Dr. Whitlock was raised in Gravel Switch, Kentucky, and he was raised on a small tobacco and dairy farm, which is awesome. I was raised on a dairy farm too, so that's awesome.

He attended Campbellsville University, where he received his BS in Chemistry, and he went to Michigan State University for his MS in Animal Science. And then he got his DVM from Auburn, where he worked for a while and did his residency at Auburn and became a Diplomat of the American College of Ethereal Genealogists. Then he earned his PhD also from Auburn with an emphasis in reproductive neuroendocrinology.

And of course, as most of you probably know, he is now a professor here at the University of Tennessee in the College of Vet Med. Currently, most of his time is spent with field services, teaching and mentoring students in the areas of ethereogeneology, large animal medicine, surgery, and production medicine. His current research interests include reproductive neuroendocrinology and physiology with a special emphasis on the use of kispeptin in large domestic animals. It's really cool because he actually can put a catheter into the ventricles of sheep, and he can study the regulation of reproduction.
appetite metabolism inflammation and the interaction of these systems in ruminants and I've actually witnessed some of this work of his which is really awesome so with that I'm going to turn it over to Dr. Whitlock thank you Dr. Miller can you hear me okay yes okay well the hardest part of that surgery is finding the sheep's brain after that it gets easy so it's all fine um I appreciate that introduction and I also appreciate the opportunity to speak to the one health lunch and learn I'm not sure how questions are handled but if you put them in the chat or afterwards we'll get to them or there may be some opportunities to voice questions verbally over Zoom also I just asked that if you have your uh your mic on you might want to turn it off and if you if you do have it on please turn your phones off so I've turned mine off and I'm grateful for that because I'm on clinics right now so I'm happy for the next hour I don't take any phone calls um so without further ado I'm gonna take probably the first half of the time I have in the lunch and learn hour and give you a background of kispeptin this is a molecule that if you've known me for any amount of time I've probably bored you with all the fun facts about this molecule and its history and then we'll talk a little bit about the candy neurons
which are a um the research for that
came after the kiss peptin work
and we now know more about those neurons
and
i hope you you too will know more about
them by the time we finish
and last but certainly not least i'm
going to touch on stress
inflammatory stress and how
that stress and its effects on
reproduction
may be mediated through this
candy neuron systems the kmdy neuron
system
also i just want to take a second to
again thank the
one health initiative and
thank the the group that reviewed grants
allison renwick and myself and dr sharma
and dr daniel
for seeing one of the small grants
allison has finished that as part of her
first project
we're in the process of analyzing
samples and getting tissue analyzed and
analyzing a lot of data
we've already have two abstracts will be
presented at the animal science meeting
in louisville soon
and we have plans for many more
abstracts and publications to come
so we're looking forward to sharing that
information from the first project that
allison completed
uh just about a month or two ago at
least the animal portion
so uh with no further ado i'll get into
our hour of lunch and learn
oops i think it's i don't
know if it's going to advance right okay
there we go
i just want to take a second and um
remind you how um
how the the hypothalamus works to a
degree and really I want you to consider how little time we've really known about hypothalamus and there's some really good quotes from some really important people with in the area of endocrinology so cushing this is the same cushing that that came up with cushing disease uh cushing said in the late 1920s here in this well concealed spot almost to be covered with a thumbnail eyes the very mainspring of primitive existence vegetative emotional and reproductive on which with more or less success man has come to superimpose a cortex of inhibition and then uh plum and vanit said this bit of brain only four grams of weight integrates almost all higher physiologic functions and so the hypothalamus it is literally this small part of the sheep brain yet it controls so much of all the physiology distal to it and this image was taken or made this diagram was made in the late 1920s 1930s and it was some of the first work that showed uh what were thought to be the nuclei of these control centers within the epidemics that regulated things downstream uh history of pituitary hypothalamus this uh claudius gallon of pergamum that's in asia minor that's uh um it's the third church that john referenced in uh revelation two in pergamum it's ismar now or it's close to ismar but
galius claudius galleon
he described the hypothalamus and the
infundibulum in the pituitary gland
as really its only role was to help
remove mucus from the brain from the
ventricles they thought that's what that
was for
and they thought the pituitary actually
facilitated movement of that
uh further end to be to be
expressed and that really was the
dominant scientific thought
until the 14th century that the
hypothalamus and the pituitary
in essence were just conduits through
which mucus was uh relieved
released from the brain
it wasn't until about 1508 50 1509
when da vinci here actually started
making some amazing drawings of the
ventral surface of the brain
and this is actually a lot of the blood
vessels that leave the pituitary
and actually drew part of the ventricles
so he started drawing things
with respect to the human body and some
of these were the pituitary and
ventricles
and it wasn't until the 18th and 19th
centuries that
pituitary tumors led to our
understanding that the pituitary was
important for some
physiology and we started understanding
diabetes insipidus and diabetes mellitus
and then if you know anything about
endocrinology raithke's pouch
was really kind of discovered with
respect to the way the pituitary
developed and that connection it has to
the hypothalamus
and then they started doing hypothectomy
research in
animal models and it wasn't until 1910
when cushing actually made a link between the pituitary and the hypothalamus and its role in uh in reproductive organs out in the body so we really it's just been a little over a hundred years since we've made a connection between pituitary reproduction and that connection to hypothalamus it wasn't until about 1977 that these three individuals uh uh scallion uh gilman and and um dr rosalyn yellow just earned the nobel prize and was for a lot of the work they did in the late 1960s these two guys their their reason that they got the nobel prize that year their portion of the nobel prize was because they created these large columns that could actually extract very really small molecules from hundreds or thousands of hypothalamic tissue samples from pigs and sheep and they actually were the first ones to discover these neuropeptides in the hypothalamus that controlled cells within the pituitary and then uh rosalind yellow she she got her share that year for her um invention of the radio amino acid she's the mother of ria assays and so that really opened the door for our understanding of how hypothalamus regulates uh the pituitary and then the the physiology distal to that and so um just as a really quick general review i feel like we have to talk about this before we move into other parts of our time together we have to understand the control of the
hypothalamic pituitary
natal axis and what was known about the
time
that those nobel prize winners received
a nobel prize
some of the details may not have been
complete but this is kind of how we
thought about the system working
that we have the hypothalamus whether it
be in the female or male mammal
and that we they discovered small
molecules like
gonadotropin releasing hormone gnrh
released
from the median eminence it's produced
in the hypothalamus released into the
hypothesis of portal circulation
and that gnrh leads to the antipituitary
and their gonadotrops there and gnrh and
how it's released its pulsator release
in fact it's not
can't just be released constantly but it
needs to be released in these
pulsatile um units and those pulses
dictate
whether and how those pulses are
released dictate whether lh
luteinizing hormone or follicle
stimulating hormone uh interacts with
the gonads whether those going out to be
ovaries or testes and then those lead to
gametogenesis the production of
eggs and sperm or they lead and they
lead to steroidogenesis the production
of sex steroids that we think of
classically estrogen and progesterone
from the ovary
classically androgens from the testicles
and then there's feedback that goes up
to hypothalamus and pituitary to affect
those things
so we've known that for some time but
this has kind of been where we were
stuck for
almost 40 or 50 years so four or five
uh decades we were stuck with
understanding this and yes we we learned
a lot about this
but we didn't really understand um
more above the grh kinetochore releasing
hormone
and its role so
i love telling this story uh it starts
out with something
no one would have ever believed would
lead to what we know now
um and dr miller clearly she's a
clinically a pathologist so she could
explain this much better than i could
but um we all understand that a lot of
times
metastasis is what leads to the
many of the the morbidities and
mortalities we associate
with cancer and as a result of that
there's been a great deal of research to
try to understand
what controls metastasis of tumors and
we all know that we have the primary
tumor
and it ultimately has to leave this this
mass of cells
it has to invade local tissue
get into the blood system go or through
the lymphatic system
go to a distant tissue and and develop
secondary tumors that are away from the
primary tumor
and that again creates a lot of
morbidity and mortality we associate
with cancer
so a lot of really amazing research has
been done
trying to understand what controls
metastasis it's a really important
question
and from what i understand the way some
of the initial work was done was they
would simply take some of these tumors and they would look for changes in the expression of genes and see if they can understand what genes are related to the tumor being non-metastatic or benign or one that's highly metastatic that's going to cause a lot of again morbidity and morbidity mortality and so this this story starts with oncology research related to metastasis a group of researchers lee and others in 1996 took some melanomas this particular melanoma cell on c8161 and you can see that this melanoma cell line has a wide potential of metastasis if it's in the red boxes with the plus it has a lot of metastatic potential fits in the green box with the minus it is very very very low metastatic potential uh in research research settings and they actually discovered that there was this one particular gene that was almost exclusively expressed in the melanomas the derivatives of this melanoma cell line in the ones that had very little or no metastatic potential lee and others went a step further and after they they saw this they looked in what would be non-non-metastatic normal human tissue and they found that the greatest expression was in the placenta and if you'll just believe me here there may have been a little expression in the brain as well that'll make sense later on so they found this gene that was highly expressed more expressed than cells that didn't have much of a metastatic potential
since the gene was discovered by leonol
it was actually discovered in hershey
pennsylvania
at the school of medicine there in
pennsylvania i believe and it was known
to be a suppressor sequence
hence the ss of metastasis
and since it was discovered in hershey
pennsylvania they went ahead and added
the k and the i in front of the ss
and lo and behold they've given the name
of kiss
sometimes you'll actually in older
literature see it called metastan
as a acknowledging its initial discovery
in its role in cancer but we now call it
kiss
is the gene kiss
so lean others did the next logical
thing
they transfected various c8161 melanoma
cell lines
to express varying degrees of kiss so
they
intentionally gave kiss to varying
levels in different melanomas
and then they took those melanomas
injected them in a thymic nude mice
and lo and behold whenever they had
melanomas that had
a lot of expression of kiss these had
much
less lung metastasize so fewer
metastases
as opposed to those that had lower
expression of kiss had a much much
higher number of mets to the lungs and
there's some other work to support this
as well
so this our time together isn't
necessarily to discuss
neoplasia metastasis but i just want to
let you know and there's probably the
list is longer than this now
it turns out that kiss is a really good indicator of survivability uh survivability curves you might look at with different types of cancers and so it's it's known to be a metastasis suppressor suppressor excuse me and it's inversely uh related to the aggressiveness of breast cancer carcinomas melanomas theochromosatomas esophageal squamous cell carcinoma flatter cancers breast cancer gastric cancer pancreatic cancer etc and we do believe that for diagnostic purposes if if you look at the expression of this gene or its receptor that we'll talk about later those often if their expression is low that's usually a bad prognostic indicator that the cell is likely to metastasize or has already metastasized more quickly who knows someday it may be an important treatment for cancer but at this point it's more for diagnosis and to help explain the aggressiveness of the cancers so about the same time just a few years later a differently completely differently uh discovered in 1999 these galan-like receptors so we leave kiss for just a minute and these galan-like receptors is what this group studied and these are a lot of receptors in the brain again and they found this orphan g protein coupled receptor number 54 gpr54 and so they didn't know what ligand went with this receptor so they found that it had a about a 50
transmembrane homology it's in the rhodopsin super family but when they took i125 labeled gallium there was no specific binding uh galinin would not bind uh this what they thought was gallon receptor what they did at the same time is they looked at the expression of this the gene for this receptor and they uh they found that it's expressed widely in the brain so it's in the brain but they don't know what uh ligand is for this is for and so within titu hybridization in the mouse brain lee and others show that it is clearly in the hypothalamus there's a lot of um the mrna there's a lot of rna for this gpr54 specifically in the arctic nucleus in the paramedial nucleus and the dorsal medial nucleus of the hypothalamus so it's really interesting that there's so much of it expressed in hypothalamus but has no no gavin and uh no gallon and vitamin at all so uh just a few years later about five years later in fact it was discovered uh in in one paper that the product of the kiss one gene turned out to be this kispeptin protein so it's uh synthesized as a pre-pro hormone in its cleave there's some post-translational cleavage that occurs and usually it can be excreted in this 54 amino acid product but ultimately these minimum on the carboxy terminal in there's ten amino acids that are needed
to bind this
uh kis1r is the kis1 receptor or gpr54
and that's what's needed to bind and
activate the kispectant receptor
it's considered a gq subclass of g
protein so it increased ionized calcium
in earth one for two okay so now we know
the
the peptide product and we know the
receptor
um kotani um
looked at the amount of i believe this
was the
this was kiss mrna and bill band looked
at kiss
one r and kiss and they found that it's
also in the placenta
and that there may indeed be some role
in some similar role between how
kespepten regulates placental
and trophoblastic cells and how it might
also
regulate how cancer cells metastasize
and invade local tissues and escape
the primary tumor so they found that
gpr54
or kis1r mrna is in the placenta and
they found
that it's that kiss one is also
expressed in the placenta and there's a
lot a lot of it
so these are some really nice uh images
of the kisspeptin protein
in the trophoblasts of human
uh placentas and then the kiss one r is
also in the truffle blast of human
placenta so
not only is the protein the ligand but
also the receptor is present
and it's produced at such a high amount
in these placental cells that it can
actually escape the placenta and be
measured in plasma
so these are some really nice work in
the early 2000s that show that when they measure caspectin in non-pregnant humans the concentrations are two to three femtomoles per ml but you see by the first trimester they're up to a thousand femtomoles by the third trimester they're up to ten thousand femtomoles and the half-life is quite short it can be broken down and just within a few weeks it's back down to 10 femtomoles so while this is femtomoles i know it's a really really low concentration the change is quite great and so this is kind of an aside but there's even some work that suggests that maybe we could use kispeptin and its concentrations at the very least in human plasma as an indicator of placental health so it might be a way an assay by which we can determine if placentas are healthy or not we don't really know in other species yet unfortunately so work by bill band in 2004 showed that when they took in vitro truffle blast cells and they have control cells and they look at how these cells are expanding in culture that there's really a nice increase in the in the spread of these cells but whenever kispeptin is put in these culture systems it really inhibits migration distance of these cells from the primary culture cells so an even higher concentrations suppress that even more and so this is just graphically represented here on the left so it really affects migration distance
compared to the control
of cells so it really does affect how
these trophoblasts migrate
so we believe again that kisspeptin might
inhibit cell migration
much like it inhibits metastasis we know
it down regulates this type 4
collagenase mmp2 it induces some
phosphorylation of focal adhesion kinase
and this paxillin and it might actually
induce some microptosis
in malignant cells so um
it may be that in the future a greater
understanding of
how kiss peptin does this and regulates
the different types of placentation that
different species have and different
pathologies of placentation mold
pregnancies uh placentas that invade the
tissue too aggressively
and go through the endometrium and into
the into the into the abdomen
maybe if we can understand that
pathology we can have a greater
understanding of control and metastasis
so if we leave the story there we have
to remember
that um come back to lee and others
in situ hybridization work and just
remember that
the kiss peptan ligand the gene for kiss
and the gene for kiss 1r or gpr54
is really well suited to regulate
neuroendocrine function
there that had to be on lee's mind
when they saw there was so much of this
gene expressed in the hypothalamus
not only the gene but if you take a step
further and look with the
immunohistochemistry
in 2005 and the rat brain this is a near
the arctic nucleus there's a lot of
kispeptin
being expressed this is imminent
chemistry for kiss baptism
in the hypothalamus and so again it's
well suited for
a neuron having a neuro under control
so um in 2003 there were actually three
groups that discovered this
within the same year these different
groups were studying
um a a clinical condition
called hypogonadotropic hypogonadism
and we've known for years that there
were forms of it called
common syndrome that have to deal with
the migration
of the generation neurons to
the to the hypothalamus and when that
migration is disrupted not only to those
individuals
not only do they never go through
puberty and they never become
reproductive mature
they can also they're in osmic so they
can never smell
um so that's classically called common
syndrome
and then there's other forms of
hypogonadotropic hypogonadism in humans
that's non-analysmic so these
individuals can clearly still smell
so it can't technically be coleman
syndrome so there were these individuals
that had at the time was called
idiopathic hypogenitropic hypogenitism
these individuals are absent or partial
spontaneous sexual maturation
some of the males have hypophallus so
they have uh
small micro microphyllite uh
they don't have typically what we
consider secondary sex characteristics
of humans
and so that never happens outside of
exogenous hormonal therapy for those
individuals the plasma concentrations of
gonadotropins like luteinizing hormone and follicle stimulating hormone very very low and they really don't have the pulses that we need to bring about normal um gametogenesis especially ovulation and such and um again for the longest time these defects were in gnrh synthesis secretion or activation but it's more related to secretion than the ones we're going to talk about these individuals would respond to exogenous gnrh so you give gonadotropin relation hormone the cells the pituitary can clearly respond to this molecule and they can release their product lh and fsh to cause again comedogenesis and steroidogenesis but there were some questions of if there were other explanations for ihh other than these common syndrome individuals so they found individuals that had consanguinous families uh intermarion interbreeding um we see this a lot in livestock species as well we see genetic mutations showing up when when there's consanguinous matings matings of the same blood as it's called and so this is a um kind of uh ancestor ancestral figure for from uh roe and seminara and the individuals in the black boxes are males that are affected and black circles or females that are affected and they would trace them back to having a common lineage and it turns out that when we see these kind of conditions they're often an autosomal recessive condition and they'll they'll end up inheriting
both alleles that are
of a mutation this is really no
different and there were several
mutations that were found not just this
one mutation that we
reported by row at all in 2003 in their
particular paper they found that there
was 155
base pair deletion in the kiswan r gene
so you can see here
this obviously this this individual is a
homozygous heterozygous carrier of the
full type of the gene for the receptor
and the the part with 155 biosphere
deletion
a homozygote normal and then these are
homozygote affected
heterozygous there's another carrier
homozygote
affected and then there's a heterozygote
so it's a classic autism recessive
condition
so this is this is the deletion they
found a subset of these in these humans
in this particular paper
so they took this base pair deletion and
they put it in the rodent they took it
into a rodent model
and they clearly showed with uh
wild type animals the testicles of the
normal size and affected animals they
have small gonads the uterus of affected
animals is quite infantile
compared to the uterus of a normal
animal in the seminiferous tubules of
the testicle there
this wild type is full of sperm and
spermatogonia and more inventory sperm
cells down closer to the sertoli cells
in the basement membrane
whereas there's none here in a in a
normal
rodent ovary there's lots of corprolutea
and
nice follicles in this affected ovary
there are no clc animals not ovulating
so they had the right phenotype
and ultimately what was discovered was that
i'm giving you the abbreviated version
believe it or not
ultimately what was discovered was that
um kispeptin
the product of uh kiss the gene
it's it what it was doing was it was
binding the kiss one receptor within the hypothalamus
and not only causing this increase in luteinizing hormone
here you see here but it was at the same time causing an increase in gonadotropin releasing hormone
so it appeared that this molecule was not having its effect on the pituitary but in fact was causing the release of gnrh
and that gene rh was then going and having its effect downstream i think anatomy
and then this this so this was the 2005 paper
out of a group in france with sheep and this was a group erwig in 2004 and they did a really nice study
where they measured plasma aluminum hormone on this axis
they gave vehicles they gave kispeptin and saline
or they gave a gnrh antagonist so they blocked
gnrh release while giving kiss peptin in essence they prevented the increase in luminescent hormone
this is really compelling research to show that kispeptin is having its effect at the generation neuron and not necessarily at the pituitary so
it meant that maybe it was
the secretog for generation or something
that was
above gnrh it was this conspecting
molecule
so it's pretty exciting um so early
again i did some other research and
showed here
that um the gnrh neurons
actually 77 of them a little more than
three-quarters
actually contain the mrna for
uh the kiss one receptor so that's again
pretty compelling evidence that
kisspeptin
is having its effect on the gnrh neurons
and i like this image
it's a immuno
immunohistochemistry with fluorescent
antibodies
showing in a rhesus monkey the
kispeptin neurons in the arcuate nucleus
and they're actually going in and not to
the body but out to the dendrites
they actually make connections with the
gnrh neurons they're about to at least
release their product into the portal
system and they’re actually coming in
very close contact with those generation
neurons
so we now know that kispeptin increases
luteinizing hormone
clearly through its effects on
generation neurons
so that was really big news about a
decade or so ago
and that really changed um how we
understood
how the hypothalamus regulated
reproduction
the other part that um and i have to
be cautious that i don't sound like a
snakehole salesperson
that um we really it kind of opened up
a lot of um new opportunities to understand what regulates the gnrh system
we knew for the longest time that they get atropine releasing hormone neurons were regulated by sex steroids it's very obvious that for instance when a cow is going into the estro cycle the progesterone concentration drops estrogen concentrations are going to rise and we clearly knew that that ultimately resulted in a gnrh surge that caused the lupine hormone surge that caused ovulation but we knew for a fact that generated neurons didn't have the right type of sex steroids receptors to respond well it turned out that the kispeptin neurons have those estrogen receptors on them and so this is uh er alpha mrna uh in the white and the red or kispeptin neurons in the argument and then this is um ihc this is actually protein so the brown are kispec neurons stain uh with immunohistochemistry and the black are actually nuclear stains uh for er alpha so these have the right types of receptors and we won't go into it but whenever the different stages of the estrous cycle we see these neurons be turned on more or less depending on the sex steroids that are present and this is just more evidence these kids spectrum neurons also express progesterone receptors so it turns out that these same neurons respond appropriately
to changes in the sex steroid made by the corpus luteum
so we see the appropriate changes in kiss expression and kispeptin the protein
depending on the stage of the estral cycle in uh all mammalian species in response to this
so if we go back to uh what i wrote earlier the hypothalamus pituitary genital axis
it's the key for regulating reproduction and before we started with the nitrogen releasing hormone
now we understand that kiss peptin above those generation neurons kiss peptide and depending on which nuclei
it's going to regulate the generation neurons which then have their effect at the pituitary which then go ahead and affect the gonads and then we have the sex steroids feeding back to the kefta neurons and affecting the output from that system so it gave us a much greater understanding for how that system is regulated um but it doesn't stop with the sex steroid component so when we think about we're going to talk about narrow inflammation in the minute inflammatory processes and how these processes are affected how the system is is an integrator of that i want to take a second and go back to the castellano paper from 2005 and just let you see here in this paper they did a couple of things they had rats and they they they had animals that were just fed at libitum
when the animals were fed at libitum
they used vaginal opening
to know when the animals go through
puberty so this is normal
these rats go through puberty about mid
30 days and when animals received a 30
reduction in their food product that
food that they offered them so they
really had a much
they were much lighter these animals
never went through puberty in this group
and if you kept following maybe they
went out then in puberty later but much
much later
when they gave kispec these animals they
could force them to go through puberty
so that was pretty powerful that they
could
make the hypothalamus uh ignore this
signal
of malnutrition and force it to go
through puberty
that tells you how powerful the signal
is but then costellano and them did
one thing greater they did a 72 hour
fast in rodents and they collected
their hypothalamus and they noticed a
couple of things
when the animals were fasted they had
about half as much
kiss expression in the hypothalamus and
they had a concomitant rise
about a 50 to 70 percent increase in the
receptor for kisspeptin
so this is likely because there was less
kispep in ligand so there was a
classic increase in the kispectin
receptor but it's really amazing that
this
metabolic stress caused the kiss
expression to be reduced so much
one of my mentors in the past said
reproduction is a luxury
i mean many of you probably heard that
before animals that are very thin
whether that be a dairy cow or
or a human that's a a runner that's very
very
has very very little body fat they won't
they won't have a normal reproductive
activity and in part that could be
because of the
metabolic signal turning the kiss system
off just to show you that you can
recover that and there's some
controversy over the
leptin story with kiss and whether or
not the kiss peptan neurons actually
have the lepton receptor or not
but this is mrna for kiss and a wild
type mouse
in an ob mouse that has a leptin
mutation
there's much much much less kiss
expression and when leptin is given back
exogenous leptin is given back you
recover some of the kiss expression
so it can be recovered by the fat
cytokine or out of a cone so now when we
go back to this figure
um we can think about the kisfecting
neuron as integrating a lot of the
different signals
whether that's photoperiod whether
that's physiologic stress
uh from cortisol whether that's body
condition
and metabolic state or whether that's
sex steroids a lot of these signals may
be going through the kispeptin neurons
to then have effects downstream to the
gnrh neurons
but it turns out it's a little more
complicated than that than just these
neurons being transpeptic neurons
and so i want to take a step back for a
second and explain to you uh
two more aspects of these neurons one
they're not simply kiss spectrum neurons in the arcuate nucleus in which that's the part that actually controls the pulse generating activity of gnrh so if you look at some work done in 2009 this group again had another ihh so a hypochondriac hypogenetism family but this time instead of a mutation in the spectrum receptor these individuals had a mutation in a tachycardine and these tachycardians include molecules like substance p and they also include molecules like neurocone and beta and so they have these 20 genes of interest but it turns out that the tachycardina was the one that was probably causing the ihh so it's actually the they were had a defect in the receptor for neurocontinent beta so this turns out that this neuroconan beta is really important for reproduction so it was not a very there was a couple different mutations in the receptor that were in the membrane transmembrane components and a loss of function of this mutation um for the neurocontinent iii receptor for neurocone and beta they ended up discovering that it it was necessary for the under control of reproduction and another group uh just about eight or nine years ago they showed that when they made mice they transfected this mutation into mice they actually knocked this receptor out and these mice had a small reproductive
tract
they had smaller gonads especially the
males the females have a smaller uterus
the males had smaller testicles
they had the females had abnormal estro
cycles this is these are wild types on
this side
and they're having normal estrous and
metaestrous and diastasis cycles or what
these figures are showing
but in the animals that were knockouts
some of the animals had in essence no um
no uh estrus they were all either
metaestrous or diastrous
and other animals they it was not
completely knocked out their ability to
have
ester cycles so some of them did have
some estrous periods but
it certainly wasn't normal like the wild
types
so it turns out that neuroconden beta is
really important for the reproductive
neuron to control as well
and um a little before that i was
talking about allison this morning that
some of the work by goodman and others
had actually given opioid
peptides endogenous opioid antagonist so
things like mew and kappa they'd
actually give an antagonist and they
found that the antagonists that were
specific
for the kappa opioid receptor it turns
out that these were really important for
reproduction as well and if you
if you gave an agonist to these
receptors you could clearly
suppress lh well work done by chad four
dory chad's now at auburn but he'd
worked with dr goodman
and others also but this is a really
nice paper by chad
uh they gave dynorphin which is a part
of the endogenous opioid families
and most neurons actually have uh input
from the north and containing terminals
and these endogenous opioid receptors
like kappa receptors
if they gave antagonists they could
clearly increase loot vessels
so for example in these studies
uh they show that the the
administration of um excuse me the
the increase in the north and clearly
causes this increase in luminosity
hormone
and then when they gave progesterone
that could actually inhibit
not only that orphan but also the peak
of the lh
so they showed that there was a
relationship between the dinorphin
concentration
and the lh concentration and it turns
out that progesterone increases the
hypothalamic
csf expression of that north and so it
looks like these sex steroids regulate
that orphan
and then last before i go to the candy
neurons um
they showed that when they knocked out
neurocon and beta and mice
they could knock out nk and k beta but
they could give an opioid antagonist
naloxone and when they did that uh these
are again this is in
a mouse model and clearly these are
adult models that have these lh pulses
and when they had uh these knockouts of
neurocannon beta they had
very very few lh pulses when they gave
naloxone which is an
opioid antagonist they could increase lh
so they could increase it and that's
working probably or it's working through
the dynorphin
receptors on these these neurons within the hypothalamus
so um the reason that's important is uh about a decade ago a little less they discovered that not only are these neurons within the hypothalamus producing kisspeptin but these neurons almost all of them are producing neuroconan and beta and also producing dinorphin so these neurons 80 90 of the neurons within the arcuate nucleus the arcuate nucleus of hypothalamus are not simply making kisspeptin but are making three things at one time kisspeptin neuroconan beta this tachycardium and dinorphin and these are some beautiful images of individual cells where the cells blue is kisspeptin green is neurocon and beta and red is dinorphin and then you can see this one particular neuron where the not the colors are all merged and so you can see a few neurons here and again this would be true for 80 or more of the neurons here's one a neuron that's just making mrna from nkb but all the rest of these are making a combination here's one that's again an nkb but the other ones are making all all three uh genes mrna for all three genes that are important so it confirms that almost all these are making all three things at once so the working model we have now for the candy candy neuron hypothesis is that within the argument nucleus we have these candy neurons and um kisspeptin is going down to the generation neurons
and it's causing generates to release its product into the portal system neuroconan beta is coming out of the candy neurons and causing the candy neurons to release kiss peptide and this is starting an lh pulse in the articulation and then some of that neurocyto beta goes to neurons outside the system and that can feed back on to um excuse me kiss peptide can go outside this these canning neurons and that can give feedback to stimulate more kispeptin release okay so nkb is going back here these neurons do not have spectrum receptors on them at all because spectrum receptors are here on generation neurons and then possibly on some cells outside the canon neuron system but nkb is feeding back because these candy neurons actually have the nkb receptor on them and then to terminate the the gnrh pulse dinorphin is coming on and inhibiting the candy neurons and again inhibiting these that are stimulating and then the dinorphan is also inhibiting gnrh release so we we believe that this system is what's regulating generation pulses in the onset of those pulses these candy neurons are doing that by kispeptin causing generator release neuroconan and beta stimulating candy neurons and then ultimately dinorphin turning the system off by negative feedback so this is uh this is a figure brought that was published by the group that allison worked with in nc state
and this just takes it a step further to talk about these neurons that were orange up here so these are really the neurons on the outside here would be equivalent to these these other neurons and these are neurons that regulate uh there are rexogenic and um and you have satiety signals satiety signals and appetite regulating signals and it turns out that these candy neurons in their product whether that be kispeptin or uh dinorphin so this would be dinorphin going to this npy neurons and turning this off or to inhibit the uh palm c system and those systems then feed back to the candy system so we believe that we're just beginning to understand better and this is casey nestor's lab at nc state is beginning to understand better how nutrition affects the system and it's an interplay between these neurons that regulate metabolism and food intake and how they work with the candy neurons to eventually have output on the generation neurons distally to affect lh pulse activity so for the last couple minutes um if i can just stress to you that this story it does tie into the inflammation story so if you don't believe me you haven't been alive for the last year animals and humans experience inflammation we've all heard of the coconut 19 pandemic and this is a nice schematic describing the cytokine storm that happens as a result of individuals being infected with the sars kobe 2 and we don't we haven't talked about the
reproductive consequences yet
to this virus but i suspect that will be
discussed in the not too distant future
so inflammation is part of our life as
humans
and not only is inflammation part of our
life as humans but inflammation is part
of the life of
almost all animals that we might work on
as veterinarians
the animals i work on most are food
animals so animals experience mastitis
and metritis
clearly have inflammatory cascades that
are going on that
affect these animals so this is a real
problem
and there could be long-term
consequences to these inflammatory
pathways that are happening
maybe we don't know how long how long
they're gonna last
also we know that there's an obesity
pandemic that's been going on for years
uh different countries experience it
differently and have different degrees
of obesity
this is more chronic inflammation as
opposed to maybe sarge toe v2
or an about of mastitis in a dairy cow
but there's no doubt that there's
impaired reproductive function and this
may be a consequence of
gram-negative bacteria that we believe
this undertone of inflammation
may be a consequence of the endotoxin
released from grain negative bacteria
even in
individuals that are experiencing
obesity
so if we think about how this gets to
the neuroinflammation signal we have the
damps and pants and lps coming into the
pants is ultimately
going to and i'm going right to the 
source it's ultimately going to cause 
more microglia one type cells which are 
pro-inflammatory cells within the brain 
so there's lots of different cells 
within the brain 
but these make up the microglia i 
believe the glial cells make up more 
than half of the cells within the brain 
and these cells can either be 
pro-inflammatory or anti-inflammatory 
and we believe that these pathways 
distal to these um 
these m1 microglial cells increase 
all these cytokines whether it be i1 
beta tnf alpha or interleukin-6 
and they're going to cause 
pro-inflammation and cause acute and 
chronic inflammation in different parts 
of the brain 
[Music]
so this is no small thing this is 
coolest paper as a review paper written 
just last year's really great paper 
it shows in early lactation it's hard to 
see on this image but 
i promise you these cows are an early 
lactation experience 
more information there's more active 
microglia cells here 
than there are in a late lactation cow 
so there's more inflammation within 
hypothalamus 
and we believe that this inflammation 
again can push our pathway 
to effect look these same cells that are 
that are the palm c 
cells within the brain so we think 
there's long-term hypothyroid 
consequences 
going back to nestor's lab so these same 
neurons if you look at hula's 
figure here these same um minor 
coordinate systems that are affected by
inflammation in the
dairy cow these same systems are part of
what we consider the under nutrition
um stress model maybe these are also
part of the inflammatory stress model
in ruminants and primates alike so we'll
go back through this again
but i just want you know all this is
going to tie together and it may be
that a lot of this explains why we see
endotoxin disrupting the ester cycle
work done by caution others years ago
showed that endotoxin delays
and when animals have their estrus and
have lhps
and what's really amazing is that a high
dose of endotoxin not only does it
suppress luteinizing hormone here but
look it really suppresses gnrh pulses
so the nanotropic releasing hormone is
much much much lower there's hardly any
pulses
and that's what's causing the lh pulse
to be really lower
at a lower dose of endotoxin it might
not be that the effect is at
the generate neuron it may be just at
the pituitary this is lh
or lower and when they gave a
non-steroidal anti-inflammatory
they eliminated that effect and maybe
that's because they're reducing the
cytokines and the hypothalamus and
that's affecting
these inner neurons that communicate
with conspecting neurons
so just the last couple of slides um
there has been some work that showed
that the kiss gene
is changed with low doses of endotoxin
in the rats
so control in lps animals here there was
a significant drop
in the expression of kiss in the arctic
nucleus costellano in the male rat showed there was less immunoreactive kiss peptide neurons whenever they gave endotoxin and then more recently i was in 2014 showed that six and 24 hours after excuse me six and 24 hours after they gave lps that kiss is much reduced but this is about the only work done the last one is forgotten in 2014 the only work done looking at the candy neurons in the hypothalamus of these of sheep and ruminants so we're really hoping over the next few years to understand the system better understand how we can prevent these cytokines to be increased and maybe uh change how we uh can select from microglia to go to type 2 so type 1 to effect have the benefit of reducing inflammation in the brain i know that was a lot of information i hope you understand the the history of kisspeptin and why we have or how we came to discover these candy neurons and they're actually a class of neurons that secrete all three things produce all of these proteins and how at once we felt the hypothalamus pituitary was simply a way to drain snot from our brains but now we understand hypothalamus regulates reproduction and how we're getting closer and closer to understanding how this stress uh is um can be abrogated through maybe blocking these heart attacks with that i don't know if there's any time for questions or not dr miller
there is time for questions uh well at least a couple of minutes um well if anything was in the chat so you can go ahead and stop sharing your screen there you go awesome and people can either put their questions into the chat or they can um just unmute themselves and and ask questions dr whitlock this is mark caldwell just a quick question do you think um the timing of inflammation is more important to disruption of and i'm afraid this in a lactating dairy cows uh perspective do you think the timing of inflammation is more important than disrupting her cyclicity or is it more about duration or severity of inflammation in that disruption of cyclicity yeah i think it might be all three and that's kind of a cheap way to answer that um clearly i think cars and others showed that with endotoxin the anna the sheep still went on and had an lh surge but if dr edwards on edwards a lot of edibles on this she would tell you that that dominant follicle if the oocyte didn't get released at the right time that's really going to have a big impact on the fertility of that animal so a dairy cow is experiencing subclinical mastitis or maybe even clinical mastitis about the same time she's going to have her lh surge that's really blunted or it's delayed
and that really affects the downstream effects of the oocyte not being released at the proper time or meiosis that the pause of dios of meiosis being released so that oocyte can go on and mature so i think that's really important the timing but one thing we don't know the answer to yet is if they experience inflammation how long does it take an animal to recover so they have normal lh release after that so that could get to your severity question dr caldwell um and it may be that the duration of the inflammatory event the animal has um and the magnitude of that inflammatory process may have long long-term effects um there's lots of work uh looking at pain response uh and it looks like people that experience chronic inflammation that the pain and fear the there may be changes within the amygdala that affects all of that so unfortunately i don't think we have a lot of answers yet but i think uh it could be all three there is one question in the chat the question is how is kiss how does kiss prevent metastasis of tumors does it strengthen the capsule formation around the tumor cell or does it prevent tumor from becoming malignant and makes it stay benign so i think it has more to do with matrix metalloproteinase so i think it doesn't even allow the those cells to leave their primary tumor site much like it does with the trophoblasts so if you treat a trophoblast with gustapton
it can't get away from the initial uh primary site of where it's at so i don't think it allows the collagen to be broken down i'm speaking out of turn i'm not a collagen or collagenase or mmp9 or mmp2 expert but i think it really does prevent them from getting through the local tissue um we might think that extravas leaving the tissue getting into a blood vessel and then getting back out later is complicated but it's probably even more complicated to fight your way through that local tissue and the collagen that's there awesome okay we're right at one o'clock um can we give dr um whitlock a round of applause zoom applause thanks so much um i really appreciate you doing this if there are any other questions you guys can put them in the chat or can speak up like right now otherwise i think that we are good thanks so much that was that was awesome i really love how you're taking this one um this approach to uh to do something that's going to be applicable to humans to animals and it you just have a lot uh one a true one health approach there that's really awesome thanks and i appreciate again you're all support i can't wait to share what allison finds in this first study and then we've already got we got another small grant to do for the fall and then we have some cows that we hope
to do some work on too
related to inflammation in the system so
we're hoping this this time next year
we'll have a lot more to share
excellent wonderful thank you thanks
English (auto-generated)
AllSeminarsListenableRecently uploadedWatched