im excited to introduce dr matthew hall

leader in ncats here at the nih he

joined ncats in 0 as a biology group

leader in the ncats chemical genomics

center dr hall earned both his

undergraduate degree with first class

honors and his phd from the university

of sydney

prior to joining incats dr hall worked

at the national cancer institute in the

laboratory of cellular biology under dr

michael godisman studying multidrug

resistance

dr hall is currently a leader leads a

team developing and optimizing both

biochemical and cellularbased assays

for highthroughput drug screening im

sure you will enjoy the presentation

hi my name is matthew hall and im a

group leader at the ncats chemical

genomics center and ill be talking to

you today about abc transporters at the

bloodbrain barrier

and following from michael godzmans

discussion about the the basic concepts

behind abc transporter biology and their

important functional role in drug

transport really critical to drug

development and our understanding of

drug action as well

so

as an example were going to talk about

the brain and the blood brain barrier

and delivering drugs to the brain is a

massive challenge and you can see

theres a a list of things here that to

im going to speak about it in this

presentation so were going to talk

about the fact that that abc

transporters like pgp transport drugs

out of the cells in multiple locations

and for multiple purposes the placenta

the brain test is really critical organs

that the body needs to protect the

integrity of

were going to talk about the

bloodbrain barrier how its what its

structure and function is composed of

and the way that pglycoprotein and

other abc transporters protect the brain

at the bloodbrain barrier

as an example were going to talk about
uh imaging and directly imaging the
function of abc transporters at the
bloodbrain barrier and that will really
uh be a nice tool to convey to you the
sense of just how powerful and
efficacious drug transporters are in
protecting the brain
and they really are critical so when we
think about pharmacokinetics and
pharmacodynamics there are four
important parts there absorption
distribution metabolism and excretion
for pk and abc transporters play a role
at all of those as michael to some
degree has discussed in his earlier
presentation so absorption its
absolutely critical abc transporters are
expressed in the gut and mediate the
bioavailability of drugs distribution
well be talking about the brain today
so distribution on an organ by organ
basis abc transporters can play a really
critical role in regulating what drugs
and other small molecules can and cant
enter certain organs metabolism there is

a relationship between drug metabolism
and transporter expression and in fact
the expression of metabolic and drug
transporter enzymes are part of the the
same
um protective operon if you will and
theyre coregulated
and of course excretion so these as
michael mentioned these transporters are
expressed in the kidneys and in the
liver in the liver and partly through
the gastrointestinal tract they can
prefer excretory action as well to
remove drugs from the body and from
blood plasma so all abc transporters are
really critical for drug action every
step of the way so lets talk a little
bit about the brain and and drug
development and delivering drugs to the
brain as i say its a big challenge and
this slide has a couple of really nice
figures from some some papers by uh bill
partridge that you can see the
references to on the bottom right there
um and really the takeaway from this if
nothing else comes from this slide is

that 9 of small molecule drugs do not

cross the bloodbrain barrier and you

can imagine therefore that the failure

rate is really profound so if youre

involved in a drug development program

you have a cns disorder youd like to

develop a small molecule towards you

might understand the target you might

not you that part of that medchem

program is understanding whether or not

a small molecule is susceptible to

transport by the abc transporters can be

transported across the bloodbrain

barium

and im not going to talk you through

any examples of it but i could refer you

to the literature and youll see again

and again stories of failure of

experimental therapeutics that either

cant get into the brain of the animal

models that are being utilized or when

they get into humans the brain

penetrance is inadequate and the program

is abandoned and if you look at large

pharma companies most of them do not

have

strong programs in developing

drugs against cns targets or cns

disorders anymore because

the the valley of death that exists

there in drug development is so profound

and you can blame the bloodbrain

barrier for that

so for the the range of cns disorders

that people try to treat uh many of them

are refractory to small molecule drug

therapy uh not currently drugged or

treated and and as i say it may be

because of an understanding of the

target it may be because of a lack of

understanding of how to deliver a small

molecule therapeutic across the

bloodbrain barrier and this figure on

the right here is is is quite a

remarkable example of just how powerful

the bloodbrain barrier can be in it and

it its a modern relatively modern

autoradiogram

of radiolabel histamine thats been

injected into this rodent and you can

see that its distributed throughout the

entire body and the way this is achieved

is that after the radiolabeled molecule

is injected into the animal its

sacrificed fixed sliced and put down on

film and so where you see dark signal

thats actually radiation exposing the

film just like the original ranchon

experiments over 00 years ago

this experiment

demonstrates that this radiolabeled

histamine is distributed everywhere

through the body of this animal except

for the the brain brain stem and spinal

cord and the reason for that is that the

bloodbrain barrier prevents it from

distributing to that part of the body so

this is a modern example with a

radiolabeled small molecule but in fact

it it perfectly mirrors the original

experiments that were performed by paul

ehrlich who discovered the bloodbrain

barrier and was awarded the nobel prize

in part for that work he injected

rabbits with dyes such as trypan blue

and notice the dye distributed

everywhere in the body on when he

examined them on necropsy didnt get

into the brain and so he did the reverse

experiment he injected the dye straight

into the brain and he saw that the dye

was distributed in the brain and spinal

column of the animals but it had an

egress back out into the parenchyma of

the body and so he recognized that there

was a barrier between the brain and the

rest of the body that must be playing a

protective role for the brain

so 9 of small molecules dont cross the

bloodbrain barrier

and

biotherapeutics experimental

therapeutics such as antibody directed

therapeutics enzyme therapies there are

very limited strategies for delivering

those to the brain as well and theyre

usually quite invasive

there are emerging technologies

addressing that at the moment

and so as a result very few companies

have bloodbrain barrier targeting

programs um and academic neuroscience

programs um also have difficulties in

addressing this area so its very

underserved this is an example of of

just how difficult it can be to deliver

a drug to the brain so lets take a look

here weve got heroin and as we know

heroin is a drug of abuse

its an opioid receptor agonist

and highly addictive

and its got these two acetyl groups

its actually also known as diacetyl

morphine and the reason is that this

very lip relatively lipophilic molecule

has reasonable brain uptake um certainly

high uptake relative to the other

molecules that were showing here once

heroin crosses the bloodbrain barrier

and enters the brain being relatively

lipophilic its actually metabolized

by through deacetylation into morphine

and morphine is actually the active drug

that binds to the mu opioid receptor

heroin is a pro drug and i know in

earlier parts of this course weve

youve talked a little bit about pro

drugs so heroin is a pro drug that can

cross the bloodbrain barrier once its

across the bloodbrain barrier its

metabolized into the active morphine

morphine itself if its directly

injected has relatively low brain uptake

and and so has a a very modulated uh

neuroactive effect as a result

and so this is an example of how you can

imagine from a medicinal chemistry or a

drug development point of view having

some insight into the into the fact that

modification to a small molecule can

change brain penetrance is a really

powerful observation codeine is here in

the middle as an example of an

overthecounter opioid receptor agonist

its slightly modified its not as

powerful an agonist and it also has

intermediate brain uptake so it works

so these are just some examples here but

but there are there are a number and

people have understood for a significant

period of time that basic

physicochemical properties can regulate

brain penetrance

on the previous slide with heroin we

said we said it was more lipophilic so

it had a higher log p a log of the

partition coefficient this is a small

sample of molecules again measuring

brain uptake from a really classic paper

from the early 90s looking at a dopamine

receptor agonists and measuring brain

uptake and you can see theres a almost

an inverse parabolic relationship here

where we have log p

across the xaxis looking at brain

uptake and you can see as log p

increases brain penetrance

increases as well and that this is

really at around considered to be as

a rule of thumb the optimal log p for a

small molecule that you want to enter

the brain unfortunately the formula is

not that simple and straightforward but

but its a good rule of thumb and

medicinal chemists tend to use it

um increase log p

even higher and and brain uptake is

reduced again so why is this occurring

so if you have a lower log p you have

poor uh lipid bilayer permeability and

so you dont have poor penetrance or

permeability

look at the optimal log p its just

right its almost like a goldilocks

scenario increase the log p too high you

tend to have very high protein binding

the higher the log p of a molecule the

higher the protein binding in blood

plasma and theres very little available

to enter the brain and theres also a

theory that that you may end up having a

very high residence time in lipid

bilayers and so the molecules dont move

across into the into the brain

so again log p can really have an

important impact on penetrance into the

brain and i put this slide in here

because its not directly related to abc

transporters but i ive mentioned

passive diffusion and permeability a

couple of times now and theres actually

uh ive been a reboot of a very uh

active debate about whether passive

diffusion really exists as a mechanism

for drugs to enter into cells um its a

its a very um wellmannered debate

thats occurring back and forth in

reviews and there are really two groups

and it initially started with this

classic review that was published a

number of years ago now um that

described the hypothesis that in fact

no drug diffuses across lipid bilayers

to enter the cell and all cell

penetrance is the result of promiscuous

uptake through multiple drug uptake

transporters and i know kathy jacomeni

has a whole class dedicated to the

importance of of permeability

transporters and drug action

but a counter argument came out and if

you follow these papers youll see

theres multiple subsequent back and

forth papers about this arguing that

indeed there is passive diffusion and

carriermediated drug transport does

occur in some situations but not all um

and theyre really theyre really worth

reading and investigating to understand

realize and recognize that that even

even today theres a large amount of

debate about even simple

aspects of drug action like how do drugs

enter the cell generally is passive

diffusion real

i think passive diffusion is real i

think the bloodbrain barrier helps

support that argument as well this is

another classic piece of data this one

is generated in the early 0s and in

fact it was generated before p

glycoprotein was understood as a protein

and as a drug transporter and so some of

the outlying data points that ive

circled here were actually mysterious at

the time the data was generated the

understanding wasnt really well

recognized but ill explain to you

exactly why

these outliers occur so these

investigators had taken a fairly large

group of small molecules theyd measured

permeability and uptake into the brain

cns permeability and you can see for

most of these molecules that are these

white dots here theres a good

correlation in this window between

increasing log p and increasing

permeability into the brain so increase

lug p

higher brain penetrance

so i think weve established fairly well

that that relationship exists over the

last few slides but we see something

else occurring here as well lets take a

look up the top here and you can see

dglucose very hydrophilic molecule does

not diffuse across lipid bilayers in

fact some people use it as a negative

control for diffusion experiments

and its got higher uptake into the

brain than you would expect based on its

log p how is that occurring well we all

know now in 0 that of course glucose

uptake transporters are very highly

expressed at the bloodbrain barrier and

they facilit and they are highly

expressed to facilitate

maximal absorption of glucose into the

brain because its critical for for

energy generation

and so as a result we have a transporter

mediated uptake

that that brings the uptake of this

molecule away from away from what you

would expect to see based on the

the relationship that were looking at

here

conversely there are a number of

molecules in this early paper bleomycin

adriamycin epirubison cyclosporin

vincristen that had much lower uptake

into the brain than one would anticipate

based on their log p alone

again as i say at the time this paper

was published 90 it wasnt understood

exactly why this was but we now know

that pglycoprotein is expressed at the

bloodbrain barrier and it can prevent

the uptake of these molecules into the

brain

one thing id comment on here and

michael would have referred to the fact

that that most of uh a lot of small

molecules that are recognized by drug

transport is a natural products and in

fact

the drug transporters evolved as we did

as organisms to recognize toxic natural

products and prevent them from entering

the body thats exactly whats happening

with these drug transporters here all of

these drug examples are derived from

natural products they were discovered

from natural product screening and

pglycoprotein evolved to recognize

these small molecules and preclude them

from entering the brain and other sites

that might be expressing a lot of drug

transporter and michael talked about the

fact that he was critical in discovering

pig like a protein by studying

multidrug resistance and that was where

p glycoprotein was first discovered and

understood it was only later once the

role of abc transporters like pgp were

identified in drug resistant cancers

that the physiologic role of abc

transporters was recognized and thats

what were looking at here this is a a

set of diagrams that explain the role of

these drug transporters and how they

work at the bloodbrain barrier so lets

take a look down here and this is a

schematic from a review we wrote a few

years ago that shows a crosssection of

a capillary in the brain

and we can see theres the luminal space

where the blood flow is occurring and

and the capillaries aligned as one would

expect with endothelial cells

these endothelial cells make up the make

up the capillary

theyre flanked on the basal lateral

side by a basal lateral membrane and and

and touching that basolateral membrane

are astrocytes and parasites that are

now very well understood to to

very tightly regulate and control the

function of the bloodbrain barrier

through cell signaling

lets zoom in and take a look at one of

these endothelial cells and whats

happening here right on the surface the

interface of the the luminal blood

interacting with the endothelial cells

of the bloodbrain barrier and theres

two things that are taking place here

that really constitute the bloodbrain

barrier the first are tight junctions

there are a series of protein protein

contacts that occur between these

endothelial cells that really create a

zipperlike structure and prevent

anything from diffusing through

paracellular transport between cells

and into the brain thats the way a lot

of small molecules and proteins can

enter into organ space through the

vasculature in the periphery of the body

but thats not the case at the

bloodbrain barrier because of these

tight junctions and the other active

defense mechanism thats thats

occurring here are a number of abc

transporters including p glycoprotein

and two others will talk about abc g

and the mrp family of transporters that

are oriented towards the lumen

their blood facing to intercept any

small molecule that tries to diffuse

into endothelial cells theyre really

intercepted and knee fluxed at the

luminal surface of the bloodbrain

barrier they never even get to cross the

vasculature let alone enter the brain

on the righthand side here im showing

a a separate area of the brain the

choroid plexus which is really critical

for generating cerebral spinal fluid and

those drug transporters also play an

important protective role

at the choroid plexus to make sure drugs

cant enter the cerebral spinal fluid

and have a sort of a back door into the

brain through csf penetrance so abc

transporters are playing a protective

role everywhere we can zoom in here and

this is actually a model derived from a

crystal structure and its to scale and

you can see p glycoprotein embedded in

the lipid bilayer and this small

molecule this is doxorubicin a common

anticancer drug its drawn to scale as

well and so you can see on the

extracellular space the apical or

luminal side the small molecule is at a

high concentration

can diffuse across a lipid bilayer under

normal circumstances but abc

transporters intercept it bind it and

use atp to pump that small molecule back

out to the extracellular space against

the concentration gradient and because

its

atp dependent its an energy dependent

process it can work against the

concentration gradient and result in

very little if any drug entering the

brain well be showing with our imaging

examples some some really nice case

studies

so there are three abc transporter

classes that we saw in the scheme on the

last slide the glycoprotein the mrp

family and abc g theyre all expressed

in endothelial cell and together they

limit drug delivery to the brain

they also limit

xenobiotic penetrance to the brain small

molecule toxins that might be ingested

in diet they play a general protective

role

that means that when the pharmaceutical

the modern pharmaceutical industry came

along we were basically primed to make

life as difficult as possible for people

trying to develop drugs to tackle

neurologic disorders

theres also an association between the

overexpression of these abc

transporters and a number of of

disorders in the brain and so for
example theres a suggestion that in
drugresistant epilepsy and
overexpression of these abc
transporters works to further decrease
the amount of antiepileptic drug
getting to the epileptogenic focus and
therefore the patient stops responding
to their the antiepileptic medication
that theyre on um hiv infection of the
brain is one way that that hiv can evade
antiviral drug therapy and total cures
and of course ive mentioned multidrug
resistance in cancer and that can also
play out in brain cancers and ill show
you an example of that
so there this venn diagram on the right
shows three abc transporters and a
number of substrates that have been
tested against all three transporters
and what you can see here is that most
of the drug substrates that have been
studied here
are transported by more than one abc
transporter so theres actually a
redundancy in substrate specificity or

selectivity of these abc transporters so

theres amazing chemical coverage

and multiple drugs are transported by

multiple abc transporters so its an

extremely efficient system thats set up

to prevent molecules from entering the

brain of course there are lots of

examples of drugs that do enter the

brain and drugs of abuse that enter the

brain as well

we talked about heroin a little earlier

and thats a really obvious drug of

abuse example but there are whole

classes of drugs such as antiepileptic

drugs or antidepressants that have been

developed and optimized based on brain

penetrance

so

as i mentioned there are a remarkable

number of drug transporters that are

expressed at the bloodbrain barrier

and a few years ago we

analyzed some data that had been

published in quantitative proteomics

studies to compare the expression of

these transporters side by side you can

see the the three main drug transporter

families

that i mentioned p glycoprotein the mrps

mrp and abc g which is also called

bcrp as michael mentioned that are

expressed at the bloodbrain barrier to

protect and prevent drug ingress into

the brain and theyre expressed at

reasonable concentrations

in femtomoles per microgram of protein

uh abcg and abc b peak like a protein

are relatively equally expressed and

play

a a strong role in protection at the

brain and and kathy geckomeni will

address uh probably not discussing the

bloodbrain barrier but

discuss the role of solute carrier

transporters and many of those are

expressed at the bloodbrain barrier to

selectively facilitate the uptake of

important um metabolites and nutrients

that are needed in the brain so there

are two glucose uptake transporters and

and theres also uh an amino acid

coordinated transporter as well thats

expressed at very high levels of the

bloodbrain barrier

that can also facilitate the uptake of

glutamine down on the bottom left here

lets talk about the volume of blood

flow thats in the brain and how is it

regulated by the bloodbrain barrier so

five percent of the brain is vascular

volume at any point in time so that five

percent of the brain volume is blood

volume but when you narrow that down to

the volume thats actually the capillary

bed in the brain two percent of the

total brain volume is protected by

capillaries and this is an electron

micrograph of of cat blood uh

blood vasculature of all things um and

and images like this we use to to

quantify so this is a plastic cast of a

human brain showing the vasculature this

is electron micrograph zooming in and it

said that that no cell is more than 00

microns from the nearest capillary and

so really critical for oxygen glucose

supply to the brain that theyre very

proximal to capillaries highly dense

network

if we take a look at a crosssection of

electron micrograph of of the

bloodbrain barrier you can see that the

majority of a capillary is of course

blood

but but its surrounded by these

volume the cell endothelial uh layer

here and in fact there was a really neat

study done in the in the 90s where

they took a projection of these

cross sections

literally cut them out with scissors and

paper weighed the piece of paper and

determined that the capillary makes up

about by weight about 0 of the volume

of the bloodbrain barrier and if you do

the math on all the numbers that ive

thrown at you you learn that the brain

is composed only 0 percent of the

total brain volume are endothelial cells

and so 0 percent of all the brain the

cells in the brain are tasked with the

job of protecting the brain by

performing that functional blood brain

barrier so its a remarkably efficient

system thats not overtaxing for the

brain

now there are ive mentioned a number of

pathologies that are associated with

bloodbrain barrier dysregulation and

brain tumors are probably one of the

better known and in fact

when a brain tumor is forming in the

brain the vasculature is disrupted and

we can we can see that here on on the

left through a sort of classic mri the

patient has received a gadolinium

contrast agent now gadolinium contrast

agents are negatively charged uh they

dont easily diffuse and they cant

cross the bloodbrain barrier so if you

look at the normal regions of the brain

in this patient you can see very low

contrast levels if you look over here to

the left this patient has a glioblastoma

and you can see very high contrast

because the blood brain barrier is

broken down and the contrast agent can

enter in into that gbm and so

thats one way that a that a surgical

neurologist in in collaboration um with

radiology colleagues can diagnose a a brain tumor and decide on surgical intervention you can also see here theres a lowgrade glioma where the bloodbrain barrier really hasnt been compromised and theres not strong contrast there and that would require further followup and so the bloodbrain barrier

and its breakdown is actually used as part of diagnosis of brain tumors using contrast agents and this of course is translated into lots of animal models that are used using contrast agents to monitor things like the size of brain tumors and how and the amount of penetrance

that takes place there and for sophisticated followup studies

so

we had decided to to try and image the drug transporter function at the bloodbrain barrier and we had some really fantastic uh collaborative investigators at the national institutes of mental health bob ennis and victor

pike who were part of a positron

emission tomography imaging team pet

imaging and together we were thinking

about how can we study pgp function at

the bloodbrain barrier so

the conceptual idea was very simple a

radiolabeled substrate that was

injected into an animal would be

intercepted by transporters and it

wouldnt be able to enter the brain and

so in fact if you entered a radio tracer

much like the gadolinium contrast agent

on the previous slide you wouldnt

really see a lot of brain intensity

however if you coinject an inhibitor a

pharmacologic blocker of abc

transporters like pgp and several of

those do exist you would stop the drug

transporter from working and when you

inject that radiotracer itll be able to

diffuse into the brain and you should

see nice brain signal and and

im sort of giving away the story here

because this is experimental data down

the bottom that that proves the

hypothesis that im putting to you

so there are there was a precedent for

this but not studying the brain and

thats that for a long time given that

transporters were known to play a role

in drug resistant cancer

studies had been done here at the nih

clinical center particularly by susan

bates and tudor fojo whod been working

with a technician radio labeled drug

transporter substrate that they would

inject into patients this patient has a

metastasis to the thigh and you can see

that after this

radiolabeled compound called sestami is

injected you can see a faint trace of

uptake into the tumor it stands out

against the muscle here and the thigh

you can also see by the way the

excretory pathway for this radiotracer

because these drug transporters do play

a role and build up in the bladder

when the same patient was coinjected

with territor which pharmacologically

blocks the drug transporters you can see

that this drug resistant tumor could no

longer pump out the radio tracer and so

it builds up an initial injection time
to pretty high levels and then over time
it diffuses out again but but you can
see the difference between this drug
resistant patient without and with a
blocker of the drug transporter and
theres an increase in signal here and
so this patient can was known to have
cancer and can be diagnosed with the
drug resistant form you can also see
because of the blocker a really reduced
amount of bladder
tracer as a result of inhibited
excretion of this radio tracer and so
you can actually watch the excretion of
these radio traces conveniently at the
same time because that was sort of a
below the waist image that was being
collected there and so given that there
was some precedent for imaging generally
and we wanted to develop a radio tracer
there were some criteria that were set
as part of the study the first is a rule
of thumb for pet radiochemical purity
would be needed in the brain and so you
need to be studying

and imaging a molecule that isnt broken
down and metabolized very quickly
because otherwise youll be imaging as
many metabolites as the parent tracer
the second from our point of view
imaging transport is we needed something
that was highly selective for peak like
a protein we didnt want to study all
the transporters at once that would be a
difficult study to interpret
so we needed to identify a molecule only
transported by pgp and if you think back
to the venn diagram that i showed a few
slides ago there were a few molecules
there so there were some clues about how
we could pursue that and the third rule
for pet generally is you need a high
magnitude of signal what you want to
measure needs to stand out from the
background and in fact for us that was
pretty straightforward because i already
showed you that we should expect no
brain signal under normal conditions and
if we block the transporter we should
get high signal so we had an advantage
there over uh normal pet imaging people

and so the the lead traces that were

selected for this study

were uh leperamide and a dmethylated

form of the pyramid called

desmethylpyramide

so why why was this picked it was picked

because its been an overthecounter

drug for many many years now and and

some of you watching this video may

fortunately or unfortunately know of it

its sold under the name imodium its an

opioid receptor agonist in fact and its

used to prevent diarrhoea in patients

suffering from acute diarrhea

um i mentioned its an opioid receptor

agonist

that pharmacologic mechanism of action

is exerted at the gut to prevent

diarrhoea so

its an opioid but its sold over the

counter why can it be sold over the

counter its all over the counter

because its known to be

a very strong peak p glycoprotein

substrate that cannot cross the

bloodbrain barrier and get into the

brain if it could and if it did have

central opioid agonist effects it

wouldnt be an overthecounter compound

at all and so we took a look at this

small molecule um uh dr spike uh

and innis had stutt and recognized that

there was a way to radio label this

small molecule so that it could be used

for in vivo study doc dr innis had had

done some preliminary studies and

recognized that that it did indeed

didnt enter the brain and so in

collaboration um with with doctors

picking innus we set about trying to

understand its metabolic stability its

specificity at drug transporters and

whether it would be a useful radio

transporter and really the two key

studies were an in vitro and in vivo

set of transporter studies heres the

cell work we radiolabeled theres

methyl the pyramid

we would expose it to cells that do not

and do express peak like a protein and

you can see these cells that express pgp

very little drug very little radio

labeled the paramide gets into these

cells because of the drug the action of

peak like a protein on the pyramid so

that is what we expected the really nice

thing that we saw with this particular

radiotracer when we examined it is that

if we took cells expressing abc g and

one of the mrp transporters they didnt

have any effect on the entry of

loperamide into those cells and so over

expression systems show that they g and

the mrps had no effect on the paramide

so that looks like weve got a pretty

specific radio tracer

we could also do in vivo pet studies

looking at brain signal and in fact when

radio labeled the paramide was injected

to wildtype mice or my sweat that had

abcc the mrp transporter or abc g

knocked out there was still very little

uptake into the brain and and this

signal is measured over time and this is

called a time activity curve however

when we took

um

a mouse model where the abc

b transporter peak like a protein and

in fact mice

through the vagaries of nature have two

p glycoproteins but when theyre both

genetically deleted you can see very

high brain penetrance and very prolonged

stable brain penetrance as well and so

these genetic studies reinforce what

weve seen in our cellbased studies

the pyramid is a specific substrate of

human and mouse peak like a protein and

we could go about doing cell studies and

so heres some nice sample data here in

the mouse very little brain uptake if

you coinject these animals with a

blocker of pig like a protein or if you

use the genetic knockout you see quite

high brain penetrance and this is an mri

side by side to so you can see that this

signal corresponds to the to the brain

region we could do the same thing in in

monkeys in the rhesus in this case and

you can see very low signal in the

monkey when the monkey there are no

genetic knockout studies of course but

when you coinject with the

pharmacologic blocker of the transporter

very high uptake into the brain

corresponding to the um the mri image

here now there is a big hot spot of

signal that you see under here arrowed

in these studies that signal from the

pituitary the pituitary is outside the

bloodbrain barrier and so you can see

the pituitary gives very high signal

along with mri signals this is a really

nice way to help the radiologist

interpreting these images from orienting

the images and orienting themselves when

theyre looking at these signals so

theres a nice we actually have a

outside the bloodbrain barrier control

built into the images through the

pituitary heres the

some further studies um trying to

understand what was happening in the

monkey brain again

high pituitary lone brain signal until

pgp blockade takes place with

pharmacologic inhibition for the monkey

studies we were using another

pharmacological blocker called dcpq and

the nice thing is that at a

pharmacologically achieved

pharmacologically achievable dose of

inhibitor we get very high uptake of

leperamide

and its very stable in the brain and

very low baseline signal when there

isnt a pharmacologic blocker

pglycoprotein can really keep this

transporter out of the brain

so these studies are important as well

because

one of the things ive talked about is

how hard it is to get drug trans drugs

transport substrates into the brain and

so theres a very active field of study

trying to understand whether

coinjecting or coadministering an

adjuvant blocker of pglycoprotein might

be a way to facilitate increased drug

uptake of a small molecule drug

candidate and this is proof perhaps that

thats achievable and theres multiple

groups that are studying that at the

moment

so going from the animal models we went

into the human and these are just some

sidebyside studies at minutes 0

minutes and 00 minutes of a patient

thats been injected with dlop the

first thing you can see i know you

wanted to look there is the brain thats

what were talking about very low uptake

into the brain however if we take a look

below the neck at this patient you can

see quite high signal in other organ

sites of course there are protective

mechanisms in the lung and you can see

pretty reasonable uptake here

the kidneys are the excretory pathway

and you can see very strong kidney

signal as a result and in fact as the

kidney single signal goes down in the

kidneys you see a little bit of buildup

in the liver probably because of

metabolic action but also you see

increased bladder signal because of the

urinary bladder excretion through the

kidneys of this radiotracer so really

nice images there of whats happening

but lets take a little closer look at

the brain here so this is a

three minute or zero to ten minutes

summed image of brain signal and what

youre looking at is initially nothing

if you look at where the brain is

the brain is evidenced by the absence of

any signal at all and we can rotate this

a few times here to take a look at this

this video

i mentioned the choroid plexus earlier

and in fact you can see

signal from the blood pool in the

choroid plexus and you can see the

venous sinus the strong venous sinus

drainage thats occurring here and if

you look really closely my favorite is

that behind the eye there seems to be

some blood blood pooling thats taking

place and you can see the back of the

eyes in fact

the the eye has its own barrier called

the blood retinal barrier that also

protects the eye from from entry and

its very similarly constructed to the

bloodbrain barrier and it also presents

its particular pharmacologic challenge

for treating some drugs such as dry eye

disease that require drug penetrance to

the eye

where the eye has an advantage is eye

drops so we can do direct topical

application to the eye of a drug

to for experimental experimental or

pharma for pharmacologic administration

um in an fda approved setting as well we

dont quite have that advantage in the

brain although as i mentioned earlier

there are some very

invasive methodologies that are used for

direct drug delivery to the brain under

certain circumstances

so i mentioned we did human studies

lets take a look now at a

cross section of a pet study yet again

very low uptake and what were looking

at here these hot spots at this level

within the brain and you can see it in

the mri as the choroid plexus

in the ventricles and so very high

signal again choroid plexus is outside

the bloodbrain barrier this is a lower

dose of a pharmacologic blocker

called tariquida and you can see

that some blockade of peak like a

protein has occurred and theres some

increased signal into the brain again

validation

first of all imaging of the profound

efficiency of peak like a protein at

preventing drug ingress into the brain

and validation of pharmacologic blockade

to try and improve drug penetrance into

the brain and its an area of study so

peak like a protein that was fun

theres another important drug

transporter the bloodbrain barrier abc

g and weve begun to study abc g and

its role at the bloodbrain barrier and

theres quite a lot of literature

arguing that it played a a minor role in

in protection and so we wanted to see if

we could directly study

drug transport at the bloodbrain

barrier it had never been achieved

before but

i mentioned that one of the three

criteria for imaging that we set down

and maybe the most important one from a

drug transport point of view is

specificity we needed a specific drug

transport substrate of abc g

and we couldnt find one we did quite a

lot of work on it

and in our reading we accidentally read

and noted that luciferin here

is a sp was an abc g transport

substrate and it was one of many that we

decided to follow up on and examine

some of you watching this may not know

much about luciferin but luciferin is

well known in the in in the assay

development and experimental biology

fields because its the substrate from

the firefly the fight the enzyme fly

luciferase that uses dioxygen and atp

acts on luciferin produces oxyluciferin

and it also produces light and so this

enzyme substrate system is responsible

for the fact that a fireflys

rear end glows in the nighttime and

thats that enzymatic system has been

used in experimental biology for a range

of different studies

and as i say we we read that that

luciferin was a specific abc it was a

abc g substrate we didnt know whether
or not it was specific and unfortunately
our colleagues in pet radio chemistry
informed us that it was unable to be
radio labeled in this form and so we
couldnt do a pet study with this but in
reading some old literature and you can
see a 9 paper here and some data on
the left hand side there so its okay to
read papers from before you were born
sometimes he can re learn really
important things from old papers and and
heres a really great example what we
found when we were reading is that yes
we all know now that that luciferin is a
reporter
that can produce bioluminescence with
luciferase so thats a readout we have
right there we could possibly take
advantage of bioluminescence but its
also fluorescent and thats a really
useful labbased uh tool for studying a
molecule um it could be excited or in
the uv range 0 nanometers and itll
emit at 0 so its fluorescent in its
own right without needing an enzyme

system to create bioluminescence the

other thing when we looked at the

literature we saw and you always see

should look for this paper in

experimental therapeutic studies

somewhere somebody did a

biodistribution study where theyll

literally administer a radiolabeled

form of a drug to an animal perform a

necropsy and theyll measure the radio

the radioactivity levels in each of the

organs to see where this drug goes and

you can see

very low brain levels

so we have an abc g substrate very low

brain levels maybe weve got something

we can study the bloodbrain barrier

with so lets take advantage of that

fluorescence you can see here some

straightforward pictures heres a cell

over expressing abc g and dluciferin

is not getting in however if we look

again using our fluorescence microscopy

with a pharmacologic blocker of

dluciferin which is called ko

high fluorescence block the transporter

luciferin gets into these cells so it

looks like were on the right track so

we set up a quantitative assay using

flow cytometry cells that dont express

g accumulated very high levels of

luciferin measuring its fluorescence

however when you express abc g

very low fluorescence very low

accumulation into these cells block the

transporter and we get high expression

okay so lets do those studies in

triplicate and lets study cells

expressing different transporters and

you can see just like the pyramid with

pgp when we do quantitative accumulation

a cell line thats expressing abc g had

very low levels of accumulation if we

blocked g they became very high this is

the data were looking at qualitatively

here now its in a quantitative fashion

but when we look at pgp theres very

high accumulation in pgp expressing

cells very high accumulation in mrp

expressing cells so we have a specific

abc g transport substrate here only

abcg stops it from entering the cells

but how are we going to image it and how were going to study it and so we set up on a hypothesis that could we could use in a mass imaging context to study the bloodbrain barrier

we could inject an animal that that with luciferin and if we could find a transgenic model that only expressed luciferase the enzyme that luciferin needs in astrocytes in the brain then under normal conditions abc g would not let this luciferin get into the bloodbrain barrier and keep it out but if we coinjected with a blocker of abc g and ill show you that again if we coinject with the blocker of abc g now lucifer can get into the brain find that luciferase in the brain and we should get light bioluminescence produced in the brain and we can image that and we can quantify it

so how are we going to achieve that well luckily for us there were a number of models that people had reported where they did express luciferase specifically in the brain

using the promoter for gfap which is a

marker for astrocytes and when we

express injected luciferin into the

brain under baseline levels we saw very

low levels and so what were looking at

here is the underside of the mouse head

if we turn the mouse over the signal is

lower because of the skull so we put it

on its back in a supine position very

low signal and then if we inject with

blockers of abc g you can see the more

the higher the dose the higher the

signal a dose effect response its

exactly what youd expect based on

everything youve done in this class so

far we can generate time activity curves

here to follow this

very low levels of bioluminescence with

luciferin we can inject ko our

blocker we get increasing brain signal

we take the area under the curve

something else youve already done in

this class to integrate that signal and

you can see by a milligram per

kilogram dose weve basically saturated

and fully inhibited abc g and we have

maximal signal

we can derive the ed0 the effective

dose for 0 of the maximal response

again part of your clinical pharmacology

class and and get an ed 0 value of

about milligrams per kilogram and

one of the important experiments we had

to do here was make sure were really

imaging brain signal and not mouse

signal and so here weve taken the brain

out we have a brainless mouse and you

can see theres no more signal the brain

is still producing bioluminescence and

so all that signal that youre looking

at up the top here is coming from the

brain its not coming from the tongue or

the nose or the ear or somewhere else

so we developed there in those two

stories ways to study p glycoprotein and

abcg with the bloodbrain barrier and i

told you i wasnt going to

parade a litany of drug development

failures in front of you to emphasize

how important

these drug transporters are for the

development of drugs that need to get

into the brain but lets flip it and

ill just give you an interesting

example of how pharma has to tackle the

bloodbrain barrier and some and peak

like a protein and this is the opposite

of the normal example so the next couple

of slides im just going to very briefly

walk you through a nice set of studies

from merck where some

assay biologists and medicinal chemists

are working side by side as part of a

team science project to develop blockers

of a potassium ion channel and the goal

of doing that in the heart

was to regulate cardiac arrhythmia so

they had a target kv potassium

channel and along with that target

they had an expected pharmacologic

response the regulation of a cardiac

arrhythmia so they had a target they

conducted a high throughput screen

against the potassium channel of

interest

and they had a hit from that high

throughput screen this small molecule

here thats labeled number one so you

have a hit from a screen and now you
need to do medicinal chemistry as youll
hear about in later classes to improve
the activity of this molecule and
optimize its property for in vivo use in
animal models and in humans
you can see the authors of this paper
acknowledge that there was prior art
something else youll talk about in drug
discovery prior art that already existed
in the literature that can be used to
validate a hypothesis around a target
but they had their hit they wanted to
make a new chemical entity something
they could patent get
protection or intellectual coverage for
as part of a drug development program
and they wanted to march this forward
but they had a specific criteria as part
of their hits
they really liked the activity of this
molecule but
they wanted to have a molecule that
could interact with potassium channel in
the heart but not get into the brain
where it would interact with potassium

channels in the brain and cause toxicity

so they actually wanted a pgp substrate

that couldnt get into the brain what

other drug development programs suffer

from is something that they really

wanted

and so when they started studying their

hit from the screen they found that

wouldnt you know it they had a drug

that did get into the brain the thing

that everybody else is desiring is was

actually their curse and so they

actually went through a medcam campaign

and um doing variations on their

molecule to retain their activity

against the potassium channel target

but to install

substrate susceptibility to peak like a

protein so it could be kept out of the

brain and so as they went from this

analog that had a carboxylic acid they

installed a number of substituted amines

here these amines unfortunately

were expected to make the molecule a pgp

substrate it didnt happen it could

still get into the brain it was still

active against the target so they

werent destroying their own target

activity but they needed to make sure

they had a substrate finally through

some subtle variations with medchem you

can see

they had a primary amine here they now

had a pgp substrate it didnt get into

the brain but they destroyed their

potassium channel antagonism and so

needed to do some further sar

modifications to get what they finally

needed

a substrate of pgp that couldnt get

into the brain but that was active

against the target in the periphery in

the heart so they had their their

combination of desirable activities here

uh its a really nice paper i encourage

you to look to what the reference was on

the previous slide the titles at the top

here

ive checked as part of putting together

this presentation about what merc has

done with that lead and in fact theyre

still advancing this series several

years later through medicinal chemistry

programs to optimize the drug

development programs and in the papers

that ive ive listed the titles for up

here and the references are down the

bottom you can see theyre going through

further second and third generation

iterations theyve improved activity

further they still

emphasize that they need molecules that

are pgp substrates that cant get into

the brain and theyre now in clinical

trials with these molecules and theyre

in humans and so this is an example how

understanding biodistribution and pgp

transport susceptibility was a really

important part of of a a cardiac target

not not a brain target

so theres really universal uh

importance here for understanding the

importance of drug transporters in the

brain theres another important site

here i wanted to mention we did some

imaging work on weve really focused on

the blood brain barrier but there are

other sites like the blood retinal

barrier that i mentioned the blood

testes barrier thats very important and

the blood placental barrier that also

forms um in pregnant women

it forms as part of the placenta that

provides to a number of roles including

supplying nutrients um to the forming

fetus and embryo as well as a protective

role just like at the bloodbrain

barrier to make sure no toxins can cross

the placenta and enter the the

circulatory system of the embryo or

fetus and we generated a very analogous

system here where we in fact took a male

mouse that just that was just a

transgenically

expressing a lot of luciferase crossed

it with a wild type female mouse so she

wouldnt express any luciferase at all

but the pups in the develo in the in

behind the blood placental barrier would

be expressing luciferase and so under

this model you could inject the ho the

mother with luciferin it wouldnt cross

the blood placental barrier under normal

cyst situations however if we

pharmacologically blocked abc g

luciferin would be able to cross the

blood placental barrier um and by the

way if you really like these pictures

im recording this talk at the part of

the medical arts facility at the nh its

exactly the same place that generates

these kind of beautiful figures so id

really encourage anyone out there in the

intramural program thats thats trying

to create really nice artwork to convey

the research that theyre doing to talk

to medical arts they do a really

beautiful job this is one of my favorite

figures ive ever been involved in

you can see down the bottom here that

this played out in the animal studies

heres here is a male mouse lots of

luciferase here is a female mouse here

is a female mouse thats impregnated and

you can see the signal thats occurring

here and thats specific to the pups

behind the placenta and in fact

similar to the time activity curves that

i showed you on the previous slides you

can see low bioluminescence

in in the pregnant female however once

we coinject an abc g blocker we get

very high signal in fact this was the

first demonstration of drug transport

activity through imaging studies at the

blood placental barrier

so ive told you that these abc

transporters have play a really

important role in understanding drug

action and in the early stages of the

drug discovery and development process

um of course when youre doing high

throughput screening and and as we do at

ncats and identifying small molecules

from program to program we need to

understand whether our molecules are

drug transport substrates

how do we do this in the lab so ive

showed you a lot of animal studies ive

showed you a lot of sophisticated

imaging studies these are obviously

oriented towards understanding basic

physiology or pathology of drug

transport action but if youve got small

molecules and you need to study this the

normal system people will use is a

transwell system so you actually place

your cells in a transwell insert they

actually form a nice cell layer and they

form those tight junctions just like at

the bloodbrain barrier if you put a

nonpermeable molecule here like a

protein a small molecule die like

lucifer yellow it actually cant cross

and diffuse across these cells and so it

stays here in the apical chamber

however if you have a normal small

molecule that can diffuse across cells

it should diffuse across to the

basolateral

side of this chamber where theres also

media if pgp transfected cells are here

they will keep the drug

on the apical surf side of this chamber

and you can add a blocker and obviously

encourage cells to go down and so people

will add a drug or an experimental

compound of interest and theyll measure

at the end of that incubation time how

much is up here in the apical side and

how much got to the basal side and that

ratio will give them insight into

whether pgp transport susceptibility is

playing a role you can do that

experiment in a 9 well plate um and so

its relatively scalable and thats the

kind of experiment would an nsa biology

group like mine will do in partnership

with the medicinal chemistry group as

part of a development program and so

when youre doing high throughput

screening and discovery youve got a

target you do a high throughput screen

and when the medicinal chemists are

making those modifications um were

coordinating with them on on

modifications to those drugs and

understanding things like p glycoprotein

transport susceptibility

so ive got a few conclusions here for

you based on what ive told you about

they might be a little bit obvious and

and they really are reinforced by dr

goddessmans talk that preceded this one

in the in the talk order so weve used

noninvasive imaging as a way here to

really drive home just how important abc

drug transporters are for regulating

drug action and and and i think a

knockon of that is what a really

profound pain they can be from a drug

development and experimental

therapeutics point of view as well

the kind of mouse model and cell line

models that people have generated can

really help the drug development process

they can help understand physiology that

they can help medicinal chemists get

where they need to get in the drug

development program collaboratively with

people like

pharmacometricians people who study

pharmacokinetics to understand the

biodistribution of drugs and

understanding that target site

and of course abc g is is a really

important drug transporter as well and

were were beginning to understand that

its just as important as peak like a

protein

in protecting and playing a physiologic

role and making life difficult for drug

development programs as well so i do

have an acknowledgement slide here based

on my work when i was at the laboratory

of cell biology and the national cancer

institute dr goddessmans lab and i had

a really fantastic and large i suppose

set of students who worked with me

and studying and understanding the role

of the bloodbrain barrier and drug

development my collaborators the

national institutes of mental health

without whom this collaborative program

could not have been successful and also

the karolinska institute in in in sweden

who are part of a graduate partnership

program with the national institutes of

health really fantastic graduate program

that we have as well and my colleagues

at

ncats where i currently work where we

still continue to try and understand the

important role of drug transporters in

in drug development and experimental

therapeutics design i really appreciate

you listening and if you have any

questions delight to contact us please

get in touch with the program

coordinator youd be happy to pass on

your questions to me to answer thank you

very much

you