross-sensitisation. Western blot analysis is an effective proteomic tool to confirm and validate differences in protein expression because this method examines the molecular weight and the total expression level of a specific protein (Liebler, 2002, p.168). The combined results obtained from Proteomic analyses and Western blotting can address the key effects that are driving behavioural cross-sensitisation, allowing for enhanced knowledge of preventative strategies and the development of targeted treatments to alleviate environmental impacts, such as diet and drug abuse, on mental health.

4.7 Conclusion

The weight of the evidence suggests that acute METH treatment induces significant alterations in the expression and functions of the proteins within the NAc. These alterations in protein expression were distinctly different from the sugar-METH-treated animals, suggesting that sugar pre-exposure induces long-term adaptations in the NAc that influenced the behavioural and neurobiological effects of METH administration. The differential expression of proteins in the NAc between the METH-treated animals and sugar-METH-treated animals triggered distinct neuroadaptations through various signalling pathways, including the actin cytoskeleton, ephrin receptors, the DARPP-32 cascade, GABA receptors, the ERK/MAPK pathway and mTOR, which likely contribute to distinct stages in the development of reward-

motivated behaviours. Additionally, the proteomic profile the METH and sugar-METH treated groups determined differentially altered functioning of the mitochondria between the groups to suggest a modulatory role of prior sugar consumption on METH-induced oxidative stress. Given the extensive nature of this study, the findings may provide an avenue to the development of targeted therapies and preventative strategies to combat against maladaptive neural and behavioural processes produced by the adolescent environment and interactions with future experiences as an adult.

Reference

Alsio, J., Olszewski, P. K., Levine, A. S., & Schioth, H. B. (2012). Feed-forward mechanisms: Addiction-like behavioral and molecular adaptations in overeating. *Frontiers in Neuroendocrinology, 33*, 127-139. doi: 10.1016/j.yfrne.2012.01.002.

Alsio, J., Olszewski, P. K., Norback, A. H., Gunnarsson, Z. E., Levine, A. S., Pickering, C., & Schioth, H. B. (2010). Dopamine D1 receptor gene expression decreases in the nucleus accumbens upon long-term exposure to palatable food and differs depending on diet-induced obesity phenotype in rats. *Neuroscience, 171*(3), 779-787. doi: 10.1016/j.neuroscience.2010.09.046.

Antion, M. D., Christie, L. A., Bond, A. M., Dalva, M. B., & Contractor, A. (2010). Ephrin-B3 regulates glutamate receptor signaling at hippocampal synapses. *Molecular and Cellular Neurosciences, 45*(4). 378-388. doi: 10.1016/j.mcn.2010.07.011.

Australian Institute of Health and Welfare. (2012). *Australia's Food and Nutrition 2012*. Canberra: AIHW. Retrieved from http://www.aihw.gov.au/WorkArea/DownloadAsset.aspx?id=10737422837.

Avena, N. M., & Hoebel, B. G. (2003). A diet promoting sugar dependency causes behavioural cross-sensitization to a low dose of amphetamine. *Neuroscience, 122*, 17-20. doi: 10.1016/S0306-4522(03)00502-5.

Avena, N. M., & Hoebel, B.G. (2003). Amphetamine-sensitized rats show sugar-induced hyperactivity (cross-sensitization) and sugar hyperphagia. *Pharmacology, Biochemistry and Behaviour, 74*(3), 635-639. doi: 10.1016/S0091-3057(02)01050-X.

Avena, N. M., Bocarsly, M. E., Rada, P., Kim, A., & Hoebel, B. G. (2008). After daily bingeing on a sucrose solution, food deprivation induces anxiety and accumbens dopamine/acetylcholine imbalance. *Physiology and Behavior, 94*(3), 309-315. doi: 10.1016/j.physbeh.2008.01.008.

Avena, N. M., Long, K. A., & Hoebel, B. G. (2005). Sugar-dependent rats show enhanced responding for sugar after abstinence: Evidence of a sugar deprivation effect. *Physiology and Behaviour, 84*(3), 359-362. doi: 10.1016/j.physbeh.2004.12.016.

Avena, N. M., Rada, P., & Hoebel, B. G. (2008). Evidence for sugar addiction: Behavioural and neurochemical effects of intermittent, excessive sugar intake. *Neuroscience and Biobehavioural Reviews, 32*, 20-39. doi: 10.1016/j.neubiorev.2007.04.019.

Avena, N. M., Rada, P., & Hoebel, B. G. (2008). Underweight rats have enhanced dopamine release and blunted acetylcholine response in the nucleus accumbens while bingeing on sucrose. *Neuroscience, 156*(4), 865-871. doi: 10.1016/j.neuroscience.2008.08.017.

Avena, N. M., Rada, P., & Hoebel, B. G. (2009). Sugar and fat bingeing have notable differences in addictive-like behaviour. *The Journal of Nutrition, 139*(3), 623-628. doi: 10.3945/jn.108.097584.

Baldo, B. A., & Kelley, A. E. (2007). Discrete neurochemical coding of distinguishable motivational processes: Insights from the nucleus accumbens control of feeding. *Psychopharmacology, 191*(3), 439-459. doi: 10.1007/s00213-007-0741-z.

Baldo, B. A., Sadeghian, K., Basso, A. M., & Kelley, A. E. (2002). Effects of selective dopamine D1 or D2 receptor blockade within nucleus accumbens subregions on ingestive behaviour and associated motor activity. *Behavioural Brain Research, 137*(1-2), 165-177. doi: 10.1016/S0166-4328(02)00293-0.

Baldwin, A. E., Sadeghian, K., Holahan, M. R., & Kelley, A. E. (2002). Appetitive instrumental learning is impaired by inhibition of cAMP-dependent protein kinase within the nucleus accumbens. *Neurobiology of Learning and Memory, 77*, 44-62. doi: 10.1006/nlme.2000.4002.

Bamford, N. S., Zhang, H., Joyce, J. A., Scarlis, C.A., Hanan, W., Wu, N. P., Andre, V. M., Cohen, R., Cepeda, C., Levine, M. S., Harleton, E., & Sulzer, D. (2008). Repeated exposure to methamphetamine causes long-lasting presynaptic corticostriatal depression

that is renormalized with drug readministration. *Neuron, 58,* 89-103. doi: 10.1016/j.neuron.2008.01.033.

Barbano, M. F., & Cador, M. (2006). Differential regulation of the consummatory, motivational and anticipatory aspects of feeding behaviour by dopaminergic and opioidergic drugs. *Neuropsychopharmacology, 31*(7), 1371-1381. doi: 10.1038/sj.npp.1300908.

Barberis, L., Wary, K. K., Fiucci, G., Liu, F., Hirsch, E., Brancaccio, M., Altruda, F., Tarone, G., & Giancotti, F. G. (2000). Distinct roles of the adaptor protein Shc and focal adhesion kinase in integrin signaling to ERK. *The Journal of Biological Chemistry, 275*(47), 36532-36540. doi: 10.1074/jbc.M002487200.

Barrot, M., Olivier, J. D. A., Perrotti, L. I., Di Leone, R. J., Berton, O., Eisch, A. J., Impey, S., Storm, D. R., Neve, R. L., Yin, J. C., Zachariou, V., & Nestler, E. J. (2002). CREB activity in the nucleus accumbens shell controls gating of behavioural responses to emotional stimuli. *Proceedings of the National Academy of Sciences, 99*(17), 11435-11440. doi: 10.1073/pnas.172091899.

Bedford, F. K., Kittler, J. T., Muller, E., Thomas, P., Uren, J. M., Merlo, D., Wisden, W., Triller, A., Smart, T. G., & Moss, S. J. (2001). $GABA_A$ receptor cell surface number and subunit stability are regulated by the ubiquitin-like protein Plic-1. *Nature Neuroscience, 4*(9), 908-916. doi: 10.1038/nn0901-908.

Bello, N. T., Lucas, L. R., & Hajnal, A. (2002). Repeated sucrose access influences dopamine D2 receptor density in the striatum. *Neuroreport, 13*(12), 1575-1578. doi: 10.1097/00001756-200208270-00017.

Bello, N. T., Sweigart, K. L., Lakoski, J. M., Norgren, R., & Hajnal, A. (2003). Restricted feeding with scheduled sucrose access results in an upregulation of the rat dopamine transporter. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology,284*(5), (R1260-R1268). doi: 10.1152/ajpregu.00716.2002.

Benton, D. (2010). The plausibility of sugar addiction and its role in obesity and eating disorders. *Clinical Nutrition, 29*(3), 288-303. doi: 10.1016/j.clnu.2009.12.001.

Berridge K. C., & Robinson T. E. (1998). What is the role of dopamine in reward: Hedonic impact, reward learning or incentive salience. *Brain Research Reviews, 28*(3), 309-369. doi: 10.1016/S0165-0173(98)00019-8.

Bibb, J. A., Snyder, G. L., Nishi, A., Yan, Z., Meijer, L., Fienberg, A. A., Tsai, L. H., Kwon, Y. T. Girault, J. A., Czernik, A. J., Huganir, R. L., Hemmings, H. C., Nairn, A. C., Greengard, P. (1999). Phosphorylation of DARPP-32 by Cdk5 modulates dopamine signalling in neurons. *Nature, 402*(6762), 669-671. doi: 10.1038/45251.

Blumenthal, D. M., & Gold, M. S. (2010). Neurobiology of food addiction. *Current Opinion in Clinical Nutrition and Metabolic Care, 13*(4). 359-365. doi: 10.1097/MCO.0b013e32833ad4d4.

Braithwaite, S. P., Adkisson, M., Leung, J., Nava, A., Masterson, B., Urfer, R., Oskenberg, D., & Nikolich, K. (2006). Regulation of NMDA receptor trafficking and function by striatal-enriched tyrosine phosphatase (STEP). *European Journal of Neuroscience, 23*(11), 2847-2856. doi: 10.1111/j.1460-9568.2006.04837.x.

Braithwaite, S. P., Paul, S., Nairn, A. C., & Lombroso, P. J. (2006). Synaptic plasticity: One STEP at a time. *Trends in Neurosciences, 29*(8), 452-458. doi: 10.1016/j.tins.2006.06.007.

Brandon, E. P., Logue, S. F., Adams, M. R., Qi, M., Sullivan, S. P., Matsumoto, A. M., Dorsa, D. M., Wehner, J. M., McKnight, G. S., & Idzerda, R. L. (1998). Defective motor behavior and neural gene expression in RIIβ-protein kinase A mutant mice. *The Journal of Neuroscience, 18*(10), 3639-3649.

Brickley, S. G., Revilla, V., Cull-Candy, S. G., Wisden, W. & Farrant, M. (2001). Adaptive regulation of neuronal excitability by a voltage-independent potassium conductance. *Nature, 409*(6816), 88-92. doi: 10.1038/35051086.

Brown, J. M., & Yamamoto, B. K. (2003). Effects of amphetamines on mitochondrial function: Role of free radicals and oxidative stress. *Pharmacology and Therapeutics, 99*, 45-53. doi: 10.1016/S0163-7258(03)00052-4.

Cadet, J. L., Brannock, C., Ladenheim, B., McCoy, M. T., Krasnova, I. N., Lehrmann, E., Becker, K. G., & Jayanthi, S. (2014). Enhanced upregulation of CRH mRNA expression in the nucleus accumbens of male rats after a second injection of methamphetamine given thirty days later. *PLOS ONE, 9*, e84665. doi: 10.1371/journal.pone.0084665.

Cameron, C. M., & Carelli, R. M. (2012). Cocaine abstinence alters nucleus accumbens firing dynamics during goal-directed behaviours for cocaine and sucrose. *European Journal of Neuroscience, 35*(6), 940-951. doi: 10.1111/j.1460-9568.2012.08024.x.

Chan, P., Di Monte, D. A., Luo, J. J., DeLanney, L. E., Irwin, I., & Langston, J. W. (1994). Rapid ATP loss caused by methamphetamine in the mouse striatum: Relationship between energy impairment and dopaminergic neurotoxicity. *Journal of Neurochemistry, 62*(6), 2484-2487.

Chao, J., & Nestler, E. J. (2004). Molecular neurobiology of drug addiction. *Annual Review of Medicine, 55*, 113-132. doi: 10.1146/annurev.med.55.091902.103730.

Chen, C. H., Hwang, S. L., Howng, S. L., Chou, C. K., & Hong, Y. R. (2000). Three rat brain alternative splicing dynamin-like protein variants: Interaction with the glycogen synthase kinase 3b and action as a substrate. *Biochemical and Biophysical Research Communications, 268*(3), 893-898. doi: 10.1006/bbrc.2000.2197.

Chen, P. C., & Chen, J. C. (2005). Enhanced Cdk5 activity and p35 translocation in the ventral striatum of acute and chronic methamphetamine-treated rats. *Neuropsychopharmacology, 30*(3), 538-549. doi: 10.1038/sj.npp.1300604.

Chiang, Y. C., & Chen, J. C. (2007). The role of the cannabinoid type 1 receptor and down-stream cAMP/DARPP-32 signal in the nucleus accumbens of methamphetamine-sensitized rats. *Journal of Neurochemistry, 103*(6), 2505-2517. doi: 10.1111/j.1471-4159.2007.04981.x.

Colantuoni, C., Schwenker, J., McCarthy, J., Rada, P., Ladenheim, B., Cadet, J. L., Schwartz, G. J., Moran, T. H., & Hoebel, B. G. (2001). Excessive sugar intake alters binding to dopamine and mu-opioid receptors in the brain. *Neuroreport, 12*(16), 3549-3552. doi: 10.1097/00001756-200111160-00035.

Colombini, M., Blachly-Dyson, E., & Forte, M. (1996). VDAC, a channel in the outer mitochondrial membrane. *Ion Channels, 4*, 169-202. doi: 10.1007/978-1-4899-1775-1_5.

Cooper, M. A., Kobayashi, K., & Zhou, R. (2009). Ephrin-A5 regulates the formation of the ascending midbrain dopaminergic pathways. *Developmental Neurobiology, 69*, 36-46. doi: 10.1002/dneu.20685.

Couve, A., Thomas, P., Calver, A. R., Hirst, W. D., Pangalos, M. N., Walsh, F. S., Smart, T. G., & Moss, S. J. (2002). Cyclic AMP-dependent protein kinase phosphorylation facilitates $GABA_B$ receptor-effector coupling. *Nature Neuroscience, 5*(5), 415-424. doi: 10.1038/nn833.

Dadke, S., & Chernoff, J. (2002). Interaction of protein tyrosine phosphatase (PTP) 1B with its substrates is influenced by two distinct binding domains. *Biochemical Journal, 364*, 377-383. doi: 10.1042/BJ20011372.

Dalva, M. B., Takasu, M. A., Lin, M. Z., Shamah, S. M., Hu, L., Gale, N. W., & Greenberg, M. E. (2000). EphB receptors interact with NMDA receptors and regulate excitatory synapse formation. *Cell, 103*(6), 945-956. doi: 10.1016/S0092-8674(00)00197-5.

David, H. N. (Ed.). (2008). *The nucleus accumbens: Neurotransmitter and related behaviours*. Karala: Research Signpost.

Davidson, C., Lee, T. H., & Ellinwood, E. H. (2005). Acute and chronic continuous methamphetamine have different long-term behavioural and neurochemical consequences. *Neurochemistry International, 146*(3), 189-203. doi: 10.1016/j.neuint.2004.11.004.

Davis, C., Levitan, R. D., Kaplan, A. S., Carter, J., Reid, C., Curtis, C., Patte, K., Hwang, R., & Kennedy, J. L. (2008). Reward sensitivity and the D2 dopamine receptor gene: A case-control study of binge eating disorder. *Progress Neuropsychopharmacolgy and Biological Psychiatry, 32*(3), 620-628. doi: 10.1016/j.pnpbp.2007.09.024.

de Araujo, M. E. G., Stasyk, T., Taub, N., Ebner, H. L., Furst, B., Filipek, P., Weys, S. R., Hess, M. W., Lindner, H., Kremser, L., & Huber, L. A. (2013). Stability of the endosomal scaffold protein LAMTOR3 depends on heterodimer assembly and proteasomal degradation. *The Journal of Biological Chemistry, 288*(25), 18228-18242. doi: 0.1074/jbc.M112.349480.

De Sousa, N. J., Bush, D. E. A., & Vaccarino, F. J. (2000). Self-administration of intravenous amphetamine is predicted by individual differences in sucrose feeding in rats. *Psychopharmacology, 148*, 52-58. doi: 10.1007/s002130050024.

del Castillo, C., Morales, L., Alguacil, L. F., Salas, E., Garrido, E., Alonso, E., Perez-Garcia, C. (2009). Proteomic analysis of the nucleus accumbens of rats with different vulnerability to cocaine addiction. *Neuropharmacology, 57*, 41-48. doi: 10.1016/j.neuropharm.2009.04.005.

Detmer, S. A., & Chan, D. C. (2007). Functions and dysfunctions of mitochondrial dynamics. *Nature Reviews Molecular Cell Biology, 8*(11), 870-879. doi: 10.1038/nrm2275.

Dhavan, R., & Tsai, L. H. (2001). A decade of Cdk5. *Nature Reviews Molecular Cell Biology, 2*(10), 749-759. doi: 10.1038/35096019.

Di Chiara, G. (2002). Nucleus accumbens shell and core dopamine: Differential role in behaviour and addiction. *Behavioural Brain Research, 137*(1-2), 75-114. doi: 10.1016/S0166-4328(02)00286-3.

Dos Remedios, C. G., Chhabra, D., Kekic, M., Dedova, I. V., & Tsubakihara, M., Berry, D. A., & Nosworthy, N. J. (2003). Actin binding proteins: Regulation of cytoskeletal microfilaments. *Physiological Reviews, 83*(2), 433-473. doi: 10.1152/physrev.00026.2002.

Farrant, M., & Nusser, Z. (2005). Variation on an inhibitory theme: Phasic and tonic activation of $GABA_A$ receptors. *Nature Reviews Neuroscience, 6*(3), 215-229. doi: 10.1038/nrn1625.

Fernandez, E., Schiappa, R., Girault, J. A., & Le Novere, N. (2006). DARPP-32 is a robust integrator of dopamine and glutamate signals. *PLOS Computational Biology, 2*(12), 1619-1933 (e176). doi: 10.1371/journal.pcbi.0020176.

Fingar, D. C., Richardson, C. J., Tee, A. R, Cheatham, L., Tsou, C., & Blenis, J. (2004). mTOR controls cell cycle progression through its cell growth effectors S6K1 and 4E-BP1/eukaryotic translation initiation factor 4E. *Molecular and Cellular Biology, 24*, 200-216. doi: 10.1128/MCB.24.1.200–216.2004.

Fornai, F., Lenzi, P., Ferrucci, M., Lazzeri, G., di Poggio, A. B., Natale, G., Busceti, C. L., Biagioni, F., Giusiani, M., Ruggieri, S., & Paparelli, A. (2005). Occurrence of neuronal inclusions combined with increased nigral expression of alpha-synuclein within dopaminergic neurons following treatment with amphetamine derivatives in mice. *Brain Research Bulletin, 65*(5), 405-413. doi: 10.1016/j.brainresbull.2005.02.022.

Fortuna, J. L. (2010). Sweet preference, sugar addiction and the familial history of alcohol dependence: Shared neural pathways and genes. *Journal of Psychoactive Drugs, 42*(2), 147-151. doi: 10.1080/02791072.2010.10400687.

Fowler, J. S., Volkow, N. D., Logan, J., Alexoff, D., Telang, F., Wang, G. J., Wong, C., Ma, Y., Kriplani, A., Pradhan, K., Schyler, D., Jayne, M., Hubbard, B., Carter, P., Warner, D., King, P., Shea, C., Xu, Y., Muench, L., & Apelskog, K. (2008). Fast uptake and long-lasting binding of methamphetamine in the human brain: Comparison with cocaine. *Neuroimage, 43*(4), 756-763. doi: 10.1016/j.neuroimage.2008.07.020.

Francis, H. M., Mirzaei, M., Pardey, M., Haynes, P. A., & Cornish, J. L. (2013). Proteomic analysis of the dorsal and ventral hippocampus of rats maintained on a high fat and refined sugar diet. *Proteomics, 13*, 3076–3091. doi: 10.1002/pmic.201300124.

Fukushima, S., Shen, H., Hata, H., Ohara, A., Ohmi, K., Ikeda, K., Numachi, Y., Kobayashi, H., Hall, F. S., Uhl, G. R., & Sora, I. (2007). Methamphetamine-induced locomotor activity and sensitization in dopamine transporter and vesicular monoamine transporter 2 double mutant mice. *Psychopharmacology, 193*, 55-62. doi: 10.1007/s00213-007-0749-4.

Fumagalli, F., Gainetdinov, R. R., Wang, Y. M., Valenzano, K. J., Miller, G.W., & Caron, M. G. (1999). Increased methamphetamine neurotoxicity in heterozygous vesicular monoamine transporter 2 knock-out mice. *The Journal of Neuroscience, 19*(7), 2424-2431.

Gambarana, C., Masi, F., Leggio, B., Grappi, S., Nanni, G., Scheggi, S., De Montis, M. G., & Tagliamonte, A. (2003). Acquisition of a palatable-food-sustained appetitive behaviour in satiated rats is dependent on the dopaminergic response to this food in limbic areas. *Neuroscience, 121*, 179-187. doi: 10.1016/S0306-4522(03)00383-X.

Gammulla, C. G., Pascovici, D., Atwell, B. J., & Haynes, P. A. (2010). Differential metabolic response of cultured rice (*oryza sativa*) cells exposed to high- and low-temperature stress. *Proteomics, 10*(16), 3001-3019. doi: 10.1002/pmic.201000054.

Gerjikov, T. V., Ross, G. M., & Beninger, R. J. (2004). Place preference induced by nucleus accumbens amphetamine is impaired by antagonists of ERK or p38 MAP kinases in rats. *Behavioral Neuroscience, 118*(4), 740-750. doi: 10.1037/0735-7044.118.4.740.

Giorgetti, M., Hotsenpiller, G., Froestl, W., & Wolf, M. E. (2002). In vivo modulation of ventral tegmental area dopamine and glutamate efflux by local $GABA_B$ receptors is altered after repeated amphetamine treatment. *Neuroscience, 109*(3), 585-595. doi: 10.1016/S0306-4522(01)00510-3.

Goldstein, R. Z., Craig, A. D. B., Bechara, A., Garavan, H., Childress, A. R., Paulus, M. P., & Volkow, N. D. (2009). The neurocircuitry of impaired insight in drug addiction. *Trends in Cognitive Sciences, 13*(9), 372-380. doi: 10.1016/j.tics.2009.06.004.

Greengard, P. (2001). Neuroscience – The neurobiology of slow synaptic transmission. *Science, 294*(5544), 1024-1030. doi: 10.1126/science.294.5544.1024.

Grimm, J. W., Fyall, A. M., & Osincup, D. P. (2005). Incubation of sucrose craving: Effects of reduced training and sucrose pre-loading. *Physiology and Behaviour, 84*, 73-79. doi: 10.1016/j.physbeh.2004.10.011.

Grimm, J. W., Shaham, Y., & Hope, B. T. (2002). Effect of cocaine and sucrose withdrawal period on extinction behaviour, cue-induced reinstatement, and protein levels of the dopamine transporter and tyrosine hydroxylase in limbic and cortical areas in rats. *Behavioural Pharmacology, 13*(5-6), 379-388.

Guan, J. L. (1997). Role of focal adhesion kinase in integrin signaling. *The International Journal of Biochemistry and Cell Biology, 29*(8-9), 1085-1096. doi: 10.1016/S1357-2725(97)00051-4.

Hajnal, A., & Norgren, R. (2002). Repeated access to sucrose augments dopamine turnover in the nucleus accumbens. *Neuroreport, 13*(17), 2213-2216. doi: 10.1097/01.wnr.0000044213.09266.38.

Hanlon, A. C., Baldo, B. A., Sadeghian, K., & Kelley, A. E. (2004). Increases in food intake or food-seeking behaviour induced by GABAergic, opioid, or dopaminergic stimulation of the nucleus accumbens: Is it hunger? *Psychopharmacology, 172*(3), 241-247. doi: 10.1007/s00213-003-1654-0.

Hay, N., & Sonenberg, N. (2004). Upstream and downstream of mTOR. *Genes and Development, 18*(16), 1926-1945. doi: 10.1101/gad.1212704.

Hayashi, T., Ishimori, C., Takahashi-Niki, K., Taira, T., Kim, Y., Maita, H., Maita, C., Ariga, H., & Iguchi-Ariga, S. M. M. (2009). DJ-1 binds to mitochondrial complex I and maintains its activity. *Biochemical and Biophysical Research Communications, 390*(3), 667-672. doi: 10.1016/j.bbrc.2009.10.025.

He, W., Lu, Y., Qahwash, I., Hu, X. Y., Chang, A., & Yan, R. (2004). Reticulon family members modulate BACE1 activity and amyloid-β peptide generation. *Nature Medicine, 10*(9), 959-965. doi: 10.1038/nm1088.

Hester, R., & Garavan, H. (2004). Executive dysfunction in cocaine addiction: Evidence for discordant frontal, cingulate, and cerebellar activity. *The Journal of Neuroscience, 24*(49), 11017-11022. doi: 10.1523/JNEUROSCI.3321-04.2004.

Heyser, C. J., McNaughton, C. H., Vishnevetsky, D., & Fienberg, A. A. (2013). Methylphenidate restores novel object recognition in DARPP-32 knockout mice. *Behavioural Brain Research, 253*, 266-273. doi: 10.1016/j.bbr.2013.07.031.

Hoebel, B. G., Avena, N. M., Bocarsly, M. E., & Rada, P. (2009). Natural addiction: A behavioral and circuit model based on sugar addiction in rats. *Journal of Addiction Medicine, 3*, 33-41. doi: 10.1097/ADM.0b013e31819aa621.

Hoeffer, C. A., & Klann, E. (2009). mTOR signaling: At the crossroads of plasticity, memory and disease. *Trends in Neurosciences, 33*(2), 67-75. doi: 10.1016/j.tins.2009.11.003.

Hou, H., Sun, L., Siddoway, B. A., Petralia, R. S., Yang, H., Gu, H., Nairn, A. C., & Xia, H. (2013). Synaptic NMDA receptor stimulation activates PP1 by inhibiting its phosphorylation by Cdk5. *The Journal of Cell Biology, 203*(3), 521-535. doi: 10.1083/jcb.201303035.

Hu, Y., Lund, I. V., Gravielle, M. C., Farb, D. H., Brooks-Kayal, A. R., & Russek, S. J. (2008). Surface expression of $GABA_A$ receptors is transcriptionally controlled by the interplay of cAMP-response element-binding protein and its binding partner inducible cAMP early repressor. *The Journal of Biological Chemistry, 283*(14), 9328-9340. doi: 10.1074/jbc.M705110200.

Huang, J., Hamasaki, H., Nakamoto, T., Honda, H., Hirai, H., Saito, M., Takato, T., & Sakai, R. (2002). Differential regulation of cell migration, actin stress fiber organization, and cell transformation by functional domains of crk-associated substrate. *The Journal of Biological Chemistry, 277*(30), 27265-27272. doi: 10.1074/jbc.M203063200.

Hyman, S. E., Malenka, R. C., Nestler, E. J. (2006). Neural mechanisms of addiction: The role of reward-related learning and *memory*. *Annual review of neuroscience, 29*, 565-598. doi: 10.1146/annurev.neuro.29.051605.113009.

Ikemoto, A., Bole, D. G., & Ueda, T. (2003). Glycolysis and glutamate accumulation into synaptic vesicles: Role of glyceraldehyde phosphate dehydrogenase and 3-phosphoglycerate kinase. *The Journal of Biological Chemistry, 278*(8), 5929-5940. doi: 10.1074/jbc.M211617200.

Ikemoto, S., & Panksepp, J. (1996). Dissociations between appetitive and consummatory responses by pharmacological manipulations of reward-relevant brain regions. *Behavioural Neuroscience, 110*(2), 331-345. doi: 10.1037/0735-7044.110.2.331.

Ikemoto, S., & Panksepp, J. (1999). The role of nucleus accumbens dopamine in motivated behaviour: A unifying interpretation with special reference to reward-seeking. *Brain Research Reviews, 31*, 6-41. doi: 10.1016/S0165-0173(99)00023-5.

Irrcher, I., Aleyasin, H., Seifert, E., Hewitt, S. J., Chhabra, S., Phillips, M., Lutz, A. K., Rousseaux, M. W. C., Bevilacqua, L., Jahani-Asl, A., Callaghan, S., MacLaurin, J. G., Winklhofer, K. F., Rizzu, P., Rippstein, P., Kim, R. H., Chen, C. X., Fon, E. A., Slack, R. S., Harper, M. E., McBride, H. M., Mak, T. W., & Park, D. S. (2010). Loss of the Parkinson's disease-linked gene DJ-1 perturbs mitochondrial dynamics. *Human Molecular Genetics, 19*(19), 3734-3746. doi: 10.1093/hmg/ddq288.

Isao, T., & Akiyama, K. (2004). Effect of acute and chronic treatment with methamphetamine on mRNA expression of synaptotagmin IV and 25 KDa-synaptic-associated protein in the rat brain. *Psychiatry and Clinical Neurosciences, 58*(4), 410-419. doi: 10.1111/j.1440-1819.2004.01276.x.

Iwazaki, T., McGregor, I. S., & Matsumoto, I. (2006). Protein expression profile in the striatum of acute methamphetamine-treated rats. *Brain Research, 1097*, 19-25. doi: 10.1016/j.brainres.2006.04.052 .

Iwazaki, T., McGregor, I. S., & Matsumoto, I. (2007). Protein expression profile in the striatum of rats with methamphetamine-induced behavioural sensitization. *Proteomics, 7*(7), 1131-1139. doi: 10.1002/pmic.200600595.

Jaworski, J., & Sheng, M. (2006). The growing role of mTOR in neuronal development and plasticity. *Molecular Neurobiology, 34*(3), 205-219. doi: 10.1385/MN:34:3:205.

Jaworski, J., Spangler, S., Seeburg, D. P., Hoogenraad, C. C., & Sheng, M. (2005). Control of dendritic arborization by the phosphoinositide-3´-kinase–Akt–mammalian target of rapamycin pathway. *The Journal of Neuroscience, 25*(49), 11300-11312. doi: 10.1523/JNEUROSCI.2270-05.2005.

Jayanthi, S., McCoy, M. T., Ladenheim, B., & Cadet, J. L. (2002). Methamphetamine causes coordinate regulation of Src, Cas, Crk, and the Jun N-terminal kinase–Jun pathway. *Molecular Pharmacology, 61*(5), 1124-1131. doi: 10.1124/mol.61.5.1124.

Kalivas, P. W., Volkow, N. D., & Seamans, J. (2005). Unmanageable motivation in addiction: A pathology in prefrontal accumbens glutamate transmission. *Neuron, 45*(5), 647-650. doi: 10.1016/j.neuron.2005.02.005.

Kawahara, E., Maenaka, S., Shimada, E., Nishimura, Y., & Sakurai, H. (2013). Dynamic regulation of extracellular signal-regulated kinase (ERK) by protein phosphatase 2A regulatory subunit B56c1 in nuclei induces cell migration. *PLOS ONE, 5*, e63729. doi: 10.1371/journal.pone.0063729.

Kelley, A. E. (2004). Memory and addiction: Shared neural circuitry and molecular mechanisms. *Neuron, 44*, 161-179. doi: 10.1016/j.neuron.2004.09.016.

Kelley, A. E., Bakshi, V. P., Haber, S. N., Steininger, T. L., Will, M. J., & Zhang, M. (2002). Opioid modulation of taste hedonics within the ventral striatum. *Physiology and Behaviour, 76*(3), 365-377. doi: 10.1016/S0031-9384(02)00751-5.

Kelley, A. E., Baldo, B. A., Pratt, W. E., & Will, M. J. (2005). Corticostriatal-hypothalamic circuitry and food motivation: Integration of energy, action and reward. *Physiology and Behavior, 86*, 773-795. doi: 10.1016/j.physbeh.2005.08.066.

Kent, C. B., Shimada, T., Ferraro, G. B., Ritter, B., Yam, P. T., McPherson, P. S., Charron, F., Kennedy, T. E., & Fournier, A.E. (2010). 14-3-3 proteins regulate protein kinase A activity to modulate growth cone turning responses. *The Journal of Neuroscience, 30*(42), 14059-14067. doi: 10.1523/JNEUROSCI.3883-10.2010.

Kirkham, T. C., Williams, C. M., Fezza, F., & Di Marzo, V. (2002). Endocannabinoid levels in rat limbic forebrain and hypothalamus in relation to fasting, feeding and satiation: Stimulation of eating by 2-arachidonoyl glycerol. *British Journal of Pharmacology, 136*(4), 550-557. doi: 10.1038/sj.bjp.0704767.

Klein, R. (2009). Bidirectional modulation of synaptic functions by Eph/ephrin signaling. *Nature Neuroscience, 12*, 15-20. doi: 10.1038/nn.2231.

Klongpanichapak, S., Govitrapong, P., Sharma, S. K., & Ebadi, M. (2006). Attenuation of cocaine and methamphetamine neurotoxicity by coenzyme Q10. *Neurochemical Research, 31*(3), 303-311. doi: 10.1007/s11064-005-9025-3.

Koob, G. F. (1992). Drugs of abuse: Anatomy, pharmacology and function of reward pathways. *Trends in Pharmacological Sciences, 13*(5), 177-184. doi: 10.1016/0165-6147(92)90060-J.

Koob, G. F., Sanna, P. P., & Bloom, F. E. (1998). Neuroscience of addiction. *Neuron, 21*(3), 467-476. doi: 10.1016/S0896-6273(00)80557-7.

Koylu, E. O., Couceyro, P. R., Lambert, P. D., & Kuhar, M. J. (1998). Cocaine- and amphetamine-regulated transcript peptide immunohistochemical localization in the rat brain. *The Journal of Comparative Neurology, 391*, 115-132. doi: 10.1002/(SICI)1096-9861(19980202)391:1<115::AID-CNE10>3.0.CO;2-X.

Krasnova, I. N., Justinova, Z., Ladenheim, B., Jayanthi, S., McCoy, M. T., Barnes, C., Warner, J. E., Goldberg, S. R., & Cadet, J. L. (2010). Methamphetamine self-administration is associated with persistent biochemical alterations in striatal and cortical dopaminergic terminals in the rat. *PLOS ONE, 5*, e8790. doi: 10.1371/journal.pone.0008790.

Kumar, V., Zhang, M. X., Swank, M. W., Kunz, J., & Wu, G Y. (2005). Regulation of dendritic morphogenesis by Ras–PI3K–Akt–mTOR and Ras–MAPK signaling pathways. *The Journal of Neuroscience, 25*(49), 11288-11299. doi: 10.1523/JNEUROSCI.2284-05.2005.

Lai, K. O., & Ip, N. Y. (2009). Synapse development and plasticity: Roles of ephrin/Eph receptor signaling. *Current Opinion in Neurobiology, 19*(3), 275-283. doi: 10.1016/j.conb.2009.04.009.

Le Cozannet, R., Markou, A., & Kuczenski, R. (2013). Extended-access, but not limited-access, methamphetamine self-administration induces behavioural and nucleus accumbens dopamine response changes in rats. *European Journal of Neuroscience, 38*, 3487-3495. doi: 10.1111/ejn.12361.

Le Moal, M., & Simon, H. (1991). Mesocorticolimbic dopaminergic network: Functional and regulatory roles. *Physiological Reviews, 71*, 155-234.

Li, H., Chen, Y., Jones, A. F., Sanger, R. H., Collis, L. P., Flannery, R., McNay, E. C., Yu, T., Schwarzenbacher, R., Bossy, B., Bossy-Wetzel, E., Bennett, M. V. L., Pypaert, M., Hickman, J. A., Smith, P. J. S., Harwick, J. M., & Jonas, E. A. (2008). Bcl-xL induces Drp1-dependent synapse formation in cultured hippocampal neurons. *Proceedings of the National Academy of Sciences – PNAS, 105*(6), 2169-2174. doi: 10.1073/pnas.0711647105.

Liebler, D. C. (2002). *Introduction to proteomics: Tools for the new biology*. New Jersey: Humana Press.

Lien, L., Lien, N., Heyerdahl, S., Thoresen, M., & Bjertness, E. (2006). Consumption of soft drinks and hyperactivity, mental distress, and conduct problems among adolescents in Oslo, Norway. *American Journal of Public Health, 96*(10), 1815-1820. doi: 10.2105/AJPH.2004.059477.

Lise, M. F., Srivastava, D. P., Arstikaitis, P., Lett, R. L., Sheta, R., Viswanathan, V., Penzes, P., O'Conner, T. P., & El-Husseini, A. (2009). Myosin-Va-interacting protein, RILPL2,

controls cell shape and neuronal morphogenesis via Rac signaling. *Journal of Cell Science 122*(20), 3810-3821. doi: 10.1242/jcs.050344.

Liu, F., Hill, D. E., & Chernoff, J. (1996). Direct binding of the proline-rich region of protein tyrosine phosphatase 1B to the Src homology 3 domain of $p130^{Cas}$. *The Journal of Biological Chemistry, 271*(49), 31290-31295.

Liu, Q., Pu, L., & Poo, M. (2005). Repeated cocaine exposure in vivo facilitates LTP induction in midbrain dopamine neurons. *Nature, 437*(7061), 1027-1031. doi: 10.1038/nature04050.

Lominac, K. D., Sacramento, A. D., Szumlinski, K. K., & Kippen, T. E. (2012). Distinct neurochemical adaptations within the nucleus accumbens produced by a history of self-administered vs non-contingently administered intravenous methamphetamine. *Neuropsychopharmacology, 37*(3), 707-722. doi: 10.1038/npp.2011.248.

Lubec, G., Krapfenbauer, K., & Fountoulakis, M. (2003). Proteomics in brain research: potentials and limitations. *Progress in Neurobiology, 69*(3), 193-211. doi: 10.1016/S0301-0082(03)00036-4.

Luscher, B., & Keller, C. A. (2004). Regulation of $GABA_A$ receptor trafficking, channel activity, and functional plasticity of inhibitory synapses. *Pharmacology and Therapeutics, 102*(3), 195-221. doi: 10.1016/j.pharmthera.2004.04.003.

Luscher, B., Fuchs, T., & Kilpatrick, C. L. (2011). $GABA_A$ receptor trafficking-mediated plasticity of inhibitory synapses. *Neuron, 70*(3), 385-409. doi: 10.1016/j.neuron.2011.03.024.

Lyvers, M. (2000). “Loss of control” in alcoholism and drug addiction: A neuroscientific interpretation. *Experimental and Clinical Psychopharmacology, 8*(2), 225-249. doi: 10.1037/1064-1297.8.2.225.

Mark, K. A., Quinton, M. S., Russek, S. J., & Yamamoto, B. K. (2007). Dynamic changes in vesicular glutamate transporter 1 function and expression related to methamphetamine-

induced glutamate release. *The Journal of Neuroscience, 27*(25), 6823-6831. doi: 10.1523/JNEUROSCI.0013-07.2007.

Martin, T. A., Jayanthi, S., McCoy, M. T., Brannock, C., Ladenheim, B., Garrett, T., Lehrmann, E., Becker, K. G., & Cadet, J. L. (2012). Methamphetamine causes differential alterations in gene expression and patterns of histone acetylation/hypoacetylation in the rat nucleus accumbens. *PLOS ONE, 7*(3), e34236. doi: 10.1371/journal.pone.0034236.

Mazzucchelli, C., Vantaggiato, C., Ciamei, A., Fasano, S., Pakhotin, P., Krezel, W., Welzl, H., Wolfer, D. P., Pages, G., Valverde, O., Marowsky, A., Porrazzo, A., Orban, P. C., Maldonado, R., Ehrengruber, M. U., Cestari, V., Lipp, H. P., Chapman, P. F., Pouyssegur, J., & Brambilla, R. (2002). Knockout of ERK1 MAP kinase enhances synaptic plasticity in the striatum and facilitates striatal-mediated learning and memory. *Neuron, 34*(5), 807-820. doi: 10.1016/S0896-6273(02)00716-X.

McClung, C. A., Ulery, P. G., Perrotti, L. I., Zachariou, V., Berton, O., & Nestler, E. J. (2004). ΔFosB: A molecular switch for long-term adaptation in the brain. *Molecular Brain Research, 132*(2), 146-154. doi: 10.1016/j.molbrainres.2004.05.014.

McDaid, J., Graham, M. P., & Napier, C. (2006). Methamphetamine-induced sensitization differentially alters pCREB and ΔFosB throughout the limbic circuit of the mammalian brain. *Molecular Pharmacology, 70*(6), 2064-2074. doi: 10.1124/mol.106.023051.

Meng, Y. Zhang, Y., Tregoubov, V., Falls, D. L., & Jia, Z. (2003). Regulation of spine morphology and synaptic function by LIMK and the actin cytoskeleton. *Reviews in Neuroscience, 14*(3), 233-240. doi: 10.1515/REVNEURO.2003.14.3.233.

Mirzaei, M., Pascovici, D., Atwell, B. J., & Haynes, P. A. (2012). Differential regulation of aquaporins, small GTPases and V-ATPases proteins in rice leaves subjected to drought stress and recovery. *Proteomics, 12*(6), 864-877. doi: 10.1002/pmic.201100389.

Mitsuhashi, S., Shima, H., Tanuma, N., Matsuura, N., Takekawa, M., Urano, T., Kataoka, T., Ubukata, M., & Kikuchi, K. (2003). Usage of tautomycetin, a novel inhibitor of protein

phosphatase 1 (PP1), reveals that PP1 is a positive regulator of Raf-1 in vivo. *The Journal of Biological Chemistry, 278*, 82-88. doi: 10.1074/jbc.M208888200.

Miyazaki, I., & Asanuma, M. (2008). Dopaminergic neuron-specific oxidative stress caused by dopamine itself. *Acta Medica Okayama, 62*(3), 141-150.

Miyazaki, M., Noda, Y., Mouri, A., Kobayashi, K., Mishina, M., Nabeshima, T., & Yamada, K. (2013). Role of convergent activation of glutamatergic and dopaminergic systems in the nucleus accumbens in the development of methamphetamine psychosis and dependence. *International Journal of Neuropsychopharmacology, 16*(6), 1341-1350. doi: 10.1017/S1461145712001356.

Moss, S. J., Smart, T. G., Blackstone, C. D., & Huganir, R. L. (1992). Functional modulation of $GABA_A$ receptors by cAMP-dependent protein phosphorylation. *Science, 257*(5070), 661-665. doi: 10.1126/science.1323140.

Murayama, K. S., Kametani, F., Saito, S., Kume, H., Akiyama, H., & Araki, W. (2006). Reticulons RTN3 and RTN4-B/C interact with BACE1 and inhibit its ability to produce amyloid β-protein. *European Journal of Neuroscience, 24*(5), 1237-1244. doi: 10.1111/j.1460-9568.2006.05005.x.

Muscat, R., & Willner, P. (1989). Effects of dopamine receptor antagonists on sucrose consumption and preference. *Psychopharmacology, 99*, 98-102. doi: 10.1007/BF00634461.

Nada, S., Hondo, A., Kasai, A., Koike, M., Saito, K., Uchiyama, Y., & Okada, M. (2009). The novel lipid raft adaptor p18 controls endosome dynamics by anchoring the MEK–ERK pathway to late endosomes. *The EMBO Journal, 28*(5), 477-489. doi: 10.1038/emboj.2008.308.

Nairn, A. C., Svenningsson, P., Nishi, A., Fisone, G., Girault, J. A., & Greengard, P. (2004). The role of DARPP-32 in the actions of drugs of abuse. *Neuropharmacology, 47*, 14-23. doi: 10.1016/j.neuropharm.2004.05.010.

Nakajima, H., Amano, W., Kubo, T., Fukuhara, A., Ihara, H., Azuma, Y. T., Tajima, H., Inui, T., Sawa, A., & Takeuchi, T. (2009). Glyceraldehyde-3-phosphate dehydrogenase aggregate formation participates in oxidative stress-induced cell death. *The Journal of Biological Chemistry, 284*(49), 34331-34341. doi: 10.1074/jbc.M109.027698.

Narita, M., Akai, H., Kita, T., Nagumo, Y., Narita, M., Sanagawa, N., Hara, C., Haebe, K., Nagase, H., & Suzuki, T. (2005). Involvement of mitogen-stimulated P70-S6 kinase in the development of sensitization to the methamphetamine-induced rewarding effect in rats. *Neuroscience, 132*(3), 553-560. doi: 10.1016/j.neuroscience.2004.12.050.

Narita, M., Akai, H., Nagumo, Y., Sunagawa, N., Hasebe, K., Nagase, H., Kita, T., Hara, C., & Suzuki, T. (2004). Implications of protein kinase C in the nucleus accumbens in the development of sensitization to methamphetamine in rats. *Neuroscience, 127*(4), 941-948. doi: 10.1016/j.neuroscience.2004.06.017.

Neilson, K. A., Ali, N. A., Muralidharan, S., Mirzaei, M., Mariani, M., Assadourian, G., Lee, A., van Sluyter, S. C., & Haynes, P. A. (2011). Less label, more free: approaches in label-free quantitative mass spectrometry. *Proteomics, 11*(4), 535-553. doi: 10.1002/pmic.201000553.

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

Nestler, E. J. (2005). Is there a common molecular pathway for addiction? *Nature Neuroscience, 8*(11), 1445-1449. doi: 10.1038/nn1578.

Nestler, E. J., & Aghajanian, G. K. (1997). Molecular and cellular basis of addiction. *Science, 278*(5335), 58-63. doi: 10.1126/science.278.5335.58.

Nestler, E. J., Barrot, M., & Self, D. W. (2001). ΔFosB: A sustained molecular switch for addiction. *Proceedings of the National Academy of Sciences, 98*(20), 11042-11046. doi: 10.1073/pnas.191352698.

Nguyen, T. H., Liu, J., & Lombroso, P. J. (2002). Striatal enriched phosphatase 61 dephosphorylates Fyn at phosphotyrosine 420. *The Journal of Biological Chemistry, 277*(27), 24274-24279. doi: 10.1074/jbc.M111683200.

Nishi, A., Bibb, J. A., Snyder, G. L., Higashi, H., Nairn, A. C., Greengard, P. (2000). Amplification of dopaminergic signaling by a positive feedback loop. *Proceedings of the National Academy of Sciences – PNAS, 97*(23), 12840-12845. doi: 10.1073/pnas.220410397.

Orr, M. E., Salinas, A., Buffenstein, R., & Oddo, S. (2014). Mammalian target of rapamycin hyperactivity mediates the detrimental effects of a high sucrose diet on Alzheimer's disease pathology. *Neurobiology of Ageing, 35*(6), 1233-1242. doi: 10.1016/j.neurobiolaging.2013.12.006.

Padgett, C. L., Lalive, A. L., Tan, K. R., Terunuma, M., Munoz, M. B., Pangalos, M. N., Martinez-Hernandez, J., Watanabe, M., Moss, S. J., Lujan, R., Luscher, C., & Slesinger, P. A. (2012). Methamphetamine-evoked depression of $GABA_B$ receptor signaling in GABA neurons of the VTA. *Neuron, 75*(5), 978-989. doi: 10.1016/j.neuron.2011.12.031.

Palzkill, T. (2002). *Proteomics*. Boston: Kluwer Academic Publishers.

Panenka, W. J., Procyshyn, R. M., Lecomte, T., MacEwan, G. W., Flynn, S. W., Honer, W. G., & Barr, A. M. (2013). Methamphetamine use: A comprehensive review of molecular, preclinical and clinical findings. *Drug and Alcohol Dependence, 129*(3), 167-179. doi: 10.1016/j.drugalcdep.2012.11.016.

Parisiadou, L., Yu, J., Sgobio, C., Xie, C., Liu, G., Sun, L., Gu, X. L., Lin, X., Crowley, N. A., Lovinger, D.M., & Cai, H. (2014). LRRK2 regulates synaptogenesis and dopamine receptor activation through modulation of PKA activity. *Nature Neuroscience, 17*(3), 367-376. doi: 10.1038/nn.3636.

Parsegian, A., & See, R. E. (2014). Dysregulation of dopamine and glutamate release in the prefrontal cortex and nucleus accumbens following methamphetamine self-

administration and during reinstatement in rats. *Neuropsychopharmacology, 39*(4), 811-822. doi: 10.1038/npp.2013.231.

Patel., J., Wang, X., & Proud, C. G. (2001). Glucose exerts a permissive effect on the regulation of the initiation factor 4E binding protein 4E-BP1. *Biochemical Journal, 358*, 497-503. doi: 10.1042/0264-6021:3580497.

Pratt, W. E., Blackstone, K., Connolly, M. E., A., & Skelly, M. J. (2009). Selective serotonin receptor stimulation of the medial nucleus accumbens causes differential effects on food intake and locomotion. *Behavioural Neuroscience, 123*(5), 1046-1057. doi: 10.1037/a0016882.

Rada, P., Avena, N. M., & Hoebel, B. G. (2005). Daily bingeing on sugar repeatedly releases dopamine in the accumbens shell. *Neuroscience, 134*(3), 737-744. doi: 10.1016/j.neuroscience.2005.04.043.

Raghavan, A., Sheiko, T., Graham, B. H., Craigen, W. J. (2012). Voltage-dependant anion channels: novel insights into isoform function through genetic models. *Biochimica et Biophysica Acta Biomembranes, 1818*(6), 1477-1485. doi: 10.1016/j.bbamem.2011.10.019.

Rando, O. J., & Ahmed, K. (2007). Rules and regulation in the primary structure of chromatin. *Current Opinion in Cell Biology, 19*(3), 250-256. doi: 10.1016/j.ceb.2007.04.006.

Rauggi, R., Scheggi, S., Cassanelli, A., De Montis, M. G., Tagliamonte, A., & Gambarana, C. (2005). The mesolimbic dopaminergic response to novel palatable food consumption increases dopamine-D1 receptor-mediated signalling with complex modifications of the DARPP-32 phosphorylation pattern. *Journal of Neurochemistry, 92*(4), 867-877. doi: 10.1111/j.1471-4159.2004.02920.x.

Reddy, P. H. (2013). Is the mitochondrial outermembrane protein VDAC1 therapeutic target for Alzheimer's disease? *Biochimica et Biophysica Acta Molecular Basis of Disease, 1832*, 67-75. doi: 10.1016/j.bbadis.2012.09.003.

Reith, M. E. A. (Ed.). (2002). *Neurotransmitter transporters: Structure, function, and regulation* (2nd ed.). New Jersey: Humana Press.

Ren, R., Zhang, Y., Li, B., Wu, Y., & Li, B. (2011). Effect of β-amyloid (25–35) on mitochondrial function and expression of mitochondrial permeability transition pore proteins in rat hippocampal neurons. *Journal of Cellular Biochemistry, 112*(5), 1450-1457. doi: 10.1002/jcb.23062.

Richter, J. D., & Klann, E. (2009). Making synaptic plasticity and memory last: Mechanisms of translational regulation. *Genes and Development, 23*, 1-11. doi: 10.1101/gad.1735809.

Ritz, M. C., Lamb, R. J., Goldberg, S. R., & Kuhar, M. J. (1987). Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science, 237*(4819), 1219-1223. doi: 10.1126/science.2820058.

Robbins, T. W., & Everitt, B. J. (1996). Neurobehavioural mechanisms of reward and motivation. *Current Opinion in Neurobiology, 6*(2), 228-236. doi: 10.1016/S0959-4388(96)80077-8.

Robinson, T. E., & Berridge, K. C. (1993). The neural basis of drug craving: An incentive sensitisation theory of addiction. *Brain Research Reviews, 18*(3), 247-291. doi: 10.1016/0165-0173(93)90013-P.

Robinson, T. E., & Berridge, K. C. (2001). Incentive-sensitization and addiction. *Addiction, 96*, 103-114. doi: 10.1046/j.1360-0443.2001.9611038.x.

Robinson, T. E., & Kolb, B. (1999). Alterations in the morphology of dendrites and dendritic spines in the nucleus accumbens and prefrontal cortex following repeated treatment with amphetamine or cocaine. *European Journal of Neuroscience, 11*(5), 1598-1604. doi: 10.1046/j.1460-9568.1999.00576.x.

Sakaba, T., & Neher, E. (2003). Direct modulation of synaptic vesicle priming by $GABA_B$ receptor activation at a glutamatergic synapse. *Nature, 424*(6950), 775-778. doi: 10.1038/nature01859.

Salamone, J. D., & Correa, M. (2002). Motivational views of reinforcement: Implications for understanding the behavioural functions of nucleus accumbens dopamine. *Behavioural Brain Research, 137*, 3-25. doi: 10.1016/S0166-4328(02)00282-6.

Salamone, J. D., Correa, M., Farrar, A., & Mingote, S. M. (2007). Effort-related functions of nucleus accumbens dopamine and associated forebrain circuits. *Psychopharmacology, 191*(3), 461-482. doi: 10.1007/s00213-006-0668-9.

Salamone, J. D., Correa, M., Mingote, S. M., & Weber, S. M. (2005). Beyond the reward hypothesis: Alternative functions of nucleus accumbens dopamine. *Current Opinion in Pharmacology, 5*, 34-41. doi: 10.1016/j.coph.2004.09.004.

Salter, M. W., & Kalia, L. V. (2004). Src kinases: A hub for NMDA receptor regulation. *Nature Reviews Neuroscience, 5*(4), 317-328. doi: 10.1038/nrn1368.

Sampson, M. J., Lovell, R. S., & Craigen, W. J. (1996). Isolation, characterization, and mapping of two mouse mitochondrial voltage-dependent anion channel isoforms. *Genomics, 33*(2), 283-288. doi: 10.1006/geno.1996.0193.

Schmitt, K. C., & Reith, M. E. A. (2010). Regulation of the dopamine transporter: Aspects relevant to psychostimulant drugs of abuse. *Annals of the New York Academy of Sciences, 1187*, 316-340. doi: 10.1111/j.1749-6632.2009.05148.x.

Shamah, S. M., Lin, M. Z., Goldberg, J. L., Estrach, S., Sahin, M., Hu, L., Bazalakova, M., Neve, R. L., Corfas, G., Debant, A., & Greenberg, M. E. (2001). EphA receptors regulate growth cone dynamics through the novel guanine nucleotide exchange factor ephexin. *Cell, 105*(2), 233-244. doi: 10.1016/S0092-8674(01)00314-2.

Shen, H., Toda, S., Moussawi, K., Bouknight, A., Zahm, D. S., & Kalivas, P. W. (2009). Altered dendritic spine plasticity in cocaine-withdrawn rats. *The Journal of Neuroscience, 29*(9), 2876-2884. doi: 10.1523/JNEUROSCI.5638-08.2009.

Shi, Y., Pontrello, C. G., De Fea, K. A., Reichardt, L. F., & Ethell, I. M. (2009). Focal adhesion kinase acts downstream of EphB receptors to maintain mature dendritic spines

by regulating cofilin activity. *The Journal of Neuroscience, 29*(25), 8129-8142. doi: 10.1523/JNEUROSCI.4681-08.2009.

Sieber, B. A., Kuzmin, A., Canals, J. M., Danielsson, A., Paratcha, G., Arenas, E., Alberch, J., Ogren, S. O., & Ibanez, C. F. (2004). Disruption of EphA/ephrin-A signaling in the nigrostriatal system reduces dopaminergic innervation and dissociates behavioral responses to amphetamine and cocaine. *Molecular and Cellular Neurosciences, 26*(3), 418-428. doi: 10.1016/j.mcn.2004.03.009.

Silvestri, L., Caputo, V., Bellacchio, E., Atorino, L., Dallapiccola, B., Valente, E. M. & Casari, G. (2005). Mitochondrial import and enzymatic activity of PINK1 mutants associated to recessive parkinsonism. *Human Molecular Genetics, 14*(22), 3477-3492. doi: 10.1093/hmg/ddi377.

Smith, K. S., & Berridge, K. C. (2007). Opioid limbic circuit for reward: Interaction between hedonic hotspots of nucleus accumbens and ventral pallidum. *The Journal of Neuroscience, 27*(7), 1594-1605. doi: 10.1523/JNEUROSCI.4205-06.2007.

Spanagel, R., Herz, A., & Shippenberg, T. S. (1992). Opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathway. *Proceedings of the National Academy of Sciences, 89*(6), 2046-2050. doi: 10.1073/pnas.89.6.2046.

Spangler, R. Wittkowski, K. M., Goddard, N. L., Avena, N. M., Hoebel, B. G., & Leibowitz, S. F. (2004). Opiate-like effects of sugar on gene expression in reward areas of the rat brain. *Molecular Brain Research, 124*(2), 134-142. doi: 10.1016/j.molbrainres.2004.02.013.

Spear, L. P. (2000). The adolescent brain and age-related behavioural manifestations. *Neuroscience and Biobehavioural Reviews, 24*(4), 417-463. doi: 10.1016/S0149-7634(00)00014-2.

Steinkellner, T., Freissmuth, M., Sitte, H. H., & Montgomery, T. (2011). The ugly side of amphetamines: short- and long-term toxicity of 3,4-methylenedioxymethamphetamine

(MDMA, ‘Ecstasy’), methamphetamine and D-amphetamine. *Biological Chemistry, 392*(1-2), 103-115. doi: 10.1515/BC.2011.016.

Stork, P. J. S., & Schmitt, J. M. (2002). Crosstalk between cAMP and MAP kinase signaling in the regulation of cell proliferation. *Trends in Cell Biology, 12*(6), 256-266. doi: 10.1016/S0962-8924(02)02294-8.

Svenningsson, P., Nishi, A., Fisone, G., Girault, J. A., Nairn, A. C., & Greengard, P. (2004). DARPP-32: An integrator of neurotransmission. *Annual Review of Pharmacology and Toxicology, 44*, 269-296. doi: 10.1146/annurev.pharmtox.44.101802.121415.

Taira, T., Saito, Y., Niki, T., Iguchi-Ariga, S. M., Takahashi, K. & Ariga, H. (2004). DJ-1 has a role in antioxidative stress to prevent cell death. *EMBO Reports, 5*(2), 213-218. doi: 10.1038/sj.embor.7400074.

Takasu, M. A., Dalva, M. B., Zigmond, R. E., & Greenberg, M. E. (2002). Modulation of NMDA receptor-dependent calcium influx and gene expression through EphB receptors. *Science, 295*(5554), 491-495. doi: 10.1126/science.1065983.

Thomas, M. J., Beurrier, C., Bonic, A., & Malenka, R. C. (2001). Long-term depression in the nucleus accumbens: a neural correlate of behavioral sensitization to cocaine. *Nature Neuroscience, 4*(12), 1217-1223. doi: 10.1038/nn757.

Tilelli, C. Q., Martins, A. R., Larson, R. E., & Garcia-Cairasco, N. (2003). Immunohistochemical localization of myosin Va in the adult rat brain. *Neuroscience, 121*(3), 573-586. doi: 10.1016/S0306-4522(03)00546-3.

Toda, S., McGinty, J. F., & Kalivas, P. W. (2002). Repeated cocaine administration alters the expression of genes in corticolimbic circuitry after a 3-week withdrawal: a DNA macroarray study. *Journal of Neurochemistry, 82*(5), 1290-1299. doi: 10.1046/j.1471-4159.2002.01083.x.

Toriumi, K., Kondo, M., Nagai, T., Hashimoto, R., Ohi, K., Song, Z., Tanaka, J., Mouri, A., Koseki, T., Yamamori, H., Furukawa-Hibi, Y., Mamiya, T., Fukushima, T., Takeda, M., Nitta, A., Yamada, K., & Nabeshima, T. (2014). Deletion of SHATI/NAT8L increases

dopamine D1 receptor on the cell surface in the nucleus accumbens, accelerating methamphetamine dependence. *International Journal of Neuropsychopharmacology, 12*(3), 443-453. doi: 10.1017/S1461145713001302.

Touzani, K., Bodnar, R., & Sclafani, A. (2008). Activation of dopamine D1-like receptors in nucleus accumbens is critical for the acquisition, but not the expression, of nutrient-conditioned flavour preferences in rats. *European Journal of Neuroscience, 27*(6), 1525-1533. doi: 10.1111/j.1460-9568.2008.06127.x.

Tsokas, P., Ma, T., Iyengar, R., Landau, A. M., & Blitzer, R. D. (2007). Mitogen-activated protein kinase upregulates the dendritic translation machinery in long-term potentiation by controlling the mammalian target of rapamycin pathway. *The Journal of Neuroscience, 27*(22), 5885-5894. doi: 10.1523/JNEUROSCI.4548-06.2007.

Tsujimoto, Y., & Shimizu, S. (2002). The voltage-dependent anion channel: An essential player in apoptosis. *Biochimie, 84*(2-3), 187-193. doi: 10.1016/S0300-9084(02)01370-6.

Tukey, D. S., Ferreira, J. M., Antione, S. O., D'amour, J. A., Ninan, I., Cabeza de Vaca, S., Incontro, S., Wincott, C., Horwitz, J. K., Hartner, D. T., Guarini, C. B., Khatri, L., Goffer, Y., Xu, D., Titcombe, R. F., Khatri, M., Marzan, D. S., Mahajan, S. S., Wang, J., Froemke, R. C., Carr, K. D., Aoki, C., & Ziff, E. B. (2013). Sucrose ingestion induces rapid AMPA receptor trafficking. *The Journal of Neuroscience, 33*(14), 6123-6132. doi: 10.1523/JNEUROSCI.4806-12.2013.

United Nations Office on Drugs and Crime. (2014). *Global Synthetic Drugs Assessment: Amphetamine-type stimulants and new psychoactive substances*. New York: UNODC. Retrieved from http://www.unodc.org/documents/scientific/2014_Global_Synthetic_Drugs_Assessment_web.pdf.

Valjent, E., Corbille, A. G., Bertran-Gonzalez, J., Herve, D., & Girault, J. A. (2006). Inhibition of ERK pathway or protein synthesis during reexposure to drugs of abuse erases previously learned place preference. *Proceedings of the National Academy of*

Sciences of the United States of America, 103(8), 2932-2937. doi: 10.1073/pnas.0511030103.

Valjent, E., Pascoli, V., Svenningsson, P., Paul, S., Enslen, H., Corvol, J. C., Stipanovich, A., Caboche, J., Lombroso, P. J., Nairn, A. C., Greengard, P., Herve, D., & Girault, J. A. (2005). Regulation of a protein phosphatase cascade allows convergent dopamine and glutamate signals to activate ERK in the striatum. *Proceedings of the National Academy of Sciences of the United States of America, 102*(2), 491-496. doi: 10.1073/pnas.0408305102.

van den Oever, M. C., Spijker, S., Wan Li, K., Jimenez, C. R., Koya, E., Van der Schors, R. C., Gouwenberg, Y., Binnekade, R., De Vries, T. J., Schoffelmeer, A. N. M., & Smit, A. B. (2006). A proteomics approach to identify long-term molecular changes in rat medial prefrontal cortex resulting from sucrose self-administration. *Journal of Proteome Research, 5*, 147-154. doi: 10.1021/pr050303y.

van Heusden, G. P. H. (2005). 14-3-3 proteins: Regulators of numerous eukaryotic proteins. *IUBMB Life, 57*(9), 623-629. doi: 10.1080/15216540500252666.

Vanderschuren, L. J. M. J., & Kalivas, P. W. (2000). Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioural sensitization: A critical review of preclinical studies. *Psychopharmacology, 151*(2-3), 99-120. doi: 10.1007/s002130000493.

Vendruscolo, L. F., Gueye, A. B., Darnaudery, M., Ahmed, S. H., & Cador, M. (2010). Sugar overconsumption during adolescence selectively alters motivation and reward function in adult rats. *Plos One, 5*(2), e9296. doi: 10.1371/journal.pone.0009296.

Volkow, N. D., Wang, G. J., Fowler, J. S., & Telang, F. (2008). Overlapping neuronal circuits in addiction and obesity: Evidence of systems pathology. *Philosophical Transactions of the Royal Society B-Biological Sciences, 363*(1507), 3191-3200. doi: 10.1098/rstb.2008.0107.

Vossler, M. R., Yao, H., York, R. D., Pan, M. G., Rim, C. S., & Stork, P. J. S. (1997). cAMP activates MAP kinase and Elk-1 through a B-Raf- and Rap1-dependent pathway. *Cell, 89*, 73-82. doi: 10.1016/S0092-8674(00)80184-1.

Wakana, Y., Koyama, S., Nakajima, K., Hatsuzawa, K., Nagahama, M., Tani, K., Hauri, H. P., Melancon, P., & Tagaya, M. (2005). Reticulon 3 is involved in membrane trafficking between the endoplasmic reticulum and Golgi. *Biochemical and Biophysical Research Communications, 334*(4), 1198-1205. doi: 10.1016/j.bbrc.2005.07.012.

Wakida, N., Kiguchi, N., Saika, F., Nishiue, H., Kobayashi, Y., & Kishioka, S. (2014). CC-chemokine ligand 2 facilitates conditioned place preference to methamphetamine through the activation of dopamine systems. *Journal of Pharmacological Sciences, 125*. 68-73. doi: 10.1254/jphs.14032FP.

Wallace, D. L., Vialou, V., Rios, L., Carle-Florence, T. L., Chakravarty, S., Kumar, A., Graham, D. L., Green, T. A., Kirk, A., Iniguez, S. D., Perrotti, L. I., Barrot, M., Di Leone, R. J., Nestler, E. J., & Bolanos-Guzman, C. A. (2008). The influence of ΔFosB in the nucleus accumbens on natural reward-related behaviour. *Journal of Neuroscience, 28*(41), 10272-10277. doi: 10.1523/JNEUROSCI.1531-08.2008.

Wallace, T. L., Gudelsky, G. A., & Vorhees, C. V. (1999). Methamphetamine-induced neurotoxicity alters locomotor activity, stereotypic behaviour, and stimulated dopamine release in the rat. *The Journal of Neuroscience, 19*(20), 9141-9148.

Wang, X., Luo, Y., He, Y., Li, F., Shi, H., Xue, L., Xue, Y., & Lu, L. (2010). Nucleus accumbens core mammalian target of rapamycin signaling pathway is critical for cue-induced reinstatement of cocaine seeking in rats. *The Journal of Neuroscience, 30*(38), 12632-12641. doi: 10.1523/JNEUROSCI.1264-10.2010.

Watari, A., & Yutsudo, M. (2003). Multi-functional gene ASY/Nogo/RTN-X/RTN4: Apoptosis, tumor suppression, and inhibition of neuronal regeneration. *Apoptosis, 8*, 5-9. doi: 10.1023/A:1021639016300.

Weingarten, H. P., & Elston, D. (1991). Food cravings in a college population. *Appetite, 17*(3), 167-175. doi: 10.1016/0195-6663(91)90019-O.

Wise, R. A. (2002). Brain reward circuitry: Insights from unsensed incentives. *Neuron, 36*(2), 229-240. doi: 10.1016/S0896-6273(02)00965-0.

Woods, S. C., & Ramsay, D. S. (2011). Food intake, metabolism and homeostasis. *Physiology and Behavior, 104*, 4-7. doi: 10.1016/j.physbeh.2011.04.026.

Xing, H., Zhang, S., Weinheimer, C., Kovacs, A., & Muslin, A. J. (2000). 14-3-3 proteins block apoptosis and differentially regulate MAPK cascades. *The EMBO Journal, 19*(3), 349-358. doi: 10.1093/emboj/19.3.349.

Yamamoto, B. K., & Raudensky, J. (2008). The role of oxidative stress, metabolic compromise, and inflammation in neuronal injury produced by amphetamine-related drugs of abuse. *Journal of Neuroimmune Pharmacology, 3*(4), 203-217. doi: 10.1007/s11481-008-9121-7.

Yamamoto, B. K., Moszczynska, A., & Gudelsky, G. A. (2010). Amphetamine toxicities: Classical and emerging mechanisms. *Annals of the New York Academy of Sciences, Addiction Reviews 2, 1187*, 101-121. doi: 10.1111/j.1749-6632.2009.05141.x.

Yang, T., & Terman, J. R. (2012). 14-3-3ε couples protein kinase A to semaphorin signaling and silences plexin RasGAP-mediated axonal repulsion. *Neuron, 74*, 108-121. doi: 10.1016/j.neuron.2011.12.034.

Yoon, Y., Krueger, E. W., Oswald, B. J., & McNiven, M. A. (2003). The mitochondrial protein hFis1 regulates mitochondrial fission in mammalian cells through an interaction with the dynamin-like protein DLP1. *Molecular and Cellular Biology, 23*(15), 5409-5420. doi: 10.1128/MCB.23.15.5409–5420.2003.

York, R. D., Yao, H., Dillon, T., Ellig, C. L., Eckert, S. P., McCleskey, A. W., & Stork, P. J. S. (1998). Rap1 mediates sustained MAP kinase activation induced by nerve growth factor. *Nature, 392*(6676), 622-626. doi: 10.1038/33451.

Yue, Y., Widmer, D. A. J., Halladay, A. K., Cerretti, D. P., Wagner, G. C., Dreyer, J. L., & Zhou, R. (1999). Specification of distinct dopaminergic neural pathways: Roles of the Eph family receptor EphB1 and ligand ephrin-B2. *The Journal of Neuroscience, 19*(6), 2090-2101.

Zhang, M., & Kelley, A. E. (2002). Intake of saccharin, salt, and ethanol solutions is increased by infusion of a mu opioid agonist into the nucleus accumbens. *Psychopharmacology, 159*(4), 415-423. doi: 10.1007/s00213-001-0932-y.

Zhang, M., Balmadrid, C., & Kelley, A. E. (2003). Nucleus accumbens opioid, GABAergic, and dopaminergic modulation of palatable food motivation: Contrasting effects revealed by a progressive ratio study in the rat. *Behavioural Neuroscience, 117*(2), 202-211. doi: 10.1037/0735-7044.117.2.202.

Zhou, Q., Homma, K. J., & Poo, M. (2004). Shrinkage of dendritic spines associated with long-term depression of hippocampal synapses. *Neuron, 44*(5), 749-757. doi: 10.1016/j.neuron.2004.11.011.

Zybailov, B., Mosley, A. L., Sardiu, M. E., Coleman, M. K., Florens, L., & Washburn, M. P. (2006). Statistical analysis of membrane proteome expression changes in *saccharomyces cerevisiae*. *Journal of Proteome Research, 5*(9), 2339-2347. doi: 10.1021/pr060161n.

Appendix A

Ethics Approval Form

MACQUARIE UNIVERSITY

ANIMAL RESEARCH AUTHORITY (ARA)

AEC Reference No.: 2011/029-5 **Date of Expiry: 31 July 2014**

Full Approval Duration: *01 August 2011 to 31 July 2014 (36 months)*

This ARA remains in force until the Date of Expiry (unless suspended, cancelled or surrendered) **and will only be renewed upon receipt of a satisfactory Progress Report before expiry.**

Principal Investigator (PI) :
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In case of emergency, please contact:
the Principal Investigator / Associate Investigator named above
or Manager, CAF: 9850 7780 / 0428 861 163 and **Animal Welfare Officer**: 9850 7758 / 0439 497 383

The above-named are authorised by MACQUARIE UNIVERSITY ANIMAL ETHICS COMMITTEE to conduct the following research:

Title of the project: **Sensitisation to methamphetamine challenge following treatment through adolescence with caffeine, sucrose or their combination**

Purpose: 4 – Research: human or animal biology

Aims: To investigate whether a diet high in sucrose and caffeine through adolescence can change gene expression and behavioural responses to methamphetamine exposure in adulthood.

Procedures category: 3 (Minor Conscious Intervention)
All procedures must be performed as per the AEC-approved protocol, unless stated otherwise by the AEC and/or AWO.

Maximum numbers approved (for the Full Approval Duration):

Species	Strain	Sex	Weight	Age	Total	Supplier/Source
Rat	Sprague Dawley	Male	80g	PND 26	120	ARC
Rat	Sprague Dawley	Male	250g	Young adult	120	ARC
Rat	Sprague Dawley	Male	80g	PND 26	8	ARC
Rat	Sprague Dawley	Male	250g	Young adult	8	ARC
				TOTAL	**256**	

Location of research:

Location	*Full street address*
Central Animal House Facility	Building F9A, Research Park Drive, Macquarie University NSW 2109

Conditions of Initial Approval:
1. *AEC meeting16 June 2011: The ARA issued subject to the adolescent animal experiments being conducted concurrently with the adult animal experiments. Please submit an amendment form documenting this change to sections2.2a and 2.2b, detailing any changes to animal numbers per year that might be required because of this alteration to the protocol.*

Amendments approved by the AEC since initial approval:
1. *Approved 11 August 2011: Concurrent experimentation on adolescent and adult rats.*
2. *Approved 8 September 2011:*
   a) *Increase the number of animals to add 8 adolescent and 8 adult rats*
   b) *Add an initial experiment to test the new water delivery system and to identify the peak stimulatory effect for each treatment.*
3. *Executive approved & AEC ratified 16 May 2013: Extension to approval duration by 1 year*

Being animal research carried out in accordance with the Code of Practice for a recognised research purpose and in connection with animals (other than exempt animals) that have been obtained from the holder of an animal suppliers licence.

Professor Mark Connor (Chair, Animal Ethics Committee) **Approval Date:** 15 August 2013

Adapted from Form C (issued under part IV of the Animal Research Act, 1985)

Appendix B

Energy Intake and Weight Results Output

Energy Intake (kj) throughout the experiment period for all three treatment groups

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Acclimation	Water/Saline	10	276.5490	39.42776	12.46815	248.3441	304.7539	224.25	342.03
	Water/METH	9	287.8200	27.65817	9.21939	266.5600	309.0800	255.97	329.03
	Sugar/METH	12	260.6717	37.23796	10.74967	237.0118	284.3315	218.92	348.92
	Total	31	273.6752	36.18306	6.49867	260.4031	286.9472	218.92	348.92
Day 1 Treatment (kj)	Water/Saline	10	301.6000	34.55816	10.92825	276.8786	326.3214	234.00	338.00
	Water/METH	9	316.3333	57.03727	19.01242	272.4906	360.1761	169.00	364.00
	Sugar/METH	12	374.1000	33.36838	9.63262	352.8987	395.3013	328.00	418.50
	Total	31	333.9419	52.16159	9.36850	314.8089	353.0750	169.00	418.50
Day 5 Treatment (kj)	Water/Saline	10	347.1000	22.97075	7.26399	330.6677	363.5323	312.00	377.00
	Water/METH	9	355.3333	18.38478	6.12826	341.2015	369.4651	325.00	377.00
	Sugar/METH	12	438.0583	41.57771	12.00245	411.6411	464.4756	346.90	489.70
	Total	31	384.7000	52.45100	9.42048	365.4608	403.9392	312.00	489.70
Day 15 Treatment (kj)	Water/Saline	10	365.3000	64.40506	20.36667	319.2274	411.3726	286.00	520.00
	Water/METH	9	385.6667	26.00000	8.66667	365.6813	405.6520	338.00	416.00
	Sugar/METH	12	472.6750	25.79105	7.44524	456.2881	489.0619	418.00	508.50
	Total	31	412.7774	63.83982	11.46597	389.3608	436.1941	286.00	520.00
Last Day Treatment (kj)	Water/Saline	10	338.0000	68.51602	21.66667	288.9866	387.0134	260.00	442.00
	Water/METH	9	378.4444	71.03892	23.67964	323.8391	433.0498	273.00	468.00
	Sugar/METH	12	406.6583	103.51389	29.88188	340.8887	472.4279	297.10	628.70
	Total	31	376.3194	86.84438	15.59771	344.4646	408.1741	260.00	628.70
METH Challenge (kj)	Water/Saline	10	370.5780	38.36746	12.13286	343.1316	398.0244	313.69	420.55
	Water/METH	9	380.8856	32.19321	10.73107	356.1397	405.6314	335.27	420.55
	Sugar/METH	12	413.8333	20.31280	5.86380	400.9272	426.7395	387.27	433.29
	Total	31	390.3145	35.31362	6.34251	377.3614	403.2677	313.69	433.29

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Acclimation	Between Groups	3912.368	2	1956.184	1.549	.230
	Within Groups	35364.057	28	1263.002		
	Total	39276.425	30			
Day 1 Treatment (kj)	Between Groups	32602.615	2	16301.308	9.311	.001
	Within Groups	49022.340	28	1750.798		
	Total	81624.955	30			
Day 5 Treatment (kj)	Between Groups	56064.551	2	28032.275	29.654	.000
	Within Groups	26468.669	28	945.310		
	Total	82533.220	30			
Day 15 Treatment (kj)	Between Groups	72208.632	2	36104.316	20.195	.000
	Within Groups	50057.063	28	1787.752		
	Total	122265.694	30			
Last Day Treatment (kj)	Between Groups	25769.817	2	12884.908	1.799	.184
	Within Groups	200488.591	28	7160.307		
	Total	226258.408	30			
METH Challenge (kj)	Between Groups	11333.066	2	5666.533	6.084	.006
	Within Groups	26078.491	28	931.375		
	Total	37411.556	30			

Test Results

Dependent Variable: Day 1 Treatment (kj) W/S v S/M

Source	Sum of Squares	df	Mean Square	F	Sig.
Contrast	28670.455	1	28670.455	16.376	.000
Error	49022.340	28	1750.798		

Test Results

Dependent Variable: Day 1 Treatment (kj) W/M v S/M

Source	Sum of Squares	df	Mean Square	F	Sig.
Contrast	17161.651	1	17161.651	9.802	.004
Error	49022.340	28	1750.798		

Test Results

Dependent Variable: Day 5 Treatment (kj) W/S v S/M

Source	Sum of Squares	df	Mean Square	F	Sig.
Contrast	45127.737	1	45127.737	47.739	.000
Error	26468.669	28	945.310		

Test Results

Dependent Variable: Day 5 Treatment (kj) W/M v S/M

Source	Sum of Squares	df	Mean Square	F	Sig.
Contrast	35194.760	1	35194.760	37.231	.000
Error	26468.669	28	945.310		

Test Results

Dependent Variable: Day 15 Treatment (kj) W/S v S/M

Source	Sum of Squares	df	Mean Square	F	Sig.
Contrast	62887.585	1	62887.585	35.177	.000
Error	50057.063	28	1787.752		

Test Results

Dependent Variable: Day 15 Treatment (kj) W/M v S/M

Source	Sum of Squares	df	Mean Square	F	Sig.
Contrast	38933.743	1	38933.743	21.778	.000
Error	50057.063	28	1787.752		

Test Results

Dependent Variable: METH Challenge (kj) W/S v S/M

Source	Sum of Squares	df	Mean Square	F	Sig.
Contrast	10205.585	1	10205.585	10.958	.003
Error	26078.491	28	931.375		

Test Results

Dependent Variable: METH Challenge (kj) W/M v S/M

Source	Sum of Squares	df	Mean Square	F	Sig.
Contrast	5582.860	1	5582.860	5.994	.021
Error	26078.491	28	931.375		

Weight (gm) throughout the experiment period for all three treatment groups

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Acclimation	Water/Saline	10	112.8700	11.92477	3.77094	104.3395	121.4005	98.60	129.70
	Water/METH	9	123.8333	12.32913	4.10971	114.3563	133.3103	108.30	148.60
	Sugar/METH	12	109.8750	6.09980	1.76086	105.9994	113.7506	98.90	117.80
	Total	31	114.8935	11.50014	2.06549	110.6753	119.1118	98.60	148.60
Day 1 Treatment	Water/Saline	10	132.4600	13.32251	4.21295	122.9296	141.9904	115.50	154.10
	Water/METH	9	144.2667	11.85190	3.95063	135.1565	153.3768	130.20	169.00
	Sugar/METH	12	129.9333	7.37198	2.12811	125.2494	134.6173	117.80	139.00
	Total	31	134.9097	12.19851	2.19092	130.4352	139.3841	115.50	169.00
Day 5 Treatment	Water/Saline	10	167.0500	18.03954	5.70460	154.1453	179.9547	143.40	196.50
	Water/METH	9	181.0667	14.25360	4.75120	170.1104	192.0230	161.80	210.40
	Sugar/METH	12	168.8250	13.27466	3.83206	160.3907	177.2593	150.40	189.60
	Total	31	171.8065	15.91351	2.85815	165.9693	177.6436	143.40	210.40
Day 15 Treatment	Water/Saline	10	248.6200	29.83666	9.43518	227.2761	269.9639	199.70	289.40
	Water/METH	9	263.5000	27.85067	9.28356	242.0921	284.9079	216.30	306.70
	Sugar/METH	12	240.1083	25.01409	7.22094	224.2151	256.0015	204.60	281.80
	Total	31	249.6452	28.24276	5.07255	239.2856	260.0047	199.70	306.70
Last Day Treatment	Water/Saline	10	326.0900	43.04763	13.61286	295.2956	356.8844	273.00	385.20
	Water/METH	9	340.9667	52.22157	17.40719	300.8256	381.1077	252.00	417.70
	Sugar/METH	12	364.6833	27.03196	7.80346	347.5080	381.8586	313.10	407.00
	Total	31	345.3484	42.77864	7.68327	329.6571	361.0397	252.00	417.70
METH Challenge	Water/Saline	10	471.1200	56.51639	17.87205	430.6906	511.5494	378.50	550.00
	Water/METH	9	486.1000	41.34616	13.78205	454.3185	517.8815	426.20	557.00
	Sugar/METH	12	473.7500	38.37858	11.07894	449.3654	498.1346	422.00	559.00
	Total	31	476.4871	44.65970	8.02112	460.1058	492.8684	378.50	559.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Acclimation	Between Groups	1062.455	2	531.228	5.120	.013
	Within Groups	2905.143	28	103.755		
	Total	3967.599	30			
Day 1 Treatment	Between Groups	1145.156	2	572.578	4.830	.016
	Within Groups	3318.951	28	118.534		
	Total	4464.107	30			
Day 5 Treatment	Between Groups	1104.671	2	552.336	2.382	.111
	Within Groups	6492.527	28	231.876		
	Total	7597.199	30			
Day 15 Treatment	Between Groups	2829.532	2	1414.766	1.877	.172
	Within Groups	21100.065	28	753.574		
	Total	23929.597	30			
Last Day Treatment	Between Groups	8367.732	2	4183.866	2.518	.099
	Within Groups	46532.626	28	1661.879		
	Total	54900.357	30			
METH Challenge	Between Groups	1209.629	2	604.814	.289	.751
	Within Groups	58625.026	28	2093.751		
	Total	59834.655	30			

Appendix C

Locomotor Behaviour during 26-Day Treatment Period Output

Total 24 hours locomotor activity for all three treatment groups on the first day of the 26-day treatment period

Descriptives

Total 24 hrs Locomotor Day 1 of Treatment

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Water/Saline	10	2735.5000	454.24840	143.64596	2410.5503	3060.4497	2181.00	3664.00
Water/METH	9	2815.0000	515.25067	171.75022	2418.9433	3211.0567	1715.00	3554.00
Sugar/METH	12	2653.7500	758.66092	219.00654	2171.7199	3135.7801	947.00	4327.00
Total	31	2726.9355	590.11230	105.98730	2510.4805	2943.3904	947.00	4327.00

ANOVA

Total 24 hrs Locomotor Day 1 of Treatment

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	134805.121	2	67402.560	.183	.834
Within Groups	10312170.75	28	368291.813		
Total	10446975.87	30			

Total 24 hours locomotor activity for all three treatment groups on the last day of the 26-day treatment period

Descriptives

Total 24 hours Locomotor Last Day of Treatment

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Water/Saline	10	3188.3000	739.86726	233.96657	2659.0308	3717.5692	1767.00	4458.00
Water/METH	9	2747.2222	540.11521	180.03840	2332.0529	3162.3915	1902.00	3607.00
Sugar/METH	11	3137.0000	276.14344	83.26038	2951.4843	3322.5157	2696.00	3693.00
Total	30	3037.1667	560.71494	102.37207	2827.7923	3246.5411	1767.00	4458.00

ANOVA

Total 24 hours Locomotor Last Day of Treatment

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1094656.511	2	547328.256	1.842	.178
Within Groups	8022979.656	27	297147.395		
Total	9117636.167	29			

Appendix D

Methamphetamine Locomotor Cross-Sensitisation Results Output

Total 60 minutes locomotor activity for all three treatment groups after challenge injection

Descriptives

			N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	Between-Component Variance
							Lower Bound	Upper Bound			
Locomotor 15 min Acclimation	Water/Saline		10	340.6000	207.15759	65.50898	192.4084	488.7916	180.00	890.00	
	Water/METH		9	304.5556	74.27165	24.75722	247.4653	361.6458	218.00	423.00	
	Sugar/METH		12	309.5000	66.27971	19.13330	267.3879	351.6121	214.00	432.00	
	Total		31	318.0968	127.31623	22.86667	271.3968	364.7967	180.00	890.00	
	Model	Fixed Effects			130.75080	23.48354	269.9929	366.2006			
		Random Effects				23.48354[a]	217.0553[a]	419.1383[a]			-1296.07573
Total Locomotor 1 hr after injection	Water/Saline		10	223.8000	125.82068	39.78799	133.7933	313.8067	107.00	448.00	
	Water/METH		9	801.3333	485.53295	161.84432	428.1197	1174.5470	226.00	1729.00	
	Sugar/METH		12	1245.2500	372.76342	107.60753	1008.4074	1482.0926	786.00	1831.00	
	Total		31	786.8710	555.29576	99.73406	583.1868	990.5551	107.00	1831.00	
	Model	Fixed Effects			356.41538	64.01409	655.7440	917.9979			
		Random Effects				306.21119	-530.6495	2104.3914			265139.8701

a. Warning: Between-component variance is negative. It was replaced by 0.0 in computing this random effects measure.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Locomotor 15 min Acclimation	Between Groups	7601.087	2	3800.544	.222	.802
	Within Groups	478681.622	28	17095.772		
	Total	486282.710	30			
Total Locomotor 1 hr after injection	Between Groups	5693707.634	2	2846853.817	22.411	.000
	Within Groups	3556893.850	28	127031.923		
	Total	9250601.484	30			

Tests of Between-Subjects Effects

Dependent Variable: Total Locomotor 1 hr after injection

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5693707.63[a]	2	2846853.817	22.411	.000
Intercept	17506326.16	1	17506326.16	137.810	.000
Treatment	5693707.634	2	2846853.817	22.411	.000
Error	3556893.850	28	127031.923		
Total	28444745.00	31			
Corrected Total	9250601.484	30			

a. R Squared = .615 (Adjusted R Squared = .588)

Test Results

Dependent Variable: Total Locomotor 1 hr after injection Saline v METH

Source	Sum of Squares	df	Mean Square	F	Sig.
Contrast	4301070.898	1	4301070.898	33.858	.000
Error	3556893.850	28	127031.923		

Test Results

Dependent Variable: Total Locomotor 1 hr after injection W/S v W/M

Source	Sum of Squares	df	Mean Square	F	Sig.
Contrast	1579948.821	1	1579948.821	12.437	.001
Error	3556893.850	28	127031.923		

Test Results

Dependent Variable: Total Locomotor 1 hr after injection W/S v S/M

Source	Sum of Squares	df	Mean Square	F	Sig.
Contrast	5691055.105	1	5691055.105	44.800	.000
Error	3556893.850	28	127031.923		

Test Results

Dependent Variable: Total Locomotor 1 hr after injection W/M v S/M

Source	Sum of Squares	df	Mean Square	F	Sig.
Contrast	1013461.750	1	1013461.750	7.978	.009
Error	3556893.850	28	127031.923		

Appendix E

Figures of Behavioural Data

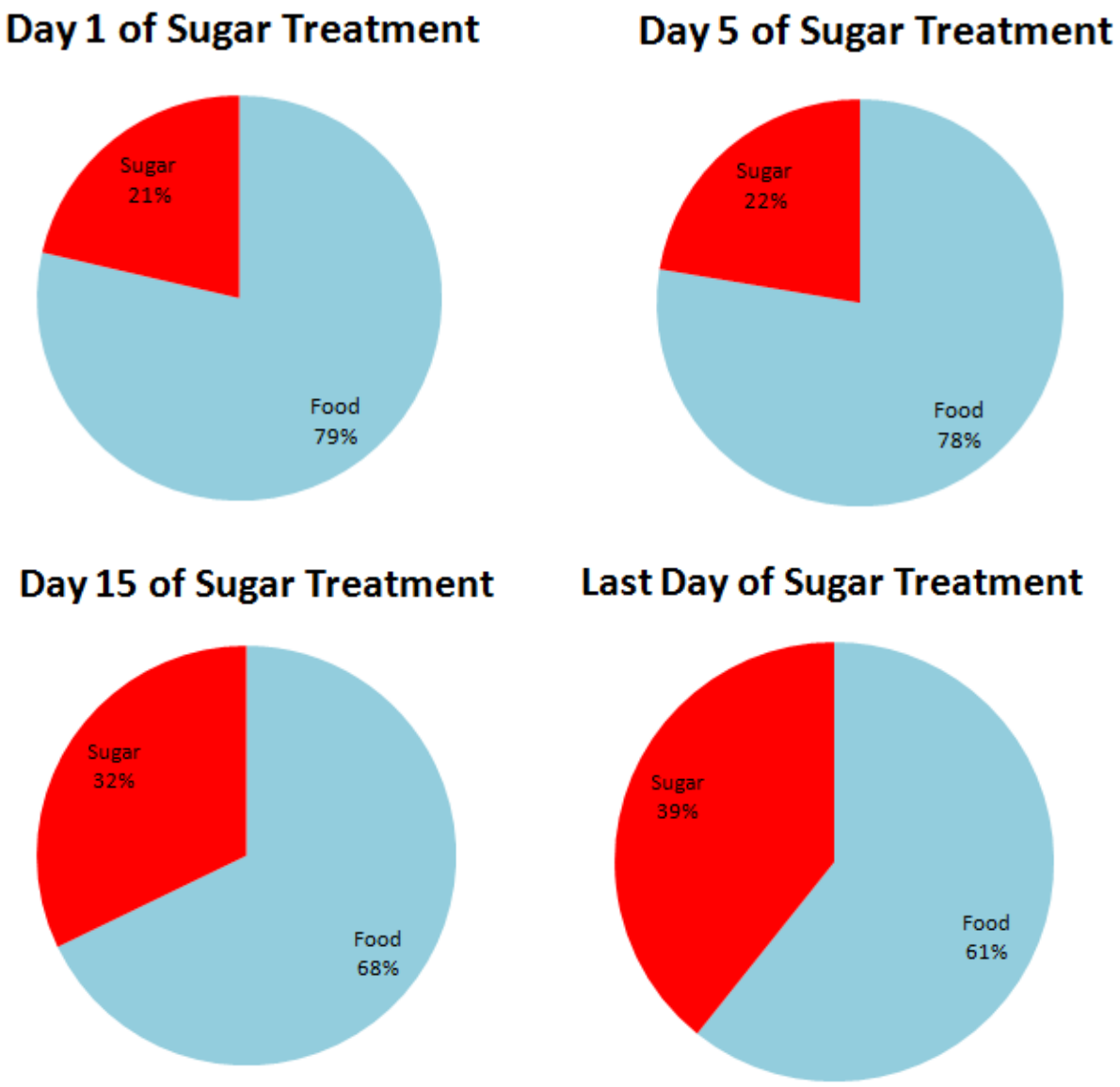

Figure 1. Charts of the proportion of total energy intake (measured in kj) consumed from food and fluid treatment in sugar-METH treatment group (Sugar/METH) during the 26-day treatment period

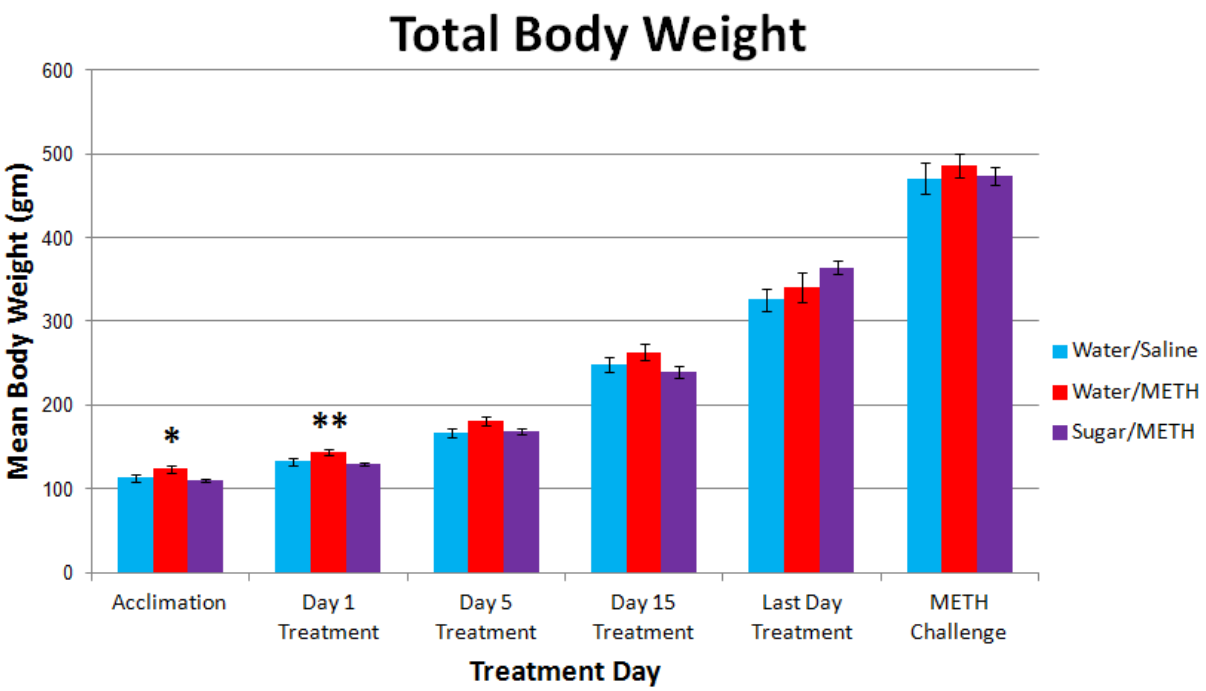

Figure 2. Bar chart of total body weight (measured in grams) between the three treatment groups throughout the experiment. There were signficant differences in body weight between the treatment groups during acclimation (*$p < 0.05$) and on the first day of the 26-day treatment period (**$p < 0.05$)

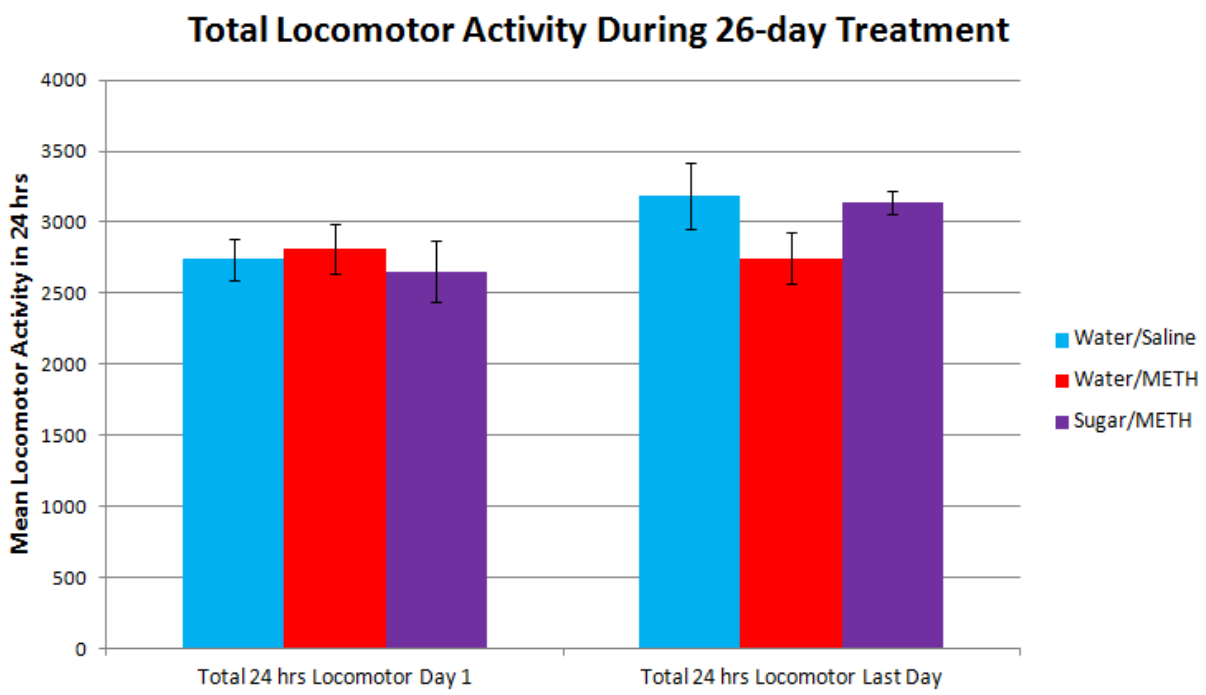

Figure 3. Bar chart of total 24 hours locomotor behaviour during the 26-day treatment period on the first day and the last day of the treatment period. There were no significant differences in locomotor activity between the treatment groups on first and last day of sugar treatment.

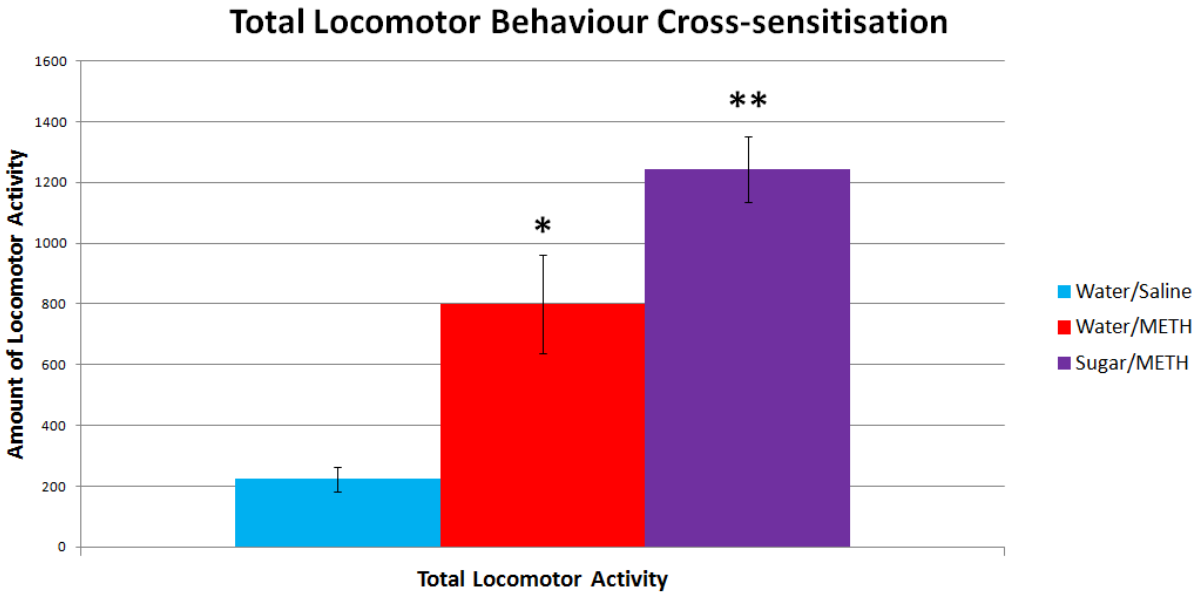

Figure 4. Bar chart of total locomotor behaviour for 60 minutes after challenge injection between the three treatment groups. There were significant differences in locomotor activity between the Water/Saline and Water/METH groups (*$p < 0.05$), and between the Water/METH and Sugar/METH groups (**$p < 0.05$) after the challenge injection