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Effects of Body Temperature and General Anesthetics on Intraocular Pressure in Rats

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Effects of Body Temperature and General Anesthetics on Intraocular Pressure in Rats

by

Aditi Pillai

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biomedical Engineering
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DEDICATION

I dedicate this work to my family. Their constant support and encouragement have helped me follow my dreams.
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I would like to thank Dr. Chris Passaglia, my thesis advisor and major professor, for his time, guidance, patience and constructive feedback to ensure my success. I would also like to acknowledge my lab teammates for all the time and effort they put into assisting me. Thank you for all your support.
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ABSTRACT

Ocular hypertension has been identified as the fundamental risk factor in glaucoma which is the leading cause for irreversible blindness in the world. Understanding the different factors that affect IOP is of utmost importance in clinical management as IOP is considered as the fundamental factor in assessing the efficiency of glaucoma medications. Several studies have attempted to assess factors that could affect IOP including age, body position, blood pressure, anesthetics commonly used during eye operations, etc. However, in most of these studies IOP is measured under anesthesia using rodent models and these anesthetics could affect the IOP measurements directly or indirectly. The use of tonometry in such experiments also includes certain limitations like acquiring IOP at discrete moments in time, human error while handling the instrument and stress induced spikes in IOP while handling awake animals. This study uses a wireless continuously monitoring device to eliminate these limitations while also acquiring IOP at a higher rate.

Anesthesia induction is known to lower body temperature. However, previous studies on the effects of various anesthetic agents fail to take into account this drop in body temperature which could potentially lead to erroneous results. This thesis focuses
on studying the effects of two commonly used anesthetic agents, isoflurane and ketamine while accounting for loss in body temperature. The effects of changing body temperature on intraocular pressure was also studied to help understand the effects of these factors accurately. There was a statistically significant drop \( p<0.001 \) in intraocular pressure post isoflurane induction with no heat support across several animals. The addition of heat support in the next set of experiments resulted in an almost steady pressure throughout the experiment. Since the body temperature was maintained constant throughout the experiment, there was no statistically significant difference \( p>0.05 \) among IOP’s for the awake and anesthetized condition. This conclusion was then confirmed by obtaining a direct effect of changing body temperature on IOP. There was a rise in IOP while the animal was placed on a 42 degree Celsius heating pad and a drop in IOP while the animal was placed on a 20 degree Celsius surface with no heat support. The corresponding changes in body temperature were confirmed using a rectal thermometer. There were no significant changes in the IOP measured by the sensor while measuring pressure with the iCare tonolab. Applanation tonometry however produced an average mean intraocular pressure increase of \( 2.11 \pm 1.62 \) mmHg.
CHAPTER 1:
INTRODUCTION

1.1 Background

An estimated 11.1 million people will suffer from blindness due to primary glaucoma by the year 2020 (Quigley, HA et al. 2006). This makes glaucoma the leading cause for irreversible blindness worldwide. Glaucoma may be asymptomatic until a later stage; thus, it’s important for us to study the pathophysiology of the disease for early diagnosis and treatment. Glaucoma is characterized by progressive damage to the eye’s optic nerve leading to retinal ganglion cell death. Although several risk factors have been identified to induce the onset of glaucoma, elevated intraocular pressure (IOP) is the most recognized and documented risk factor as the extent of retinal ganglion cell damage is closely associated with the extent of intraocular pressure elevation (Guo, L, et al. 2005). While research is underway to study and improve the understanding of glaucomatous changes, challenges arise in producing, maintaining and measuring intraocular pressure in animal models.

Intraocular pressure is determined by the rate of aqueous humor production and the rate at which it exits the eye. IOP is necessary to inflate the eye and maintain the shape
and optical properties of the globe (Goel, G Picciani et al. 2010). Aqueous humor is produced by the ciliary body and exits the eye via two possible outflow pathways; the conventional pathway includes the trabecular meshwork and the unconventional pathway includes all the pathways other than the trabecular meshwork such as the uveoscleral pathway (Goel, G Picciani et al. 2010). Thus, an increase in IOP is caused due to lack of effective drainage via these pathways. As the eye is unable to drain out the excess fluid from the anterior chamber over time, pressure rises. In the case of angle closure glaucoma, this pressure rise is rapid and large as compared to open angle glaucoma where the rise in intraocular pressure is gradual and subtle. In the case of open angle glaucoma, there is an increased resistance to outflow via the trabecular meshwork (Abu-Hassan, Diala W, et al. 2014).

Intraocular pressure can cause mechanical stress and strain on the posterior structures of the eye, such as the lamina cribosa and adjacent tissues (Quigley, H A, et al. 1981). The sclera is perforated at the lamina where the optic nerve fibers i.e. the retinal ganglion axons exit the eye. As a result, IOP induced stress and strain can cause deformation, compression and remodelling of the lamina cribosa with subsequent mechanical axonal damage and axonal transport that disrupts the retrograde delivery of the essential trophic factors to the retinal ganglion cells from their brainstem target (Fechtner, R D, and R N Weinreb. 1994; Burgoyne, C F, et al. 2005). Disrupted axonal transport occurs early in the pathogenesis of glaucoma and similar ultrastructural changes in optic nerve fibers are seen in the post mortem of human eyes that have glaucoma (Quigley, H A, et al. 1981).
In order to understand glaucomatous conditions, elevated IOP animal models are studied. These models aim to recreate the disease to attempt to establish the behaviour and possible treatment routes. The established methods of achieving elevated intraocular pressure involve intracameral injections of a viscous solution of polystyrene beads (Smedowski, Adrian, et al. 2015), laser photocoagulation of the trabecular meshwork (Yun, Hongmin, et al. 2014), or using a hypertonic saline solution to scleros the aqueous humor outflow pathways (Jia, L, et al. 2000). While effective at producing elevated IOP, there are still incidents of failure as these methods are subject to the natural IOP fluctuations that happen in an eye.

1.2 Motivation

There are numerous physiological factors that can contribute to fluctuations in intraocular pressure. These fluctuations, long-term or short-term could cause severe damage to the eye’s optic nerve thus giving rise to a host of other diseases including neurological disorders. These factors also pose a serious issue in clinical management since the level of intraocular pressure is one of the most fundamental ways clinicians use as an indicator to measure the efficacy of glaucoma medications.

Due to the importance of studying and understanding the dynamics of IOP, there are numerous studies that have been conducted so far that have attempted to recognize the effects of some of these factors on intraocular pressure. There are studies that draw a very close correlation between intraocular pressure and both systolic and diastolic pressure (Klein, B E, et al. 2005). Some studies have looked into the effects of transient
physiological factors like exercise and have deduced that exercise can lead to reduced intraocular pressure (Yan, Xiaoqin, et al. 2016). Several studies indicate that pharmacological agents like anaesthetics can affect IOP as well, depending on the anaesthetic agent used (Ding, Chun, et al. 2011). Previous studies report that isoflurane produces a significant reduction in IOP (Mirakhur, R. K., et al. 1990, Ding, Chun, et al. 2011). Ketamine is one of the most widely used dissociative anaesthetics that works by primarily blocking the N-methyl D-aspartate receptor. Since it is routinely used as a procedural sedation in children, several studies have attempted to identify the effect of this drug on IOP. Some studies suggest that ketamine elevates IOP (Yoshikawa, K, and Y Murai. 1971), whereas some report no effect (Peuler, Martin. 1975; Drayna, Patrick C., et al. 2012; Blumberg, Dana, et al. 2006). While research so far look into the direct impact of anaesthetic agents on the intraocular pressure, there are no studies to our knowledge that attempt to do so while maintaining the body temperature constant. This is crucial while performing experiments in order to avoid the effects of reduced body temperature on intraocular pressure.

Measuring IOP in an animal model is challenging. Many current protocols require the animals be anesthetized to allow for accurate tonometric readings to be taken. There has been some work into developing a method of measuring IOP in animals that are awake. This involves the production/modification of Goldman applanation tonometer (Cohan, and Bohr DF. 2001). However, this method has major flaws as well due to the fact that it can measure IOP only at discrete moments of time and has the added disadvantage of operator error. In order to recognize the onset and development of
glaucoma in a more appropriate and extensive method, we need to use technology that gives us comprehensive IOP data over time.

Recently, there has been the development of a novel direct pressure monitor that serves the dual function of regulating intraocular pressure as well. This device has been shown to accurately measure IOP for months without altering ocular physiology (Bello, S A, et al. 2016). The ability to accurately and directly measure IOP, then to actively control the IOP at a set point has the potential to dramatically reduce the incidence of blindness secondary to glaucoma.

1.3 Aims and Objectives

This thesis aims to study factors that affect intraocular pressure while overcoming the limitations of current IOP studies. The study was focussed on determining the effects of two commonly used anaesthetic agents, isoflurane and ketamine.

The first part of the study included anaesthesia induction without accounting for body temperature and found the results to be in agreement with previously done studies. The second part of the study attempted to repeat the experiments while maintaining the body temperature constant. The results obtained were contradictory to the first study. In order to confirm the conclusion that body temperature has a direct effect on IOP, a third study was performed to determine the effects of temperature on intraocular pressure. A rebound tonometer (iCare Tonolab) and an applanation tonometer (Tonopen XL were
used to measure IOP in anesthetized rats to measure the effect of possible discomfort of the tonopen action on the corneal surface of the eye.

In this study, we used Brown Norway rats for all the experiments due to their genetic and physiological similarities to humans.
CHAPTER 2:
MATERIALS AND METHODS

2.1 Animals and Cannula Implantation

Adult male Brown Norway rats weighing 300-400g were used for this study. Animals were used in accordance with the ARVO statement for the Use of Animals in Ophthalmic and Vision Research and NIH guidelines. The use of animals in this study was approved by The Institutional Animal Care and Use Committee (IACUC), University of South Florida. The rats used in this study were anesthetized with an intraperitoneal (IP) bolus of ketamine (75 mg/kg) and xylazine (7.5 mg/kg) along with supplemental IP injections of (25 mg/kg) of ketamine given during the duration of the implantation procedure. The body temperature of the rats was maintained by resting the animal on a thermal pad and a pre-emptive subcutaneous bolus of carprofen (5 mg/kg) was given to the rats for post-operative pain.

The scalp was cut along the midline and the head mount was affixed to the skull. The device was then attached to a rodent jacket that the animal wore for the duration of the experiment. A sterile catheter filled with artificial aqueous fluid was tunnelled under the skin from the device, guided out a hole made in the conjunctiva, inserted into the anterior chamber, and secured to the sclera with nylon sutures and surgical glue. Wound
edges are closed over the implanted device with nylon sutures and secured with surgical glue. The animal was then allowed to wake from anaesthesia, and returned to the housing. The animal was inspected daily and for the first-postoperative week it was given carprofen (5 mg/kg) every 12 hrs for the first 96 hrs. A drop of 1% prednisolone acetate and 1% cyclopentolate were instilled in both eyes every 12 hrs as needed for up to 5 days to resolve inflammation and relieve miosis, respectively. Any signs of pain or infection were referred to the veterinarian and, if the situation could not be managed, the animal was euthanized with carbon dioxide.

2.2 Measurement of Isoflurane Effects on IOP

The intraocular pressure of the animals was monitored beginning 10 minutes prior to induction to establish a baseline. Each animal was placed in an induction chamber connected to a scavenger-equipped isoflurane vaporizer set at a flow rate of 3% and 2L/minute of oxygen (with or without heat support). The animal was left in the chamber for 10 minutes, at which point the animal was then moved to a heating pad outside the induction chamber. Anaesthesia was continuously maintained via nose cone. Temperature was monitored using a rectal thermometer and was maintained constant. Once the animal was stable on the heating pad, the anaesthetic depth was varied by changing the percent dosage of isoflurane every 10 minutes. The scheme by which anaesthesia was changed was: 1%/3%/5%. Anaesthesia was varied in this manner for no longer than 1 hour. The animal was then allowed to wake from anaesthesia and was monitored to detect any adverse effects to the sedation.
2.3 Measurement of Temperature Effects on IOP

The intraocular pressure of the animals was monitored beginning 10 minutes prior to induction to establish a baseline. Each animal was placed in an induction chamber connected to a scavenger-equipped isoflurane vaporizer set at a flow rate of 3% and 2L/minute of oxygen. Upon anesthetization, the animal was then placed on a heating pad set to 42 degree Celsius or a cold surface measured at ~20 degree Celsius. Temperature was continuously monitored via a rectal thermometer. Anaesthesia was maintained by a nose cone with an isoflurane flow rate of 3% at 2L/min of oxygen. Temperature was varied by moving the animal between the two pre-established surfaces for ten minute periods each. IOP was measured continuously while varying the temperature. Upon conclusion of temperature variation, the animal was allowed to wake from anaesthesia and was monitored to detect any adverse effects to the sedation.

2.4 Measurement of Ketamine Effects on IOP

The intraocular pressure of the animals was monitored beginning 10 minutes prior to induction to establish a baseline. Each animal was placed in an induction chamber connected to a scavenger-equipped isoflurane vaporizer set at a flow rate of 3% and 2L/minute of oxygen (with heat support). The animal was left in the chamber for two to three minutes and then moved outside to a heating pad and allowed to wake up. An intraperitoneal (IP) bolus of ketamine (75 mg/kg) was given as the animal was waking up. Body temperature and IOP was monitored throughout the experiment until animal was awake. The animal was monitored to detect any adverse effects to the sedation.
2.5 Measurement of Tonometry Effects on IOP

A rebound tonometer (Tonolab iCare) and an applanation tonometer (Tonopen XL) was used to measure the intraocular pressure non-invasively and verify the results obtained via the implanted sensor. The animal was placed under anaesthesia using an isoflurane induction chamber set at a flow rate of 3% and 2L/min of oxygen. Upon anesthetization, the animal was removed from the induction chamber and placed on a heating pad set to 42 degree Celsius. Anaesthesia was maintained at the same rate throughout the experiment via nose cone. Once the IOP stabilized and remained constant, the rebound tonometer and the applanation tonometer was used on the animal to study effects.

2.6 Data Analysis

Off line data analysis and statistical analysis of the IOP recordings were carried out on a PC computer using the software Systat SigmaPlot. Student’s t-test and analysis of variance (ANOVA) was used to test the intraocular pressures across awake and anesthetized conditions with a significance level of 0.05.
3.1 Effect of Isoflurane on IOP

Awake IOP's of all the animals ranged from 10-22 mm Hg. The first set of experiments were performed without heat support in the isoflurane induction chamber which resulted in a drop in intraocular pressure as seen in the figure below. Fig 3.1 displays the intraocular pressure measured by the sensor and the body temperature measured by the rectal thermometer over time while changing the percentage dosage of isoflurane being administered to a single animal.
Figure 3.1 Effect of isoflurane on IOP on single animal with no heat support. The IOP measured by the sensor and body temperature measured by the rectal thermometer is plotted over time for a single animal. The isoflurane dosage administered is changed throughout the course of the experiment.
This experiment was then repeated across several animals and was found to show similar results. The awake IOP for each animal was calculated prior handling to avoid stress induced spikes in intraocular pressure. The percentage dosage was varied every 10 minutes and the mean IOP’s at each concentration dosage was calculated. As IOP was measured with no heat support during isoflurane induction, there was a significant reduction (p<0.001, t-test) in intraocular pressure across all isoflurane dosages compared to the awake IOP as seen in figure 1.

The lack of heat support during isoflurane induction resulted in a drop in body temperature which subsequently lead to the drop in eye pressure. On an average, the IOP decreased by 38.77% when there was no heat support during isoflurane induction. Fig 3.2 plots the mean and standard deviations of the measured IOP’s at all four conditions i.e. awake, 1%-3%-5% isoflurane at 2L/min of O2. Each of the means for all the conditions were calculated for a period of ten minutes.
Although there was a significant drop in intraocular pressure when anesthetized with isoflurane, the eye pressure stayed steady throughout the rest of the experiment while increasing the dosage on isoflurane being administered. In order to identify if the drop in intraocular pressure is dose dependant, the change in IOP measured for each percentage dosage of isoflurane was averaged across all the animals. Fig 3.3 plots the mean and standard deviations of the change in intraocular pressure measured across the three different percentage dosages across all animals.
The experiment was then repeated with the added heat support in the induction chamber and found to have no significant drop in the intraocular pressure post isoflurane induction ($p > .05$). The animals were placed on heat support during the entire duration of the experiment and body temperature was maintained constant. This method proved to be useful to avoid the sudden drop in body temperature during isoflurane induction. Fig 3.4 illustrates the effects of changing the isoflurane dosage administered on a single animal with heat support.
Figure 3.4 Effect of isoflurane on IOP on single animal with heat support. The IOP measured by the sensor and body temperature measured by the rectal thermometer is plotted over time for a single animal. The isoflurane dosage administered is changed throughout the course of the experiment.
The experiment was repeated on the same animal and was found to have similar results. The difference in the awake IOP’s for the same animal could be due to the difference in the time of the day that the experiments were performed. Diurnal variations and natural fluctuations in IOP due to various factors like diet, fluid intake, variations in systemic blood pressure, etc. cause the same animal to have variations in IOP. There was no statistically significant difference between awake and anesthetized IOP. Fig 3.5 illustrates the mean and standard deviations of awake IOP and the anesthetized IOP across different days on the same animal.

![Graph](image)

Figure 3.5 Effect of isoflurane on IOP with heat support across trials. Each bar represents the mean IOP calculated for a duration of 30 minutes. Error bars give the standard deviation.
The experiment was then repeated for several animals and was found to have similar results. Adding heat support to the experiments and maintaining body temperature resulted in the intraocular pressure staying steady. There was no drop in eye pressure and isoflurane had no effect. This can be explained as the lack of drop in body temperature as seen in the first experiment.

There was no statistically significant difference (p>0.05) among the IOP’s for the three anesthetized conditions thus indicating that the change in IOP is not dose dependant. Fig 3.6 plots the mean and standard deviations of the change in intraocular pressure measured across the three different percentage dosages across several animals.
Figure 3.6 Change in IOP across isoflurane dosage with heat support. Each bar represents the mean change in IOP measured at each percentage dosage of isoflurane across several animals. Error bars give the standard deviation.

While performing the above experiments, there was notable rise in intraocular pressure in all the animals while being handled during transport from the cage to the isoflurane chamber while awake. This significant increase (p<0.001) in intraocular pressure can be explained as a stress effect. The figure below plots the mean intraocular pressure measured before the animal was handled and the corresponding mean
intraocular pressure while the animal was being transported into the isoflurane induction chamber.

Figure 3.7 Effect of stress on IOP across animals. Each bar represents the mean intraocular pressure calculated for a duration of two minutes. Error bars give the standard deviation.
3.2 Effect of Temperature on IOP

Intraocular pressure and body temperature were measured throughout the experiment while the animal was varied between the two surfaces. There was a significant drop in the intraocular pressure when the animal was placed on a cold metal surface measured at 20 degree Celsius. This was due to the resultant drop in body temperature as measured by a rectal thermometer. Similarly, the intraocular pressure increased while the animal was placed on a heating pad due to the resultant increase in body temperature. The animal was placed on each surface for 10 minutes each. Fig 3.8 illustrates the effects of changing the position of the animal across two surfaces thereby raising or dropping the body temperature. The subsequent intraocular pressure is plotted over time for a single animal.
Figure 3.8 Effect of temperature on IOP on single animal. The IOP measured by the sensor and the body temperature measured by the rectal thermometer is plotted over time for a single animal. The animal is moved across surfaces measured at two different temperatures throughout the course of the experiment.
The experiment was repeated on the same animal and was found to have similar results. The mean IOP was calculated for the start and end of each of the 10 minute periods. The difference in these means were then used to calculate the change in intraocular pressure. The figure below illustrates the absolute change in the intraocular pressure where the positive change indicates the increase in the IOP while on a heating pad and the negative change indicates the drop in IOP without heat support.

Figure 3.9 Effect of temperature on IOP on single animal across trials. Each bar represents the mean change in IOP. The positive bar is the mean change in IOP calculated while the animal was placed on a 42 degree Celsius heating pad. The negative bar is the mean change in IOP calculated while the animal was placed on a 20 degree Celsius surface. Error bars give the standard deviation.
The experiment was then repeated for several animals and was found to have similar results. An increase in body temperature resulted in a rise in IOP while a drop in body temperature resulted in a fall in intraocular pressure. The difference in changes seen among the animals can be explained as the differences in the thermoregulatory capabilities of each animal. The figure below illustrates the absolute change in the intraocular pressure where the positive change indicates the increase in the IOP while on a heating pad and the negative change indicates the drop in IOP without heat support seen across several animals.

Figure 3.10 Effect of temperature on IOP across animals. Each bar represents the mean change in IOP. The positive bar is the mean change in IOP calculated while the animal was placed on a 42 degree Celsius heating pad. The negative bar is the mean change in IOP calculated while the animal was placed on a 20 degree Celsius surface. Error bars give the standard deviation.
A control experiment was performed on a euthanized animal to confirm that the changes in intraocular pressure seen in the previous experiments was in fact due to the changes in body temperature and not a result of the sensor picking up the change in body temperature of the two surfaces. The euthanized animal was placed on the two pre-established surfaces for ten minutes each and the resulting IOP is plotted below.

Figure 3.11 Effect of temperature on IOP on euthanized animal. The intraocular pressure measured by the sensor is plotted over time.
3.3 Effect of Ketamine on IOP

The awake IOP’s ranged from 10-20 mm Hg. On animal 1, post ketamine administration, intraocular pressure increased and then stayed steady throughout the experiment at a value higher than the awake IOP prior anesthesia. As the animal was placed on a heating pad throughout the experiment, there was no loss in body temperature post ketamine induction. The animal regained consciousness around ~25 minutes post the ketamine shot. The figure below displays the effect of ketamine experienced by animal 1 on a single day.
Figure 3.12 Effect of ketamine on IOP on animal one. The IOP measured by the sensor and body temperature measured by the rectal thermometer is plotted over time for a single animal.
The experiment was then repeated on the same animal and was found to have similar results. There was no drop in intraocular pressure post ketamine induction. The figure below illustrates awake IOP and the subsequent IOP post ketamine induction at 10 minute intervals for a single animal across two trials. The mean and standard of deviations are calculated for 10 minute periods post ketamine induction.

Figure 3.13 Effect of ketamine on IOP on animal one across trials. Each bar represents the mean IOP calculated for a period of 10 minutes across both trials. Error bars give the standard deviation.
The experiment was repeated on Animal 2 and was found to have contradictory results. On animal 2, post administration of ketamine, IOP rapidly dropped 5-10 mm Hg compared to the awake IOP and then stabilized ~30 minutes post induction. Once the animal was awake, the intraocular pressure returned to the measured awake IOP range prior ketamine induction. While the animal was placed on a heating pad throughout the experiment, it was difficult to avoid a slight drop in body temperature immediately post ketamine injection. There was a 43.22% drop in measured IOP at the 40 minute interval post ketamine induction. The animal regained consciousness at around ~40 minutes post the ketamine shot. The figure below displays the effect of ketamine experienced by animal 2 on a single day.
Figure 3.14 Effect of ketamine on IOP on animal two. The IOP measured by the sensor and the body temperature measured by the rectal thermometer is plotted over time for a single animal.
The experiment was then repeated on the same animal and was found to have similar results. There was a 32.38% drop in intraocular pressure at the 40 minute interval compared to awake IOP across the two trials. Animal gained consciousness at ~45 minutes post ketamine induction each time. The figure below illustrates awake IOP and the subsequent IOP post ketamine induction at 10 minute intervals for a single animal across two trials. The mean and standard of deviations are calculated for 10 minute periods post ketamine induction.

Figure 3.15 Effect of ketamine on IOP on animal two across trials. Each bar represents the mean IOP calculated for a period of 10 minutes across both trials. Error bars give the standard deviation.
3.4 Effect of Tonometry on IOP

IOP was continuously monitored by the implanted sensor throughout the experiment to demonstrate the effects of the applanation tonometer on the eye. The Tonopen XL was used for a period of two-three minutes to get more than five readings of intraocular pressure while the animal was under anesthesia. During this period of tonometry measurements, the intraocular pressure of the animal was higher than the IOP prior tonometry. This rise in eye pressure can be explained as the stress induced on the corneal surface of the animal while attempting to obtain valid readings. The deformation of the corneal surface produced by the Tonopen XL leads to a rise in intraocular pressure. Applanation tonometry produced a mean intraocular pressure increase of $2.119 \pm 1.621$ mmHg. The figures below display the IOP measured by the implanted sensor over time compared to the IOP readings measured by the Tonopen XL across different animals.
Figure 3.16 Effect of applanation tonometry on IOP. IOP measured by the sensor and Tonopen XL are plotted over time across three different animals.

IOP was continuously measured by the iCare rebound tonometer for a period of one minute while the animal was under anesthesia. The corresponding IOP measured by the sensor was then used to determine the effect of the tonopen action on the corneal surface of the eye. There was no change in the intraocular pressure while taking measurements using the rebound tonopen. The IOP measured by the sensor stayed...
constant during the entire duration of the tonopen action. These experiments were performed across several animals during the course of this study and were found to have similar results. There was no statistically significant difference in IOP readings by the sensor before and during rebound tonometry measurements. The figures below display the IOP measured by the implanted sensor over time compared to the IOP readings measured by the iCare rebound tonometer.

Figure 3.17 Effect of rebound tonometry on IOP. IOP measured by the sensor and iCare Tonopen are plotted over time across three different animals.
As the rebound tonometer did not produce an effect on intraocular pressure readings while taking measurements, a control experiment was performed to measure temperature effects on an anesthetized animal using the iCare tonometer. Intraocular pressure and body temperature were measured throughout the experiment to validate the effects of changing body temperature on intraocular pressure. The tonometer measurements were higher while the animal was placed on the 42 degree Celsius surface as compared to the readings obtained while the animal was placed on the 20 degree surface. There was a strong correlation between body temperature and tonometer readings (0.8) using the Pearson’s Moment Correlation test.
Figure 3.18 Effect of body temperature on IOP using rebound tonometry. The IOP measured by the rebound tonometer and the body temperature measured by the rectal thermometer is plotted over time.
CHAPTER 4:
DISCUSSION AND FUTURE WORK

4.1 Conclusion

Post decades of research on intraocular pressure, various studies have yielded contradictory results on the numerous factors affecting IOP. IOP is one of the most important factors that needs to be studied to help detect the onset of open angle glaucoma and other neurological defects. In the case of glaucoma, ocular hypertensive conditions severely damage the cells responsible for delivering visual information to the brain. Yet, very little is known about the dynamics of intraocular pressure. Most of the time, the success of experiments involving IOP depend on the instruments used to measure IOP, the method of the experiments and the ability to successfully isolate the factors being studied. Since numerous surgical and anaesthetic factors can affect IOP, an understanding of the physiology of IOP and the ways that it may be altered during ophthalmological operations is of utmost importance.

The results obtained in this project clearly indicate that isoflurane itself does not have an effect of intraocular pressure. Isoflurane produces a drop in body temperature which results in the fall in IOP. When the body temperature is controlled and maintained constant, IOP remains stable with isoflurane induction. This result was reproducible in
several animals and at different times of the day. Varying the percentage dosage of isoflurane administered also showed no significant effect on IOP. Thus, isoflurane can be safely used without risking changes in IOP if controlled for fall in body temperature during induction.

The first set of ketamine experiments performed on animal one resulted in no changes in the intraocular pressure post ketamine induction. The IOP stayed steady throughout the experiment and the animal was under sedation for a short period of time (~20 minutes). Ketamine produced a drop in intraocular pressure in the animal in the second set of experiments that could be the direct result of the drug or due to secondary factors like drop in body temperature that could not be controlled for. The animal stayed under sedation for a longer period of time and took an average of 45 minutes to regain consciousness. These contradictory results could be explained as the inability to maintain the body temperature in the second animal. Factors like age, body mass, genetics, metabolic activity, hepatic function and the overall health of the animal determines the time taken for each animal to regain consciousness post anaesthesia induction. Further studies need to be performed to obtain conclusive results on how ketamine affects intraocular pressure by successfully maintaining body temperature.

Change in body temperature produces a direct effect on IOP, with a rise in temperature producing a rise in IOP and a fall in body temperature resulting in a drop in
IOP. This result was reproducible in several animals and during different times of the day. Thus body temperature is a physiological factor that must be taken into consideration while taking intraocular pressure measurements.

The rebound tonometer can be considered as a more reliable alternative to applanation tonometer without causing an increase in intraocular pressure during measurement of intraocular pressure in rat eyes.

4.2 Experimental Issues

Some of the issues and difficulties encountered during the course of this project are described below.

There were multiple unsuccessful cannula insertion surgeries due to poorly made head mount device leading to leaks and inability to proceed with the completion of the surgery. Successfully implanted surgeries also lead to experimental failures due to the clogging of the cannula in the anterior chamber of the eye. Attempts were made to avoid the clogging by dousing the cannula overnight in heparin solution but failed to do so. Further attempts were made to disconnect the sensor and flush the cannula every couple of days post-surgery with saline solution to get rid of clogging. In certain cases, the animal failed to completely recover from the surgery and thus had to be euthanized.

The ability to maintain body temperature constant during the experiments was difficult in certain animals and thus resulted in drop in intraocular pressure. Due to the
use of a rectal thermometer to measure body temperature, there was a lack of body temperature data prior anaesthesia induction while the animal was awake.

4.3 Future Work

While the cannula used in this study was adequate for the scope of this project, alternate methods and materials need to be studied in order to avoid having the cannula clogged in the anterior chamber of the eye. This would result in longer lasting experiments that could potentially improve the results obtained while studying the dynamics of intraocular pressure.

While this study focussed on healthy Brown Norway rats, this could be replicated in several other animals as well. Once normal IOP dynamics are well understood, this study can also be replicated on glaucomatous eyes or in different sets of animals set at different IOP levels.

A tail cuff can be incorporated in similar studies in order to get continuous measurements of several other physiological factors like systolic and diastolic pressure, heart rate and blood flow.
REFERENCES


Cohan, and Bohr DF. “Eye Research Fund Laboratory, Medical School, University of Michigan, Ann Arbor, Michigan 48104, USA.” Health Communication, Oxford PharmaGenesis, Oxford, europepmc.org/abstract/med/11581198.


APPENDIX A:

IACUC APPROVAL DOCUMENTATION

Procedures and protocols such as cannula implantation surgeries performed on rats were carried out in accordance with Institutional Animal Care and Use Committee (IACUC) of the University of South Florida of Protocol Number R1425. The following pages are scans of IACUC approval documentation for the one year this study was conducted.
MEMORANDUM

TO: Christopher Passaglia,

FROM: Farah Mouli, MSPH, IACUC Coordinator
Institutional Animal Care & Use Committee
Research Integrity & Compliance

DATE: 6/27/2017

PROJECT TITLE: Structure and function of healthy and glaucomatous eyes

FUNDING SOURCE: National Eye Institute American Health Assistance Foundation

IACUC PROTOCOL #: RIS00001425

PROTOCOL STATUS: APPROVED

Your request for continuation of this study was received and will be reported to the Institutional Animal Care and Use Committee (IACUC). The IACUC acknowledges that this study is currently ongoing as previously approved. Please be advised that continuation of this study is in effect for a one-year period beginning 8/30/2017.

Please take note of the following:

- IACUC approval is granted for a one-year period at the end of which, an annual renewal form must be submitted for years two (2) and three (3) of the protocol through the eIACUC system. After three years all continuing studies must be completely re-described in a new electronic application and submitted to IACUC for review.

- All modifications to the IACUC-Approved Protocol must be approved by the IACUC prior to initiating the modification. Modifications can be submitted to the IACUC for review and approval as an Amendment or Procedural Change through the eIACUC system. These changes must be within the scope of the original research hypothesis, involve the original species and justified in writing. Any change in the IACUC-approved protocol that does not meet the latter definition is considered a major protocol change and requires the submission of a new application.