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 we won't hold
that against you
and then he uh worked for a while and he
uh got his um did his residency at
auburn and became
a diplomat of the american college of
ethereal genealogists
and 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 and it's
really cool because he
actually can put a catheter into the um
ventricles of of sheep uh
the ventricles in their brains 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
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 uh 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

percent
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 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 kispeptin 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 estra
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 kispeptin
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 kispeptin 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 kispeptin
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 kispeptin 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 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
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
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