im excited to introduce todays lecture
molecular imaging branch at the national
institute of mental health dr ennis
holds a bs degree from yale in molecular
biophysics and biochemistry he received
his md and phd in pharmacology from
johns hopkins university
his research focuses on the use of pet
imaging to study neuropsychiatric
disorders please enjoy the presentation
uh welcome uh my name is uh dr robert
dennis and im very glad to speak to you
today about my area of research which is
in positron emission tomography
and i want to explain it in terms of its
principles and what it can do of
relevance to pharmacology and in
particular in that regard how pet
positron emission tomography can be used
to study pharmacokinetics
and also to facilitate therapeutic drug
development
what i will tell you today ill
summarize at the beginning and the first
is to say that pet positron emission

tomography is an in vivo technique with
very high sensitivity and specificity it
can measure compounds present at very
low concentrations in the body and it
can measure specific proteins labeled
with radioligans
ill explain how pet is currently used
in therapeutic drug development
primarily to determine the appropriate
dose to be given on phase one studies
ill also try to explain in words and by
by simple analogies what pharmacokinetic
modeling provides
and its
basically measuring the concentration of
drug in the plasma the plasma
concentration and the tissue uptake and
if you do both of those you can measure
the density of the target of the
receptor that the drug is working at
ill show examples of how a pet can be
used to study distribution of drugs
distribution by the way is the
reversible transfer of drugs from the
plasma to the tissue and back so if its
distributed the drug goes to the tissue

and then comes back to the plasma and

the specific example is one where you

can block the distribution to the

periphery and increase the distribution

to the brain

ill also show an example how pet can

study metabolism where metabolism is

making a new bond or breaking a bond

destroying the in some ways the original

chemical structure of the drug in this

particular case it will be in regard to

inhibiting

defluorination

okay

pat has been around for several decades

going back to the

late 90s

and the general strategy is shown here

a

cyclotron is used to produce unstable a

radioactive atom for example carbon

can be made in a cyclotron it has a

halflife of only 0 minutes so you have

to use it very quickly

so it might come out of the cyclotron as

c labeled co

and then its incorporated into some

drug

rapidly

made appropriate for human injection

and then the subject is injected in is

in a pet camera which is like a sort of

a doughnut hole and the head or the

whole body can go in

the result of that for example would be

this image a tomograph where a tomograph

is a slice this is a slice through the

brain where we have

the front of the brain the back of the

brain and the two sides and you can see

that this c labeled drug in this case

raclopride which binds to dopamine

receptors is present in the internal

portion of the brain

so this uh

cartoon comes from the mid90s when

the resolution of pet was on the order

of about millimeters the resolution

has gotten a lot better since then

and the pictures are less blurry

i like this next movie clip made by

simon sherry from uc davis and it shows

the principles of pet imaging for the

most common clinical use currently

and that is a radiolabeled form of

glucose or sugar is injected with

patients with cancer most cancers many

of them

and it so happens that

tumors

as well as their metastases burn up a

lot of sugar and take up a whole lot so

you can localize the tumor and its

metastases by increased uptake of a

radiolabeled sugar analog

okay

so in this movie clip youll see the do

knight doughnut shaped pet camera and we

have the iv ready to inject the compound

here

the radioactive f fluorodeoxyglucose

is scintillating so its within a

glucose analog it travels through the

body and then it will be taken up by a

tumor

in the lung

what happens with this unstable

radioactive atom is that theres a

nuclear decay a particle is emitted from

the nucleus of the atom and

after a certain per that particle is a

positron and at a certain time the

positron will annihilate will meet with

an electron so thats a matter an

antimatter a positron and an electron

meeting which gives off pure energy in

the form of two gamma rays which go out

at 0 degree angles and then can be

detected by the pet camera and

reconstruct it into slices

throughout the body or throughout the

brain in this case

i i like that because its

im still fascinated by this ever since

i was a medical student at hopkins in

the late 90s and i first heard about

pet it was matter and antimatter

combining giving off pure energy in the

form of two gamma rays and being a star

trek fan i said scotty board me bring me

aboard beam me aboard because thats

what i want to do

and it is amazing

sometimes for students i will

ask them if i give you the mass

of the electron and the mass of the

positron i want you to calculate the

frequency of the

gamma rays that are emitted and you can

do that and you have to use einsteins

equation e equals m c squared because

the mass is converted into pure energy

its amazing that were able to do this

now

okay another way to understand pet is by

a comparison with magnetic resonance

imaging which is a far better known and

that the spatial resolution of mri is

much much better less than one

millimeter for the mri

uh whereas pet well pet debt is down to

about its limit now of about one

millimeter

but the way in which pet really stands

out is its sensitivity

whatever mri or mrs is measuring has to

be present in vivo at 0 to the minus

fourth molar

whereas pet has a sensitivity down to 0

minus perhaps 0 to the minus th

molar

so it can see that radioactive atom

carbon or f if its present at very

low concentrations

it turns out that many of the proteins

that were interested in the body and in

the brain that might be involved in

pathophysiology are present at ten to

the minus ninth molar one nanomolar so

theres no way that mri would be capable

of measuring proteins at such low

concentrations whereas pet is certainly

able to do so

so the radionuclide the unstable atom

carbon rather than the normal form

carbon can be detected in vivo with

very high sensitivity

the if that is combined with a drug like

raclopride it can have high selectivity

for the d type of dopamine receptor

you end up with a radioligand c

raclopride that has high sensitivity

because of the carbon and selectivity

because of the rackler product so this

can be used for any protein in the body

enzyme receptor transporter

and be able to measure it

in vivo with a pet device

so a radioligand then is a drug plus

some radioactivity with it and

i should mention that the

the drug is administered at tracer doses

at minuscule doses

that will have no pharmacological

effects and we can administer minor such

low doses because pet has the

sensitivity to measure it in the body

after its distributed to the body

because its a tracer dose from a

pharmacological perspective there are no

pharmacological effects it might bind to

or label you know less than one percent

of the receptors but that labeled subset

reflects the entire population so you

use the radio ligand to label a

representative subset perhaps less than

one percent of all the receptors in

order to study those receptors

as a drug the radiolike end is disposed

of in the body where disposition in this

case is referring to for example

metabolism and distribution metabolism

is where you break a bond or add a new

bond and distribution refers to the

transfer of a drug from the blood plasma

to the tissue and back and im going to

show you examples of how pet can be used

to study metabolism and distribution

the major disadvantage of pet is that

there is radiation exposure associated

with it

and the radiation exposure is a very

complex and controversial

but i think it would be fair to say

based upon large numbers of studies

particularly the survivors of

nuclear bombs in hiroshima and nagasaki

that the level of radiation that we have

here has never been documented for

example to increase the cancer rate so

it would be considered safe within our

current knowledge and it is currently

used very commonly for example in cancer

patients where you have the

radiolabeled glucose analog

but it it does it is associated with

radiation exposure

okay

well i was mentioning that the

resolution has gotten better and thats

shown here in this slide a movie clip

so in this case this was a rat that was

injected with sodium fluoride the f

the unstable isotope not f9

and

fluoride as you may know goes to the

bone particularly to the growth portions

of bone so you can see really the level

of detail that you can get here when you

inject a rat intravenously with sodium

fluoride and the f can taken up

particularly in the growth portions of

the of the bone so the resolution has

gotten a lot better we can image

rodents mice transgenic mice

not just

large humans

okay

so

i want to tell you examples of how pet

can be used in it and is being used as a

tool in therapeutic drug development

and by far the most common use is to

determine the dose that should be used

and the dosing interval how if you have

a new therapeutic drug how much do you

have to give and how often do you have

to give it

a good example of that comes from this

slide i think this must be from the

early 990s from brookhaven national

laboratory

joanna fowler nora volkow

and it looks at the uptake in the brain

of a c labeled depranol so a

radioactively labeled drug this is

actually an inhibitor of monoamine

oxidase b

an enzyme in the brain involved in the

metabolism of monoamines

so the subject here was in was scanned

on four different times one at baseline

and you can see high uptake high amounts

of monoming naob in the brain and then

after giving a blocking dose of

lizabide

which is an mao inhibitor so this is

nonradioactive lisabloid

you can see what happens how you block

the uptake at bid

0 milligrams is more but that it all

returns after about hours okay

so lets compare this to

if you used silene which is a marketed

antidepressant and also an maop

inhibitor

you can see baseline here

and then five milligrams bid wow

so its clear that celene is more potent

than lacebamide because five milligrams

did more than did

but perhaps even more striking is the

fact that this blockade of the enzyme

appears to last for several weeks

well the reason for that by the way is

that lisabemide is a reversible

inhibitor it binds to mao b and binds

and comes off and when its binding it

blocks the binding of the radial ligand

c labeled depranol

in contrast celegoline is an

irreversible inhibitor its a suicide

substrate when it binds to it also

covalently binds to mao b

so what were seeing in the bottom slide

is actually

the production the synthesis of newly

made maob replenishing that that had

been

irreversibly inhibited by cellegeline

so i think that this sort of

schematically shows how you can use a

radial label and looking at therapeutic

compounds to find well how much are you

going to have to give and how often do

you have to give it

for the latter question for example i

mean thered be no reason to give more

than milligrams twice a day of silene

because youd have a complete blockade

and theres no reason to give it every

day

because the effect lasts for at least a

week

and so this is commonly done now when a

new therapeutic drug is being considered

particularly for the brain

and its a very important question you

have a new drug it acts at receptor x

how much are you going to give in the

initial study

do you even know that the drug passes

the bloodbrain barrier and makes it

into the brain so this is a very

important question if that dose is too

much you could have a lot of toxicity

well unnecessary toxicity if you gave

more than was necessary but if you gave

too little well then youre going to

have no efficacy you think it failed but

it really wasnt that it failed it just

didnt didnt give the right dose

this so this can be like a 00 million

dollar question and you can use the

radial label

compound to say for example with this

compound i think bid is more than

enough

and i think we should give it uh you

know maybe even once a day but itll

give you some rough guidance to it and

if the milligrams bid does not treat

the depression you can say well this

drug is ineffective at acting at this

target to treat depression and you

really know that its a negative study

okay so that is commonly done in most

pharma companies that im aware of now

at least for cns drugs central nervous

system drugs if they begin to look at a

new target right at the time that they

identify some new target that they want

a therapeutic compound for they allocate

resources money people

supplies

to also make a pet radioligand for that

target so that it is ready on the

initial human studies to determine

did the drug actually get to the brain

was there target engagement

is thats the term for it and has enough

gotten there is there a high enough

receptor occupancy maybe fifty percent

youd be looking for but theres no

reason to go above a hundred percent and

theres no reason to go then less than

zero percent

another way that a pet can be used is to

identify a more homogeneous group of

patients and the example that ill give

here and in the next one has to do with

parkinsons disease

this cartoon shows a synapse or the

connection between two neurons in the

brain

and the presynaptic terminal contains

the transmitter in this case dopamine

in vesicles which are released by

exocytosis diffuse across the synaptic

gap

to the postsynaptic site it might

interact with d dopamine receptors for

example

the signal is terminated

by dopamine being taken back up into the

presynaptic terminal where it can be

recycled or metabolized

the way that cocaine works or at least

one of its mechanisms of actions is by

blocking the dopamine transport as shown

here thereby leading to elevated

concentrations of dopamine in the

synapse which are thought to mediate the

high from cocaine and also its addictive

properties

well the connection to parkinsons

disease is that for unknown reasons

theres a degeneration of the

dopaminecontaining neurons in the brain

you lose the dopamine terminals you lose

the dopamine and you also lose the

dopamine transporter

and for that reason when several years

ago when i was at yale we made a

radiolabeled analog of cocaine

in order to measure the cocaine receptor

the dopamine transporter because we

expected it would be reduced

in patients with parkinsons disease

okay this shows an image at that time we

were using a related technique i wont

go into the difference but its radial

ligand studies with a single photon

emitter

of the dopamine transporter

and you can see in this study that the

resolution of the spect in this case is

like about millimeters like the

oldtime pet

and you can see that theres high uptake

as expected in these terminal areas the

quad that im showing here and in the

putamen

so this is what a healthy subject would

look like

and we quickly went into patients with

parkinsons disease in this case this

was an individual in stage one

parkinsons disease is an idiopathic

movement disorder is very bad perhaps a

third of the patients will actually

develop fullblown dementia so its a

severe terrible

neurodegenerative disorder

and this person is in stage one which is

when you might have symptoms on where

you have symptoms on only one side of

the body maybe a tremor on just one side

has not yet progressed to hit both sides

and you can see clearly theres a

significant loss of these dopamine

transporters to the cocaine receptor

actually the symptoms begin when theres

about a 0 loss

you can also tell and this is done

quantitatively but theres a greater

loss in the putamen than in the caudate

which is consistent with the known

neuropathology at the time of death in

patients with parkinsons disease

theres a greater loss of dopamine

terminals in the butamine than the

caudate

and this also shows a right left

asymmetry and we can be certain that

this patients symptoms 00 of the time

began on the other side of the body from

where the greater decrease was found

so there are three

distinguishing features of the dopamine

transporter in parkinsons disease

theres decreased uptake symptoms

beginning at about 0 loss its

asymmetrical which always reflects the

side of the body with the initial onset

and the loss in the putamen is greater

than the loss in the quantity

so

this can be very helpful in therapeutic

trials

because

this is the really bad disease the other

alternative is where you just have a

little bit of tremor in old age its

grandma with a little bit of tremor and

thats called benign senile tremor its

like a little bit of tremor its not

going to get worse its not going to

develop into parkinsons disease

and those people with benign senile

tremor will have normal or healthy

dopamine transporters only the people

with the parkinsons disease will have

this 0 percent loss or greater

so if you have a drug that you think

could be helpful in parkinsons disease

youd want to start it as soon as

possible

and youd want to eliminate those

individuals who have benign senile

tremor

nowadays i think its fair to say that

if you go to the neurologist with a

little bit of tremor they cant tell

and they just have to follow a person

for one to two years if it progresses

its the bad disease if it doesnt

progress its the benign senile tremor

well this can be used and is actually

approved to be used to aid in the

diagnosis of parkinsons disease to um

to distinguish those two conditions it

was approved first in the european union

and first here and it can be used to get

a more homogeneous group of individuals

for this putative neuroprotective

treatment of parkinsons disease its

going to have people with parkinsonian

symptoms plus an abnormal

scan showing loss of

dopamine transporters and it has been

used for this purpose

okay in addition to identifying a group

homogeneous group of individuals more

homogeneous using it as a biomarker if

you will it can also be a biomarker for

drug efficacy

and thats shown here

these are repeat images in a patient

with parkinsons disease over a period

of about four years

and you can see that theres a

relatively rapid loss of dopamine

transporters in this individual

and thats true of the bad disease

theres a rapid deterioration

well

if you have some intervention thats

supposed to slow the progression

this can be used if its supposed to

slow the progression then it should slow

the loss of this biomarker which is on

those dopamine terminals that continue

to degenerate

it can be very hard to measure the

progression of parkinsons disease based

upon clinical symptoms because they vary

during the day and its hard to get one

measure but this is a endophenotype a

biomarker in the brain that can be used

and that certainly should not

deteriorate if the neuroprotective agent

is

effective

so

dopamine transporter imaging has been

used to assess efficacy of

neuroprotective agents looking serially

over time in patients

currently the

probably the most wellknown or

widespread

is studies being done now

in alzheimers disease

so a pet radio ligand was developed for

amyloid actually the first one was at

university of pittsburgh

pittsburgh compound b

and

theres this amyloid is an abnormal

protein which builds up in the brain

and

is associated with the death of neurons

and dementia

the um

you can see theres much higher uptake

in this patient with alzheimers disease

than in the control subject

well several um

antiamyloid different types of

antiamyloid therapy have been tested

like for example making antibodies to

amyloid and see if the antibodies would

remove amyloid and make the people

better

in order to and in

to assess whether the antibodies are

effective in removing amyloid the

patients are given serial images with

this uh pet marker or

related one

to the amyloid protein itself

so it can be used as a biomarker of drug

efficacy in this case of drugs used to

treat or slow the progression of

alzheimers disease

so far

theres no clear evidence that thats

effective

but its

being looked at very actively

okay

so

in addition to pet being used currently

in therapeutic drug development the most

common being to determine the initial

dose of a phase one study

pat

theoretically and and practically can uh

by very useful information um

uh about the target of drug action and

so

this is what comes under the term

pharmacokinetic modeling

where you measure the drug in the plasma

and concurrently in the tissue

this is a very complex area im going to

try to make it simple

by making up this story of what it was

like for me when i was a resident in

psychiatry at yale

so

as many of you know prozac is a widely

used now off patent

ssri selective serotonin reuptake

inhibitor

prozac the generic name is fluoxetine

is the blocks the serotonin transporter

just like cocaine blocks the dopamine

transporter fluoxetine blocks the

serotonin transporter

and

its an effective antidepressant

so this is the story that i make up when

i was a psychiatry resident i said well

to my professor professor moriarty

who by the way is the literary foil of

sherlock holmes i said professor i think

that fluoxetine must be effective

because ill bet that patients with

depression have too many serotonin

transporters and fluoxetine blocks them

and bring them back to normal

so in order to

test this i made f labeled fluoxetine

which has several fluorines and i did

this uh pet scan and we see much higher

uptake in the patient than in the

healthy subject and if we take the

entire extrapolated area under the curve

to infinity it was versus and i

said you see professor ive proved my

point patients with depression have too

many twice as many serotonin

transporters and he said well bob you

know thats thats pretty good but tell

me

how much did you inject the subject with

oh well in the patient i injected with

0 millicarries but the healthy subject

the radiochemists were having a bad day

and they only injected 0 millicarries

and he said bob you stupid fool of

course theres going to be more in the

patient because you injected twice as

much in the patient go back and do it

properly

so i went back and i did it again and

this time i injected a new patient a

healthy subject each got 0 millicarries

but i still got the same result and i

said now professor you see ive

definitely proved it it says well this

youre doing better but tell me how much

did they weigh

and i said oh well the healthy subject

was healthy by us standards at 00

kilograms but the patient lost the

appetite was down to 0 kilograms and he

said bob you stupid fool of course the

concentration in the brain is twice as

much in the patient because its

distributed in half the total volume as

the healthy subject go back and do it

properly so i did i went back and this

time i got two normal people quote at

00 kilograms they both got 0 millikers

but i still got the same result and i

said now ive proven it they say well

this is better but tell me

how was tell me a little bit more about

the medical history and i said oh well

the patient actually had liver disease

the patient had tried to commit suicide

a couple of times and completely blew

out the liver but the healthy subject

had a normal liver

and he said ah bob now

how do you know there wasnt more in the

patient just because the patient didnt

metabolize the drug the healthy subject

was a normal metabolizer

this is a very difficult question to

answer

and this is where pharmacokinetic

modeling comes in to answer this

question

okay so

im going to refer to something called

binding potential we want to measure the

total number of receptors or

transporters in the brain

and

it this binding potential that were

going to measure

is a product of the number of receptors

the receptor density and the affinity of

the drug for that receptor so youre

going to have more uptake in some

individual if they have more receptors

or

if their receptors happen to have higher

affinity for the ligand in general for

almost all cases everybody has the same

affinity so this binding potential is

just measuring the density

in order to answer that last question

you may have to measure in addition to

the brain

you have to measure the concentration of

drug in the plasma

and this is actually arterial plasma in

the plasma thats going to the brain

so this is the parent radioligand over

time you extrapolate both of those to

infinity this might be and this might

be two and the binding potential will be

equal to the area in the brain divided

by the area of the plasma curve so in

this case it would be eight its a

unitless number that is proportional to

the density of receptors

how does this correct for the injected

dose well those are such low doses

tracer doses that were in the linear

portion of the curve if you double the

injected dose from 0 millicarries to 0

millicarries the concentration in the

plasma will go up twofold from lets

say air into the curve two to air into

the curve four and similarly it would

also be linear in the brain

so the binding potential the first time

would be over which is the

binding potential would be over the

second time it would still be

just because weve doubled the dose we

havent changed the density of the

serotonin transporters in this subject

of course

so it corrects for injected dose

and it also corrects for body weight

because if its distributed to other

portions of the body itll be lower

concentrations in the plasma

and it also corrects for faster

metabolism because if theres faster

metabolism this will come down faster

and the area under the curve will be

lower

so thats how you do it

okay so the major point of pet

pharmacokinetics in words can be done

this way

the plasma pharmacokinetics provides a

limited view of whats happening to the

drug in plasma and you will receive many

lectures on on pharmacokinetics and what

they are talking about is the

concentration of drug in the blood over

time kinetically

but thats a very limited view of whats

happening to the drug

pet provides a limited view of whats

happening to the drug in the tissue in

my case and the brain

but if you concurrently measure the drug

in the plasma and drug in the tissue

with pret you with pet

you can quantify the target of drug

action that is the receptor

okay

so

pharmacokinetics as i mentioned is the

study of the drug in plasma and that is

often distinguished from

pharmacodynamics

which is like the effects that the drug

has after it binds to the receptor so

the dynamic effect of a drug to lower

blood pressure what effect did it have

on blood pressure

the but the very first

pharmacodynamic effect is when the drug

binds to whatever receptor the alpha

receptor or whatever that this

antihypertensive agent uses

so we are measuring the very first step

of pharmacodynamics and that its

binding of the drug at the receptor and

the combination of those two allows us

to measure receptor density which can be

important if its related to disease

um so the plasma and the drug is shown

in that slide

the

the drug and the plasma is shown in the

first slide the drug in the brain is

shown in the second one and we can

measure receptor density

and so this can be used quantitatively

to measure the

site of drug action whether its the

dopamine transporter and parkinsons

disease or the amount of amyloid in a

patient with alzheimers disease

okay

so that was a intuitive understanding of

what pharmacokinetic modeling is

let me show you now how a pet can be

used to study distribution and again

distribution in the pharmacological

sense refers to the reversible transfer

of a drug from the plasma to the tissue

and back so if it goes to the tissue and

never comes back to the

to the plasma

thats eliminate its been eliminated

but if it goes and comes back goes to

the tissue and comes back thats

distribution

and ill do this with regard to

a receptor called previously called the

peripheral benzodiazepine receptor

okay the current name for this

is the translocator protein kelly

dalton it is also known as or previously

known as the peripheral benzodiazepine

receptor

it is a mitochondrial protein thats

highly expressed in macrophages and

activated microglia which are phagocytic

inflammatory cells the microglia are in

the brain macrophages are in the

periphery but theyre very very similar

cells

it exists in both the periphery and the

brain it was first discovered in the

kidneys but then they found it was in

the brain so thats why they had to take

peripheral benzodiazepine receptor out

of there

it has multiple potential functions

one thats most talked about is steroid

synthesis

but im not going to get into its

function

im just going to you it and but i do

want to say it is distinct from the

typical benzodiazepine receptor the

valium receptor

which is the gaba a receptor in the

brain this is not the gaba a receptor

this is a transporter protein on

mitochondria highly expressed in

phagocytic cells

were using it really just as a marker

for cellular inflammation where these

cells are present in

localized or

generalized inflammation

okay

so

we developed a radio ligand a pbr

that

binds to this

tspo

and we evaluated it and these are the

typical sort of curves that i was

mentioning to before in the brain and in

the plasma so this is the concentration

of this radioligand in the monkey brain

over time

and then this is the concentration of

the radiologand parent radioligand in

arterial plasma note that this is a

log scale so its declining very rapidly

here but its still staying in the brain

because these receptors have high

affinity for the

radioligan in this case over here

before the animal was injected with

tracer doses of pbr the animal was

injected with pharmacological doses of a

blocking of a nonradioactive drug that

also acts at this receptor

and you can see in this case that

theres a much different

curve so theres an early and higher

peak its rapidly washing out of the

brain

and in the plasma the concentration is

much much higher like this is almost 0

fold higher

okay well how can we understand this

kinetics

the baseline condition whats happening

is that the drug when you have a high

affinity binding site in the brain or in

any organ the the drug binds and comes

off binds and comes off binds and comes

off and it sticks there its like a

magnet that is able to keep these iron

particles the drugs

closely associated with it and it stays

there in the brain much longer than in

the plasma

when we did gave the receptor blocking

dose of a nonradioactive compound if

the nonradioactive compound has gotten

on there and blocked the binding of the

radioligand its sort of like that

that

childrens game is it goose goose

duck duck goose or whatever it is in any

case where you have to get up and one

chair is removed and if other people are

occupying it then youre not able to get

on there and thats whats happening

here

the reason why its rapidly coming out

of the brain is that youve blocked all

of those sites youve sort of

demagnetized the magnet so it no longer

has an affinity its not going to stay

in the brain its going to wash out of

the brain as quickly as it washes out of

the plasma

so we can understand the basic

pharmacokinetics of this

why its

why its washed out much faster

but why is there so much more in the

brain why did this go up to 00

well the reason for that is because

there was more in the plasma actually

there was 0 times more in the plasma so

thats why there was more transiently in

the brain

well why was there 0 times more in the

plasma because actually we injected the

very same dose in the identical monkey

ahha

thats the issue of blocking peripheral

distribution

and i can show it to you here

oh but before i move on if you did that

calculation that i mentioned before you

did this extrapolated to infinity you

did this and extrapolate to infinity and

took the area in the brain divided by

the area in the plasma the number that

you get would be 0

if you do it on the blocked scan the

number you would get would be

which means that more than this is an

outstanding radio like and in the monkey

brain more than 99 of it is specifically

bound to receptors because we can

displace it with other pharmacological

agents from that site so the vast

majority of the uptake in the brain is

reflecting binding to the tspl

okay receptor blockade displaces a

radioligand in the lung and the kidney

and drives more to the brain but it

doesnt stick in the brain

so ive been showing you images of the

brain which you can see in this whole

body monkey scan thats just thats all

that were measuring but in the

periphery there are huge amounts of this

tsp it was first discovered in the

kidney and also in the lungs and in the

heart and the spleen

and when you give the blocking dose you

not only block in the brain but you

block in the periphery where theres

huge numbers of these sites so the

nonradioactive drug pk in this case

blocks distribution in the periphery

leading to much higher concentrations in

the plasma 0 times higher

concentrations of the radiolig end of

the plasma which transiently go into the

brain this initial blush but then wash

out because the magnets have been

demagnetized

so this is an example of showing how pet

can measure distribution

in this case by blocking the

distribution to peripheral organs that

have a very high density you can

transiently deliver

more to the brain

okay

um

id like to take a little digra oh ill

take a little digression here in two

ways

first is um

heres an example of a regular healthy

human subject here and this is the

brain time activity curve

and

this is the

and this is a

a normal monkey

so

which do you think by the way has more

tspo

a normal monkey or a normal human well

because there was much more uptake that

stayed a longer time its basically

weve done the full quantitation and the

monkey has

0 or 0 times as many tspo as the human

healthy

and this was the example that i showed

you before of a preblocked monkey there

was higher uptake and then it washes out

very quickly

well we came across this odd healthy

human subject who had a higher uptake

notice the difference in the scale and

it washed out immediately and it looked

like a preblocked monkey

so given the fact that this was a young

male in his 0s in the united states we

said hes probably on drugs and the

drugs must be blocking it but he said no

and his drug urine tox test was negative

but he did say he was taking quite a bit

of ibuprofen motrin and we thought well

maybe maybe motrins binding to the site

he stopped taking it we came back and

sure enough he had the same

thing

rapid wash out of activity from the

brain as if he had no tspf

well we said well if he has no this

receptor in the brain maybe he has none

of these receptors in the periphery

and heres a whole body uh image of this

tspo radioligand like in the monkey

theres very high uptake in the lungs

the kidneys on the spleen

but in this no binding subject which

represents about 0 of the healthy

population theres no binding there

um

it turns out to make a long story short

that these individuals do have tspo but

they have a single nucleotide

polymorphism

in a single nucleotide polymorphism

which causes this differential affinity

and theres a that causes a substitution

of an alanine to a threonine in the tspo

the allelic frequency of this is about

0 percent and therefore the presence of

the homozygous you have two genes 0

percent of thirty percent is about nine

percent so about you know ten percent of

the population

and you have individuals who have too

high affinity binding

too low affinity binding or actually a

combination of the high and the low but

the low affinity binders represent um

about five to ten percent of the

population were finding in this area

so this is a case where there are

individuals who have different affinity

for the rate for the radioligand this is

quite unusual and its caused by a

single nucleotide polymorphism we can

still use this radial ligand but we have

to correct for either statistically or

by matching

the genotype of the individuals

well if you do do that i want to show

you how this marker of how pec can be

used to study pathophysiology in this

case of tspo

so this marker of information highly

expressed in activated microglia which

are sort of the macrophages of the brain

neuroinflammation

is known to exist in alzheimers disease

at the time of death and massive

neuroinflammation

and it may be a contributor to the

disease pathology but its unclear

whether the um

alzheimers whether the inner

inflammation begins early or late in the

disorder its present at the time of

death

prior tspo pet studies have shown

conflicting results in alzheimers

disease and also in mild cognitive

impairment which is the precursor

syndrome a little bit of

memory

problems this pbr i wont go into it

but is a markedly improved much greater

signal to mars ratio much more specific

binding than the traditional one

so we used it with the genotype

correction as mentioned to look for

expected differences in tspo density

between alzheimers disease and controls

and also mci which we didnt know how

they would turn out to be

the bottom line is shown here published

in 0 i think

this so basically

theres increased tspo density and this

is a mean image of a number of subjects

this its increased in alzheimers

disease but not in mild cognitive

impairment compared to controls

i think the one of the most important

things that might have been expected but

this was not necessarily expected

so this looked at the correlation

between the binding and the disease

severity

a clinical dementia rating scale and

when on a standardized

x and y axis there was a very strong

correlation so it means

crosssectionally between individuals

the more inflammation you have the more

cognitive impairments you have so it

would appear to be a biomarker of

disease severity

and that can be understood in some ways

in this progression of various aspects

in alzheimers disease so there are

various

[Music]

markers or functioning that are shown

here and this is generally accepted

the very first thing to develop in

alzheimers disease is actually the a

beta these plaques of amyloid beta

and they reach a peak just about at the

beginning of mci and stay about the same

so it had been known from postmortem and

also from imaging pet imaging studies of

amyloid that theres no correlation

between disease severity and the amount

of amyloid and the reason for that is

that the amyloid is reaches the peak

very early and stays at that high level

while the patient these are other

markers if you look at clinical function

this one over here its deteriorated

just a little bit in mci and then it

goes to hell in the dementia well the

the function is

the dysfunction is increasing but the

amyloid is staying the same so thats

why theres no correlation between

amyloid and function

what we see with the neural inflammation

is more similar to this here

theres it its a marker of the

transition from mci to dementia and that

it correlates the more information you

have the greater the cognitive

impairment

we also followed this up with the

longitudinal study in some of these

individuals to determine if the tsbo

bind increased during the progression of

alzheimers disease and also compared it

to normal aging and subjects were

scanned

two to four years

scanned again two to four years

after

the initial scan

and what we found is that the pbr

binding increased in the patients in

every patient and there was no change in

controls over this two to four year

period of time

the individual subjects are shown here

every subject over this two to four year

period of time increased in this

neuroinflammation whereas the controls

there was no increase whatsoever

but again arguably the most important

thing is the correlation here the

increased pbr the amount of increase

in the binding correlated with the

amount of increase in clinical severity

with the change of this

cognitive marker the sum of boxes

squared

and the correlation was really quite

strong

so this would suggest that

neuroinflammation is a biomarker of

disease progression

to summarize that here a crosssectional

study in which everybody got one pet

scan inert inflammation occurs after the

conversion of mci to ad and worsens with

disease progression

it is therefore a putative biomarker of

disease severity

um the longitudinal study showed that

this tspo labeled with pbr increases

in alzheimers disease but not in

controls and that the amount of increase

correlates with disease progression and

therefore it is a putative biomarker of

disease progression in alzheimers

disease

these are small pilot studies that need

to be replicated in other centers and i

think largely they are being replicated

so this shows how a marker of

inflammation can be used in

ways that sound pretty important disease

severity and disease progression when

you consider therapies that might be

tried and antiinflammatory therapies

are are worth i think reconsideration in

patients with alzheimers disease

okay

the um

to summarize then i use tsbo as an

example of studying distribution

in this case between the periphery and

the brain and how if you block both of

them you can leave more into you can

have a higher concentration in the

plasma

and then lead transiently higher levels

the distribution to the brain

i also wanted to show that this marker

itself could be useful in alzheimers

disease and in many others were looking

at inflammation in either the brain or

in the periphery

and now i want to take another little

digression in talking about distribution

for one study that we did that i thought

was really very fascinating

and

its pet imaging of pgp permeability

glycoprotein so this is looking how pet

can show distribution in this case

blocking the distribution

it is an efflux transporter which ill

explain which for the brain at least

helps to protect the brain from toxins

but it can also cause drug resistance

permeability glycoprotein or pgp

for which there may be a separate

lecture later

by michael goddessman

it transports drugs out of cells in many

locations for example in the brain and

in the testes so that drugs lets say

potentially toxic compounds are not able

to accumulate in sensitive organs like

the brain and the testes

with regard to the brain it is a

specific component of the bloodbrain

barrier the general idea of the

bloodbrain barrier is that theres a

the endothelial the capillary walls are

very tightly linked to each other and so

in order for a drug to get in it has to

go across a lipid bilayer so it has to

be lipophilic thats considered like the

structural component but there are other

blockers at the barrier including these

efflux transporters like pgp that ill

show you

and an example of a substrate for pgp is

loperamide imodium

imodium is a potent opiate

that acts on the gut to slow motility

but it has no actions in the brain

whatsoever

so there are opiate receptors in the gut

and when the opiate binds to it the the

gut will move

will move more slowly and therefore

theres more time for the water to be

taken up and

if you have diarrhea you can take

loperamide but you wont get high if

youre a heroin addict one of your

common complaints will be constipation

because youre taking too much of it

okay

the reason why you dont get high

is because pgp blocks the entry of this

opiate but not others

in the brain

and and i should mention that it is over

expressed in half of tumors that are

resistant to chemotherapy

so these drugresistant tumors maybe

half of them overexpress pgp and other

efflux transporters which in some cases

block the entry of the cancer

chemotherapy

and it also occurs

very likely in the brain for drug

resistance epilepsy where theres over

expression of pgp blocking the entry

over time

of years of treatment of the

antiepileptic drug

so it helps protect the brain but it can

also block the entry of therapeutic

compounds like antiepileptic drugs

likely causing drug resistance and

epilepsy which is about a third of the

patients

okay

so this uh diagram which is a takeoff

from one

that michael goddessman created

looks at the blood in a ring on the

outside and then you have the tissues

here and then you have the excreted

areas here

and it looks at the distribution of pgp

which is throughout the body as

mentioned pgp is located at the

bloodbrain barrier and the capillary

endothelium

and it will transports drugs out of the

brain its also at the testis barrier

and wont let drugs like loperamide or

imodium go into the testes sofa

expressed in multidrug resistance

tumors

mdr tumors but its in other examples

also so in the placenta for example

itll block the entry of drugs that are

substrates to so it would protect the

fetus okay

so its widely distributed

pglycoprotein removes lipophilic

substrates directly from the plasma

membrane so if this is the bloodbrain

barrier blood testis barrier this out

here would be the blood and this inside

here is the brain and the substrate

loperamide or whatever any substrate it

try it begins to cross the membrane but

then pgp captures it and excludes it so

it actually if its a very potent

substrate the drug itself will never

even make it into the brain instead pgp

will pump it immediately out before it

even gets through the lipid bilayer

so we wanted to measure the function of

this efflux transporter and we made a

radioactively labeled loperamoid it was

the d just it has doesnt have a methyl

group on it for various reasons its uh

its a better

and you can see that the in a normal

brain in the wildtype brain of a mouse

theres no uptake in the mouse

brain but if you look at a pgp knockout

mice that doesnt have it then therell

be uptake in the brain so these mice

will get high and do get high from

loperamide but they

if theyre knocked out but not if

theyre wild type

and if you look at the amount it really

makes quite a big difference in the

brain the wild type has hardly anything

but the pgp knockout has a lot getting

into the brain so its getting high

going up the phylogenetic tree this

looks like looks at the uptake in

in a monkey brain this is a coronal

section through a monkey brain and

heres the pituitary for reference we

dont have a knockout monkey but we can

give a drug that will block pgp

and we see that if we block the pgp the

drug is

allowed to get into the brain and the

monkey would presumably be getting high

weve also used the oh and the amount of

uptake is really quite striking so

theres very little uptake of the

radiolabeled loperamide at baseline but

if you block the flux transporter a huge

amount manifold

can get into the brain

so this is showing in animals that this

is an appropriate substrate measuring

the function of pgp to block the entry

of the drug into the brain

heres the

distribution of c dlop in a healthy

male and

it looks at various times looking at the

radioactivity at minutes 0 minutes

and 00 minutes

on this here you can see well theres

high uptake initially like

in the kidneys the lungs and the thyroid

and the reason for that is that they

have a high blood vol theres a high

amount of blood in each of those organs

so you see initially sort of the blood

distribution with the drug in the blood

at later time points you can see

metabolites are potentially binding to

pgp sites there

i know when i uh

showed this some years ago to some

females in the lab they said oh yeah

yeah this is a typical male no brains no

uptake in the brains and no up no uptake

in the testes typical male no brains no

balls

and thats true

because the pgp blocks the entry into

the brain and into the testes

okay

looking at this early summed images from

zero to three minutes you measure you

see a lot of the blood pool so lets see

if this image would go around first

before i start this rotating this is the

site where it was injected and theres

high uptake

in the because its a lipophilic

compound in the endothelium of the vein

as it comes in heres the the thyroid

kidneys

lets take a look at that

but look in the brain well theres this

this is the for whatever reasons which i

dont really fully know there is high

uptake in the pituitary but you also see

this structure here

and i wonder if any of you know what

that is

that is the venous sinus of the brain

and which we hardly ever see in the pet

imaging studies that i do

the this drug as i mentioned uh the

radioligand is blocked from entering the

brain and it stays in the

blood

and the

blood of the brain is drained the venous

drainage goes into these very large

veins so big that they are called

sinuses so what we are seeing here is

that were able to see the blood in the

sinuses as the draining veins of the

brain

and we can see it because theres no

uptake in the brain most of the

radioligans that i

develop go into the brain so therefore

we dont we cant by contrast see it

okay

all righty so uh

this looks at a fused image this this is

the mri of lets say that individual

you can see that theres very high

uptake in the pituitary for reasons that

i wont go into

but there are some other areas of high

uptake

um

but the uptake this number is very very

low if you look in the brain itself

in addition to the pituitary there are

these other structures

that are labeled

and if anybody out there can tell me

that one

ill give you a

case of heineken

okay

so here they are in another

distribution or different tomograph what

are these sites here which are located

in the medial surface of the lateral

ventricle which are located in the roof

of the third ventricle

and

also located in the roof of the fourth

ventricle the ventricles are the csf

drainage from the brain

and

the answer is that this is the

site

where

the

theres the drainage of csf

from the brain

the uh

take one break

why am i blocking it

okay so um

this up tay ill go back one

so these uptake

is in a tissue in the brain called the

choroid plexus

the chloride plexus is a tissue that is

involved with producing the csf the

cerebral spinal fluid or the water in

the brain

and for whatever reasons

drugs that are substrates for pgp also

happen to accumulate in the chloride

plexus

which i have never seen before ive seen

the ventricle maybe a little bit so

heres an image of it you can see

the

venous drainage the venous sinus but

here now threedimensionally you can see

better this chloride plexus which is the

tissue that makes the csf um

for the brain

the water in the brain

okay

well can we increase the uptake of a

drug with a pgp inhibitor in human

subjects and we can

so you can see this is the baseline

uptake no uptake in the brain but

theres the uptake in the chloride

plexus and if we give this inhibitor at

six milligrams per kilogram we can

increase the delivery of the drug to the

brain

and thats shown here the brain uptake

is increased with this inhibitor in a

human just like it is in a monkey

so it suggests that for those tumors

that could have increased pgp you could

potentially increase the uptake of the

cancer chemotherapy agent into the tumor

or you could increase the delivery of

the drug to the brain with a pgp

blocking agent

in any case i found this a very

fascinating study to show how pet could

study an important regulator of

distribution in this case the pgp efflux

transporter which blocks the entry of

those drugs which are substrates in many

tissues in the body

okay

so

i gave some examples of how pet can

study the distribution uh in the case of

the tspo previously known as the

peripheral benzodiazepine receptor you

can look at the competitive distribution

or distribution to the brain and the

periphery you can also look at how the

distribution of drugs are blocked by

efflux transporters including pgp

i want to end with showing how pet can

also study drug metabolism

and metabolism again is where you make

or break a bond in the drug

you change the chemical structure

this radiolike and fc way is a one that

was developed at nih and it labels a

particular type of serotonin receptor in

the brain

but at two hours after injection the

vast majority of uptake is just in the

bone the skull

surrounding the brain but very little

uptake in the brain

and the reason for that is that fcwa is

relatively rapidly metabolized its

defluorinated so that you end up with

f fluoride ion

and as i showed you previously in that

rat study taken up into the bone

including into the skull

so this is the structure of fc whey

which is defluorinated

and you end up with fluoride iron here

which is taken up in bones of the body

including the brain

but it obscures the picture

and therell be some spilling of

activity from the skull into the

surrounding neocortex

so one of the mechanisms of

defluorination is a cytochrome p0

enzyme cype

ive gotten that correct

is a defluorinating enzyme in the liver

so we wanted to see if myconazole which

is known to inhibit this enzyme would be

able to inhibit radio defluorination and

decrease bone uptake

myconazole is an antifungal agent its

also advertised as quick acting tanactin

and its a potent inhibitor of cype

this looks at the uptake of fluoride ion

if a rat is injected with f fluoride

ion

in this case down below weve injected

f fc whey and initially there is a lot

of uptake uh in the skull particularly

the base of the skull which is thick in

a rat and in human

but then in these studies we gave

increasing concentrations of miconazole

and it could

decrease the uptake into the skull and

give you more delivered into the brain

and this is the hippocampus of a rat

which has the highest density of this

particular type of serotonin receptor

we wanted to see if we could do this in

human subjects but we couldnt give

myconazole intravenously instead we used

disulforam which is a

known as ant abuse disulfurium i think

is the most potent inhibitor of cype

it inhibits many different enzymes and

it is used clinically to maintain

sobriety far more off far more

frequently in europe than in the united

states but if you take this drug which

inhibits the intermediate metabolism of

alcohol you have a buildup of a

an intermediate intermediate metabolite

that makes you flush and gives you

tachycardia makes you sick and nauseous

so if you take this disulfiram every day

youre not going to take a drink because

youll get sick

so we gave the disulfiram the standard

sobriety dose 00 milligrams on the

night before and it was just a dramatic

effect we could block the defluorination

block the uptake in the skull and

deliver more into the brain

so here at the time activities for that

you can see that disulfiram looking at

in the skull just virtually blocked all

the uptake in the skull because it was

blocking the deflourination

if you look in the brain it really

delivers far more to the brain its not

metabolized so therefore theres more

radiolike antiplasma to go into the

brain and bind to these serotonin

receptors

if you look at the concentration of

fluoride ion in the plasma the

disulfiram almost completely blocks the

amount of fluoride ion that gets there

and then if you look at the parent

tracer because youre deep because

youre blocking the metabolism theres

more of the parent compound and theres

more that would go into the brain

so this shows how you can

use pet to study the metabolism in this

case of a radiolite ligand and in this

case of the defluorination

it turns out that defluorination is

fairly common there are different

mechanisms in which it occurs and can be

a problem for f labeled drugs

if it occurs via

cype you can eliminate that problem by

giving the subject just the standard

single sobriety dose of disulfiram

let me summarize then what ive told you

today

pet positron emission tomography is a

technique that has very high sensitivity

and specificity it has high sensitivity

meaning that it can measure that

radioactive atom carbon or f at

very low concentrations 0 to the minus

0 to the minus molar therefore it

has adequate sensitivity to measure many

of the proteins that were interested in

which are present at 0 to the minus

ninth molar

it has specificity in so far as if the

radioactive atom is connected with a

drug that binds specifically to the d

type of dopamine receptor then youll be

labeling specifically that receptor

pat is used in therapeutic drug

development currently in several ways

perhaps most commonly in helping to

determine the initial dose of the drug

because on the initial dose you dont

want to give too much and have

unnecessary toxicity and you wont want

to have too little and miss the

potential efficacy if you had used the

proper dose

pharmacokinetic modeling of

pet radioligans involves simultaneously

knowing the concentration and drug over

time in the plasma as well as in the

tissue and having both of those together

a pharmacokinetic measure and the very

first of the pharmacodynamic measures

you can in combination with that find

out the density of the target site of

the receptor in the tissue of interest

ive shown how pet can study

distribution which is the reversible

transfer of a drug from the blood to the

tissue and back

the case i gave of translocator protein

was one where the receptor is located in

the brain in the periphery and you can

have

sort of a competitive distribution of

the drug or the radioligand to those two

sites i also showed that that particular

target can be can provide useful

pathophysiological

information

on inflammation or neuroinflammation in

my particular case

and finally i showed how drug how pet

can study metabolism making or breaking

a bond in the radioligand in this case

it was the defluorination and the

ability to inhibit the defloor nation

and have more of the parent radio like

it

let me finish up and let me remind

everyone whos taking

this course for credit this is the time

to wake up

im going to give you all a

selfassessment quiz try to remember

this

true or false lets go over them

imaging with positron emission

tomography pet involves the injection of

a radioactively labeled drug that emits

a particle called a positron

and the answer is

true thats true the particle is

released the positron it later will

annihilate with an electron matter and

antimatter combining giving off two

gamma rays that are high enough energy

to go straight through the skull and can

be measured with a pet camera

pet shows the location of radioactivity

in a crosssection or tomograph of the

body yes thats what the t in tomography

comes from a tomograph is not just a

flat image but its a slice going

through we have the front and the back

and the left and the right so that is

true

pet can be used to quantify the density

of specific proteins in the body

the answer to this is also true

if you simultaneously measure the

concentration of the drug in the plasma

and in the brain lets say then you can

determine

how many receptors are there that is you

would have more uptake in the brain

relative to how much was delivered in

the plasma

okay compartmental modeling

which is very complex of pet data

typically uses the measurements over

time of pet images of the target tissue

and concentrations of the unchanged

parent radioligand in plasma

and the answer to that is true it has

pharmacokinetic measurements in the

plasma and the initial pharmacodynamic

measures in the tissue

and with those being measured

simultaneously and extrapolated to

infinity you can get a measure of the

density of the target in the body

thank you very much for your attention i

hope i was adequately able to

communicate my enthusiasm

for this technology positron emission

tomography which i think can be useful

for studying the brain and other organs

in the body and its based upon

pharmacology so be well do good work and

keep in touch

you