Dopamine mechanisms in learning and memory: evidence from rodent studies

Chapter 7

Summary and Conclusion

Part I: Roles of dopamine D1 and D2, opioid and glutamate NMDA receptor signaling in the acquisition and expression of fat-conditioned flavor preferences in rats and c-Fos analysis of the mesotelencephalic dopamine reward pathway and the nigrostriatal pathway following intake of sugars and fats in rats.

1. The effects of differing neurotransmitters on fat-conditioned flavor preference

Overconsumption of food is partly based on the learned preferences for specific types of food, especially those that contain sugars and fats. Fat-rich and sugar-rich foods are highly attractive and are capable of eliciting CFP's in animal models. Fats and sugars are highly palatable and lead to positive effects in the gut leading to overeating and consumption. Sugars have been extensively studied in both flavor-flavor (f/f) and flavor-nutrient (f/n) conditioning (Azzara, Bodnar, Delamater, & Sclafani, 2001; Sclafani & Ackroff, 1994; Touzani, Bodnar, & Sclafani, 2009; Yu, Silva, Sclafani, Delamater, & Bodnar, 2000a, 2000b), while the roles of fat-condition flavored preferences (CFP) in both f/f and f/n conditioning have not been fully explored. Because both fats and sugars contribute to overeating, it might be assumed that fats cause have similar reactions to specific neurotransmitters as sugars.

Chapter 2 explores two types of dopamine (DA) receptors, D1 and D2, in their role in fat-CFP through ingestion of differing corn-oil solutions and pairing them with differing flavors. Previously, it was found that systemic injections of the DA D1 antagonist (SCH23390) and to a lesser degree, the DA D2 (Raclopride) antagonist, blocked acquisition of fructose-flavor conditioning, whereas both DA D1 and D2 antagonists significantly reduced expression of fructose-CFP (Baker, Shah, Sclafani, & Bodnar, 2003) for f/f conditioning, while the DA D1, but not DA D2, blocked f/n conditioning of intragastric (IG) infusions of sucrose (Azzara et al., 2001). The current study found that the DA D1 antagonists produced a dose-limited reduction in expression of corn oil-CFP (both f/f and f/n) in contrast to SCH23390-induced eliminations of the fructose-CFP (f/f CFP) in rats (Baker et al., 2003), while the DA D2 antagonist was only blocked CS+ preference at the 200 nmol/kg dose in expression of corn oil-CFP. The DA D1 antagonist failed to block acquisition of a CS+ preference, while the DA D2 antagonist only had one significant drug acquisition effect at the 50 nmol/kg dose which resulted in a hastening of extinction of the CS+ preference. DA D1 and D2 antagonism is much less effective at

attenuating the acquisition of fat-conditioned flavor preferences. Overall, the data from the current study indicate a limited DA D1 and D2 receptor signaling involvement in acquisition and expression of a fat-CFP relative to previous robust effects for sugar-CFP.

While DA did not seem to have strong effect in fat-CFP, other neurotransmitters were also suspected to be involved. Opioids have been linked to affect the general rewarding characteristics of sugars (Arbisi, Billington, & Levine, 1999; Fantino, Hosotte, & Apfelbaum, 1986), but the opioid receptor antagonist, Naltrexone (NTX), failed to alter the expression or acquisition of sugar-CFP. NTX dose-dependently reduced fructose (CS+) and saccharin (CS-) intakes when injected during training, yet NTX was unable to reduce conditioning in either acquisition CFP or expression CFP (Baker, Li, Lee, Sclafani, & Bodnar, 2004), NTX also failed to alter either the acquisition and expression of CFP elicited by IG sucrose solutions (f/n conditioning) (Azzara, Bodnar, Delamater, & Sclafani, 2000). In the current study of **chapter 3,** NTX failed to block acquisition of corn oil-CFP and was moderately attenuated by NTX in the expression of corn-oil CFP. It can be concluded that while opioids may have a role in the ingestion of sugars and sweet palatable foods, it does not have much involvement in the learning and memory of either a sugar or a fat.

Lastly, the *N*-methyl-D-aspartate (NMDA) glutamate receptor has been shown to play a role in food-incentive learning (Kelley, 2004; Ranaldi et al., 2011). The non-competitive NMDA antagonist, MK-801, significantly increased sucrose intake in moderately food-deprived animals (Covasa, Ritter, & Burns, 2004; Qiang et al., 2000). Glutamate signaling has shown to be involved in sugar conditioned flavor preference as well. MK-801 was found to block acquisition, but not expression of fructose-CFP. The other study in **chapter 3** subsequently examined glutamate NMDA signaling in fat-CFP. Lower doses of MK-801 slightly reduced CS+ intake in expression, while high doses blocked CS+ intake. In contrast, MK-801 treatment during training blocked acquisition of corn oil-CFP. The data from both sugar and fat studies suggest that there is a critical role for NMDA signaling in the acquisition of fructose (f/f) and fat (f/f and f/n) CFP, and at best limited involvement of NMDA receptors in the expression of a previously learned preference.

2. The DA reward pathways through ingestion of sugars and fats

Many of the learned associations to flavor cues are thought to stem from the connection of the hedonic taste, the palatability of the nutrients, and the "reward" associated with dopamine (DA) release in the brain particularly from the Ventral Tegmental Area (VTA) to its major mesotelencephalic projection sites including the nucleus accumbens (NAc), amygdala (AMY) and medial prefrontal cortex (mPFC), as well as from the Substantia Nigra Pars Compacta (SNc) to the striatum (Caudate and Putamen: CP). The systemic and central pharmacological analyses of mediators of sugar and fat intake as well as preferences conditioned by these nutrients can provide important information about underlying substrates. Further, the increased release of endogenous neurochemical mediators (particularly DA) in similar brain sites (e.g., NAc, AMY, mPFC) following sugar and fat intake can provide further insight into these processes. One remaining area of inquiry concerns the spatial resolution of activation within these sites in response to fat and sugar nutrients. c-Fos is an indirect marker of neuronal activity because this protoonco-gene is often expressed when neurons fire action potentials (Dragunow & Faull, 1989; VanElzakker, Fevurly, Breindel, & Spencer, 2008). **Chapter 4** explored the differing projection sites as well as the VTA to see if ingestion of a fat or sugar produces greater activation than the controls. Ingestion of corn-oil (which conditions f/f and f/n processes) activated DA-labeled cells in the VTA as well as DA-projection fields in the AMY, NAc core, dorsal CP, and mPFC. The NAc shell failed to display activation. Glucose (which conditions f/f and f/n processes), significantly increased c-fos activation in the AMY, NAc core, and dorsal CP. However, ingestion of glucose failed to increase c-fos activation in the VTA, NAc shell or mPFC. Ingestion of fructose (which conditions only f/f processes), significantly activated cells in the AMY and dorsal striatum, and to a lesser degree, the NAc core, while failing to display activation in the VTA, mPFC and NAc shell. Lastly, ingestion of saccharin and xanthan gum suspension failed to activate in any site relative to water intake. From this study, it can be concluded different solutions activate different areas of the brain in varying ways. In those situations in which significant activation was observed, there were many instances of highly positive relationships across sites, supporting the idea of activation of a distributed brain network mediating sugar and fat intake. Interestingly, both corn-oil and glucose was found to have f/f and f/n processes, but affected the mesotelencephalic and nigrostriatal pathways in various manners. There may be more to CFP feeding studies than just f/f and f/n conditioning, but further research needs to be conducted to pinpoint what this difference may be.

3. Future perspectives

A logical next step after the CFP studies presented in this thesis would be to study microinjections of DA D1, DA D2 and NMDA antagonists into specific sites of the brain, specifically the mesotelencephalic and nigrostriatal pathways, with corn-oil CFP. These microinjection CFP studies have already been conducted with sugar-CFP (Bernal et al., 2008; Bernal et al., 2009; Malkusz et al., 2012). Since previous studies (Bernal et al., 2010) and current studies examining opioid antagonists failed to produce sugar- and fat-CFP, further investigations are not necessary. It may be intriguing to compare the DA specific site data to what was found in **chapter 4**, to see if the different brain sites correlate the same way, especially since corn-oil strongly activated many brain sites.

For the c-fos studies, it would be interesting to see activation in the brain from sham studies and intragastric infusion studies. The sham studies would examine activation only orosensory processes (f/f studies), as it was previously conducted in CFP studies (Yu et al., 2000a, 2000b), while the intragastric infusions would examine only post-ingestive processes (f/n studies), as it was previously conducted in CFP studies (Azzara et al., 2000, 2001). This would be especially intriguing with corn-oil ingestion. Since corn-oil activated the brain so strongly in so many areas, it would be interesting to see if there is a different in activation between the f/f processes and the f/n processes.

Part II. Dopamine, memory, deep brain stimulation and hypoxia

1. Dopamine, memory and deep brain stimulation

Research has shown that DA is involved in various parts of the brain to help perform various memory tasks. Reward, and reinforcement (the "stamping-in" of memory) have been linked to the brain areas talked about in the previous chapters. DA D1 and D5 receptors in the dorsal hippocampus have been found to be involved in episodic memory (Bethus, Tse, & Morris, 2010). Anterior Nucleus of the Thalamus (ANT), Mammillothalamic Tract (MTT) and Entorhinal Cortex (EC) were chosen as deep brain stimulation (DBS) sites because of the they

are all part of the Circuit of Papez. Another brain area belonging to the Circuit of Papez is the hippocampus and both are known to be connected to memory. Interestingly, our study became less about how DA is involved in memory and memory impairments and more of how differing DBS sites affect DA levels in the SNc and VTA.

Chapter 5 explored the DA synthesis sites when these memory processes are disrupted, by emitting DBS in several sites of the Circuit of Papez to reverse the memory loss caused by injection of scopolamine, a muscarinic acetylcholine antagonist. Levels of tyrosine hydroxylase (TH, a rate-limiting enzyme in the formation of DA) and c-fos (a proto-oncogene and a marker for cell activation) were explored in the two major sites of DA synthesis: the VTA and the SNc. There were no significant differences found with the c-fos marker in the VTA and the SNc, or in the TH levels of the SNc. However, DBS stimulation into the ANT induced an increase in TH cells in the VTA, while DBS stimulation into the EC and the MTT did not, relative to the controls. This seems to be in line with behavioral findings of our unpublished data and previous studies. DBS into the EC (Stone et al., 2011) reversed the effects of memory loss caused by scopolamine, while DBS into the ANT had memory loss retention (Hamani et al., 2009). It is suspected that the neurons in the VTA had a phenotypic switching from GABA cells to TH cells caused by the environmental factor of DBS into the ANT, but not by DBS in the EC or MTT. Environmental changes have previously been seen with mating rats changes GABA cells to TH cells in both the VTA and SNc (Aumann, Tomas, & Horne, 2013). ANT-DBS had been found to be effective in treating refractory focal epilepsy (Graber & Fisher, 2012), but some side effects include depression and memory loss. As dopaminergic projections from the VTA mediate reward-seeking behavior (among other functions), our current findings may imply that ANT-stimulation with DBS, such as used in the treatment of epilepsy, may induce alterations in reward-seeking and other behavioral phenotypes.

2. Dopamine and hypoxia

Besides reward-motivated behavior and memory, DA has many other roles as one of the neurotransmitters released in the brain, several of which include motor control, cognition, and release of several important hormones. DA is also known to play a key role in psychiatric disorders (van Os, Kenis, & Rutten, 2010), and is suspected to play a role in the survival

functions of hypoxia (Grunblatt, 2004; Norris & Millhorn, 1995). Our studies in **chapter 6** found a colocalization of DA with both two types of hypoxia inducible factor- 1β (HIF-1β) subunits: aryl-hydrocarbon receptor nuclear translocator (ARNT) and ARNT2 in the SNc as well as the VTA. In the midbrain DA system, neurons in the SNc and VTA expressed ARNT in the same intensity and level in TH neurons as much as other neurons, while immunoreactivitity for ARNT2 in TH neurons was low. In the SNr, ARNT2 was shown to having greater amounts of intense staining, in addition to expressing ARNT. Dopaminergic neurons may primarily form HIF-1 by using ARNT, whereas other neurons, including neurons in the SNr have both ARNT and ARNT2 available.

This is an interesting finding because ARNT and ARNT2 are known to have only partially overlapping functions in hypoxia signaling (Keith, Adelman, & Simon, 2001), and the differences between the two cellular compositions may induce differential responses to hypoxia. For example, it is estimated that ARNT2/HIF-1α accounts for half of the DNA-binding HIF complexes (Maltepe, Keith, Arsham, Brorson, & Simon, 2000). Vascularization in the *Arnt2* -/- brains was normal during development whereas deletion of *Arnt* had dramatic consequences for vascular development (Keith et al., 2001). This data suggests that ARNT2/HIF-1 alpha can regulate HIF-1 target genes, but ARNT/HIF-1 alpha complexes may be sufficient to regulate normal hypoxic responses during development in vivo. In addition, TH has been described as hypoxia regulated by involving HIF-1 peripheral dopaminergic neurons (Mannello, Medda, & Tonti, 2011). It is hypothesized that midbrain neurons may want to avoid fluctuations of TH expression due to local hypoxic events because it would impact the remote striatum. The findings from **Chapter 6** indicate both shared and non-shared expression patterns of ARNT and ARNT2 in dopaminergic neurons in the mouse midbrain patterns, thereby possibly directing distinct vulnerability to hypoxia for different types of dopaminergic neurons.

3. Future perspectives

The next logical step for the DBS study is to look at the same brain tissue from the same DBS-treated, scopolamine-injected animals, and run additional studies on them. Because it is suggested that the DA cells were previously GABA cells, it is proposed to double-stain the SNc and VTA with TH and GAD67 (an antibody that labels GABA cell bodies) to see if the populations of TH and GABA are similar to those found in the controls. Strengthen the

credibility that GABA is involved; a study similar to Aumann and colleagues (2013) can be conducted. In addition to DBS into the ANT, EC and MTT, injections of a GABA antagonist through cannula injections into the VTA and SNc can be administered. TH levels in the VTA and the SNc can be examined to observe the possible changes made from the GABA antagonist. Lastly, it would be interesting to see if the DA projection areas from the VTA and SNc were also affected. Immunohistochemistry staining of the DA fibers in the NAc and striatum could be done to see if the fibers are affected by the increase of TH levels of ANT-DBS.

For the DA and hypoxia study, the next logical step would be to induce hypoxia in mice and study the colocalization levels of ARNT/TH and ARNT2/TH in the VTA and SNc. From here, quantitate studies through stereology can compare the levels of colocalization of the wild type mice and hypoxia mice. Increased DA was already found in hypoxia studies of stem cells (Mannello et al., 2011; Panchision, 2009) and HIF-1α was found to regulate the differentiation and production of dopaminergic neurons (Panchision, 2009). An increase in DA levels and colocalization of ARNT/TH and ARNT2/TH are expected from the effects of inducing hypoxia in rodents. If this hypothesis proves true, animal models of mental disorders, such as animal models of Parkinson's disease or schizophrenia, could be included as an additional independent variable in the hypoxia studies. This would strengthen the link of gene x environment interaction hypothesis that hypoxia may be a factor in increasing the risk of a mental disorder (Schmidt-Kastner, van Os, Esquivel, Steinbusch, & Rutten, 2012).

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