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# Neurogenesis and Hippocampus- and Olfactory-Dependent Learning in the Goto-Kakizaki Rat

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# Neurogenesis and Hippocampus- and Olfactory-Dependent Learning in the Goto-Kakizaki Rat

By

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**Abstract**

Research suggests that the chronic hyperglycemia associated with type 2 diabetes impairs brain function a number of ways – including a reduction in adult neurogenesis in the dentate gyrus (Beaquis *et al.,* 2009; Lang *et al.,* 2009;) and olfactory bulb (Lang *et al.,* 2009; Ramos-Rodriguez *et al.,* 2014). To investigate these impairments, Goto-Kakizaki (GK) rats were tested in both a radial arm maze and the social transmission of food preference (STFP), behaviours that depend on the integrity of the dentate gyrus and olfactory bulb, sites of profound adult neurogenesis (Altman & Das, 1965; Galef and Wigmore, 1983; Morris *et al.,* 2012). On the radial arm maze, GKs showed increased latencies to complete the task, as well as decreased correct choices. During STFP, GKs were unable to successfully discriminate between the flavoured foods provided to them, resulting in unsuccessful establishment of a food preference following social interaction. Using immunohistochemical procedures, doublecortin- and Ki67-positive cells were quantified to provide a measure of neurogenesis, specifically cell proliferation and survival, in these regions. These findings reveal no significant differences in the number of doublecortin- and Ki67- positive cells in hyperglycaemic animals. For now, the current results limit the possible mechanisms for the behavioral impairments found in the presence of chronic hyperglycaemia.

**Acknowledgements**

I would like to thank all those whose contributions made my project possible. First and foremost, I would like to thank my supervisor Dr. Diano Marrone for allowing me to be a part of his lab. I am truly greatful for your continued support and patience throughout this entire journey. I would also like to thank my committee members, Dr. Paul Mallet, Dr. Rudy Eikelboom, and Dr. Neil Fournier, for their valuable contributions to my project. I would like to thank all the members of the Marrone Lab that helped with this project. Without their dedication to this project little of it would be possible. It was a pleasure working with all of you. A special thanks to my friend and colleague, Hailey Katzman. Together, we kept each other grounded throughout the ups and downs of our projects. I am also forever grateful for the endless support of my family throughout this journey and in understanding my extremely demanding schedule. I am also truly thankful to have had my partner throughout all of this. You helped me realize my potential as a researcher and kept me motivated to work through every difficulty I faced.

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## Chapter 1: General Introduction

Type 2 diabetes remains a public health concern as the number of cases in each year continues to rise in Canada (Messier, 2005). Research investigating the effects of type 2 diabetes has demonstrated that the disorder can result in a number of impairments including deficits in learning and memory, and patients exhibit structural degradation in a number of brain regions (Allen *et al.*, 2004; Biessels and Gispen, 2005; Messier, 2005; Moreira *et al.*, 2007; Winocur *et al.*,2005). A powerful tool to establish the mechanism for this degradation is the use of rodent models of the disease, which include: Goto- Kakizaki (GK) rats, Zucker rats, db/db mice and many others (Biessels and Gispen, 2005), since they develop many of the same deficits. One particular deficit common to all of these rodent models of diabetes is a reduction in adult neurogenesis (Beaquis *et al.*, 2009; Ho *et al.*, 2007; Lang *et al.*, 2009). The learning deficits these animals show in tasks thought to depend on reductions in neurogenesis, however, remain uncharacterized. In particular, this thesis tested GK rats on a version of the radial arm maze shown to depend on neurogenesis as well as the social transmission of food preference. By training and testing rats on these tasks examining neurogenesis in both the hippocampus and olfactory bulb, the effect of hyperglycaemia on neurogenesis and learning can be examined.

### Type 2 Diabetes

Diabetes mellitus is characterized by abnormally high glucose levels in the bloodstream and exists in two forms, type 1 and type 2. These two forms differ based on the etiology of hyperglycaemia and how the body responds to it (Ho *et al.*, 2013). Normally, when glucose is detected in the bloodstream the pancreas produces and releases insulin in order to metabolize it. This allows glucose to be distributed among cells as a source of energy (Taylor, 1999). In the

case of type 1 diabetes, hyperglycaemia arises as a result of an auto-immune response in which the insulin-producing cells are destroyed (Atkinson, 2012). In type 2 diabetes, there is either an insufficient production of insulin or a resistance to it. In either of these situations glucose cannot be metabolized, and this results in a buildup of it in the bloodstream (Biesels and Gispen, 2005; Messier, 2005; Moreira *et al.,* 2007).

Type 2 diabetes is associated with changes in the brain of both human patients and animal models (Allen *et al.,* 2004; Biessels and Gispen, 2005; Messier, 2005). Magnetic resonance imaging (MRI) shows atrophy that is generally localized to the hippocampus and amygdala in comparison to those of non-diabetic individuals (den Heijer *et al.,* 2003). Consistent with the observation of atrophy in these regions, a number of cognitive impairments have also been documented. The most common include those in verbal memory and processing speed (Brands *et al.,* 2006; Messier, 2005; Yau *et al.,* 2014). For instance, patients with type 2 diabetes have impaired performance across many measures of verbal memory when compared to control subjects (Yau *et al.,* 2014). Moreover, Brands *et al.* (2006) found significantly slower processing speed in diabetics in a number of cognitive tests, including the Stroop Colour-Word Test, Trail-Making Test, and the digit symbol substitution test. Together, these studies provide evidence for the structural and cognitive impairments observed in human patients.

Consistent with patient data, rodent models also show cognitive impairments in a number of tasks, including active avoidance, and spatial navigation (Biessels & Gispen, 2005; Li *et al.,* 2002; Moreira *et al.,* 2007; Winocur *et al.,* 2005. Moreover, GKs showed significantly lower breakpoints on a progressive ratio, suggesting that hyperglycaemia impairs motivation (Moreira *et al.,* 2007). In another study, type 2 diabetic Zucker rats and db/db mice were tested on the Morris Water task (Li *et al.,* 2002). When the platform was visible, both animals performed at

levels comparable to non-diabetic control rats, demonstrating no impairments. On trials when the platform was hidden, both animals showed significantly longer swimming distances before locating the platform, demonstrating impaired performance on the task. These results demonstrate that the animals do not have impairments in swimming or exploratory activity but do show impairments in spatial memory, a function known to depend on the hippocampus (Li *et al.,* 2002). Given the consistent observations of cognitive deficits in both human patients and rodent models of type 2 diabetes, further research can use these models to understand the mechanisms by which impaired glucose metabolism undermines cognitive function.

**Neurogenesis**

Neurogenesis is the process by which new neurons are produced from precursor stem cells (Liu and Zhao, 2009). Although at one point it was believed that new cells were only generated during early development, a considerable amount of research has provided evidence for the generation of new neurons in the adult brain as well. In particular, two brain regions produce large numbers of adult-generated neurons throughout adulthood – the dentate gyrus and the olfactory bulb (Altman and Das, 1965).

Neurogenesis occurs in three phases: proliferation, migration and differentiation (Couillard-Despres *et al.,* 2005; und Halbach, 2007). During the proliferation phase, cells are generated within the subgranular layer of the dentate gyrus and subventricular zone (SVZ). These stem cells are able to regenerate themselves and have the added ability to differentiate into specific cell types, such as neurons. Once generated, these cells migrate into their respective regions of the brain where they differentiate into mature neurons (Zhao *et al.,* 2008). In the dentate gyrus, these cells regenerate in the subgranular layer, a layer at the junction of the hilus and the granular cell layer, and then migrate into the granular cell layer where they differentiate

into granule cells (Taupin, 2007). In the olfactory bulb, newly generated cells begin in the subventricular zone and migrate through the rostral migratory stream into the granule and glomerular layers of the olfactory bulb and differentiate into mature granule cells (Alvarez-Buylla and Garcia-Verdugo 2002; Bèdard and Parent, 2004). In both these regions, the mature granule cells eventually contribute to the primary neural system and have similar properties to those of already-existing neurons within the hippocampus and olfactory bulbs (Ramirez-Amaya *et al.,* 2006; Shors, 2008).

To investigate neurogenesis, researchers have made use of a wide range of activity markers that are selectively expressed by cells during individual phases of neurogenesis. One widely used method to investigate neurogenesis is through use of bromodeoxyuridine (BrdU). Animals are injected with BrdU, which is then incorporated into the DNA of cells as they proliferate. The BrdU remains in these cells for the rest of their lives, and using immunohistochemical procedures, the BrdU expression in these cells can be visualized and quantified. This can then provide a "birthdate" for a cohort of proliferating cells within a given brain region (Kee *et al.,* 2002; Taupin *et al.,* 2007). Two other popular markers of neurogenesis, and the two being used in the current project, are Ki67 and doublecortin (DCX). These two proteins are expressed by cells in the different phases of neurogenesis. Ki67 is a known marker of cell proliferation (Gerdes *et al.,* 1984), while doublecortin acts as a marker of cell maturation (Brown *et al.,* 2003; Couillard-Despres *et al.,* 2005). Gerdes *et al.* (1984) were able to provide evidence for Ki67 as a reliable marker of cell proliferation through immunohistochemical analysis. In this experiment, cell cultures were generated and labeled for Ki67. The number of cells expressing Ki67 was then measured at different time points. As the levels of cell proliferation increased so did the expression of Ki67, which began approximately 24 hours

following the generation of these cell cultures. Therefore, these results support Ki67 as a marker of proliferation, which has also been found in subsequent experiments (Endl *et al.,* 2000; Kee *et al.,* 2002; Scholzen, *et al.,* 2000). Doublecortin has also been demonstrated to be a particularly reliable indicator of adult neurogenesis, as is it known to be selectively expressed in regions that involve continuous neurogenesis. Cells express DCX when they migrate into their respective regions and become mature neurons. Evidence has shown this that significant increases in DCX-positive cells start to appear approximately 7 days following proliferation in cells that have begun to mature into neurons (Brown *et al.,* 2003; Couillard-Despres *et al.,* 2005). As such, DCX is typically seen as a marker of cell maturation. The use of both of these markers may allow neurogenesis to be observed across a broad range of a cell's life cycle. Both of these staining procedures have also been shown to be sensitive enough to detect changes in neurogenesis as a result of (for instance) aging and physical injury (Couillard- Despres *et al.,* 2005). Thus, these two proteins will provide a measure of the levels of cell proliferation and survival to determine whether there is an alteration in neurogenesis caused by the presence of hyperglycaemia.

Evidence has shown the importance of neurogenesis to cognitive function. Newly-matured cells contribute to processing of olfactory information (Bédard & Parent, 2004; Carleton *et al.,* 2003; Sultan *et al.*2010). When neurogenesis is ablated in the olfactory bulb and further cell proliferation is blocked, mice showed impaired retention of an odour that was paired with a food reward. Similarly, decades of research have shown that manipulations that upregulate neurogenesis improve performance on a number of hippocampus-dependent tasks (e.g., Gould et al., 1999; van Praag et al, 2002, 2005; Clelland et al., 2009; Sahay et al., 2011). Inversely, manipulations which knock down neurogenesis cause a number of memory deficits (e.g., van

Praag et al., 2002, 2005; but see Frankland et al., 2013). These studies will be described in further detail in chapters 3 and 4.

**The Goto-Kakizaki Rat as a Model for Type 2 Diabetes**

One method to study type 2 diabetes and its effect on the brain is through the use of rodent models, including the Zucker rat, the GK rat, and db/db mice (Biessels & Gispen, 2005; Cefalu, 2006; King, 2012). No single model captures all of the characteristics of type 2 diabetes, but each can be used to investigate different characteristics and complications of the disease (Cefalu, 2006; King, 2012).These models have been meticulously developed to capture the many features of type 2 diabetes such as etiology and disease progression (Bertram & Hanson, 2001).

The GK rat was first created from the non-diabetic Wistar by selectively breeding for glucose intolerance (Goto & Kakizaki, 1981). Over several generations, this intolerance became significant enough for animals to express hyperglycaemia. GKs mimic the early stages of diabetes and exhibit several features that make them an appropriate model of type 2 diabetes (Sajid Hamid Akash *et al.*, 2013; Galli *et al.,* 1999). Like humans and other rodent models with type 2 diabetes, GK rats demonstrate an impaired production of insulin and a resistance to it resulting in hyperglycaemia. Additionally, they are known to be spontaneously diabetic, meaning that they start exhibiting hyperglycaemia and insulin resistance as early as one month of age. They also have genes that control for weight gain, making them non-obese (Lang *et al.,* 2009). Their body weights are approximately 10-30% lower than age-matched non-diabetic rats (Sajid Hamid Akash *et al.,* 2013). These features allow the disease to be studied in a model that closely resembles human patients.

Alterations in neurogenesis have been seen in GK rats. More specifically, GK rats have increased cell turnover – that is, they have increased cell proliferation and decreased cell survival

in both the dentate gyrus and the subventricular zone (Lang *et al.,* 2009). To ensure that these impairments were in fact associated with the presence of hyperglycaemia in the GK rats, neural stem cells were generated in these animals. The development of these stem cells was then compared with those generated in Wistars. When exposed to two different growth factors, the GK cultures showed no significant increases in growth, whereas the Wistar cultures did when exposed to the same growth factors. This provides evidence for a possible mechanism underlying the impaired proliferation and survival seen in GK rats, and other animals with type 2 diabetes. These results line up with other studies involving diabetic GK rats. For example, Beaquis *et al.* (2009) observed increased proliferation in the dentate gyrus and subventricular zone of GK rats, while there was a rapid decrease in the number of surviving cells. Consistent with Lang *et al.* (2009), this study saw no responses of GK cell cultures to growth factors. In another study involving db/db mice, both proliferation and survival were analyzed in the subventricular zone (Ramos- Rodrigues *et al.,* 2014). Although neurogenesis in the dentate gyrus was not investigated, the results regarding the SVZ support those found in GK rats. Again, an increase in proliferation and decrease in survival was observed in the db/db mice. Together, these studies provide converging evidence for the presence of altered neurogenesis in rodents with type 2 diabetes. What these studies do not explore is how these changes in neurogenesis influence cognitive performance. The evidence for alterations in neurogenesis and cognitive deficits in GKs, and other models of type 2 diabetes, provides a rationale for the current experiment which will be reviewed in the following section.

## Current Experiment

Type 2 diabetes is associated with a number of changes in the brain, including an increase in the turnover of adult-generated neurons (Beaquis *et al.,* 2009; Ho *et al.,* 2013; Lang *et al.,*

2009; Ramos-Rodriguez *et al.,* 2014). Research has demonstrated the involvement of neurogenesis in a variety of tasks, but more specifically those dependent on the dentate gyrus and olfactory bulb (Clelland *et al.,* 2009; Moreno *et al.* 2009). What research has yet to investigate is if and how hyperglycaemia associated with type 2 diabetes alters neurogenesis to influence performance on hippocampus- and olfactory- associated tasks. Therefore, the current research question is whether hyperglycaemia influences dentate gyrus- and olfactory-dependent tasks through alterations in neurogenesis.

The current thesis will address this research question by measuring performance on the radial arm maze and the social transmission of food preference (STFP). This will be followed by the investigation of neurogenesis in the dentate gyrus and olfactory bulb through immunohistochemical methods. The two hypotheses propose that GK rats will perform at a much lower level of performance than non-diabetic Wistars on both tasks, and they will also exhibit increased levels of proliferation and decreased levels of survival within the dentate gyrus and olfactory bulb. By first assessing learning on behavioural tasks, I will be able to assess the impact of hyperglycaemia on tasks known to involve the dentate gyrus and olfactory bulb. To examine its impact on neurogenesis, doublecortin- and Ki67-positive cells will be quantified to provide a measure of neurogenesis in the two regions. Using the behavioural and histological results will provide a greater understanding into the relationship between glucose intolerance, neurogenesis and learning.

## Chapter 2: General Procedure

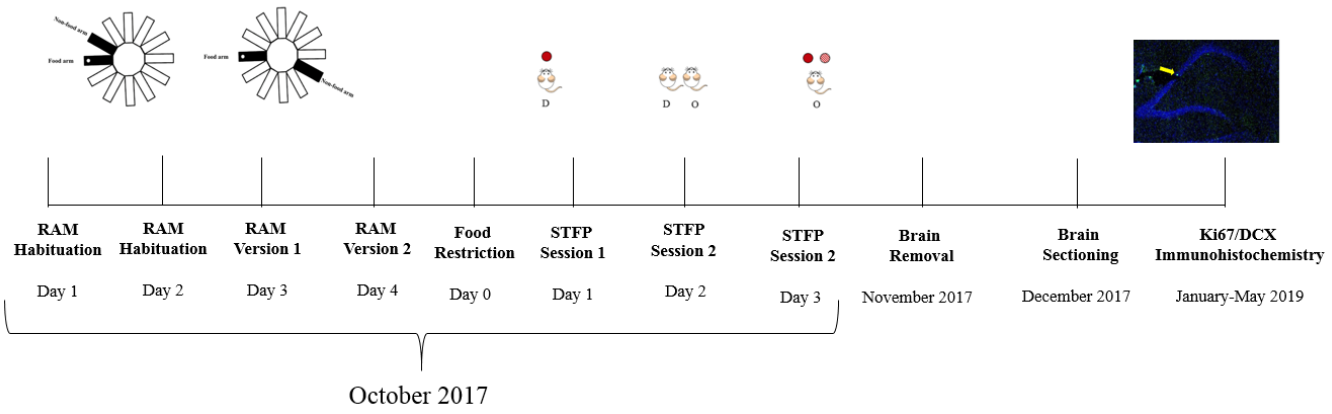



**Figure 1.** Experimental Timeline. The experiment began with testing on the radial arm maze. Animals were first habituated to the maze over the first 2 days. On day 3, animals were tested on one version of the maze (high or low separation). On day 4, animals were again tested on the version of the maze they were not tested on day 3. Animals were then tested on STFP over 4 days. STFP began with a food restriction 24 hours prior to testing. All animals then underwent 2 sessions of STFP over 2 days. Following STFP, brains were extracted and sectioned. The tissue was then processed for Ki67 and DCX using immunohistochemistry and then quantified to provide a measure of proliferation and survival.

## Subjects

All the procedures were approved by the Wilfrid Laurier University Animal Care Committee, in accordance with the standard of the Canadian Council of Animal Care. Forty-two rats were used in total, 16 GK rats and 26 Wistars. Upon testing, subjects were approximately 3-4 months of age and weighed 300-400 g. All subjects were housed in pairs upon arrival. The room was kept at a temperature of 21-22° C and on a 12-hour reverse light/dark cycle (lights on 7:00 pm, lights off 7:00 am), and all testing was done during the dark cycle. All rats were given approximately 20 g of chow daily and water was available *ad libitum.* Rats were handled 7 days prior to the start of the experiment.

## Behavioural Procedures

All animals were tested on two different behavioural tasks, the radial arm maze and STFP. Each of these tasks will be further reviewed in their corresponding chapters.

**Histology**

All rats were anaesthetized through isoflurane inhalation. The brains were removed within 3 minutes and were immediately frozen in a bath of ice and ethanol. Until sectioning, all brains were kept at -80° C. One to two days prior to sectioning, brains were removed from -80° C and kept at -20° C. Before sectioning began, brains were mounted using OCT compound (Fisher Scientific, Whitby, ON). All brains were sectioned using a cryostat at a thickness of 20 μm and mounted on Superfrost Plus slides (VWR). All slides were kept at -80° C until immunohistochemical processing.

**Ki67/DCX Immunohistochemistry**

Slides were removed from -80° C and kept at -20° C for 1-2 days prior to processing the tissue. After being thawed to room temperature, slides were fixed in 2% paraformaldehyde for 5 minutes, followed by 2-minute wash in a 0.1 M Tris-buffered saline (TBS) solution, and then a 5-minute wash in 1:1 acetone: methanol solution. Slides then underwent another TBS wash for 5 minutes, followed by a 15-minute wash in 3% hydrogen peroxide. Slides were then washed in a 0.1 M Tris-buffered saline with 0.05% Tween 20 (TBS-T) for 5 minutes, followed by a wash in TBS. Following this, slides were incubated in a TSA blocking buffer with 5% normal sheep serum for 30 minutes. Slides were then stained with anti-DCX antibody (Abcam) or anti-ki67 antibody (Abcam) at a concentration of 1:200, coverslipped and kept at 4° C overnight. Following the overnight incubation, coverslips were removed by bathing the slides in TBS. Slides then underwent one 10-minute TBS-T wash followed by two 10-minute TBS washes. The slides were then stained and incubated in an Anti-rabbit Alexa488 antibody at a concentration of 1:200, coverslipped and kept at room temperature for 2 hours. The coverslips were then removed in a TBS bath and underwent two 10-minute TBS-T washes followed by a 10-minute TBS wash.

Following thus, slides were stained and incubated in a 4'-6-Diamidino-2-phenylindole (DAPI, Sigma-Adrich) in TBS. For dentate gyrus sections, slides were stained at a concentration of 1:500, but for olfactory bulb sections, they were stained at a concentration of 1:1000. They were then coverslipped and kept at room temperature for 30 minutes. After coverslips were removed using TBS, the slides underwent three 10-minute washes in TBS before being coverslipped with Vectashield anti-fade mounting medium (Vector Laboratories, Burlington, ON). Slides were sealed using nail polish and kept at 4° C until images were collected.

### Imaging and Quantification

Images were taken of coronal sections of the dentate gyrus and olfactory bulb using an Olympus FV100 confocal microscope at 10x and 40x magnifications. Within the dentate gyrus, both the suprapyrmidal blade (SP) and infrapyramidal blade (IP) were imaged. For the dentate gyrus, three sets of images were taken. One of these sets was taken at 10x magnification to image the entire dentate gyrus. The second sets of images were taken at 40x magnification to image the granular cell layers within the SP and IP. For the olfactory bulb, two sets of images were taken. The first set was taken at 10X magnification to image the entire olfactory bulb. The second set was taken at 40X magnification to capture the granular cell layer. Additionally, images were taken only of the left hemisphere of each brain. Ki67- and DCX-positive cells were quantified using the software Fiji.

## Chapter 3: Investigating Neurogenesis in the Dentate Gyrus

### Introduction

The dentate gyrus is one sub-region of the hippocampus, a structure found within the medial temporal lobe of the mammalian brain (McHugh *et al.*, 2007). The dentate gyrus is

composed of a number of layers. These layers are the molecular layer, the subgranular layer, the granular layer, and the polymorphic layer (Amaral *et al.,* 2007). In the subgranular layer, the layer beneath the granular layer, stem cells are regenerated. Once regenerated, these cells migrate into the granular layer and differentiate into granule cells (Taupin, 2007). Within the dentate gyrus, these newly-generated granule cells become incorporated into the larger neural network that contributes to tasks dependent on the dentate gyrus (van Praag *et al.,* 2002).

Memory interference requires the input of the dentate gyrus to allow animals to differentiate between highly similar experiences and the cues that make up these experiences (Bakker *et al.,* 2008). A considerable amount of literature has demonstrated the role of the dentate gyrus in this task in both humans and rodents (Bakker *et al.,* 2008; Lee and Solivan, 2010; Morris *et al.,* 2012). For instance, a study involving the use of functional magnetic resonance imaging (fMRI) demonstrated significantly increased levels of activation in the dentate gyrus when individuals were given images with minimal differences between them. On subsequent trials, the same individuals were given unrelated images. The results of the fMRI demonstrated significantly greater activation in the dentate gyrus on trials containing foils highly similar to the study images (Bakker *et al.,* 2008). The most plausible explanation for these results is that the dentate gyrus is required to differentiate between images with minimal differences, but is not necessary when the images were more distinct. These data support the long-held idea (Marr, 1971) that the role of the dentate gyrus lies in reducing memory interference.

Just like humans, rats are susceptible to memory interference when distinguishing between similar stimuli, and preventing interference requires the dentate gyrus (Lee and Solivan, 2010; Morris et al., 2012). For instance, the radial arm maze is known to depend on the dentate gyrus when an animal is required to differentiate between locations defined by adjacent arms

(Morris et al., 2012). Due to the location and appearance of the arms, they appear very similar to each other.

Adding to the evidence that neurogenesis is critical for the dentate gyrus to reduce memory interference, knocking down neurogenesis in the dentate gyrus through irradiation has also been shown to impair discrimination between adjacent arms of a radial arm maze (Clelland *et al.,* 2009). To ensure that the radiation was successful BrdU labelling was used to label for newly-proliferated cells within the dentate gyrus.

The experimental evidence from these studies support the radial arm maze as an appropriate task to explore the contribution of neurogenesis to memory interference. The specific task that will be used in the current study is modeled after the one used by McDonald and White (1995). In this experiment, an 8-arm maze was used in which rats were given access to two arms. One was assigned as the designated food arm and the other as the non-food arm. These arms either had low or high separation between them to act as dentate- and non-dentate-dependent versions. Together, this will provide additional evidence regarding the influence of hyperglycaemia on neurogenesis and learning, two processes that are known to be associated with one another (Clelland *et al.,* 2009; Moreno *et al.,* 2009; Zhao *et al.,* 2008).

## Methods

### Subjects

All procedures were approved by the Wilfrid Laurier University Animal Care Committee, in accordance with the standard of the Canadian Council of Animal Care. Thirty-one rats were used in total for this experiment, 16 GK rats and 15 Wistars. Upon testing, subjects were approximately 3-4 months of age and 300-400 g. All subjects were housed in pairs upon arrival. The room was kept at a temperature of 21-22° C and on a 12-hour reverse light/dark cycle (lights

on 7:00 pm, lights off 7:00 am), and all testing was done during the dark cycle. All rats were given approximately 20 g of chow daily and water was available *ad libitum.* Rats were handled 7 days prior to the start of the experiment.

**Apparatus**

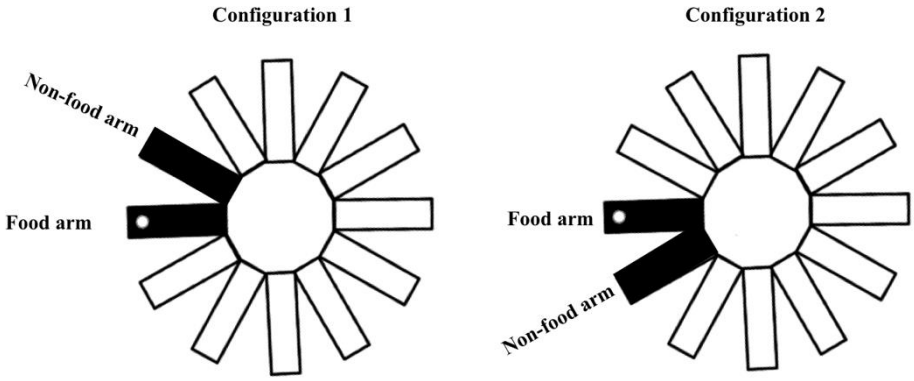
The first task was carried out in a radial arm maze consisting of 12 equidistantly spaced arms measuring 50 cm x 10 cm x 13 cm, with a total diameter of 122 cm. The maze rested on a table and was located in the centre of a room containing extra-visual cues on all walls and a computer in the corner.

**Procedure**

To begin, all rats underwent 2 days of habituation in which they were given 10 minutes of free exploration of the maze each day. During this time, Froot Loops (Kellogs) were scattered throughout the maze. On day 3, testing began. Each rat was placed into the centre of the maze and only given access to two arms. One of these arms was baited with a Froot Loop and the other was not. All other arms were blocked using Styrofoam blocks. Each rat was given 5 minutes to enter the correct arm and consume the Froot Loop. Immediately after this, the rat was removed from the maze and returned to its homecage for 60 seconds. If the incorrect arm was entered, the rat would be removed and returned to its homecage for 60 seconds. If an arm was not entered within the allotted time, the rat would be removed and returned to its homecage for 60 seconds. Following these 60 seconds, all animals would then return to the maze for the next trial. This process continued until each animal completed all 10 trials of the same version they were assigned to. This process would again be repeated on Day 4. What differed between these two training days was the version of the maze that each animal would complete. Two versions of the maze were created, a low-separation version and a high-separation version. The difference

between the two was the distance between the food arm and the non-food arm. On the low-separation version, the two arms were directly adjacent to each other (Figure 2a). On the high-separation version, the two arms were 5 arms apart from each other (Figure 2b). For both of these versions, the food arm remained the same, but the non-food arm was altered across versions. Again, all other arms were blocked using Styrofoam blocks. If a rat was assigned to the low-separation version on day 3, it would be assigned to the high-separation version on day 4, and vice versa. One additional manipulation within the maze was with the specific non-food arms within each version. On every second trial, the non-food arm would be switched between configuration 1 and 2 that corresponded to their assigned version (Figure 2). This prevented rats from using intramaze cues to locate the arms, but instead use distal cues within the room to do so. Throughout all 10 trials, animals were recorded on their latency to enter an arm, as well as whether they entered the correct/incorrect arm.

a.




b.

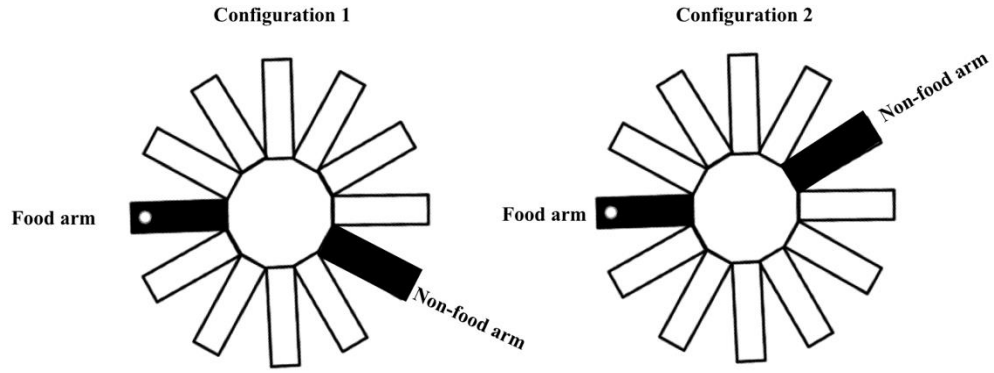



**Figure 2.** Overhead view of the radial arm maze depicting the two configurations for the low-separation version (a) and high-separation version (b). The arms labelled food arm were baited with Froot Loops and those labelled non-food arms were empty. Under both versions, animals were given 5 minutes to retrieve the Froot Loop when given access to both the food and non-food arms. After a 60-second delay they were placed back into the maze for the next trial.

## Histology

Histological processing was carried out as described previously in the General Procedure section.

## Ki67/DCX Immunohistochemistry

Immunohistochemical processing was carried out as described previously in the General Procedure section.

**Imaging and Quantification**

Images were taken of coronal sections of the dentate gyrus using an Olympus FV100 confocal microscope at 10x and 40x magnifications. Within the dentate gyrus, both the suprapyrmidal blade (SP) and infrapyramidal blade (IP) were imaged. For the dentate gyrus, 3 sets images were taken. One of these sets was taken at 10x magnification to image the entire dentate gyrus (Figure 3). The second sets of images were taken at 40x magnification to image the granular cell layers within the SP and IP (Figure 4). Additionally, images were taken only of the left hemisphere of each brain. Ki67- and DCX-positive cells were quantified using the software Fiji.

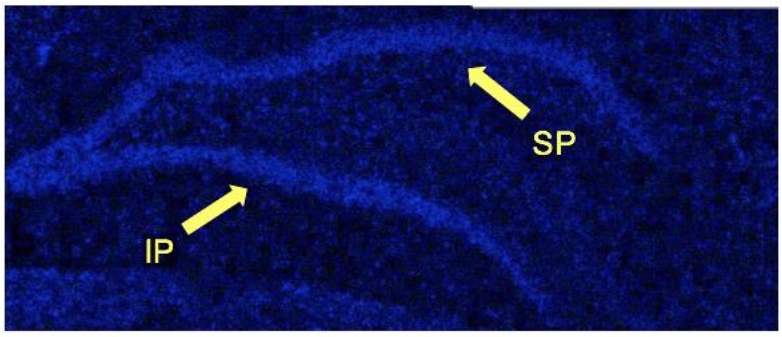



**Figure 3.** Coronal section of the dentate gyrus stained with DAPI showing Suprapyramidal (SP) and Infrapyramidal (IP) blades taken by Olympus FV100 confocal microscope.

(a) (b)

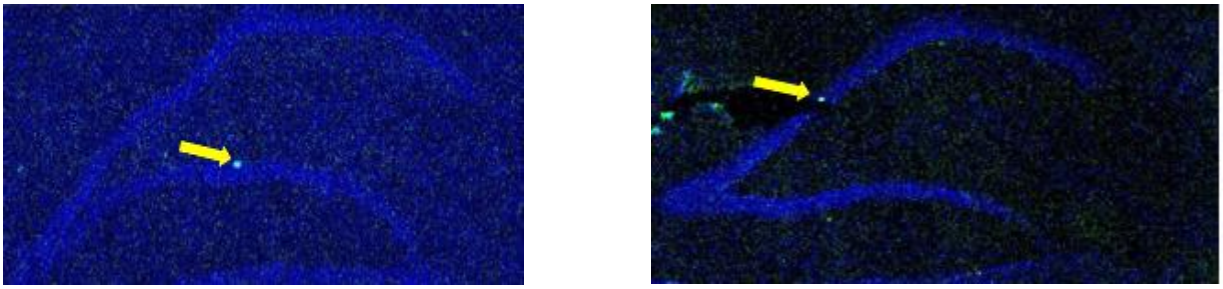


**Figure 4.** Coronal sections of the dentate gyrus with staining for Ki67 and DCX. (a) Coronal section of the dentate gyrus showing a Ki67-positive cell taken at 10x magnification by Olympus FV100 confocal microscope. (b) Coronal section of the dentate gyrus showing a DCX-positive cell taken at 10x magnification by Olympus FV100 confocal microscope.

## Results

### Behavioural Results

To begin, the first 2 trials for each day were removed from the data. These trials were treated as learning trials, as the rats were first exposed to the maze during this trial and had no prior experience with this task. The remaining 8 trials were organized into 2 different trial blocks, block 1 and block 2. The first block consisted of trials 3-6 and the second block consisted of trials 7-10. There were three possible choices that could be made on the maze. Rats could either enter a correct arm, enter an incorrect arm, or not enter an arm and remain in the centre of the maze for the full 5 minutes. Initially, this last choice, referred to as a non-engagement, was considered an incorrect choice, but it can be argued that this is not considered a choice at all. Therefore, the results were analyzed in two different ways. Figure 5 shows the mean latencies for each trial block including all non-engagements. GKs demonstrate higher mean latencies across all trials for both days (Figure 5). To determine whether there were significant differences between the GKs and Wistars a three-way ANOVA was conducted to investigate the possible effects of strain (GK or Wistar), trial block and task version (high or low). The results revealed a

significant effect of strain ($F_{1, 29}$= 37.910, $p < 0.001$) and trial ($F_{1, 29}$=7.8410, $p < 0.001$). An interaction was found between strain and trial ($F_{7, 203}$= 3.304, $p <0.01$). No significant effects were seen for task version. The mean latency across both trial blocks was also examined with all non-engagements excluded from the data (Figure 6). Again, GK rats show higher mean latencies across all trials for both days. The results of the statistical analysis revealed a significant effect of strain ($F_{1, 29}$)=, $p <0.001$). No significant effects were found for version or trial.

The mean percent of correct choices for each trial block was also calculated (Figures 7 & 8). Figure 7 illustrates that mean percent of correct choices made including all non-engagements as incorrect choices, even if trials consisted of only non-engagements. When non-engagements were included in the data, GKs demonstrated a decreased number of correct choices. There appears to be minimal differences between both versions for GKs and Wistars. For these results an ANOVA was run to investigate the effect of strain and task version. A significant effect of strain was found ($F_{1,29}$=23.457, $p < 0.001$). No significant effects were seen for version or trial. Figure 8 shows the same results, but all non-engagements were removed as they were considered neither a correct or incorrect choice. Again, GKs demonstrated decreased Mean percent of correct choices on both versions. Statistical analysis revealed a significant effect of strain ($F_{1,29}$ =1.721, $p < 0.001$). No effect was seen for any other factors.

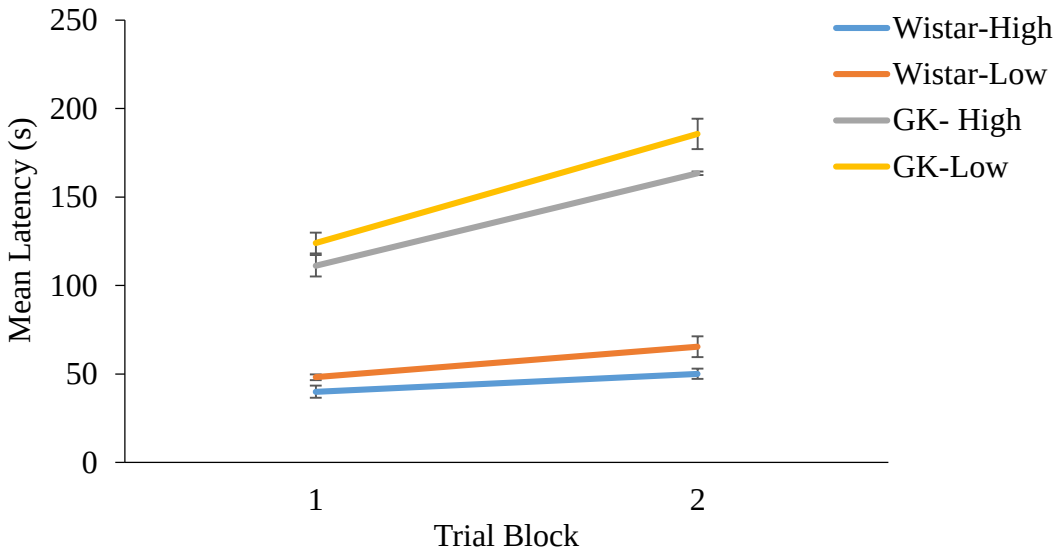



**Figure 5.** Mean Latency (±SEM) to enter correct/incorrect arms on both the high-separation ("high") and low-separation ("low") versions including all non-engagements. Trials were divided into 2 blocks, block 1 (trials 3-6) and block 2 (trials 7-10). On both versions, mean latencies of GKs were significantly higher than those for Wistars ($p < 0.001$). Significant differences were found between trials ($p < 0.001$). A significant interaction was found between strain and version ($p < 0.05$). No significant effect was seen for task version.

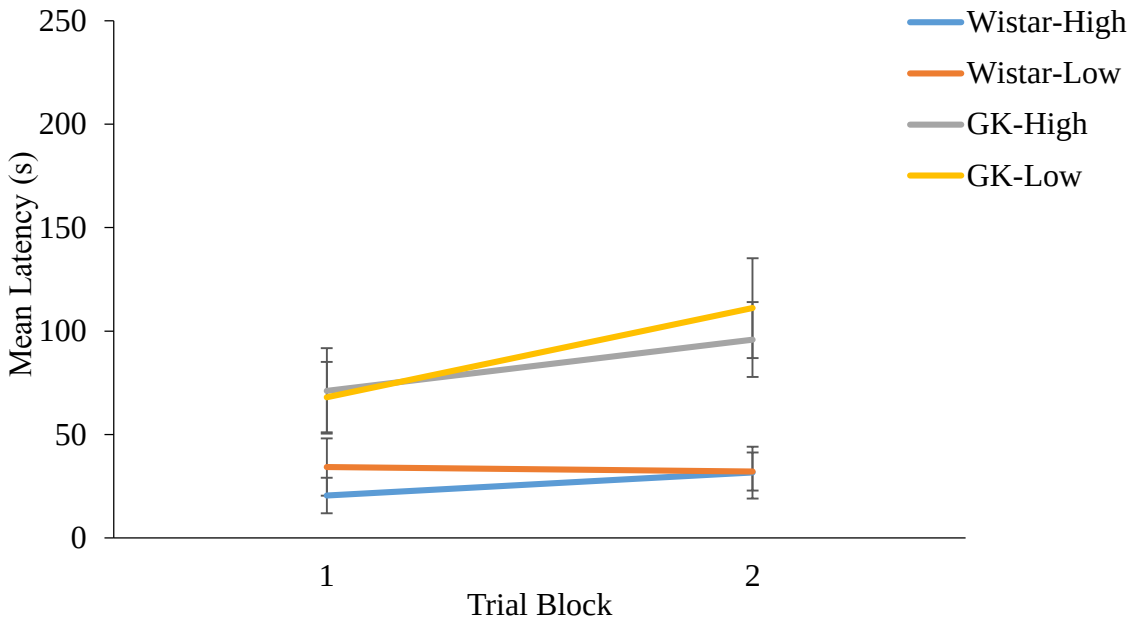



**Figure 6.** Mean Latency (±SEM) to enter correct/incorrect arms on both the high-separation ("high") and low-separation ("low") versions excluding all non-engagements. Trials were divided into 2 blocks, block 1 (trials 3-6) and block 2 (trials 7-10). On both versions, mean latencies of GKs were significantly higher than those for Wistars ($p < 0.001$). No significant differences were found between trial and version.

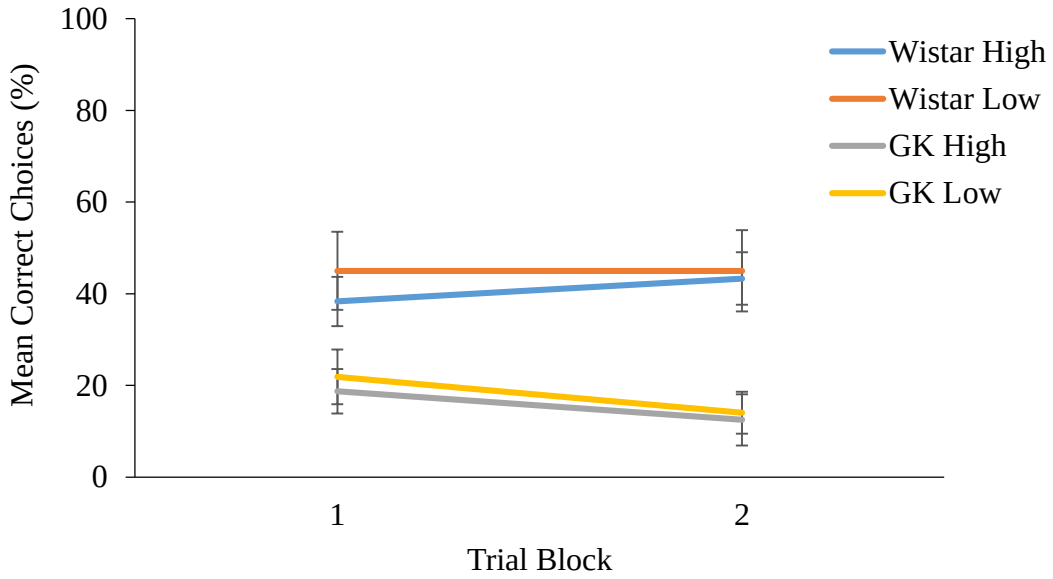



**Figure 7.** Mean percent (±SEM) of errors made on both high-separation ("high" and low-separation ("low") versions including all non-engagements. Trials were divided into 2 blocks, block 1 (trials 3-6) and block 2 (trials 7-10). GKs demonstrated significantly lower mean percent of correct choices ($p < 0.001$). No significant differences between versions were found.

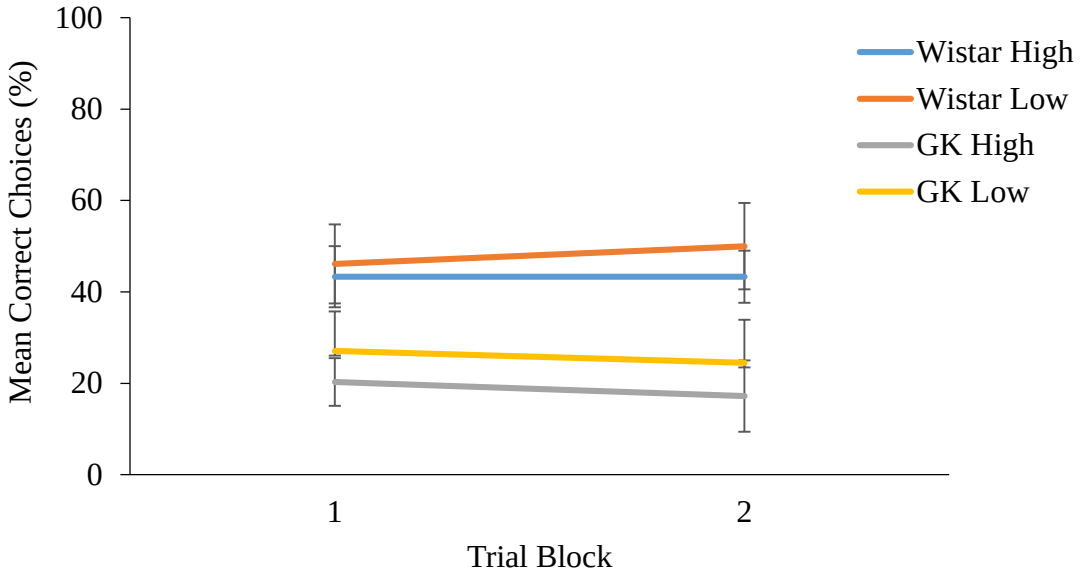



**Figure 8.** Mean percent (±SEM) of correct choices made per trial on both high- separation ("high") and low-separation ("low") versions not including non-engagements. Trials were divided into 2 blocks, block 1 (trials 3-6) and block 2 (trials 7-10). GKs demonstrated significantly lower mean percent of correct choices ($p < 0.001$). No significant differences between versions were found.

**Neurogenesis Results**

As discussed, the tissue from these animals was collected and processed to visualize neurogenesis within the dentate gyrus. Cells were stained with DAPI and quantified to provide a measure of the total cell population within the dentate gyrus (Table 1). No significant differences were found between GKs and Wistars ($t_{1.783}$=0.670, $p > 0.05$). Ki67 and DCX were used to label immature cells and surviving cells. The results obtained from this indicate minimal differences in cell counts for GK and Wistar rats (Figure 9). The statistical analysis provides further support for this through t-tests. These tests showed no significant difference in Ki67-positive cells ($t_{9.150}$= -1.1438, $p > 0.05$) or DCX-positive cells, ($t_{10.643}$=-0.14118, $p > 0.05$).

| | Mean | SD |
|---|---|---|
| Wistar | 792.062 | 411.452 |
| GK | 659.691 | 411.709 |

**Table 1.** Mean and standard deviations for the number of DAPI-positive cells for GKs and Wistars per um3. No significant differences were found between GKs and Wistars.

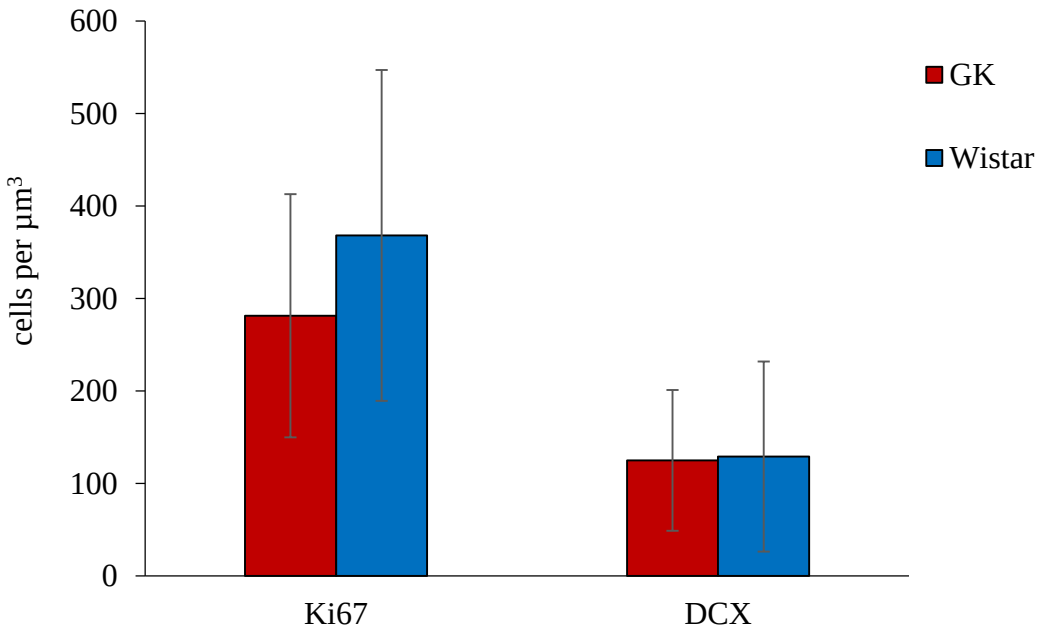



**Figure 9.** Mean number (±SEM) of DCX- and Ki67-positive cells within the dentate gyrus per $\mu m^3$. No significant differences were found between cell populations for either stain.

## Discussion

GKs were expected to demonstrate poor performance on the low separation version of the delayed nonmatch to position task conducted in the radial arm maze, as this version is dependent on the dentate gyrus (Mcdonald & White, 1995; Morris et al., 2012). This poor performance should result in increased latency times to enter an arm and an increase in incorrect choices to enter the non-baited arm selectively in low-separation trials, while comparatively little difference

was expected in the high-separation trials. The current data do not support these hypotheses. GKs showed significantly higher Mean latency times across all trials under both versions. During their trials in the maze, rats were given access to three locations. They were given access to the centre of the maze, the baited arm and the non-baited arm. Therefore, there were three different choices to be made by either remaining in the centre, entering the baited arm or entering the non-baited arm. While entering a baited vs non-baited arm are clear correct and incorrect choices, remaining in the centre of the maze can be interpreted in two different ways. It can either be interpreted as an incorrect choice or it can be interpreted as not making a choice at all. Therefore, the data was organized in two different ways. The results were first processed with all non-engagements regarded as incorrect choices. These results demonstrate significantly reduced Mean percent of correct choices for GKs under both versions of the task. The data was also processed with all non-engagements omitted. Again, GK rats show significantly reduced mean percent of correct choices for both versions.

Several interesting results were found in the current experiment. Based on the existing literature, GKs were expected to only show significantly reduced performance on the low-separation task, but instead they showed very similar performance for both versions (McDonald and White, 1995; Morris et al., 2012). Under the high-separation version, animals do not depend on the dentate gyrus to distinguish between the arms. This is presumably because they are very visually distinct and can be associated with a completely different set of distal cues. In contrast, the low-separation version requires the dentate gyrus in order for animals to distinguish between the arms. They are highly similar in physical appearance and location and have an overlapping set of distal cues in their visual field. Therefore, animals with impairments to their dentate gyri are expected to show clear impairments on this version of the maze. One possible explanation for

this could be lack of motivation, as seen in GKs by Moreira *et al.* (2007). GKs showed significant impairments in a progressive ratio lever press task. Similarly, The GKs in this experiment could have been demonstrating the same lack of motivation. Regardless of the version of the task, as trials progressed GKs may have demonstrated less motivation in retrieving the food reward. This explanation is supported by the fact that GKs showed increasing non-engagement as trials proceeded.

Another interesting finding is that GKs demonstrated performance around the 20% mark, which is significantly lower than chance. These results could be interpreted as GKs intentionally avoiding the food arm and choosing the non-food arm. A possible explanation for this is that GKs were intentionally avoiding the Froot Loop, which is a food known to be high in glucose. As GKs are unable to metabolize glucose (Goto & Kakizaki, 1975), consumption of it may result in an unpleasant experience. With continued consumption of the food reward and the continued negative reaction, animals may learn to avoid the food reward because of the negative outcome. Therefore, showing continued avoidance of designated food arm. This provides one potential explanation for the results found, which can be avoided through the use of another appetitive food reward, but not one that could initiate a negative reaction in GKs to affect the results.

An additional possible explanation for the poor performance of GKs on both versions pertains to the complication of type 2 diabetes. One such complication is a loss of vision. In human patients of type 2 diabetes develop diabetic retinopathy, a disease in which the retina is damaged due to the presence of diabetes. This has been modeled in various animals, including rodents (Robinson *et al.*, 2012). The evidence for this disease in animals may provide an explanation for the inability of GKs to distinguish between the food- and non-food arms. GKs may be showing early signs of vision loss that directly affect their ability to identify the correct

food arm. To investigate this, GKs may be tested on various tests of vision such as an object recognition task. For future testing, this investigation is strongly recommended.

Not only did GKs perform at significantly low levels, but so did Wistars. Although there were significant differences between GKs and Wistars, Wistars were still not performing significantly above chance. These results are surprising because Wistars were expected to able to complete the task. Mcdonald and White (1995) report that normal rats achieved an asymptotic performance close to 100% in a nearly identical protocol. Those with lesions to the hippocampus demonstrated performance at or below the 50% level. One potential explanation for the poor performance of wistars is that they required further training on the maze before testing began. Animals received two habituation trials and two training trials before immediately beginning testing. With further training trials to learn the configuration of the maze, wistars may have demonstrated increased performance that was seen in the study by Mcdonald and White (1995).

With regards to the histological data, no significant differences in the expression of Ki67 or DCX were found between GK and Wistar rats. Thus, the data showed no significant differences in cell proliferation or survival between GK and Wistar rats. These results are not consistent with our hypothesis. This is particularly unexpected because significant differences between the number of Ki67- and DCX- positive cells between GKs and Wistar rats have been observed in several other studies (Beaquis *et al.,* 2009; Lang *et al.,* 2009; Ramos- Rodrigues *et al.,* 2014). More specifically, GKs were expected to show a significant increase in Ki67, while DCX should have been significant decreased.

**Conclusions**

Using both a dentate-dependent task and histological evidence, this thesis explored the possible influence of type 2 diabetes on adult neurogenesis and associated cognitive function.

The results from this experiment do not match existing research. In terms of the choices made on the radial arm maze, Wistars performed near chance, which was not seen in previous research. GKs demonstrated reduced performance on the task in terms of latency and percent of correct choices, but reliable comparisons cannot be made between GKs and Wistars as there is not clear evidence that any of the animals consistently learned the task. Similarly, the histological results do not demonstrate the expected differences in neurogenesis. As mentioned, research has reported significant differences in proliferation and survival of cells within the dentate gyrus of GK rats, which could not be replicated here. These results can suggest that there may be additional mechanisms by which type 2 diabetes affects the dentate gyrus.

## Chapter 4: Investigating Neurogenesis in the Olfactory Bulb

### Introduction

The second brain region which is characterized by large-scale neurogenesis throughout the lifespan is the olfactory bulb (Carleton *et al.*, 2003). The olfactory bulb acts as the first relay station of the central olfactory system with projections to the rest of the brain (Mori *et al.*, 1999). When odour molecules enter the nasal cavity, they converge onto olfactory sensory neurons, which project their axons onto the olfactory bulb. This information is then sent to higher brain regions to be processed. These regions include the hippocampus, amygdala, and the pririform cortex (Firestein, 2001).

The olfactory bulb contains many different layers: the olfactory nerve layer, the glomerular layer, the external plexiform layer, the mitral cell layer and the granular cell layer. In the olfactory bulb, newly-generated neurons that have migrated from the subventricular zone are predominantly found within the granular and glomerular layers (Bédard & Parent, 2004). Like in the dentate gyrus, these newly-matured cells make a critical contribution to information

processing (Bédard & Parent, 2004; Carleton *et al.*, 2003). The following sections will review the contribution of the olfactory bulb to tasks involving assessing and discriminating between olfactory cues. Furthermore, the involvement of neurogenesis in such tasks will also be explored.

**The Olfactory Bulb and Olfaction**

As mentioned, the olfactory bulb is critical for the perception and discrimination of odours. Xu *et al.* (2000) were able to show that the olfactory bulb encodes different types of information about olfactory cues. Rats were presented with limonene and iso-amyl acetate at varying exposure times and concentrations. Odour concentrations ranged from 40-1500 parts per million (ppm) for iso-amyl acetate and 420 ppm for limonene. At these concentrations, they were subjected to different exposures times. Animals were either presented with a train of 10-second exposures followed by a 3.5-minute exposure or they were presented with a single 10-second exposure followed by a 10-minute exposure. Spatial activity patterns (SAPs) in the olfactory bulbs were investigated following these exposures. To do so, fMRI images were taken to visualize activity patterns. The collected images demonstrated increased activity in the olfactory bulbs, in the form of SAPs, as a result of increased odour concentrations. While there was a difference in the activity levels, the images showed that the same receptor types were active. Images also showed activation of different receptor sub-types when exposed to different odours, providing evidence for the ability of the olfactory bulb to discriminate between different odours. In terms of exposure times, SAPs differed in their reproducibility across sessions. With shorter exposure, SAPs showed low modifications across sessions, meaning that there was high reproducibility. During longer exposures, SAPs were modified across sessions, suggesting low reproducibility. To conclude, these results demonstrate the changing activity in the olfactory bulb

during varying exposure times and concentrations, which enabled the olfactory bulb to encode and discriminate between the different properties of odour.

Another task shown to involve the recruitment of the olfactory bulb is the STFP. Research has demonstrated that members of the same species are able to exchange information about food consumed during an interaction (Galef and Wigmore, 1983; Posadas-Andrews, 1983; Strupp & Levitsky, 1984). Under Galef and Wigmore's protocol (1983), a food-restricted rat, known as a demonstrator rat, is given a specifically flavoured food and allowed 30 minutes to consume it. After this initial feeding, the demonstrator rat is placed into a cage with another food-restricted rat, known as an observer rat, for a 30-minute interaction. The observer is then given a food preference test in which it is left undisturbed for 24 hours with two different flavoured foods. Although the observer rat has zero direct experience with either flavour, one food has the flavour that its paired demonstrator was exposed to and the other a different flavour that the demonstrator were not previously exposed to during the experiment. After the 24 hours, the observer consumes a significantly greater amount of the food that the demonstrator was exposed to, therefore showing a preference that was transmitted socially based on the experience of another rat. This capability allows individuals to transmit crucial information about food to other adults of the same species, as well as their own offspring (Posadas-Andrews, 1983). One known benefit of the ability to transmit information about food reference is that it helps to overcome what is known as neophobia, the natural avoidance of novel stimuli, such as food. Rats are known to avoid novel foods in order to prevent the consumption of dangerous and poisonous foods (Barnett, 1958; Posada-Andrews & Roper, 1983). In an experiment investigating the degree of neophobia in wild rats it was observed that rats developed a preference for a novel food following an interaction with another rat that was exposed to the same food (Barnett, 1958).

The establishment and transmission of such a food preference allows rats to become familiarized with novel foods that they would not normally expose themselves to (Posadas-Andrews, 1983). In addition to its benefit to neophobia, the transmission of food preference is known to be beneficial in foraging for food and weaning pups (Galef, 1977; Galef & Giraldeau, 2001). When foraging animals detect foods on the breath of conspecifics, it informs them what foods are safe to consume, thus what foods to forage for (Galef & Giraldeau, 2001). Weaning rats can be biased towards certain foods, such as those with higher nutritional value, following social interaction with adult conspecifics (Galef, 1977). The general benefit of the social transmission of food preference is to provide a larger variety of foods to consume.

Research exploring the underlying mechanisms for STFP have provided evidence for the involvement of the olfactory system and the transmission of olfactory cues. Even in the absence of all other cues, a rat will establish a preference for a flavoured food after detecting that same flavoured food on another individual (Galef and Wigmore, 1983). In one experiment, mouth pieces were attached to designated observer rats. This prevented them from engaging in any physical contact and limited them to only rely on olfactory cues to detect food preference. After an interaction with a demonstrator that had an established preference, observer rats showed a clear preference for the food given to their paired demonstrator. In comparison to rats that had no constraints preventing physical contact, these rats showed no significant differences in the amount of each food consumed. This suggests that they achieved a successful social transmission of food preference when only allowed access to olfactory cues. A second experiment by Galef and Wigmore (1983) compared rats without the ability to perceive olfactory cues, known as anosmic rats, with controls that had a functioning olfactory system. Following an interaction with a demonstrator that had a specific preference, the anosmic rats showed no significant

differences in the consumption of the two foods provided during the food preference test. Collectively, these two studies demonstrate the requirement of the olfactory system and olfactory bulb in the social transmission of food preference.

Like tasks dependent on the dentate gyrus, those dependent on the olfactory bulb also rely on the contribution of neurogenesis. For instance, the ablation of neurogenesis in the olfactory bulb has shown to produce deficits in tasks involving odour perception (Moreno *et al.* 2009). Before testing, mice were implanted with an osmotic pump to administer cytosine arabinoside, a known inhibitor of neurogenesis, directly to the subventricular zone. Mice were then tested on a perceptual learning task. All were exposed to a combination of two novel odours, which they were eventually habituated to. They were then presented with a different combination of odours to which they were not previously exposed to. On presentation of both these odour combinations over several trials, mice were recorded on the time spent investigating the source of both odour combinations. In this experiment, the mice that received vehicle injections showed significantly higher investigation times for the novel combination of odours than the familiar combination. Unlike the saline-infused animals, those with ablated neurogenesis showed non-significant differences in investigation times between the familiar and novel odours, therefore showing no preference for the novel odour. In addition to observing behaviour, neurogenesis in the olfactory bulb was also investigated using BrdU labelling. The results demonstrated a significant difference in the number of BrdU- positive cells in the granular cell layer of the olfactory bulb, with significantly lower numbers in animals that received the cytosine arabinoside. To connect this with the behavioural results, rats with ablated neurogenesis showed poor performance on the task involved in this experiment and significantly low numbers of BrdU-expressing cells. From these results, it can be proposed that neurogenesis is in fact

associated with the perception of odour, as well as other tasks that are dependent on the olfactory bulb. This is one of many studies that demonstrate the importance of neurogenesis in tasks that require the olfactory system. Many studies have used various methods to impair/disable neurogenesis in the olfactory bulbs to demonstrate its effect on tasks dependent on the olfactory bulb (Gheusi *et al.*, 2000; Nissant *et al.*, 2009; Sultan *et al.*, 2010). In line with the mentioned study, these experiments have found the same results of impaired cell proliferation/survival and impaired acquisition of olfactory-dependent tasks. From the discussed literature, it can be proposed that neurogenesis is involved in olfactory-dependent tasks. Furthermore, from what is known about the involvement of the olfactory bulb in STFP it can be proposed that it is an appropriate task to explore the involvement of neurogenesis within the olfactory bulb. This experiment will use GK rats to explore the effect of alterations in neurogenesis that are known to be influenced by hyperglycaemia on olfactory-dependent leaning.

## Methods

### Subjects

In this experiment, a total of 42 rats were used and assigned to one of two groups. The first group consisted of the same 15 Wistar and 16 GK rats that had previously been used. These rats were assigned as observer rats, which will be further explained in the following sections. The second group consisted of 11 Wistar rats that were all assigned as demonstrator rats. All observer rats underwent 2 sessions of STFP, each with a different food combination. All 31 rats were first tested with a food combination of cumin and ground cloves. Fifteen of which were given a preference of cumin and 16 ground cloves. During the second sessions of STFP, 16 of the animals were tested using a preference of cocoa and cinnamon and 15 using marjoram and

anise. Under the cocoa-cinnamon combination, 8 were given a preference for cocoa. The remaining animals were given a preference for cinnamon. Under the marjoram-anise combination, 7 were given a preference for marjoram and 7 a preference for anise. Prior to testing, one GK was removed from testing.

Upon testing, subjects were approximately 3-4 months of age and weighed 300-400 g. All subjects were housed in pairs upon arrival. The room was kept at a temperature of 21-22° C and on a 12-hour reverse light/dark cycle (lights on 7:00 pm, lights off 7:00 am), and all testing was done during the dark cycle. All rats were given approximately 20 g of chow daily and water was available *ad libitum.* Rats were handled 7 days prior to the start of the experiment.

**Apparatus**

The demonstrator feeding, interaction and food preference test were all performed in clear acrylic cages, measuring 45cm × 25 cm × 20 cm, covered by a metal lid. All cages were filled with woodchips and contained a plastic pipe for environmental enrichment. During the demonstrator feeding and food preference test, rats were provided with the flavoured foods in plastic square bowls. The plastic bowls were snapped into the container tops that were glued to larger metal bowls to allow them to be easily removed. The metal bowl was attached to a Plexiglas sheet. For the demonstrator feeding, one feeding bowl was attached to a plexiglass base. While for the observer food preference test, two feeding bowls were attached to a single plexiglass base.

**Procedure**

Before testing, three different food combinations were chosen. These combinations were: cumin and ground cloves, cocoa and cinnamon, and marjoram and anise. For each of the

feedings in the current experiment, these flavoured foods were ground and combined with ground unflavoured rat chow. The ratios of flavoured to non-flavoured food have been previously defined in existing studies (Damphousse *et al.*, 2019; Galef & Whiskin, 1992; Galef, 1993). Together, the combination of these food and specific ratios in which they are combined make them equipalatable. This means that animals are equally likely to consume the same amounts of both foods. This ensures that the preferences seen in the current experiment were due to STFP and no other possible factors, such as a difference in equipalatibility. The three combinations of flavoured foods in this experiment were: cocoa (2%) and cinnamon (1%), cumin (0.5%) and ground cloves (0.4%), and marjoram (2%) and anise (1%).

Figure 10 provides an overview of the daily procedure for STFP. All rats were food restricted 24 hours before the start of the experiment. To begin, rats were assigned as a demonstrator or observer. Demonstrator rats began with a demonstrator training. To begin, one flavoured food was ground and combined with ground non-flavoured rat chow at the specified ratio to total 60 g. The designated demonstrators were given 60 g one of two flavoured foods and left undisturbed for 20 minutes. At the end of this 20 minutes their food was weighed. If the demonstrator consumed at least 3 g of the food, it would move onto an interaction with its assigned observer rat. Otherwise, it would be given an additional 30 minutes to consume the required amount before moving onto the interaction phase. Immediately after, a demonstrator and its paired observer were placed into a novel cage in a novel environment and allowed a 30-minute interaction. During this time the animals were left undisturbed. Immediately following, both the observer and demonstrator were returned to separate cages and the observer underwent a food preference test. The observer was given two flavoured foods, each weighing 60g and prepared exactly as for the demonstrator training. They were then given 4 hours to consume

both. One flavoured food was the one provided to the demonstrator in the demonstrator feeding. Therefore, the observer rat was exposed to this food during the interaction period. The other food was a novel flavoured food that the demonstrator and observer were not exposed to. Upon completion of the 4 hours, both food bowls were measured to determine which was consumed more by the observer. For STFP to be considered successful, observers were required to consume more than 50% of the preferred food that was consumed by their paired demonstrator, as defined by Damphousse *et al.* (2019). All observer rats underwent 2 sessions of STFP, each with 2 different combinations of flavoured food. For the first session, all animals received a food combination of cumin and ground cloves (C-G). The food combinations for the second session differed between animals. Half of the animals received a combination of cocoa and cinnamon (C-N), while the other half received a combination of marjoram and anise (M-A). From these combinations, half of the animals were given a preference for one flavoured food and the other half were given a preference for the other. During these tests, all observers were naïve to the food combinations.

Following completion of the STFP procedure, all GK rats that were assigned as observers underwent an additional feeding. To begin, animals were food deprived for 24 hours. They were then given 60g of non-flavoured rat chow and left undisturbed for 4 hours. At the end of this time, the food was weighed to determine the amount consumed. The goal of this test was to determine if the unsuccessful results were due to an inability to successfully establish a food preference, and not due to external factors that may be caused by the presence of type 2 diabetes

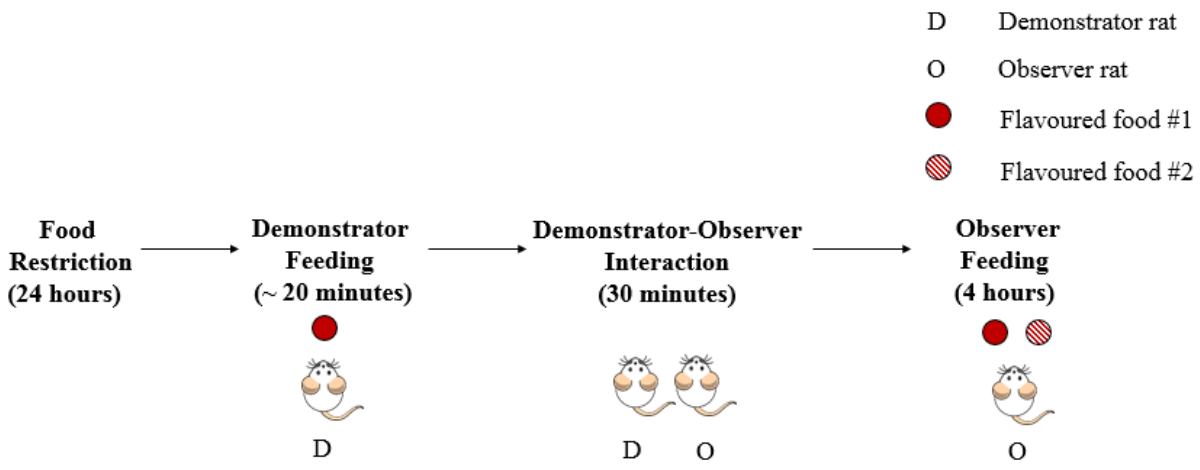



**Figure 10.** Overview of daily STFP procedure. The experiment began with food restriction 24 hours prior to testing. Day 1 began with the assigned demonstrator rat (D) undergoing a demonstrator feeding, approximately 20 minutes in length, in which they were given one of two flavoured foods from the corresponding combination. Immediately following, demonstrators and observers (O) underwent a 30-minute interaction. Observers then underwent a 4-hour feeding in which they were given 60g of the two flavoured foods of the combination they were assigned to. This procedure was repeated on day 2 for the second combination of flavoured foods.

### Histology

Histological processing was carried out as described previously in the General Procedure section.

### Ki67/DCX Immunohistochemistry

Immunohistochemical processing was carried out as described previously in the General Procedure section.

### Imaging and Quantification

Images were taken of coronal sections of the olfactory bulb using an Olympus FV100 confocal microscope at 10x and 40x magnifications. Within the olfactory bulb the main olfactory bulb (MOB) and accessory olfactory bulb (AOB) were imaged. One set of images was taken at 10x magnification to image the entire olfactory bulb and another set at 40x magnification to

image the granular cell layer. Additionally, images were taken only of the left hemisphere of each brain. Ki67- and DCX-positive cells were quantified using the software Fiji.

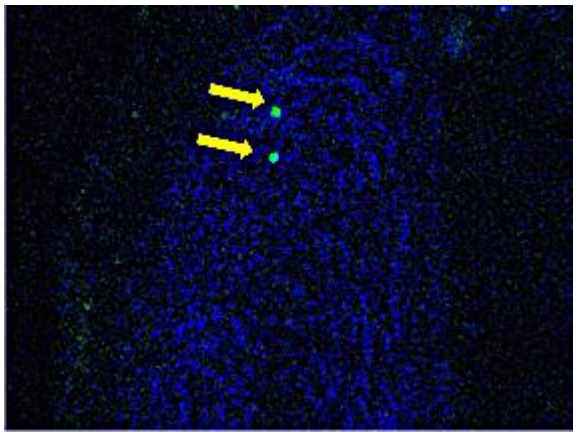


**Figure 11.** Coronal section of the upper region of the main olfactory bulb showing Ki67-positive cells (yellow arrows) taken at 10x magnification by Olympus FV100 confocal microscope.

## Results

### Behavioural Results

Across all combinations, GKs showed decreased consumption of both foods in comparison to Wistars (Figures 12 &13). As mentioned, all animals underwent two sessions of STFP. For the first combination (C-G) that all animals were tested on a t-test was conducted to examine the possible effects of strain. The results revealed a significant difference between GKs and Wistars ($t_{26.148}$=-10.656, $p < 0.05$). The next analysis compared total consumption between the food combinations that were used in the second session of STFP (C-N and M-A). A two-way ANOVA revealed a significant effect of strain ($F_{1,26} = 38.576$, $p <0.001$) and a significant effect of combination ($F_{1,26}= 7.646$, $p < 0.05$).

The second measurement taken was the percent of exposed food consumed, which was averaged across GKs and Wistars. Again, this measurement for each combination was analyzed.

GKs showed decreased mean consumption of the exposed food across all combinations (Figures 14 & 15). A t-test was once again run to analyze the possible effects of strain. An affect was seen for strain ($t_{18.716}$=-2.374, $p < 0.05$). For analysis of the remaining two combinations a two-way ANOVA revealed a main effect for strain ($F_{1,26}$=19.019, $p < 0.001$) and an interaction between strain and combination ($F_{1,26}$= 6.5100, $p < 0.05$).

For STFP to be considered successful, meaning a preference was established as a result of the interaction, animals had to consume more than 50% of the exposed food (Damphousse *et al.*, 2019). The percent of exposed food consumed was analyzed to determine whether it was significantly different from 50%. Again, different statistical tests were run for the different food combinations. For GK and Wistars, a t-test was run for each food combination, resulting in a total of 6 t-tests. First, a t-test was run to compare consumption of the exposed food for the C-G combination against 50%. The results of this test do not demonstrate a significant difference between GKs and the 50% baseline, $t_{15}$=-0.861, $p > 0.05$, indicating that STFP was not successful. For the C-N combination, no significant differences were found ($t_7$=-1.648, $p > 0.05$). Lastly, no significant differences were found for the M-A combination ($t_7$=-1.499, $p > 0.05$). The same t-tests were run for each combination for Wistars. Wistars showed the opposite results, with the percent of exposed food consumed for the C-G combination being significantly higher than 50% ($t_{14}$= -9.795, $p < 0.001$). For the next combination, C-N, Wistars again showed significant differences from 50% ($t_7$=-13.028, $p < 0.001$). For the last combination, these same results were found, ($t_5$=-4.604, $p < 0.01$).

Following the completion of STFP, all GK rats that were assigned as observers underwent a post-feeding test. The results of this test showed decreased consumption of the foods during STFP in comparison to the unflavoured food provided during this feeding (Figure

16). A t-test was conducted to compare the results of the post-STFP feeding. This t-test examined the effect of food type (flavoured VS non-flavoured) on levels of consumption. The results of this t-test demonstrated a significant difference between the amount of non-flavoured and flavoured food consumed, $t_{29.809}=2.59$, $p < 0.05$. GKs ate significantly more of their normal non-flavoured food than the flavoured foods provided to them during the observer feedings.

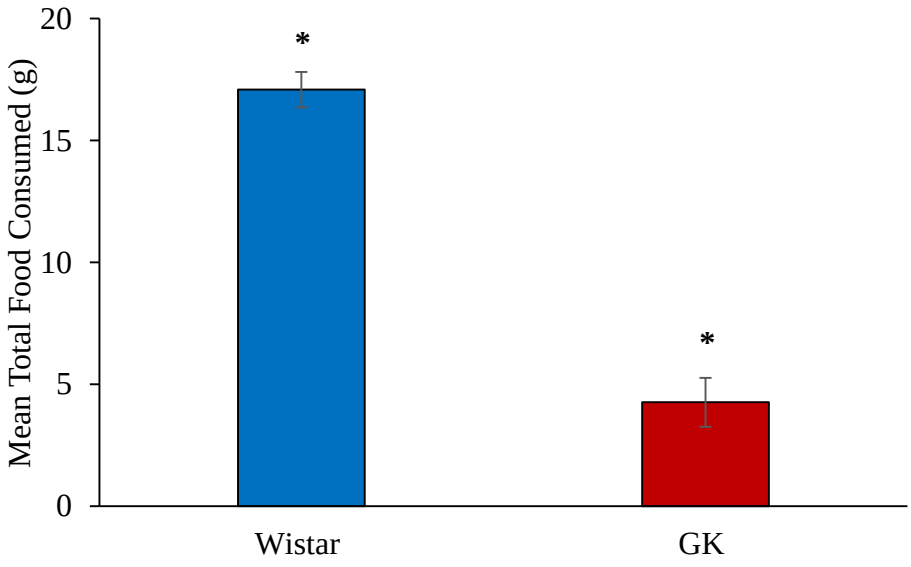



**Figure 12.** Mean total amount of food consumed (±SEM) by observer Wistar and GKs for the cumin-ground cloves combination. Significant decreases in mean total food consumed was observed in GKs ($p < 0.001$).

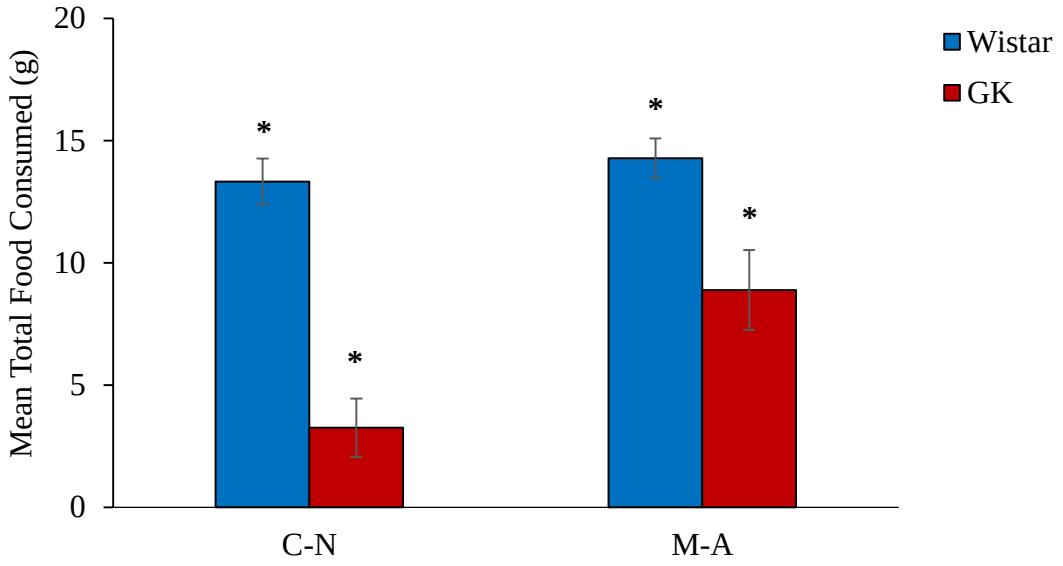



**Figure 13.** Mean total amount of food consumed (±SEM) by observer Wistar and GKs for cocoa-cinnamon and marjoram-anise combinations. Significant decreases in mean total food consumed was observed in GKs ($p < 0.001$). A significant effect was found for combination ($p < 0.05$)

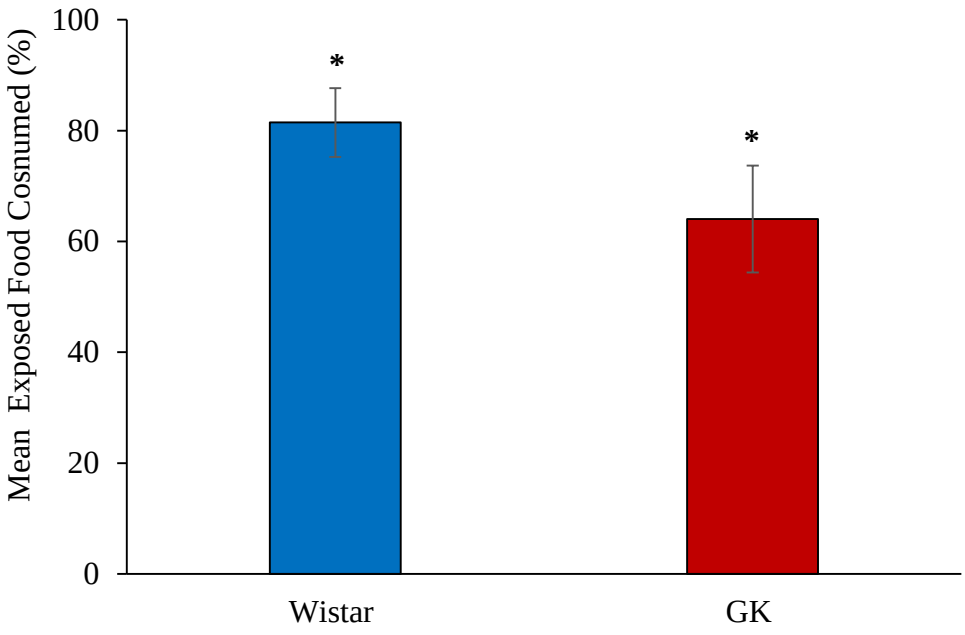



**Figure 14.** Mean percent (±SEM) of exposed food consumed by observer Wistars and GKs during food preference tests for the cumin-ground cloves combination. Mean exposed food consumed was significantly decreased in GKs ($p < 0.05$).

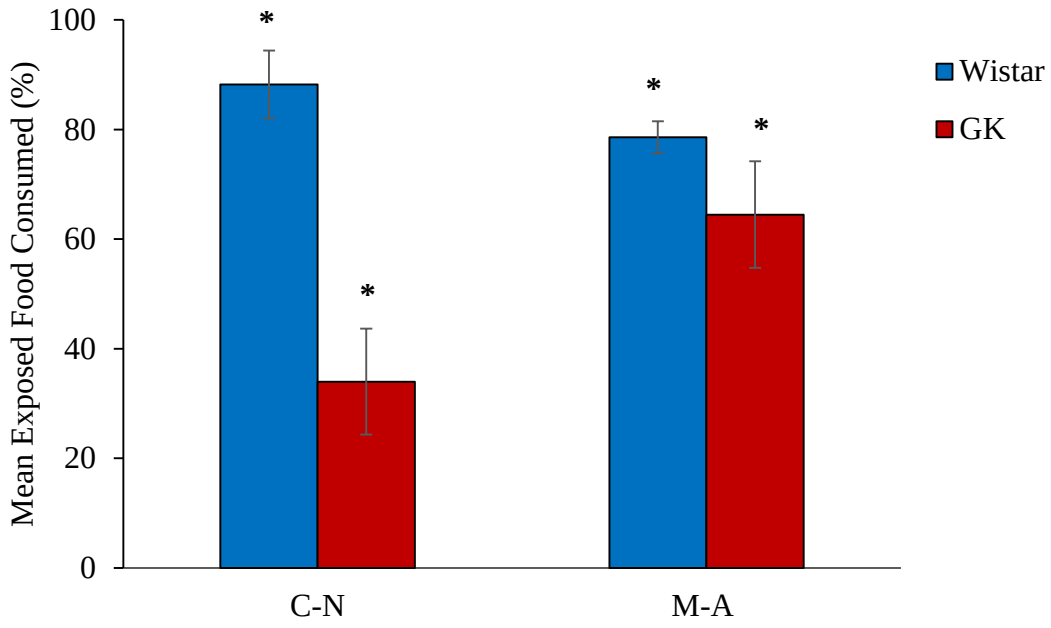



**Figure 15.** Mean percent (±SEM) of exposed food consumed by observer Wistars and GKs during food preference tests for the cocoa-cinnamon and marjoram-anise combinations. Mean exposed food consumed was significantly decreased in GKs ($p < 0.001$). An interaction was found between strain and combination ($p < 0.05$).

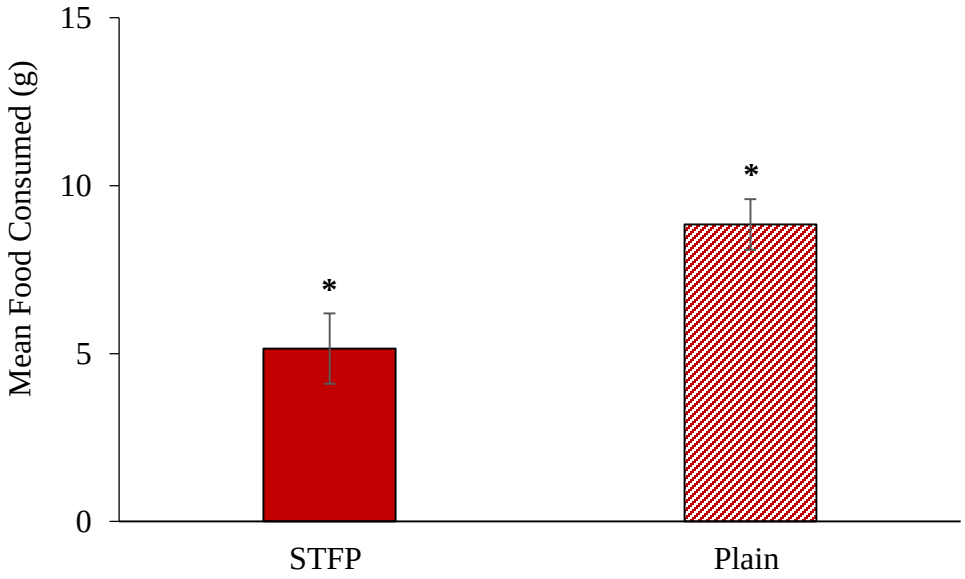



**Figure 16.** Mean amount of plain and flavoured food consumed (±SEM) by GKs. Significant increases in Mean plain food consumed was observed ($p<0.05$).

**Neurogenesis Results**

Due to unforeseen complications with tissue storage, only a portion of the olfactory bulb tissue was imaged and quantified. Due to this, there was not enough data to make reliable conclusions from the collected data. From the available data, cells that expressed DAPI were quantified (Table 2). This provided a measure of the total cell population within the olfactory bulb. To analyses these results, a t-test was run. No significant differences were found between GKs and Wistars ($t_{14.693}$=-1.419, $p > 0.05$). Ki67 counts were also taken from GKs and Wistars (Figure 17). From what was collected, the results demonstrated decreased Ki67-positive cells within the olfactory bulb of GK rats. These results are in line with what has been found in existing research. To further analyze the results, a t-test was run to investigate the effect of strain on cell counts. No significant differences were found between GKs and Wistars ($t_{22.265}$=-0.3804, $p > 0.05$).

| Strain | Mean | SD |
|---|---|---|
| Wistar | 459.626 | 220.643 |
| GK | 557.059 | 130.894 |

**Table 2.** Mean and standard deviations for the number of DAPI-positive cells within the olfactory bulb per um3. No significant differences were found between GKs and Wistars.

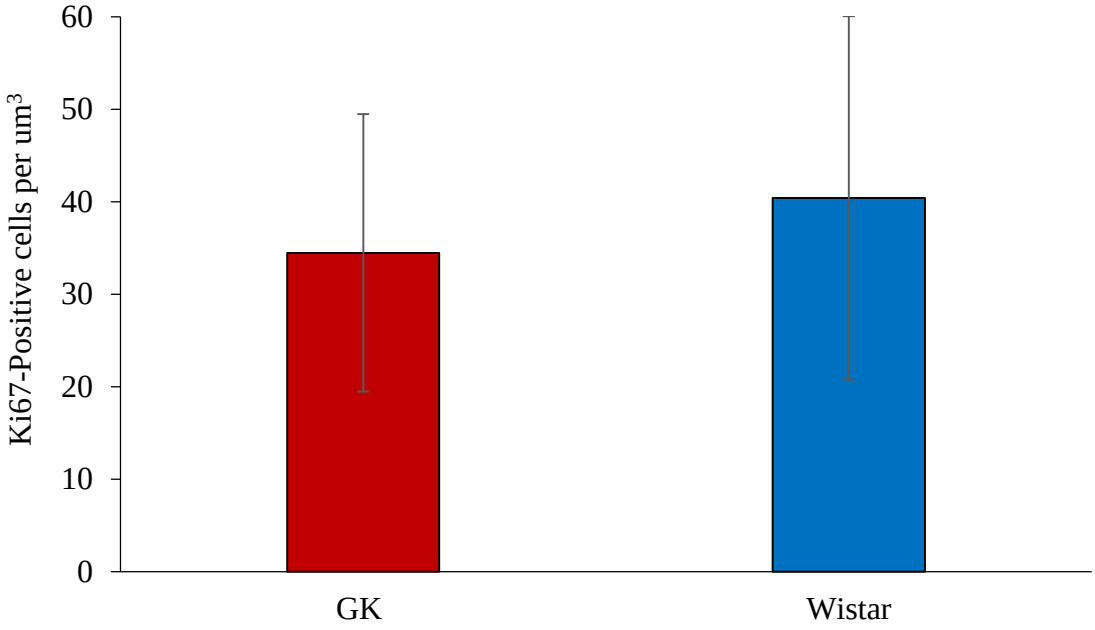



**Figure 17.** Mean number (±SEM) of Ki67-positive cells within the olfactory bulb per $\mu m^3$. No significant differences were found between Wistars and GKs.

## Discussion

The ability of GK rats to successfully transmit and perceive information about food preference was examined using STFP. It was hypothesized that GK rats would be impaired in their ability to establish a food preference on the basis of exposure to a demonstrator and this behavioural impairment would be accompanied by a decrease in neurogenesis. The results showed that Wistar rats are able to establish a preference on the basis of social learning, as demonstrated by their increased consumption of the flavoured foods in which they were exposed to only vicariously through a conspecific. Additionally, this increase was significantly higher than expected by random chance. These findings are consistent with existing research (Galef and Wigmore, 1983; Posadas-Andrews, 1983; Strupp & Levitsky, 1984). GKs were unable to establish a preference on the basis of social learning. GKs showed significantly reduced consumption of all food – that is, both the exposed and non-exposed foods combined. Beyond

the reduction in total food consumption, GKs also demonstrated significant reductions in the percentage of exposed food consumed. These reductions were significantly below Wistars and were at the 50% threshold. Therefore, the results show that GKs are unable to learn a food preference in this paradigm, consistent with the hypothesis that GK rats are impaired in establishing a food preference.

Precautions were taken to ensure the results were due to an inability to establish a preference on the basis of social learning. As mentioned, rats will naturally avoid all novel foods in the case that they may be dangerous. This avoidance is overcome when they detect novel foods on conspecifics as this indicates the food as safe to consume. When given a food they have been previously detected animals should consume significant amounts of this food, especially after being deprived (Barnett, 1958; Posada-Andrews & Roper, 1983). GKs did not demonstrate this as they showed significant reductions in the exposed food consumed. To ensure that this was due to an impairment in the ability to establish such a preference on the basis of social learning, all GKs that were previously assigned as observers were subjected to an additional feeding. During this feeding they were given their normal non-flavoured rat chow and left undisturbed for 4 hours. All rats were expected to show normal consumption, as it is not a novel food that they would normally hesitate to consume. GKs showed significantly higher consumption of their normal non-flavoured rat chow over the 4 hours in comparison to the flavoured food (Figure 7). These results indicate that GKs were still physical able to consume food and chose not to during their observer feeding due to the novelty of the foods. They were unable to detect the food on their paired demonstrator, thus unable to establish a preference for that food as a result of the interaction. Therefore, GKs suppressed their eating due to the fact that these foods still remained novel to them.

One consideration to be noted about the results is that the different combinations of flavoured foods that were used appeared to affect the results of this experiment. Three different combinations of flavoured foods were used in this experiment. All rats underwent their first STFP test with the cumin and ground cloves combination. To increase the number of observations, all animals underwent an additional round of STFP with a different food combination. This alternate combination was used for their second session of STFP because the preferences developed through STFP are known to last up to 30 days following testing (Galef and Whiskin, 2003). This same study found that rats can undergo STFP using one combination, but this will not affect performance using another combination of flavoured foods. This permits testing STFP multiple times without learning effects. For the second session, half of the animals underwent STFP with a food combination of cocoa and cinnamon and the remaining half with a combination of marjoram and anise. These combinations have been used previously for STFP successfully (Galef & Whiskin, 1992; Galef, 1993; Strupp & Levitsky, 1984). Despite these data, it is possible that the change in combination may have affected the results. Therefore, this effect was investigated. The results did find a significant effect of food combination when analyzing the total food consumed. Additionally, there was a significant interaction found between strain and combination in the analysis of the total percent of exposed food consumed. From the results collected, GKs demonstrate the least amount of food consumed when given the cocoa-cinnamon combination. With the remaining two combinations, they appear to show comparable amounts of total food consumed. This could mean that the cocoa-cinnamon combination may have been unpleasant for the GKs and therefore not be an effective combination to use in STFP. Therefore, it is suggested to test for equipalatibility in GKs. This

would ensure that the combinations known to be effective in non-hyperglycaemic animals, such as wistars, will also be effective in GKs.

Another consideration to be made is that the ability of rats to establish a food preference following social interaction has also been shown to depend on the hippocampus (Bessières *et al.,* 2017; Countryman *et al.,* 2005). The learning acquired in this task is known to result in long lasting memories, as supported in the by Galef and Whisken (2013) in which rats were still able to show a preference for a flavoured food up to one month following testing. In a review by Bessieres *et al.,* (2017), the involvement of remote memories in STFP was explored. To investigate this, animals are trained on a standard STFP protocol, such as the one by Galef *et al.,* (1983). They then received a preference test shortly following testing, as well as 30 days following testing. These different time points capture both short-term and long-term memory. The hippocampus is known to be involved in the formation of long-term memory (Countryman *et al.,* 2005). Therefore, the results from the mentioned experiment can provide further information about the proposed involvement of the hippocampus in STFP by analyzing performance at the two different time points. These principles were applied by Countryman *et al.* (2005), but this experiment went further by suppressing c-Fos within the hippocampus. To begin, assigned observer rats were administered with a hippocampal antisense that resulted in the suppression of c-Fos. Demonstrator rats were fed cocoa and placed into an interaction with its paired observer. The observer was given cocoa and one of two other flavoured foods, either cinnamon or ground ginger, and left undisturbed. Following this both foods were measured to determine the amounts of each consumed. This test investigated both short-term memory and long-term memory. To test short-term memory, rats were provided with cocoa and cinnamon and given a preference test immediately after the interaction. To investigate long-term memories, rats

underwent an additional preference test 14 days later. In this test they were given a choice between cocoa and ground ginger and left undisturbed. Both were then measured to determine the amount of each consumed. Their tissue was then processed and the entire hippocampus (CA1, CA3, and DG) was stained for c-Fos. These cells were then quantified to provide a measure of activity within the hippocampus. The inactivation of c-Fos had non-significant effects on short-term memory as seen by the significant increased consumption of the exposed food in comparison to the level of chance (50%). With regards to long-term memory, significant impairments were found following c-Fos inactivation. Animals demonstrated non-significant differences in the consumption of the exposed food compared to chance (50%). Therefore, this experiment provides evidence that the hippocampus is required for the long-term memory regarding socially-transmitted food preferences.

With this information on the contribution of the hippocampus, further analysis may need to be conducted to validate the task as one dependent on the olfactory bulb. Future experimentation could test animals on other tests of olfaction in addition to STFP. There are a wide variety of tasks involving the olfactory bulb and olfactory system that can be implemented in this experiment. For instance, the experiments by Moreno *et al.* (2009) and Xu *et al.* (2000) could be interchanged with the STFP procedure to test for cognitive impairments within the olfactory bulb. Moreno *et al.* (2009) measured mice on a habituation-dishabituation task following the ablation of neurogenesis. An osmotic pump was implanted in the SVZ in order to administer cytosine arabinoside, a mitotic blocker, to prevent cell proliferation. All mice were then exposed to a combination of two novel odours, which they were eventually habituated to. They were then presented with another novel combination, but were not habituated to this one. Mice were recorded on the time spent investigating the familiar and novel combinations. Under

normal circumstances, animals are expected to show a preference for the novel combination as shown by their increased investigation time of this combination. This was seen in control mice that were not impaired in any way. Those with ablated neurogenesis showed non-significant differences in the amount of times spent investigating each combination, thus demonstrating no preference for a specific combination. These results are in line with others that have used the same task (Gheusi *et al.,* 2000; Sanchez-Andrade *et al.,* 2005). From existing literature surrounding the role of the olfactory bulb, it was assumed that this task would require the input of the olfactory bulb to distinguish between familiar and novel odours (Gheusi *et al.,* 2000; Mori *et al.,* 1999). Therefore, ablation to neurogenesis is suggested to affect the functioning of the olfactory bulb. This in turn demonstrates that the olfactory bulb is involved in the odour discrimination required in this task. Another task that could be used is one by Xu *et al.* (2000) in which rats were presented with varying odours and the resulting activity patterns within the olfactory bulb were investigated. The resulting images revealed changing activity in the olfactory bulb as a result of varying exposure times and concentrations of the same odour. These images provide evidence for the involvement of the olfactory bulb in encoding information about different properties of odours. Together, both these studies reliably test the role of the olfactory bulb in odour discrimination and can be used to further validate the use of STFP in this experiment as a way to test olfactory bulb function.

Impairments in neurogenesis have been previously seen in the olfactory bulb in animals with hyperglycaemia, such as GK rats (Beauquis *et al.,* 2009; Lang *et al.,* 2009; Ramosa, Rodrigues *et al.,* 2014). During the proliferation phase, GK rats have demonstrated significant increases in proliferation within the SVZ. Once these newly generated cells begin to progress into the next stages of neurogenesis, they start to show significant decreases in survival in

comparison to Wistars. The expected results for the current experiment were expected to match these ones found previously. Ki67 and DCX were used to provide a measure of cell proliferation and survival. GKs were expected to show significantly increased ki67 expression and decreased DCX expression. Therefore, demonstrating increased proliferation and decreased survival in comparison to Wistars. Due to unforeseen circumstances the majority of the olfactory bulb tissue could not be processed. From what was processed, a decrease in cell proliferation was found in GKs, but this difference was not meaningful. With further analysis of this tissue, the data could provide the expected results that have already been seen in the discussed literature. From the behavioural results collected in this experiment, and the significant differences in performance on the task, the STFP procedure used in this experiment demonstrate reliability. To further validate this task, additional measurements of olfaction can be used additionally to ensure STFP is an appropriate measure of olfaction.

**Conclusions**

This chapter focused on the olfactory bulb, a region in which neurogenesis is thought to be critical for its proper function. The social transmission of food preference and histological processing were combined to provide potential evidence for the effect of hyperglycaemia on learning dependent on regions associated with neurogenesis. The behavioural results provide clear evidence for impairments on this task. Under normal circumstances, rats are able to transmit information about food preferences between one another. GK rats were unable to establish a preference for a food in which they were exposed to following an interaction with a conspecific. These results suggest a clear behavioural impairment that is associated with the presence of hyperglycaemia. The histological data that was collected demonstrated decreases in

cell proliferation, as measured through Ki67. For now, the current experiment provides clear behavioural deficits on a task that may be dependent on regions involved in neurogenesis.

## Chapter 5: General Discussion

The purpose of the current experiment was to explore the effect of type 2 diabetes on neurogenesis and learning. Based on existing research, it was hypothesized that GK rats will demonstrate impairments on both a delayed non-match to position in the radial arm maze and in STFP GK rats demonstrated impaired latencies and decision-making over the course of the 2 testing days in the radial arm maze, and were unable to establish a food preference following an interaction with another rat that had an established preference, which is uncharacteristic of normal functioning animals (Galef and Wigmore, 1983; Posadas-Andrews, 1983; Strupp & Levitsky, 1984). Together, these results demonstrate that there are clear impairments on these tasks that have been functionally linked to regions that have large quantities of neurogenesis. In terms of neurogenesis, the results collected from observing and quantifying images of the olfactory bulb do not demonstrate significant alterations in either phase of neurogenesis. For now, the results demonstrate impairments in GKs on the behavioural tasks used in the current experiment.

The current thesis provides support for the use of animal models. In investigating type 2 diabetes, researchers are limited in their use of human models. This can be due to a variety of issues, whether genetic or ethical (Cefalu, 2006). There are many factors that influence type 2 diabetes, both genetic and environmental. Examples include: diet, physical activity, family history, etc. In research involving human patients, subjects can be carefully screened to choose those that best encompass the disease, but all potential factors are difficult to be controlled for (Bertram & Hanson, 2001; Cefalu, 2006; Manschot *et al.*, 2006; Moran *et al.*, 2013). In research

surrounding type 2 diabetes, animals, such as mice and rats, are selectively bred to express specific characteristics of diabetes. These include obesity, beta cell mass, insulin deficiency, insulin resistance and several more (Bertram & Hanson, 2001). This allows researchers to further understand the genetic underpinning within these animals than would be possible in humans. Additionally, the various environmental factors known to influence diabetes can also be controlled for. Diets can be altered and physical inactivity can be prevented by providing animals with regular exercise (Bertram & Hanson, 2001; King, 2012). Another limitation in human research is the disease progression. Type 2 diabetes can take several years to develop and requires many resources, whereas in animals the progression of the disease is significantly shorter than that for humans, typically months (Cefalu, 2006). This makes the investigation of type 2 diabetes much more efficient for researchers. These issues with human patients have led to the use of animal models. Animal models allow researchers to further investigate the disease under what seem to be more optimal conditions. The goal of which is to understand the underlying mechanisms and testing therapeutic treatments (Sajid Hamid Akash *et al.*, 2013, Cefalu, 2006). With animal models, researchers have increased control around the different properties of type 2 diabetes. In the current experiment, GK rats were used to model glucose intolerance animals to investigate the potential impairments associated with type 2 diabetes. The goal of which is to apply the findings to human patients. This experiment does not only provide support for animal models, but also provides support for the use of the Goto-Kakizaki rat. From the literature review, there appeared to be very little research involving the use of GK rat to study the possible impairments associated with type 2 diabetes. The current experiment adds to the literature by providing additional tasks that can be used to test such impairments. Research involving the social transmission of food preference has not yet been applied to type 2 diabetes

and hyperglycaemia. As discussed in previous chapters, with validation from additional tests of olfaction, this task could be utilized as a test of impaired olfactory bulb functioning associated with type 2 diabetes.

One consideration to be noted deals with the glucose levels of the GK rats. In experiments involving type 2 diabetes and hyperglycaemia, glucose tolerance tests are typically conducted (Goto & Kakizaki, 1975; Andrikopoulos *et al.,* 2008). Glucose tolerance tests are a way to measure blood glucose levels and can be used to determine whether an individual is hyperglycemic. This is done by administering rats a glucose solution, whether orally or intraperitoneally, and collecting blood samples at various time points following administration. These blood samples provide a measure of the blood glucose levels (Goto & Kakizaki, 1975; Andrikopoulos *et al.,* 2008). In the current experiment, glucose tolerance tests were not conducted. From existing research, it was assumed that all GKs would demonstrate glucose intolerance once testing began, which was at approximately three to four months of age. GKs begin to develop glucose intolerance at an early age, typically as early as one month following birth (Sajid Hamid Akash *et al.,* 2013; Goto & Kakizaki, 1975; Moreira *et al.,* 2007; Lang *et al.,* 2009). Conducting glucose tolerance tests would validate the presence of glucose intolerance in all GKs at the time of testing and potentially reveal inconsistencies across animals. These data may permit stronger conclusions to be drawn regarding the effect of glucose intolerance on neurogenesis and learning. For future research involving any model of type 2 diabetes, a glucose tolerance test is recommended as an added precaution.

Another consideration to be made involves the association between neurogenesis and learning. Among research involving neurogenesis there existed debate regarding the relationship between learning and neurogenesis. One side argued that learning enhances neurogenesis within

the hippocampus and olfactory bulb (Gould *et al.*, 1999; *Alonso et al.*, 2006). On the other hand, it was argued that successful performance on such tasks depends on neurogenesis. Experimental evidence has supported this argument through the manipulation of neurogenesis through exercise (van Praag *et al.*, 1999; Sahay *et al.*, 2011). To conclude, these experiments provide evidence for both arguments concerning the relationship between neurogenesis and learning. As a precaution, the current experiment can only assume that the presence of a glucose intolerance influenced the relationship between neurogenesis and learning, but the experimental evidence cannot indicate any causal relationship.

Direct manipulation of neurogenesis can be conducted to further investigate the relationship between neurogenesis and learning. In this experiment the levels of neurogenesis within the dentate gyrus and olfactory bulb are explored, but not manipulated in anyway. By upregulating neurogenesis in the hippocampus and olfactory bulb, researchers can look at whether there is an effect on behavioral performance. The use of drugs and exercise are two methods that have been proposed to increase levels in neurogenesis (Malberg *et al.*, 2000; van Praag *et al.*, 1999). For instance, administration of anti-depressant drugs has shown positive effects on the levels of neurogenesis within the hippocampus (Malberg *et al.*, 2000). Using a variety of classes of anti-depressants, this experiment demonstrates that continuous administration of each one significantly increases the number of proliferating and surviving cells within the dentate gyrus, as visualized through the use of BrdU labelling. These results provide evidence for the effectiveness of anti-depressant drugs in increasing proliferation and survival within the dentate gyrus. As mentioned, exercise has been found to increase overall levels of neurogenesis within the dentate gyri of mice (van Praag *et al.*, 1999). Mice that were provided a running wheel in their homecage showed increased proliferation and survival in comparison to

those with no such addition to their homecages. To relate this to learning, these animals were also tested on the Morris water task, a test of spatial memory. Animals that had access to running wheels in their homecages showed significantly improved performance on the task. From these results it can be suggested that the upregulation of neurogenesis within the hippocampus through exercise contributed to their performance on the Morris water task, a hippocampus-dependent task. In further investigation of the potential influence of neurogenesis on learning these are two methods that can be utilized.

To statistically support the above statement about the nature of the relationship between neurogenesis and learning, a correlation can be conducted. In the majority of the research discussed in the chapters of this thesis, correlational tests were conducted to investigate the relationship between the different variables measured (Gold *et al.,* 2007; Lang *et al.,* 2009; Ramos-Rodriguez *et al.,* 2014). If all the histological results were collected, the relationship between the behavioural and neurogenesis could have been further investigated through a correlation. This would provide further information on whether the presence of type 2 diabetes did in fact affect this relationship and how. For the purpose of this experiment, running a correlation was not possible because of the complexity of the data, as well as missing data. Future research on this topic should consider conducting a correlational analysis to ensure accurate conclusions can be made about the results collected.

**Conclusions**

To conclude, the current thesis aimed to investigate the potential influence of hyperglycaemia associated with type 2 diabetes on the relationship between hippocampus and olfactory-dependent learning and neurogenesis. Using the GK as a model, experimental evidence sheds light upon the learning impairments observed in the presence of type 2 diabetes. Further

histological analysis can be done in the hopes of observing the expected impairments seen in existing research.

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