



**UNIVERSITY
OF TURKU**
Faculty of Medicine



Neurotransmitter systems studied with PET

Jussi Hirvonen, MD, PhD

Professor of Radiology, Tampere University

Neuroradiologist, Turku University Hospital



jussi.hirvonen@utu.fi



[@jussihirvonen](https://twitter.com/jussihirvonen)

Outline of talk

- Neurotransmission – what is it?
- The Tool Kit: molecular and multimodal brain imaging
 - Magnetic Resonance Imaging (MRI)
 - Structural MRI
 - Functional MRI
 - Positron Emission Tomography (PET)
- What makes a good radioligand for brain?
- Typical study designs
 - Studies of pathophysiology
 - Group comparison: cross-sectional vs. longitudinal
 - Imaging genetics: vulnerability vs. individual genes
 - Studies of vulnerability traits in healthy subjects
 - Studies of neurotransmission dynamics
 - Large-scale studies

Levels of analysis

Brain volume (MRI)

Brain white matter integrity (DTI)

Structure-function

Brain function (fMRI)

Plus: TSPO, beta-amyloid, tau, synaptic vesicle glycoprotein, etc.

Specific neurotransmitters (NT PET)

Neurochemistry

Neurotransmitter activation (NT PET)

Brain PET imaging: pros & cons

- What is excellent:
 - Molecule-level phenomena in living tissue = unique
 - Non-invasive = no brain biopsy needed
 - High chemical resolution = measure only one thing
 - High sensitivity = measure very small amount (nanomolar)
- What are the limitations:
 - Modest spatial resolution (typically 4–6 mm)
 - Poor time resolution (minutes)
 - Ionizing radiation (typically about 2–4 mSv per injection for ^{11}C tracers)
 - Limited availability
 - High cost

Review Article

Consensus nomenclature for *in vivo* imaging of reversibly binding radioligands

Robert B Innis¹, Vincent J Cunningham², Jacques Delforge³, Masahiro Fujita¹, Albert Gjedde⁴, Roger N Gunn⁵, James Holden⁶, Sylvain Houle⁷, Sung-Cheng Huang⁸, Masanori Ichise⁹, Hidehiro Iida¹⁰, Hiroshi Ito¹¹, Yuichi Kimura¹², Robert A Koeppe¹³, Gitte M Knudsen¹⁴, Juhani Knuuti¹⁵, Adriaan A Lammertsma¹⁶, Marc Laruelle², Jean Logan¹⁷, Ralph Paul Maguire¹⁸, Mark A Mintun¹⁹, Evan D Morris²⁰, Ramin Parsey⁹, Julie C Price²¹, Mark Slifstein⁹, Vesna Sossi²², Tetsuya Suhara¹¹, John R Votaw²³, Dean F Wong²⁴ and Richard E Carson²⁵

Major differences between *in vitro* measurements and *in vivo* PET

- *In vivo* PET: usually, tracer doses are used ($F \ll K_D$)
- Thus, receptors are not occupied at all $\rightarrow B_{\max}$ or K_D cannot be measured separately, only their ratio (BP_F)!
- *In vitro*, multiple levels of saturation is used to describe B_{\max} and K_D
- *In vivo* PET: regional blood flow, extraction, binding to plasma proteins, non-specific binding, multiple populations of specific binding sites, internal milieu (pH, ion concentrations etc), receptor trafficking (internalization), radioactive metabolites of the radiotracer, endogenous neurotransmitters, factors related to PET instrumentation...

What is neurotransmission?

- Mechanisms responsible for chemical communication between neurons in brain
- Along the signaling pathway:
 - Neurotransmitter synthesis and storage
 - Neurotransmitter release
 - Pre- and postsynaptic receptors
 - Reuptake and catabolism
 - Second messenger systems and other downstream effects

Neurotransmitter systems

- Dopamine
 - Synthesis capacity, dopamine D₂ receptors, dopamine D₁ receptors, dopamine transporter
- Serotonin
 - Pre- and post-synaptic 5-HT_{1A}R, 5-HT_{2A}R, serotonin transporter, MAO activity
- Glutamate
 - mGluR5
- Gamma-aminobutyric acid (GABA)
- Cholinergic
- Cannabinoid
 - Presynaptic CB₁ receptors, CB₂ receptors?
- Opioid
 - Mu-, kappa-, sigma receptors

Table 1. Tracers listed by target category. Descriptions of distinguishing characteristics are given as well as year of introduction (corresponding to first peer-reviewed publication in humans). Years were determined by a keyword search of <tracer name> AND (positron OR PET) using Thompson Reuters Web of Science.

Target System	Radioligand	Protein target	Description	Year of introduction	
Dopamine	[¹¹ C]raclopride	D2/3 receptor	D2/3 antagonist	1985	
	[¹⁸ F]fallypride	D2/3 receptor	D2/3 antagonist	2002	
	[¹¹ C]fallypride	D2/3 receptor	D2/3 antagonist	2009	
	[¹¹ C]FLB457	D2/3 receptor	D2/3 antagonist	1999	
	[¹¹ C]-(+)-PHNO	D2/3 receptor	D2/3 agonist	2006	
	[¹¹ C]NPA	D2/3 receptor	D2/3 agonist	2008	
	[¹¹ C]MNPA	D2/3 receptor	D2/3 agonist	2009	
	[¹¹ C]NNC112	D1 receptor	D1 antagonist	1998	
	[¹¹ C]SCH23390	D1 receptor	D1 antagonist	1991	
	[¹¹ C]PE2I	DAT	DAT antagonist	2006	
	[¹¹ C]altropane	DAT	DAT antagonist	2007	
	[¹⁸ F]CFT	DAT	DAT antagonist	1998	
	[¹¹ C]CFT	DAT	DAT antagonist	1998	
	[¹⁸ F]DOPA	DA terminals	Substrate for AADC	1986	
	[¹⁸ F]FMT	DA terminals	Substrate for AADC	1995	
	[¹¹ C]DTBZ	VMAT2	VMAT2 antagonist	1996	
	[¹⁸ F]AV133	VMAT2	VMAT2 antagonist	2009	
	Serotonin	[¹¹ C]McN5652	SERT	SERT antagonist	1995
		[¹¹ C]DASB	SERT	SERT antagonist	2000
		[¹¹ C]AFM	SERT	SERT antagonist	2011
		[¹¹ C]HOMADAM	SERT	SERT antagonist	2011
		[¹¹ C -carbonyl]WAY100635	5-HT _{1A} receptor	5-HT _{1A} antagonist	1998
		[¹⁸ F]MPPF	5-HT _{1A} receptor	5-HT _{1A} antagonist	2000
[¹⁸ F]FCWAY		5-HT _{1A} receptor	5-HT _{1A} antagonist	2003	
[¹¹ C]CUMI101		5-HT _{1A} receptor	5-HT _{1A} antagonist	2010	
[¹¹ C]MDL100907		5-HT _{2A} receptor	5-HT _{2A} antagonist	1998	
[¹¹ C]CIMBI-36		5-HT _{2A} receptor	5-HT _{2A} agonist	2014	
[¹⁸ F]Altanserin		5-HT _{2A} receptor	5-HT _{2A} antagonist	1994	
[¹¹ C]P943		5-HT _{1B} receptor	5-HT _{1B} antagonist	2010	
[¹¹ C]AZ10419369		5-HT _{1B} receptor	5-HT _{1B} antagonist	2011	
[¹¹ C]GSK215083		5-HT ₆ receptor	5-HT ₆ antagonist	2012	
Norepinephrine		[¹⁸ F]MeNER	NET	NET antagonist	2005
		[¹¹ C]MRB	NET	NET antagonist	2007
Opioid		[¹¹ C]carfentanil	μ opioid receptor	μ opioid agonist	1988
	[¹¹ C]diprenorphine	opioid receptor	Non-selective opioid antagonist	1988	
Acetylcholine	[¹¹ C]LY2795050	κ opioid receptor	κ opioid antagonist	2015	
	2-[¹⁸ F]-FA-85380	nicotinic receptor	α4β2 nAChR antagonist	2005	
Glycine	[¹⁸ F]FP-TZTP	muscarinic M2	M2 agonist	2003	
	[¹¹ C]GSK931145	GlyT1	GlyT1 antagonist	2011	
GABA	[¹¹ C]RO5013853	GlyT1	GlyT1 antagonist	2013	
	[¹¹ C]flumazenil	GABA _A receptor	GABA _A antagonist	1985	

(Continued)

Table 1. (Continued)

Target System	Radioligand	Protein target	Description	Year of introduction	
Glutamate	[¹¹ C]ABP688	mGluR5	Allosteric antagonist	2008	
	[¹⁸ F]FPEB	mGluR5	Allosteric antagonist	2013	
Substance P	[¹⁸ F]SPA-RQ	NK1 receptor	NK1 antagonist	2004	
Enzymes	[¹¹ C]deprenyl	MAO B	MAO B antagonist	1987	
	[¹¹ C]clorgyline	MAO A	MAO A antagonist	1987	
	[¹¹ C]rolipram	PDE4	PDE4 antagonist	2002	
	[¹¹ C]IMA107	PDE10	PDE10 antagonist	2014	
	[¹⁸ F]MNI659	PDE10	PDE10 antagonist	2014	
	TSPO	[¹¹ C]PK11195	TSPO	TSPO ligand	1999
		[¹¹ C]PBR28	TSPO	TSPO ligand	2008
		[¹⁸ F]PBR06	TSPO	TSPO ligand	2009
		[¹⁸ F]PBR111	TSPO	TSPO ligand	2013
		[¹⁸ F]FEPPA	TSPO	TSPO ligand	2011
Cannabinoid	[¹¹ C]OMAR	CB1 receptor	CB1 antagonist	2010	
	[¹⁸ F]MK9470	CB1 receptor	CB1 antagonist	2008	
β-amyloid ^a	[¹¹ C]CURB	FAAH	FAAH antagonist	2013	
	[¹¹ C]PiB	β-amyloid	β-sheet fibrils of β-amyloid	2004	
	[¹¹ C]AZD2184	β-amyloid	β-sheet fibrils of β-amyloid	2009	
	[¹¹ C]AZD4694	β-amyloid	β-sheet fibrils of β-amyloid	2012	
	[¹⁸ F]GE067	β-amyloid	β-sheet fibrils of β-amyloid	2009	
	[¹¹ C]SB13	β-amyloid	β-sheet fibrils of β-amyloid	2007	
	[¹⁸ F]BAY94-9172	β-amyloid	β-sheet fibrils of β-amyloid	2008	
	[¹⁸ F]AV45	β-amyloid	β-sheet fibrils of β-amyloid	2012	
	[¹⁸ F]FDDNP	β-amyloid	β-sheet fibrils of β-amyloid	2006	
	Tau	[¹⁸ F]AV-1451 ^b	Tau	PHF -tau	2013
[¹⁸ F]THK5117		Tau	PHF -tau	2013	
[¹⁸ F]THK5351		Tau	PHF -tau	2014	
[¹¹ C]-PBB3		Tau	PHF -tau	2013	

Quantitative imaging of protein targets in the human brain with PET

Roger N Gunn^{1,2,3}, Mark Slifstein^{4,5}, Graham E Searle² and Julie C Price⁶

Gunn et al. *Phys Med Biol* 2015;60:R363-R411.

Review

PET reporter systems for the brain

Sridhar Goud Nerella,^{1,*} Michael Michaelides,² Takafumi Minamimoto,³ Robert B. Innis,¹ Victor W. Pike,¹ and Mark A.G. Eldridge^{1,4,*}

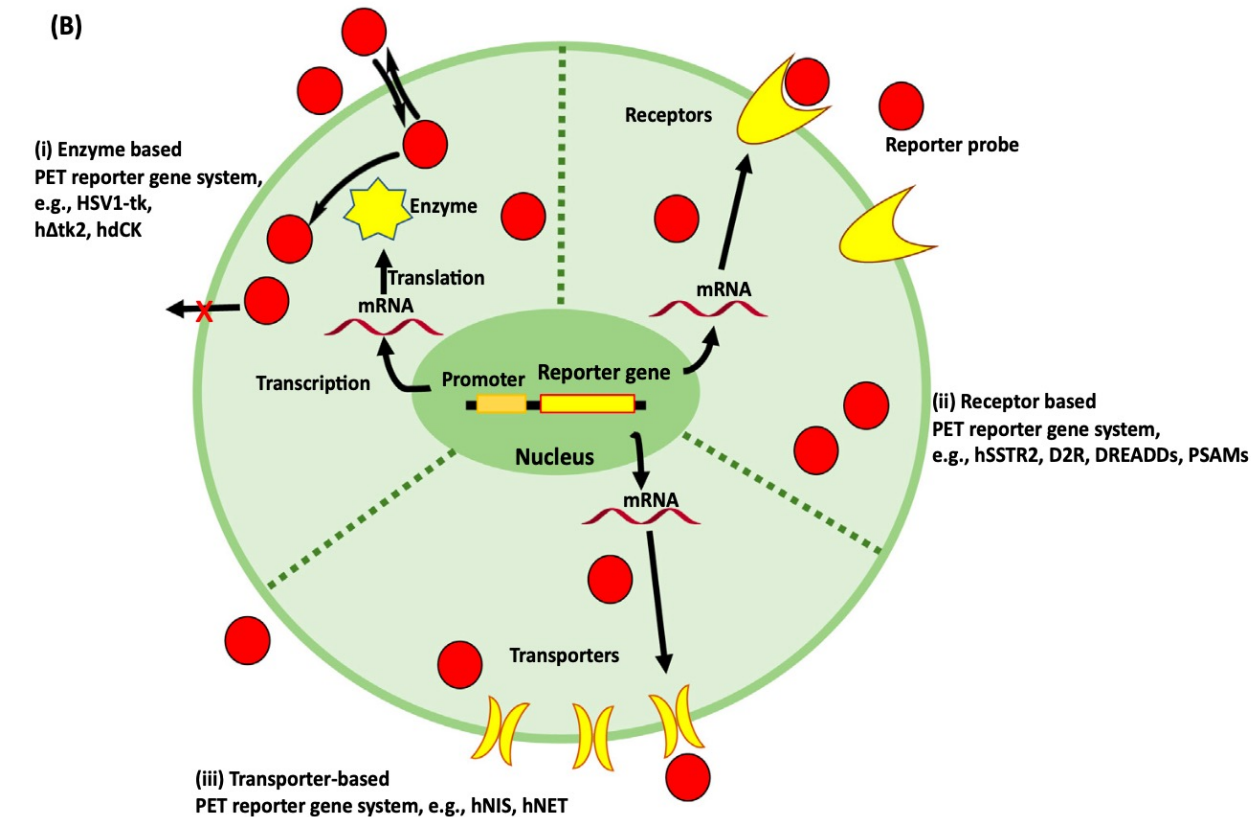


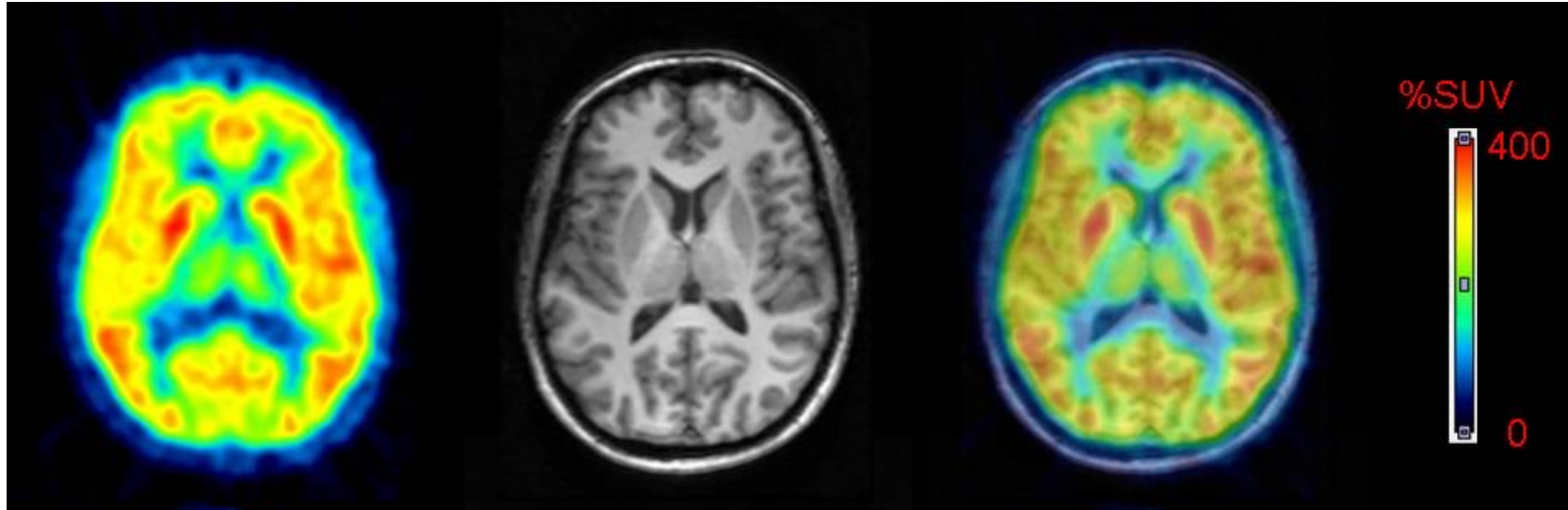
Table 1. List of PET reporter systems

Reporter system	Reporter gene(s)	Reporter probe(s)
Intracellular entrapment	HSV1-TK HSV1-sr39TK hΔTK2 hDTK2 hDCK VZV-TK	[¹⁸ F]FIAU, [¹⁸ F]FMAU, [¹⁸ F]FEAU, [¹⁸ F]FHBG
	Pyruvate kinase M2 (PKM2)	[¹⁸ F]DASA-23
	AADC TH/GAD1	6-[¹⁸ F]fluoro-L-m-tyrosine, [¹¹ C]raclopride
Protein binding	hSSTR2	[⁶⁸ Ga]DOTATOC, [⁶⁸ Ga]DOTATATE
	hD2R	[¹¹ C]raclopride, [¹⁸ F]fallypride, [¹⁸ F]spiperone derivative.
	Cannabinoid (CB2) receptor	[¹¹ C]GW405833
	ecDHFR	[¹⁸ F]FE-TMP
	Optogenetics: ChRERa	[¹⁸ F]FES
	Chemogenetics: DREADDs	[¹¹ C]CLZ, [¹¹ C]DCZ, and [¹⁸ F]JHU37107
	Chemogenetics: PSAMs	[¹⁸ F]ASEM
Transporter-based	hNIS	[¹⁸ F]tetrafluoroborate, [¹⁸ F]fluorosulfate
	hNET	[¹¹ C]ephedrine, [¹²⁴ I]meta-iodobenzylguanidine (MIBG), and [¹⁸ F]meta-fluorobenzylguanidine (MFBG), [¹¹ C]MeNER, and [¹¹ C]MRB
	hSERT	[¹¹ C]DASB, [¹¹ C]McN 5652, [¹¹ C]ADAM, [¹¹ C]DAPA, and [¹¹ C]AFM

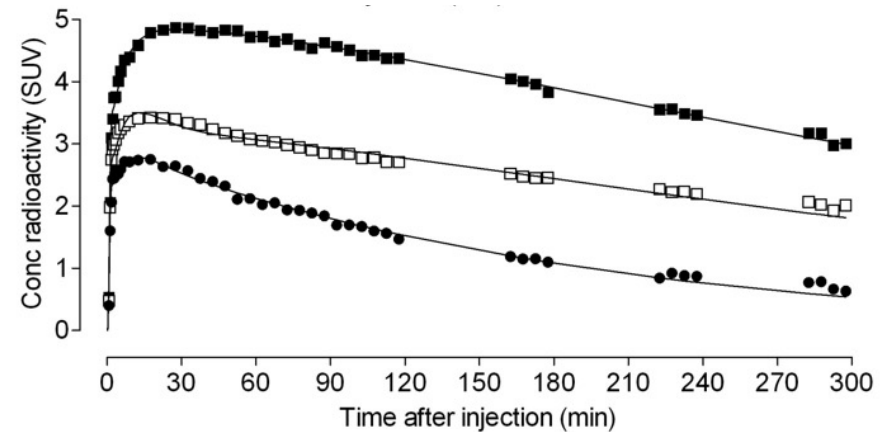
What makes a good radioligand for brain?

- Sufficient target density and ligand affinity ($B_{\max}/K_D \approx 5$)
- High brain uptake (>100%)
- High pharmacological selectivity
- Optimal lipophilicity ($\text{LogP}=2.5-4$)
 - Sufficiently high to cross blood-brain barrier
 - Not too high to cause non-specific binding
- No brain-penetrant radiometabolites
- Not substrate for efflux transporters at BBB (e.g., P-gp)
- Quantifiable plasma protein binding
- Amenability to rapid labeling with high specific activity
- Fast enough kinetics to allow measurement in a few hours

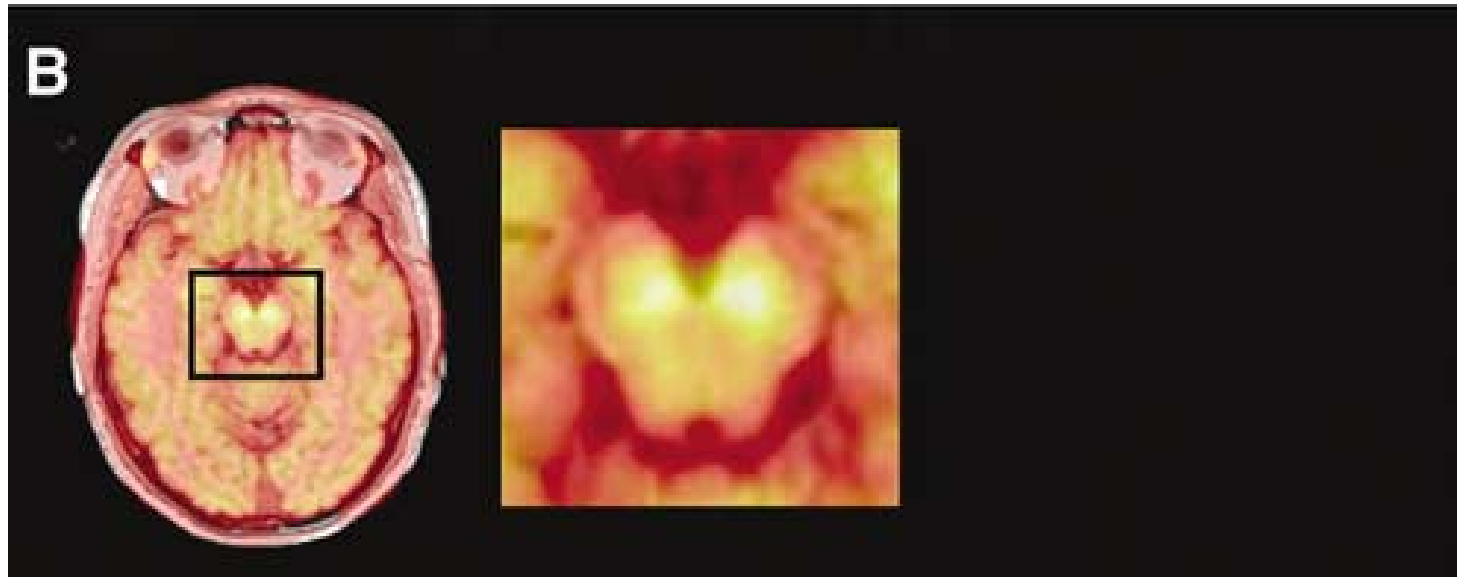
^{18}F -FMPEP- d_2 : high brain uptake despite high lipophilicity



- ^{18}F -FMPEP- d_2 for cannabinoid CB_1 receptor
- Very lipophilic ($\text{LogP} = 4.8$)
 - high protein binding
 - high non-specific binding
- However, high brain uptake and specific binding because CB_1 receptor is highest density GPCR in mammalian brain
- Monkey study shows 90% of V_T is specific binding



^{11}C -PE2I: distribution of dopamine transporter in human brain

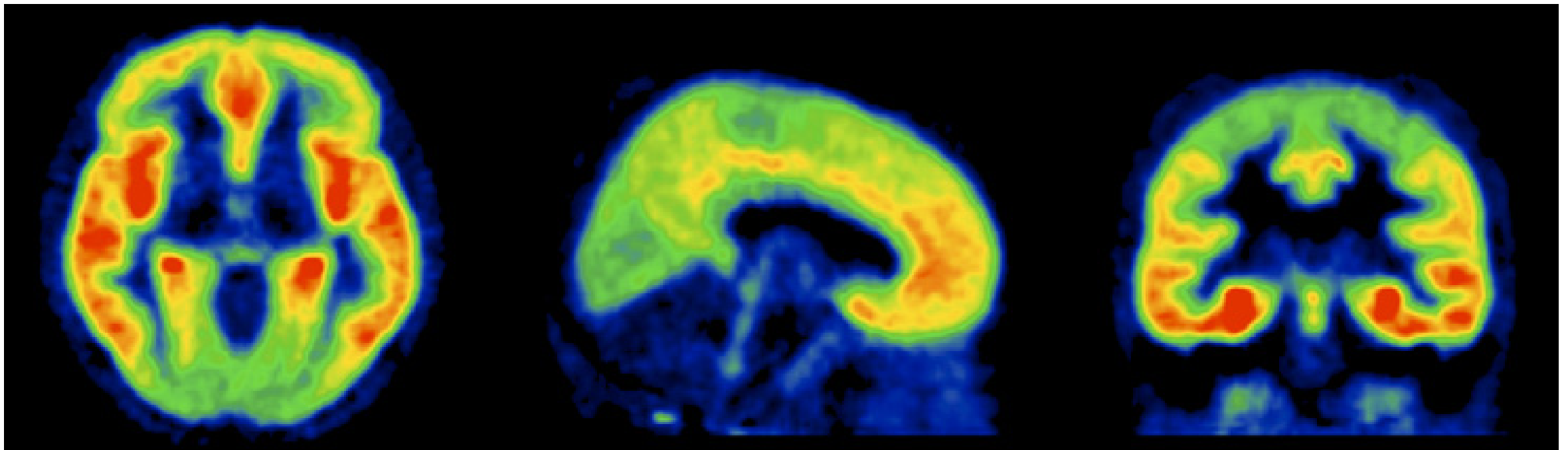


carbonyl-¹¹C-WAY-100635: distribution of serotonin
5-HT_{1A} receptors in human brain

Axial

Sagittal

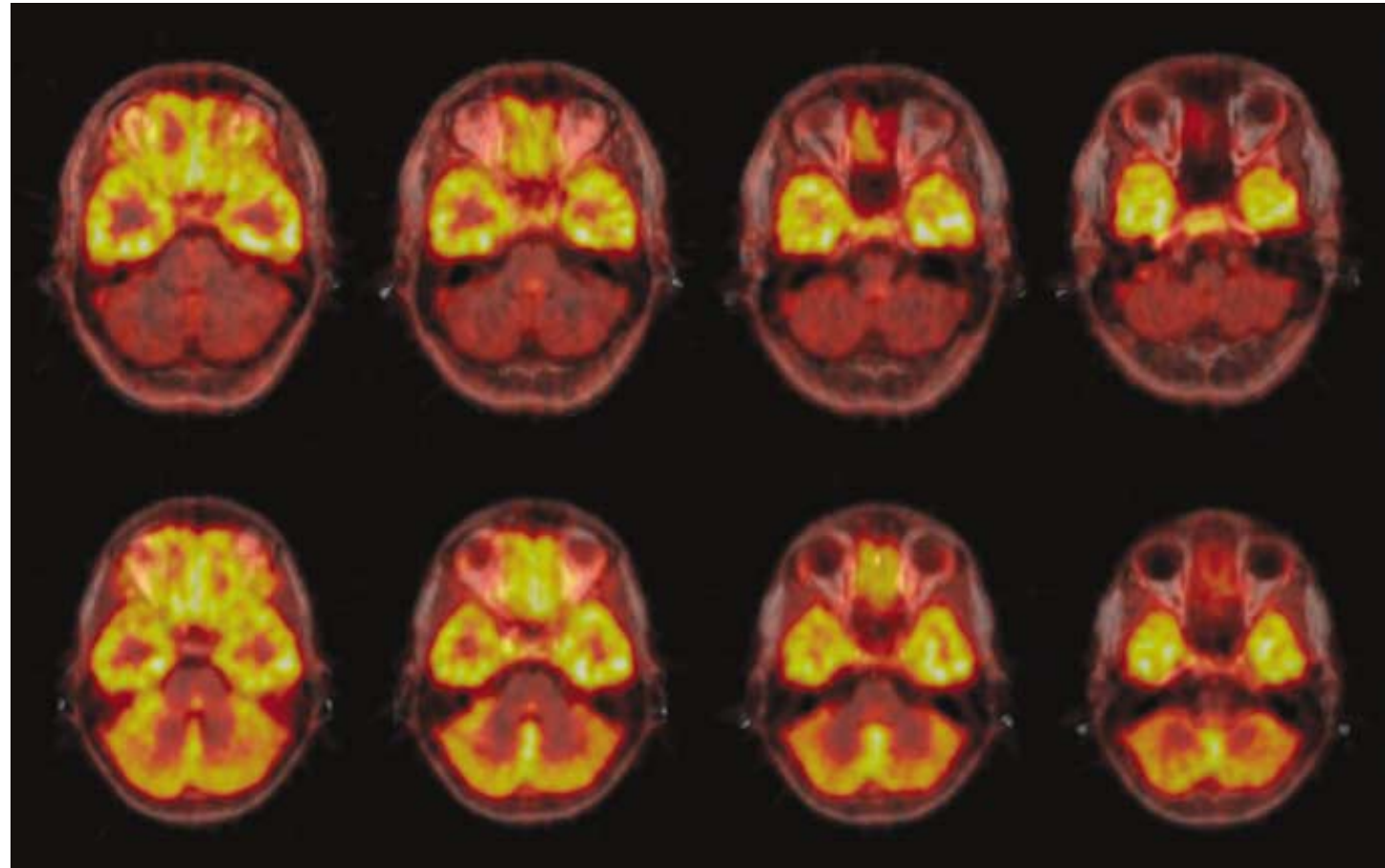
Coronal



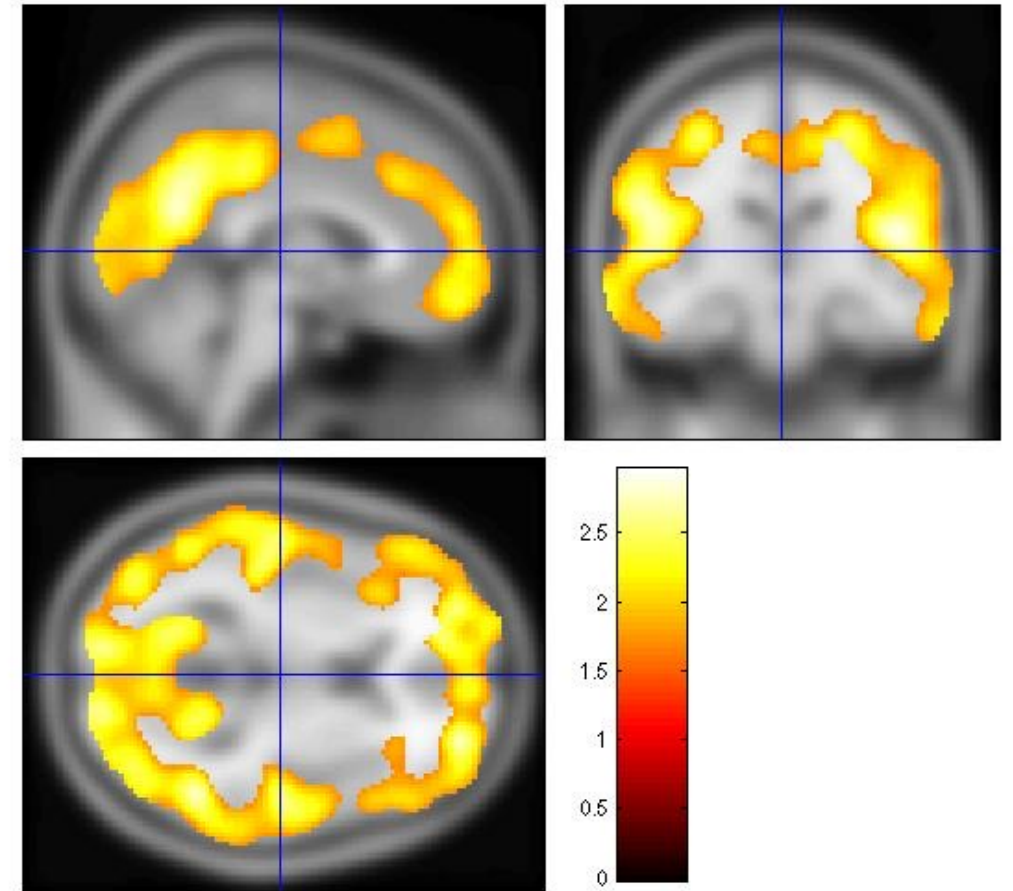
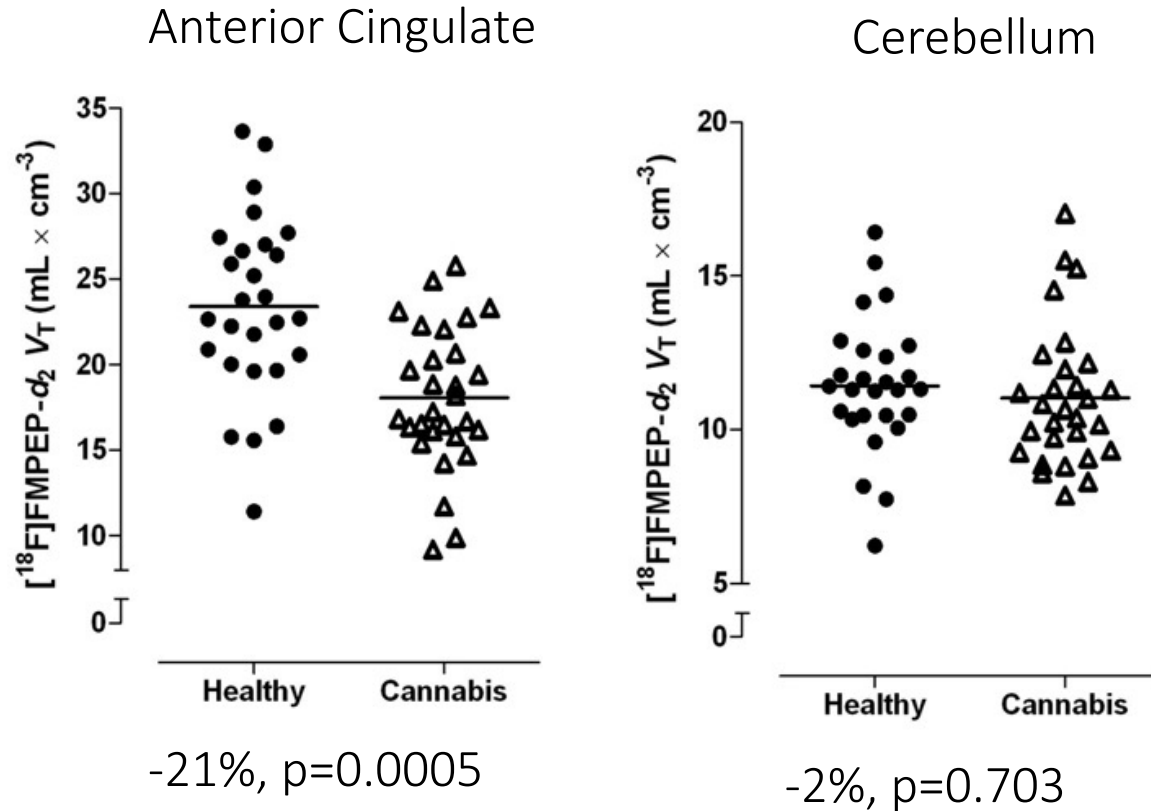
carbonyl-¹¹C-WAY-100635: distribution of serotonin 5-HT_{1A} receptors in human brain

Typical pattern
of distribution:
little uptake in
cerebellum

Atypical pattern
of distribution:
high uptake in
cerebellum



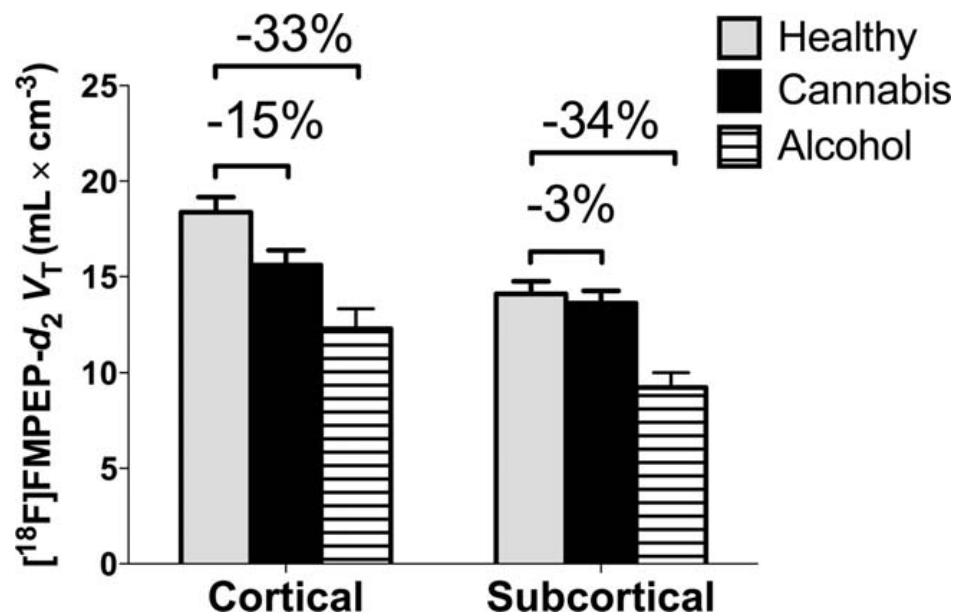
Study of pathophysiology: CB₁ receptors are region-specifically downregulated in cannabis smokers



In Vivo Imaging of the Cannabinoid CB₁ Receptor With Positron Emission Tomography

DISCOVERY

J Hirvonen^{1,2}



ORIGINAL ARTICLE

Reduced cannabinoid CB₁ receptor binding in alcohol dependence measured with positron emission tomography

J Hirvonen¹, P Zanotti-Fregonara^{1,3}, JC Umhau^{2,3}, DT George², D Rallis-Frutos¹, CH Lyoo¹, C-T Li¹, CS Hines¹, H Sun², GE Terry¹, C Morse¹, SS Zoghbi¹, VW Pike¹, RB Innis^{1,3} and M Heilig^{2,3}

Molecular Psychiatry (2012) 1–6

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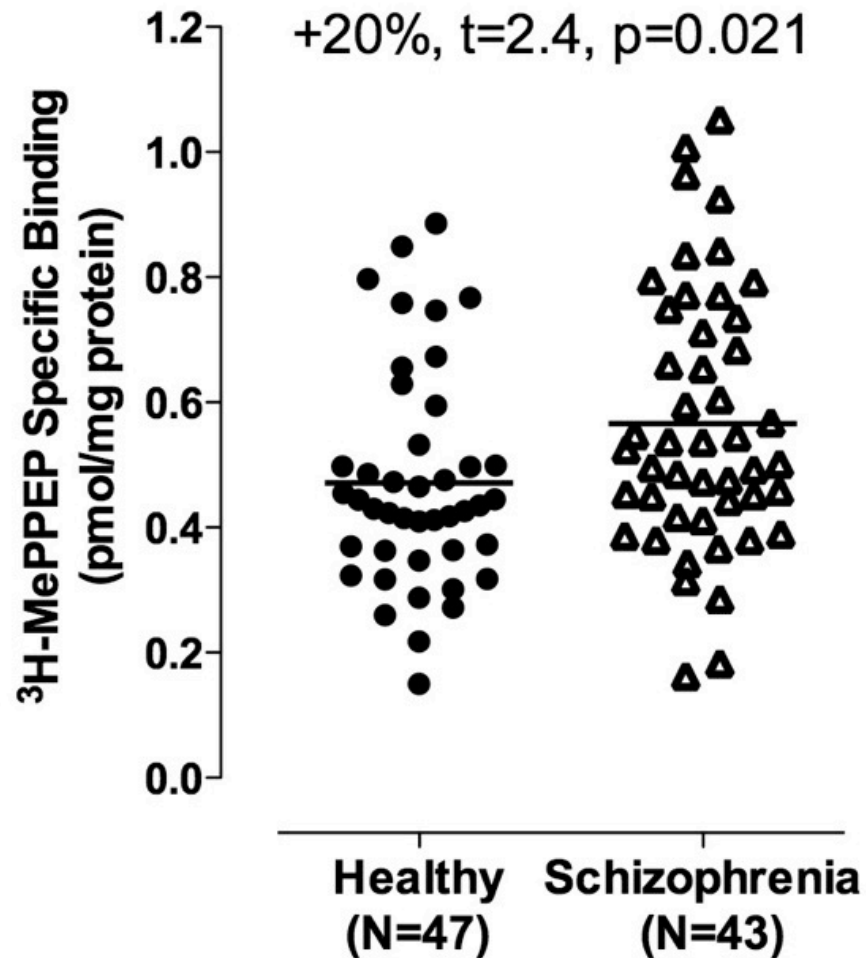
www.nature.com/mp

Decreased Cannabinoid CB₁ Receptors in Male Tobacco Smokers Examined With Positron Emission Tomography

Jussi Hirvonen, Paolo Zanotti-Fregonara, David A. Gorelick, Chul Hyoung Lyoo, Denise Rallis-Frutos, Cheryl Morse, Sami S. Zoghbi, Victor W. Pike, Nora D. Volkow, Marilyn A. Huestis, and Robert B. Innis

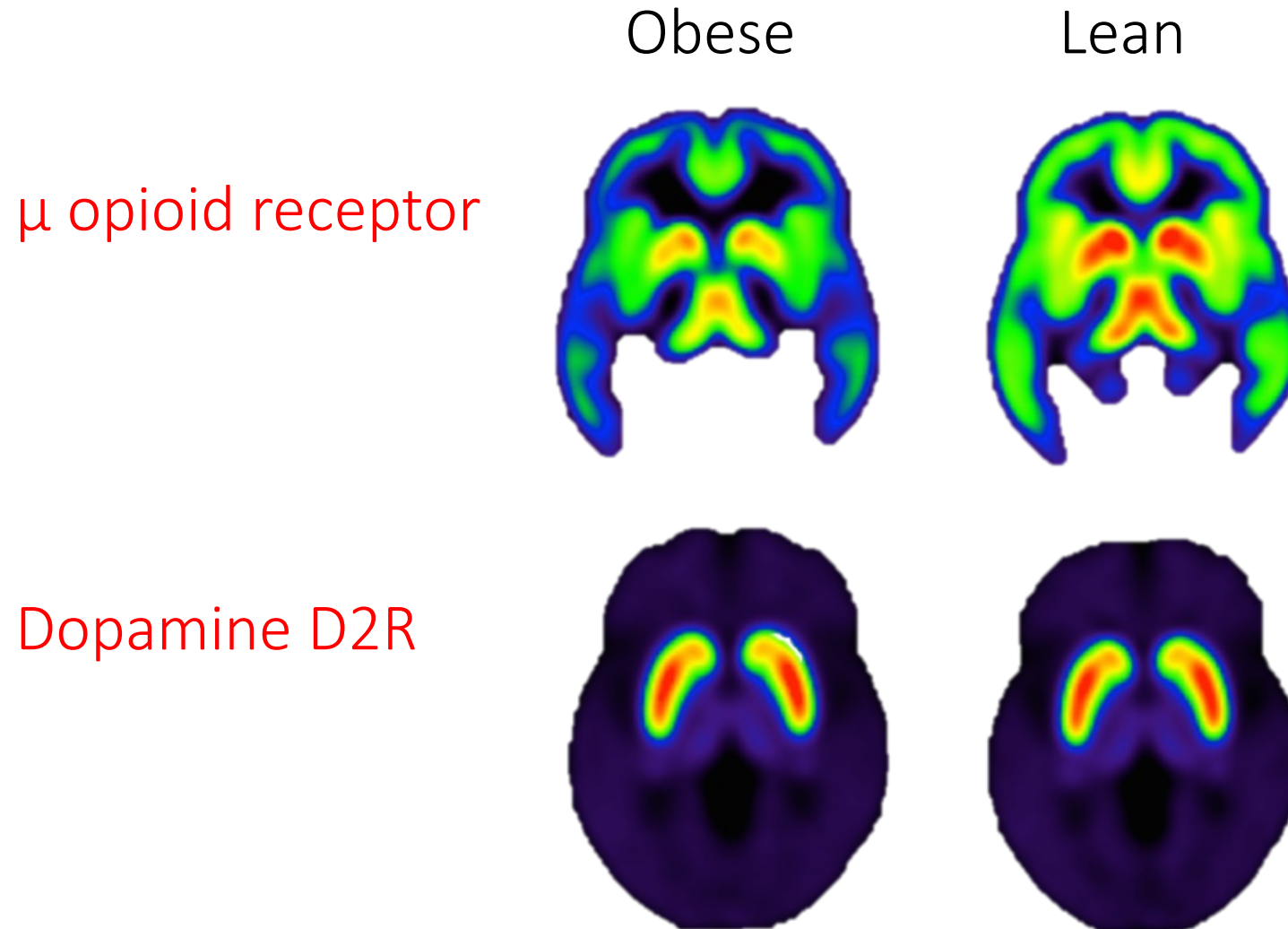
Biological
Psychiatry

Prefrontal CB₁ receptors increased in schizophrenia *in vitro*

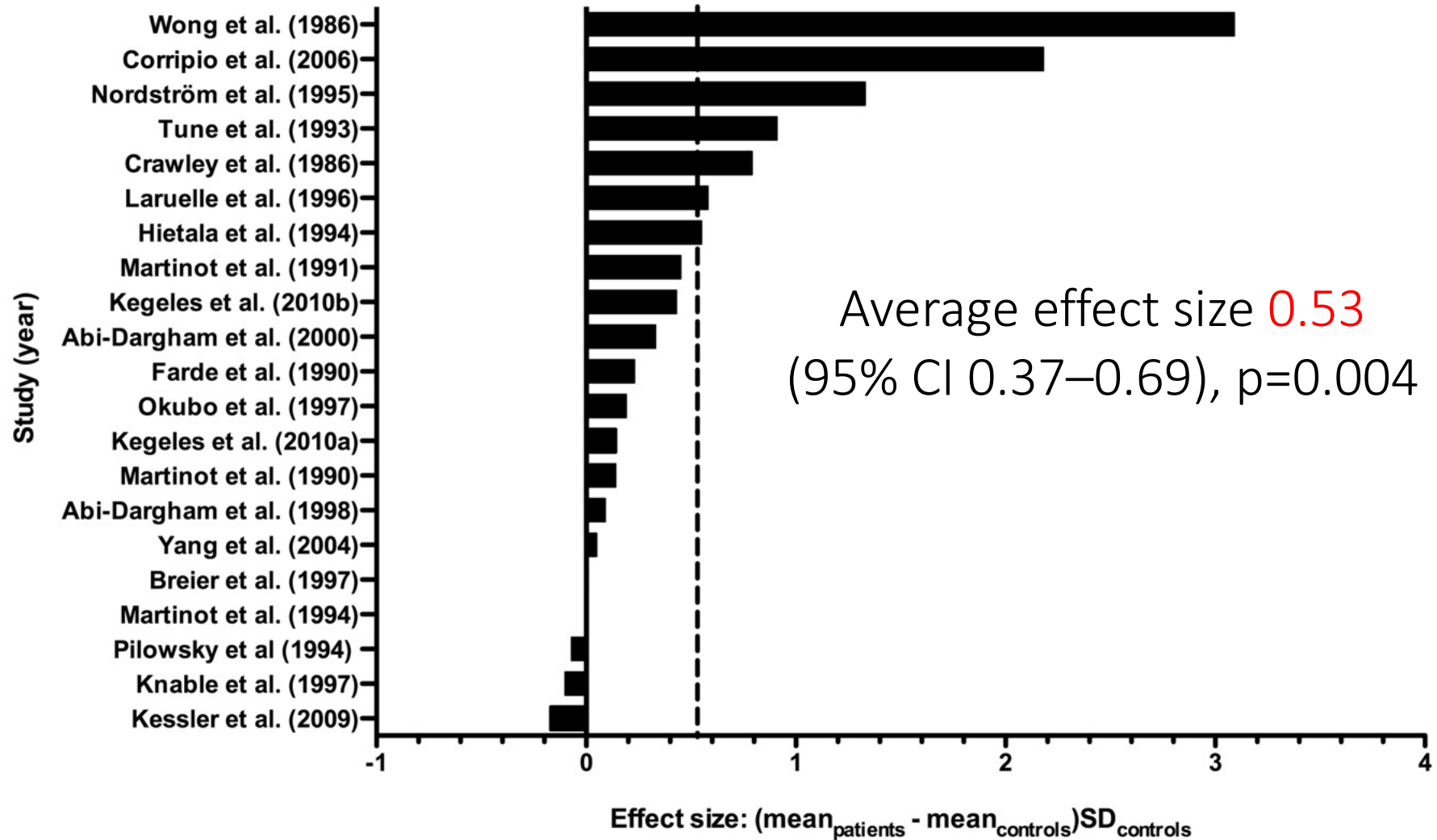


Power analysis shows 52 subjects per group needed to demonstrate difference with [¹⁸F]FMPEP-d₂ PET in vivo ($\alpha = 0.05$, $\beta = 0.20$)

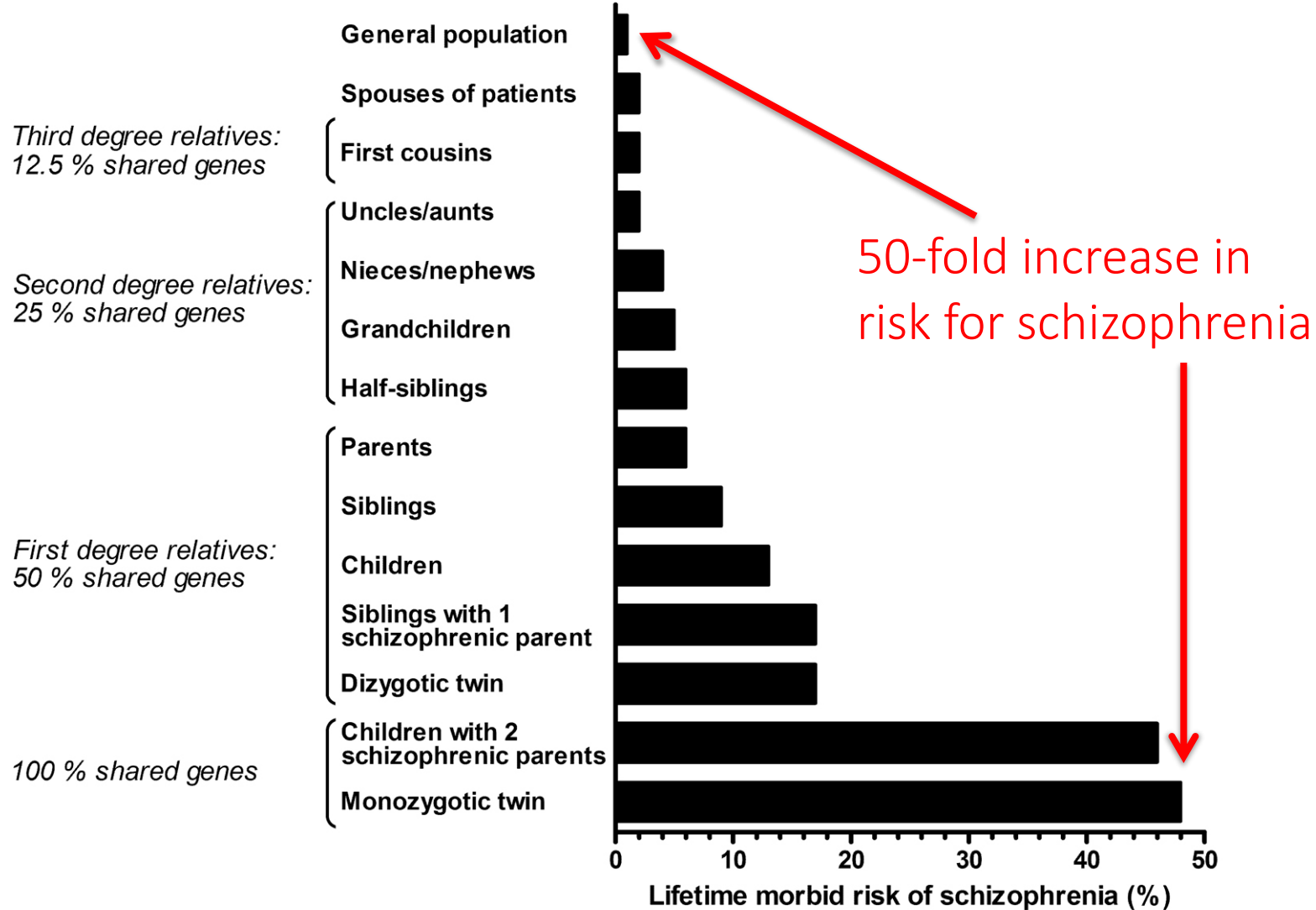
Neurochemistry in obesity: decreased μ opioid receptors, unchanged dopamine D2R



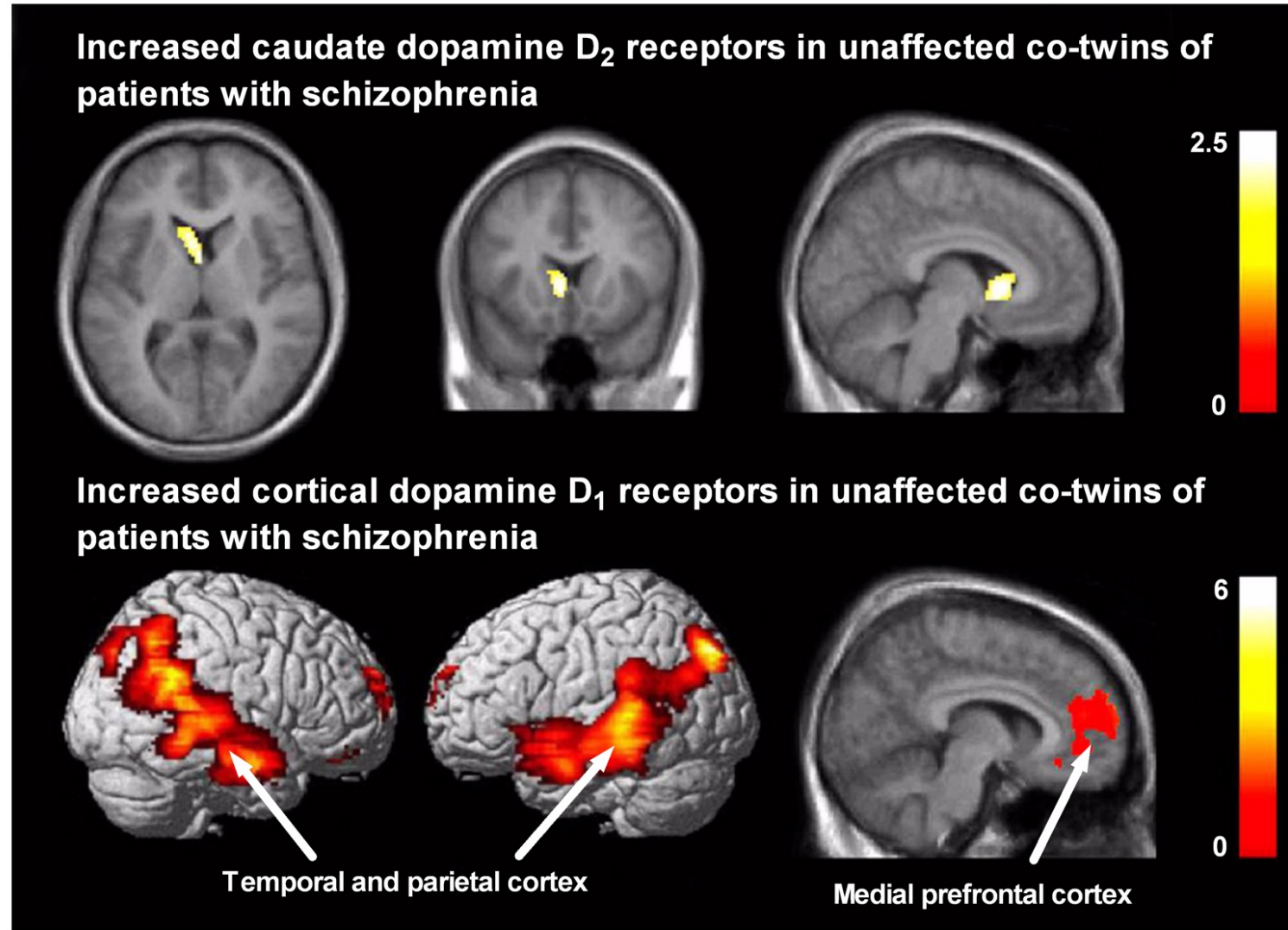
Striatal Dopamine D₂ Receptor Imaging Studies in Schizophrenia: Meta-Analysis



Study of pathophysiology: Imaging genetics: Dopamine receptors in twins with schizophrenia

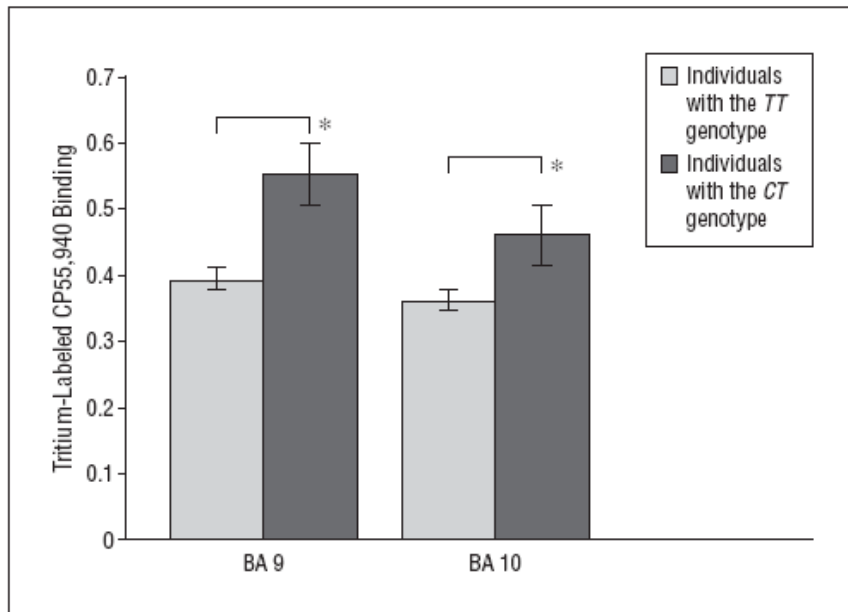


Study of pathophysiology: Imaging genetics: Dopamine receptors in twins with schizophrenia



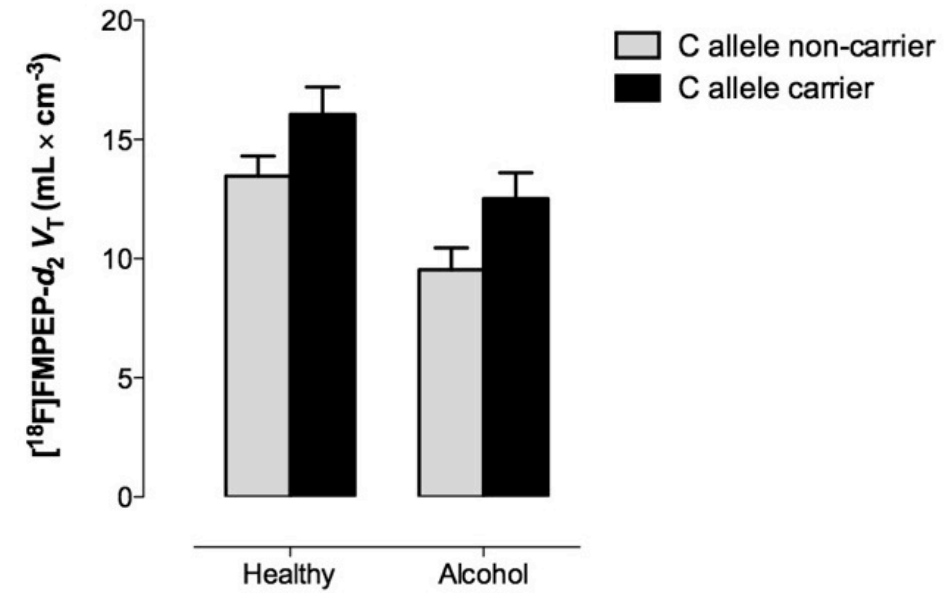
Variation at the CNR1 gene in vitro

- Polymorphisms in CNR1 associated with substance abuse
- C allele of the rs2023239 polymorphism:
 - Increased CB₁ agonist binding in prefrontal cortex
 - Increased alcohol-induced activation of brain reward system
 - Increased subjective reward from alcohol



Post mortem

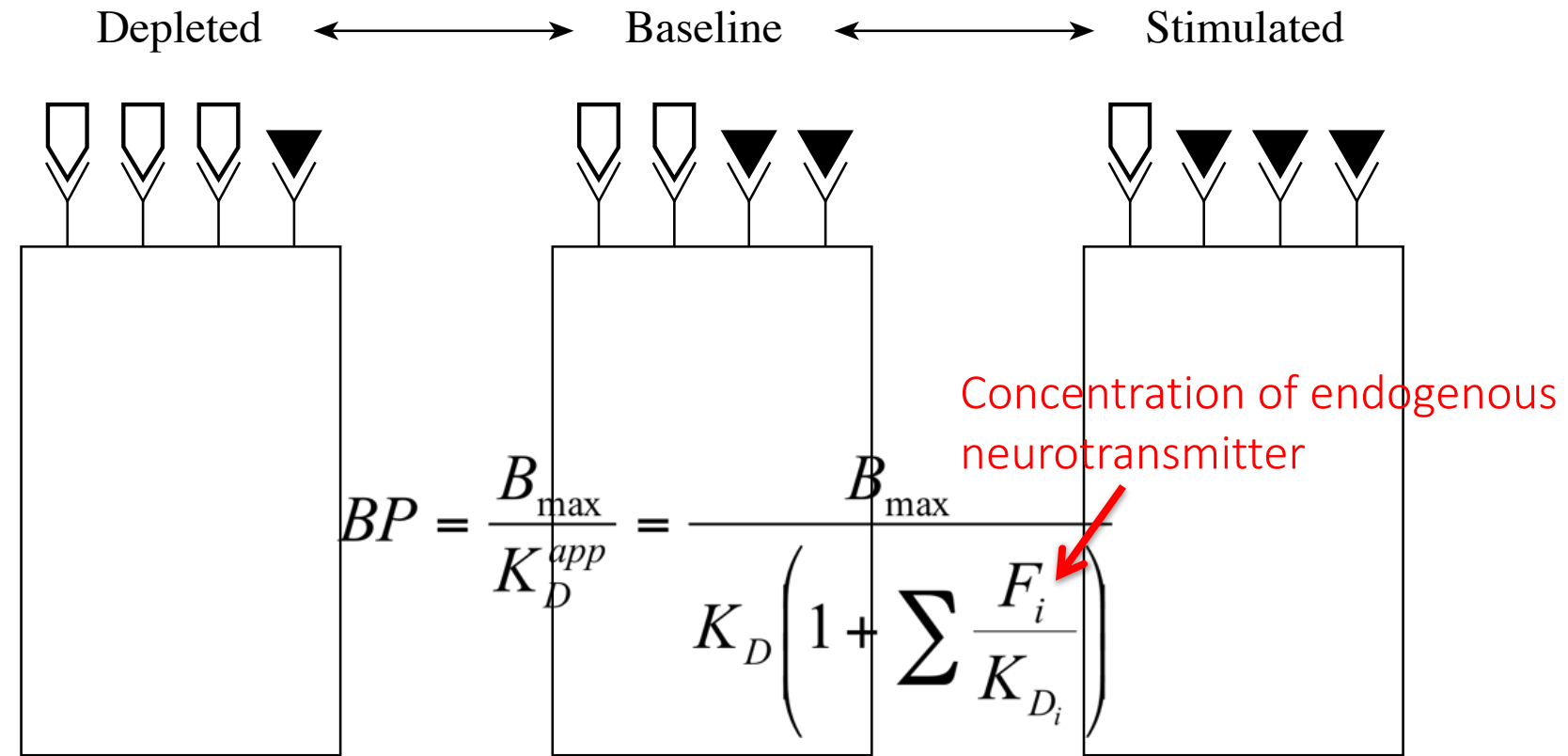
Hutchison *et al.* Arch Gen Psychiatry 2008;65:841-850.



In vivo

Hirvonen *et al.* Molecular Psychiatry 2013;18:916-921

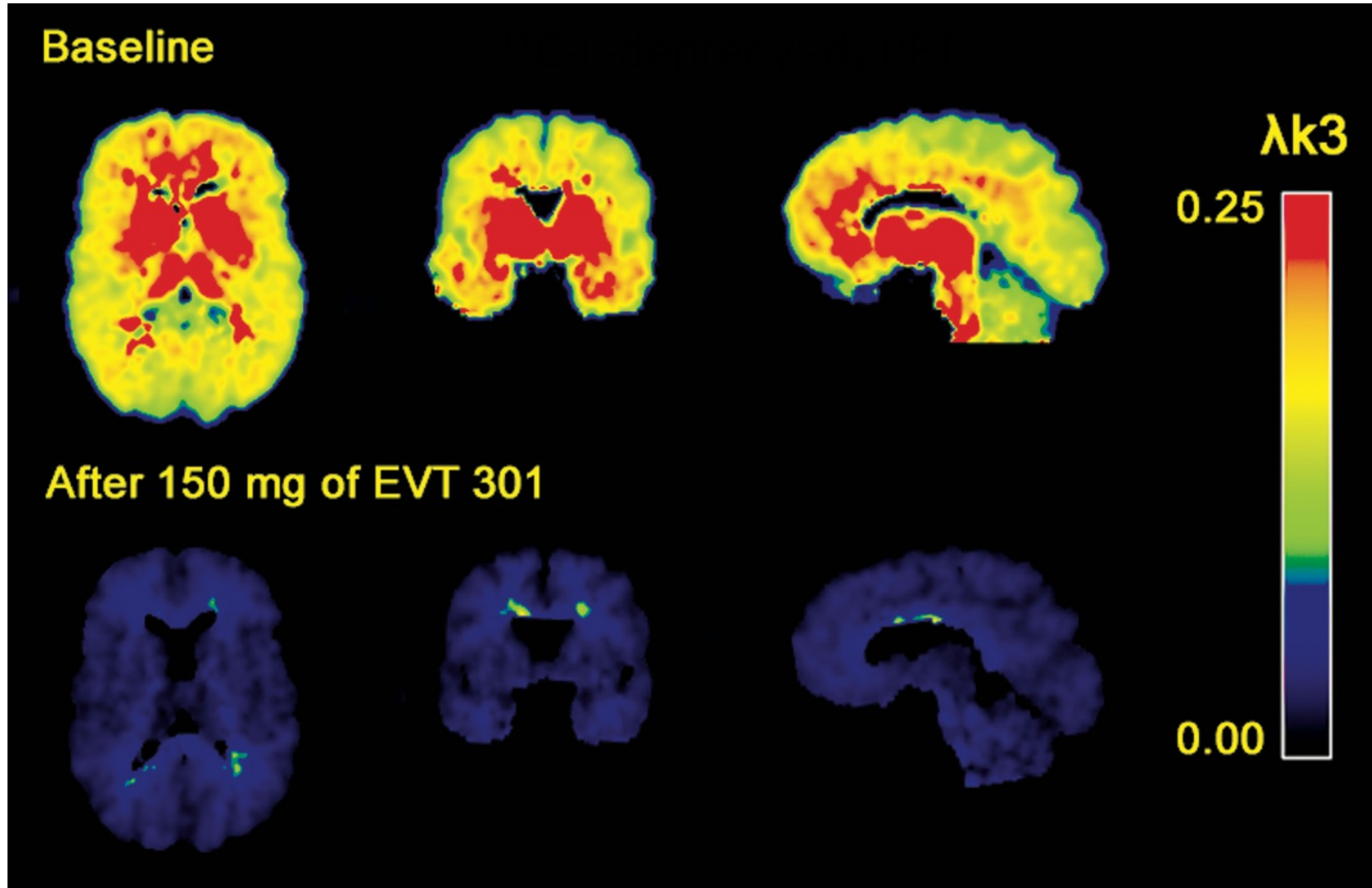
Challenge studies: Classical occupancy model



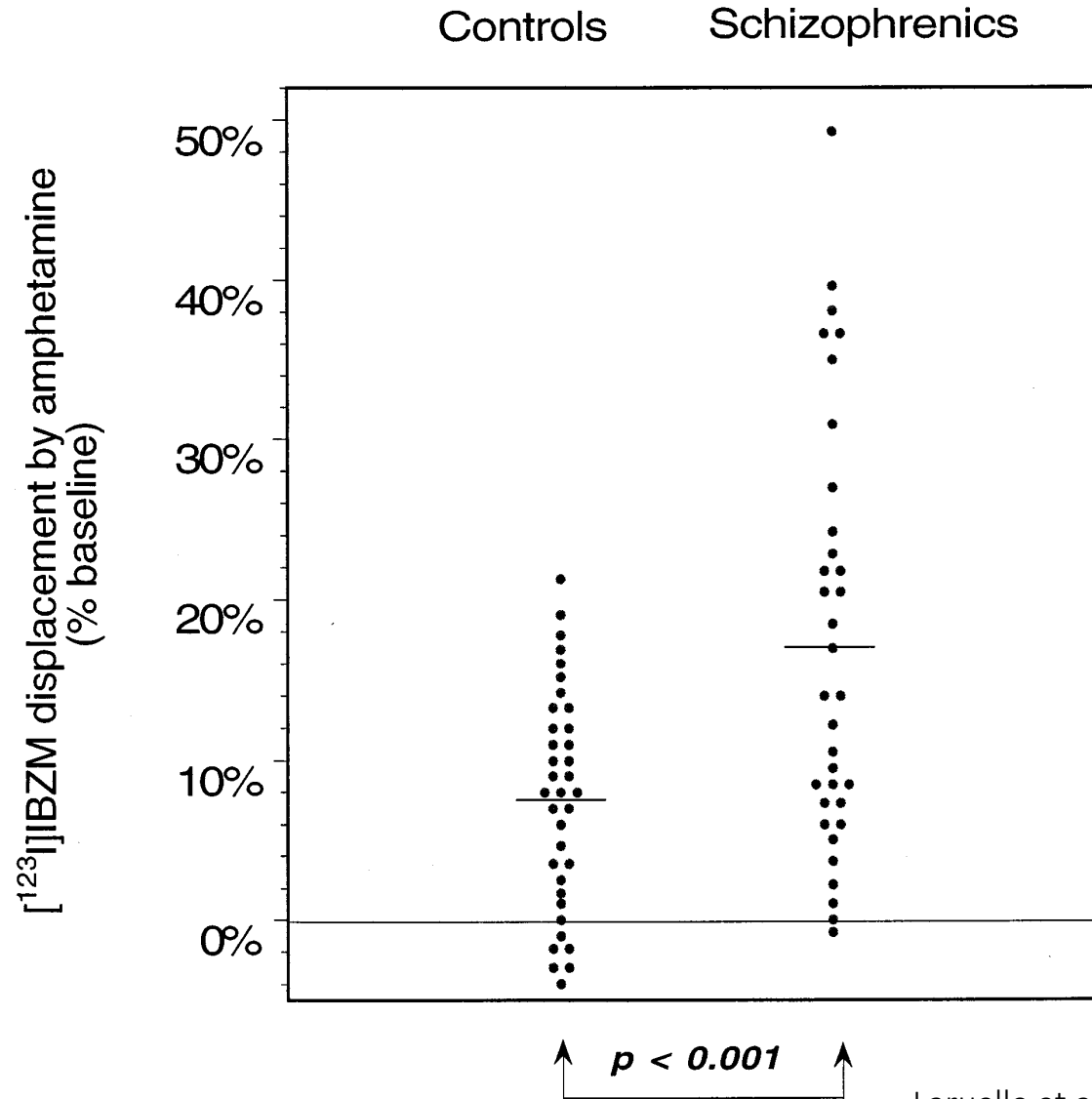
$$\text{Occupancy (\%)} = \frac{BP_{\text{BEFORE}} - BP_{\text{AFTER}}}{BP_{\text{BEFORE}}} * (100\%)$$

Υ D2 receptor [C-11]raclopride Dopamine

Study of drug action: Occupancy of EVT 301, a novel MAO-B inhibitor, in human brain

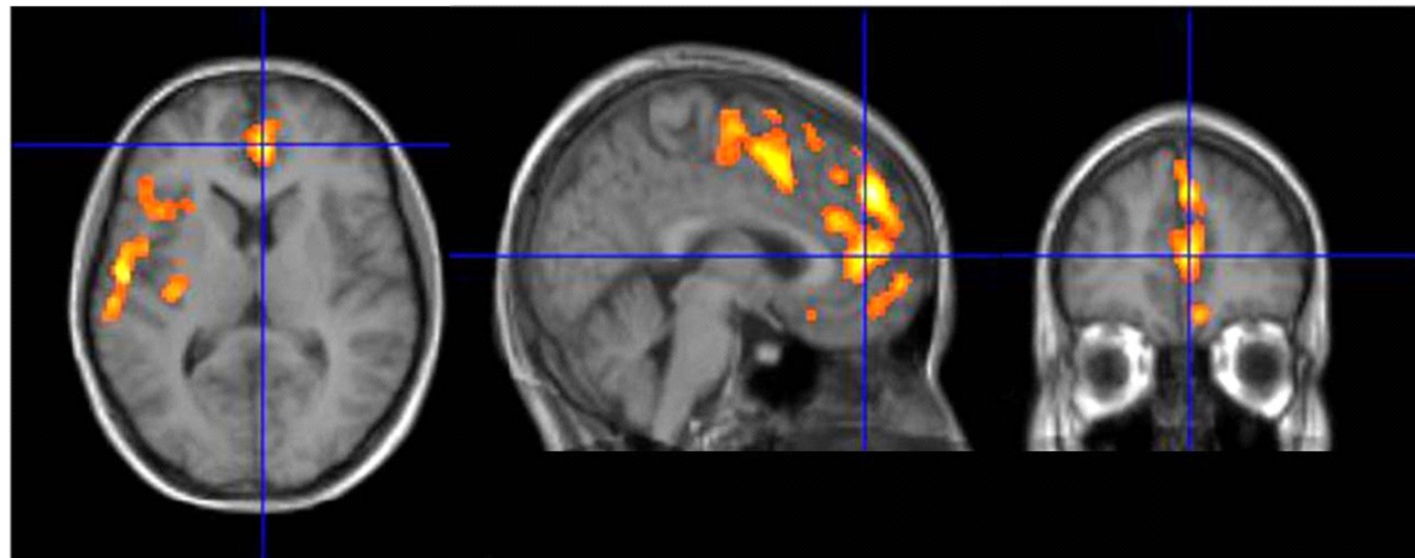
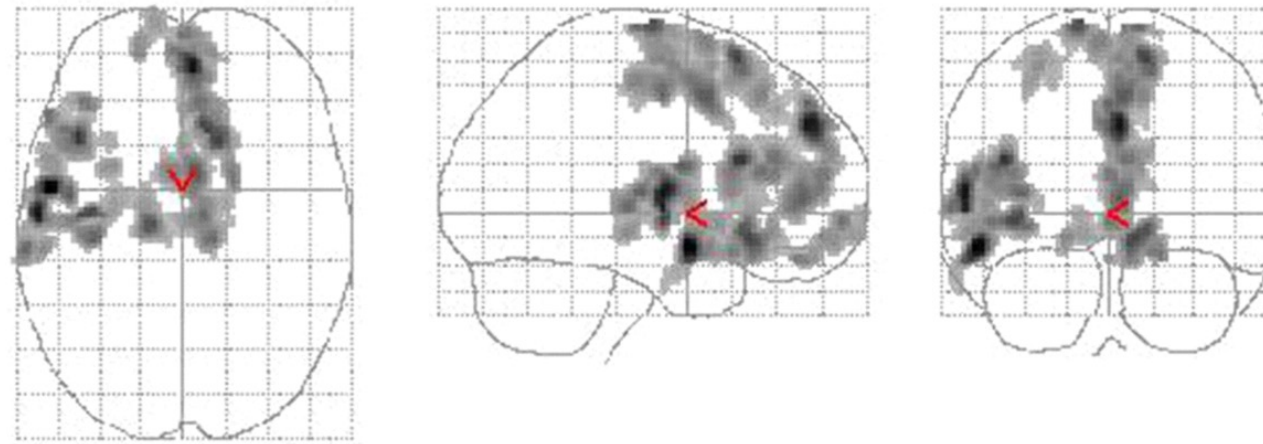


Challenge studies: Increased amphetamine-induced dopamine release in schizophrenia

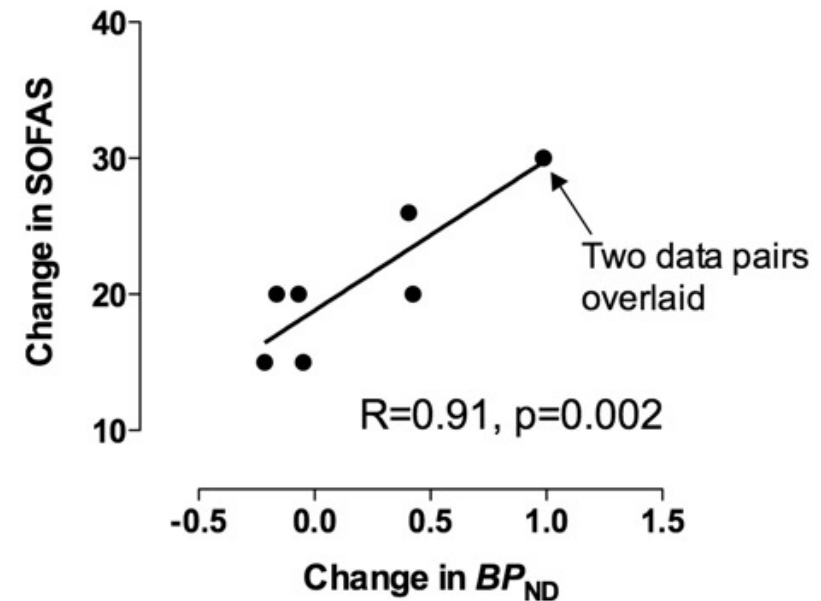
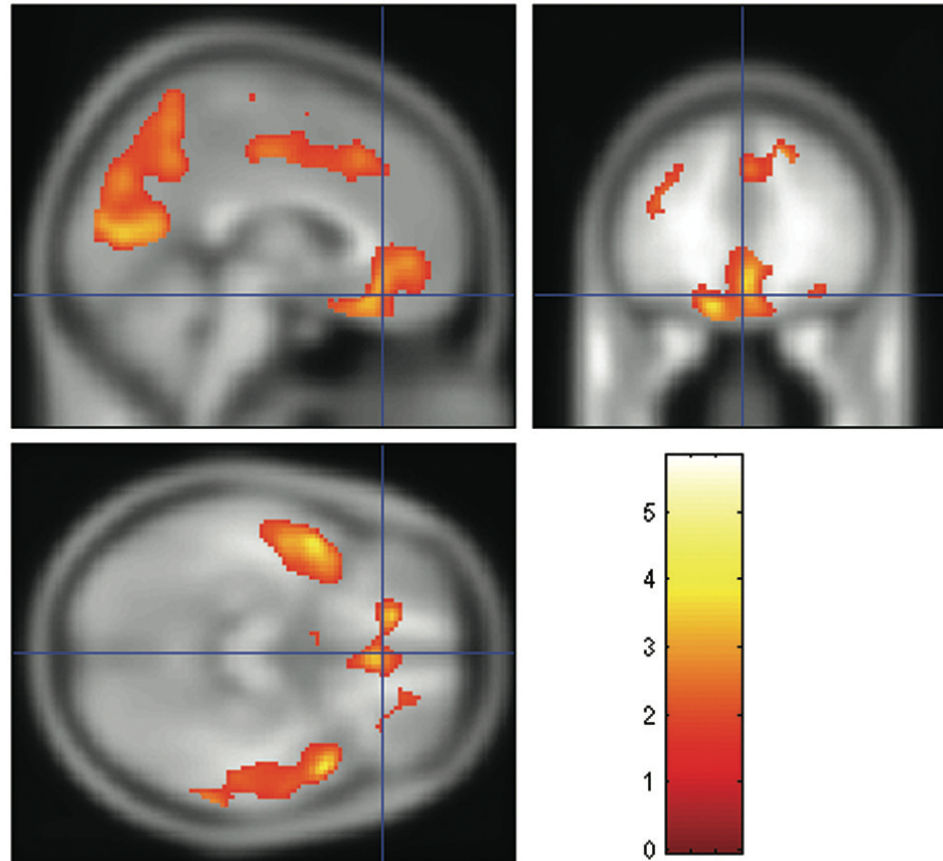


Challenge studies: Opioid release after repeated transcranial magnetic stimulation (rTMS)

^{11}C -carfentanil PET



Psychotherapy increases cortical serotonin 5-HT_{1A} receptor density in patients with major depression



[carbonyl-¹¹C]WAY-100635

Opioid Release after High-Intensity Interval Training in Healthy Human Subjects

Tiina Saanijoki¹, Lauri Tuominen^{1,2,3}, Jetro J Tuulari¹, Lauri Nummenmaa^{1,4}, Eveliina Arponen¹, Kari Kalliokoski¹ and Jussi Hirvonen^{*,1,5}

¹Turku PET Centre, University of Turku, Turku, Finland; ²Department of Psychiatry, Massachusetts General Hospital, Boston, MA, USA; ³Harvard Medical School, Boston, MA, USA; ⁴Department of Psychology, University of Turku, Turku, Finland; ⁵Department of Radiology, Turku University Hospital and University of Turku, Turku, Finland

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RESEARCH ARTICLE

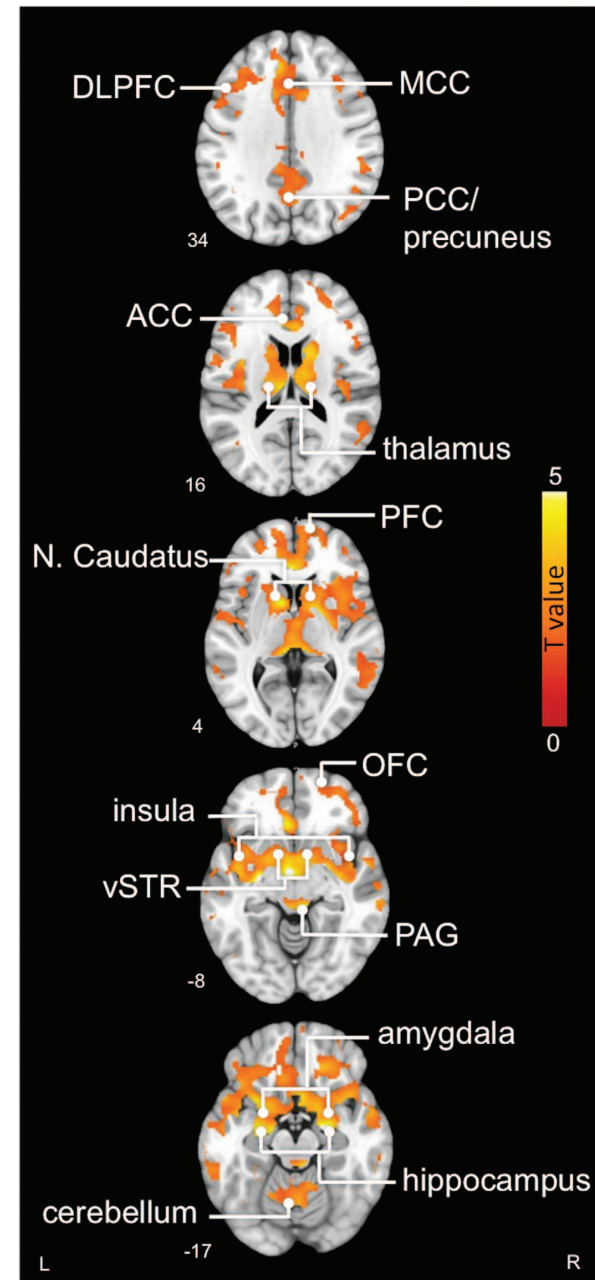
WILEY

Aerobic exercise modulates anticipatory reward processing via the μ -opioid receptor system

Tiina Saanijoki¹  | Lauri Nummenmaa^{1,2}  | Jetro J. Tuulari¹ |

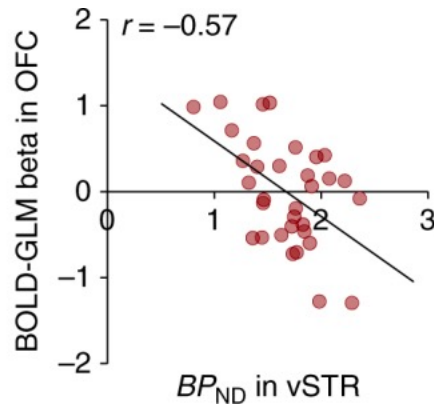
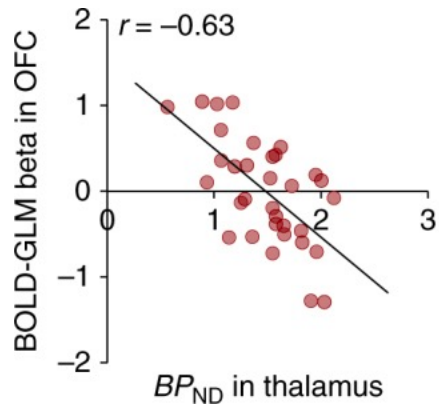
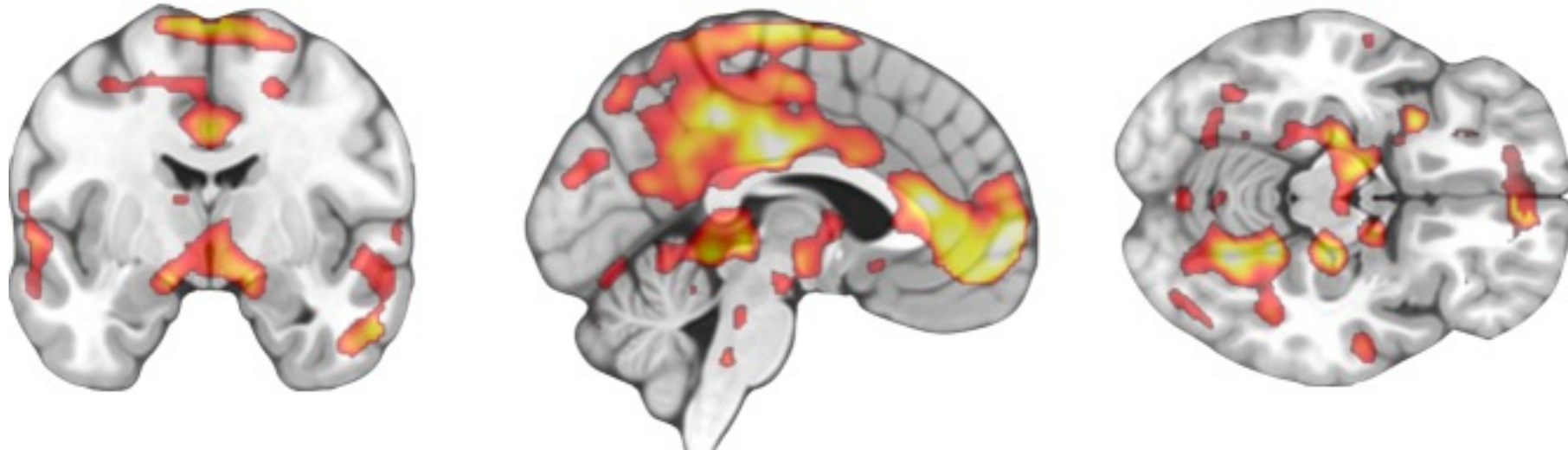
Lauri Tuominen^{1,3,4} | Eveliina Arponen¹ | Kari K. Kalliokoski¹ | Jussi Hirvonen^{1,5}

Saanijoki et al. *Neuropsychopharmacology* 2018, *Human Brain Mapping* 2018

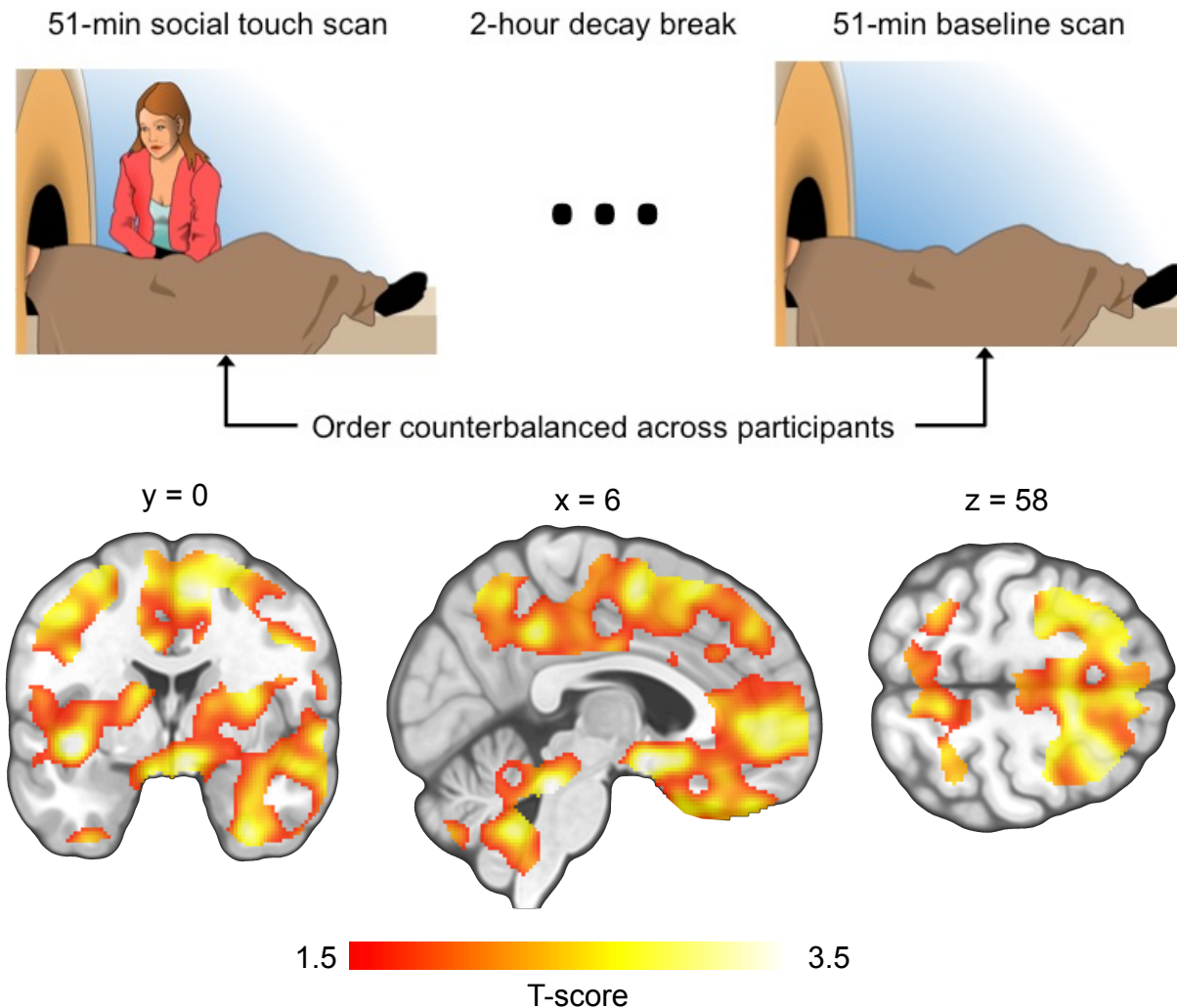


PET + fMRI: μ opioid receptors modulate responses to viewing foods

MOR-dependent responses to palatable versus non-palatable foods

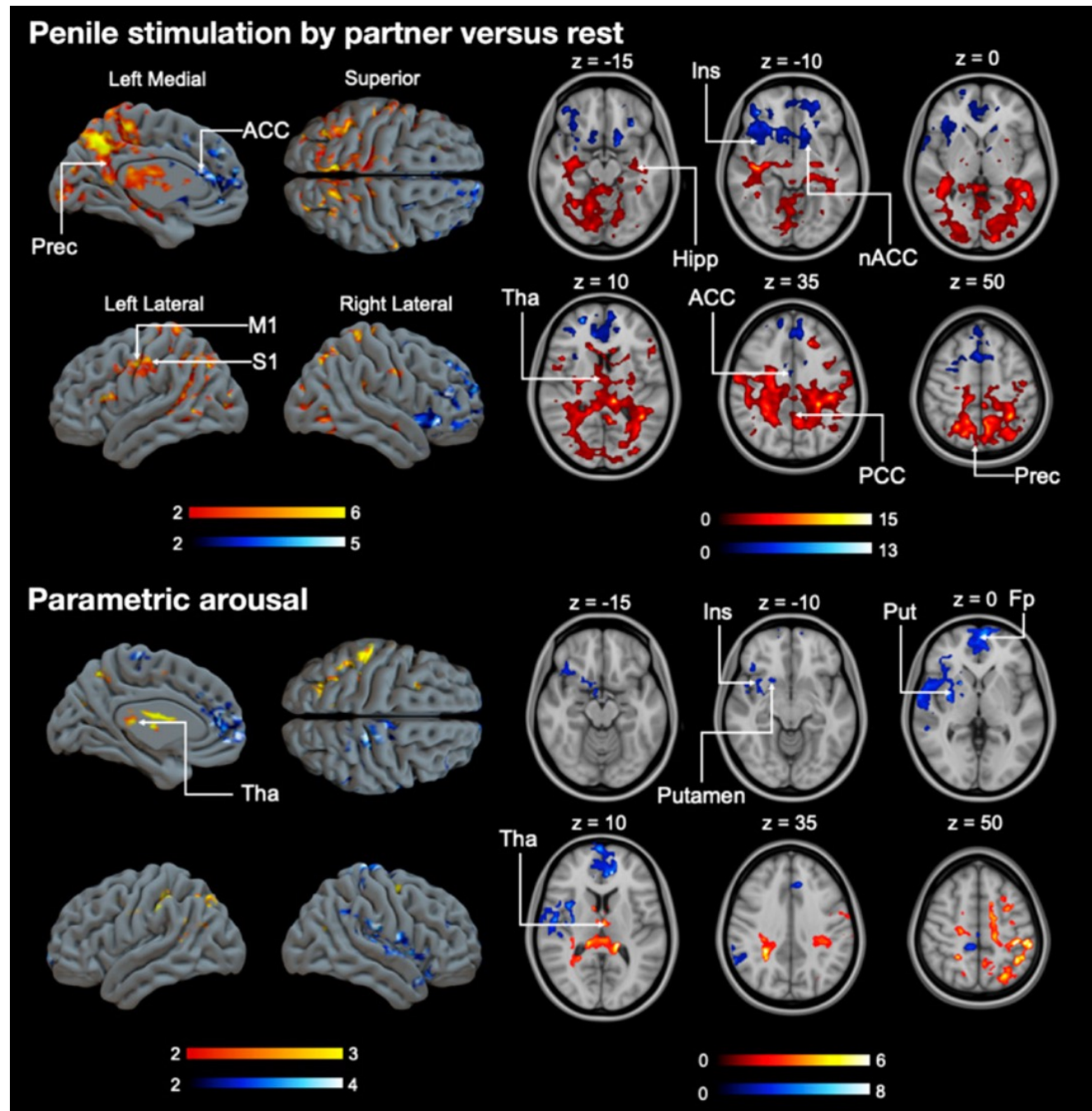
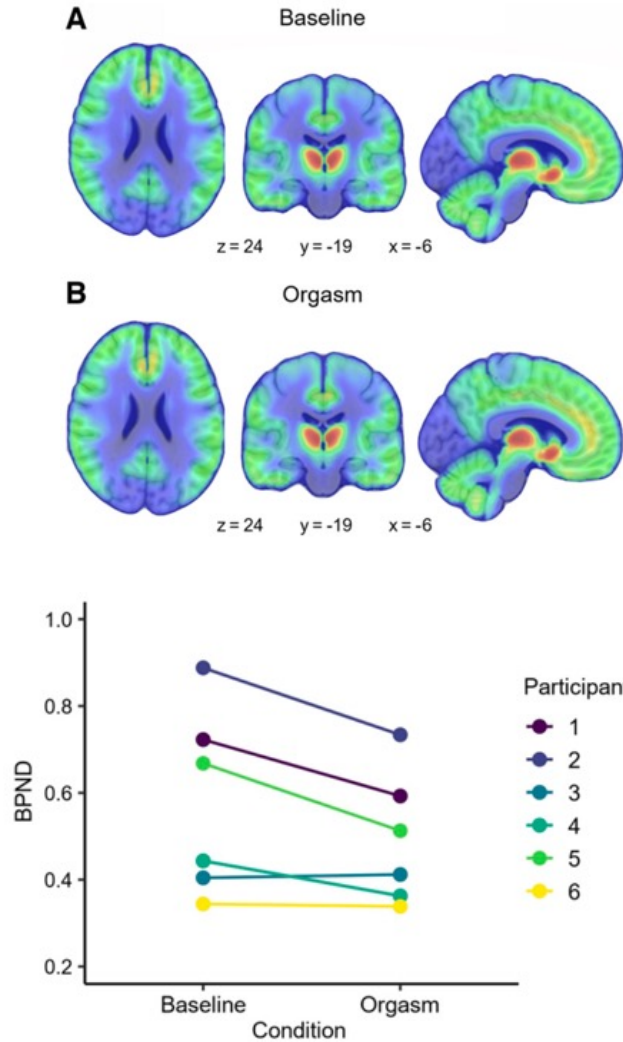


PET: Activation of μ opioid receptors after social touching in healthy subjects



Endogenous Opioid Release After Orgasm in Man: A Combined PET/Functional MRI Study

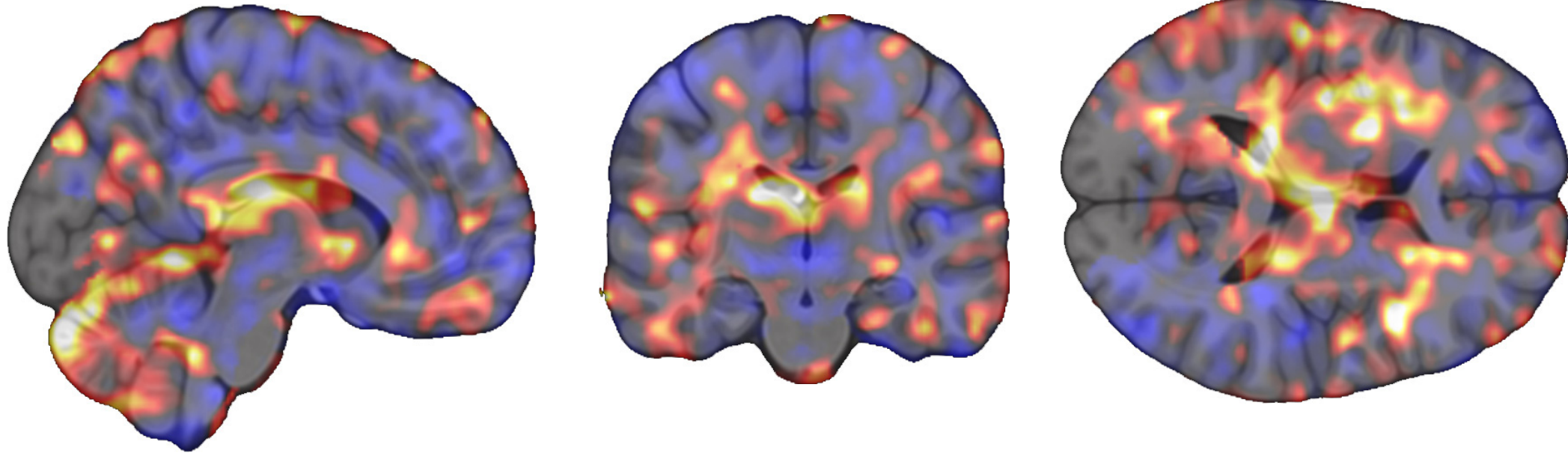
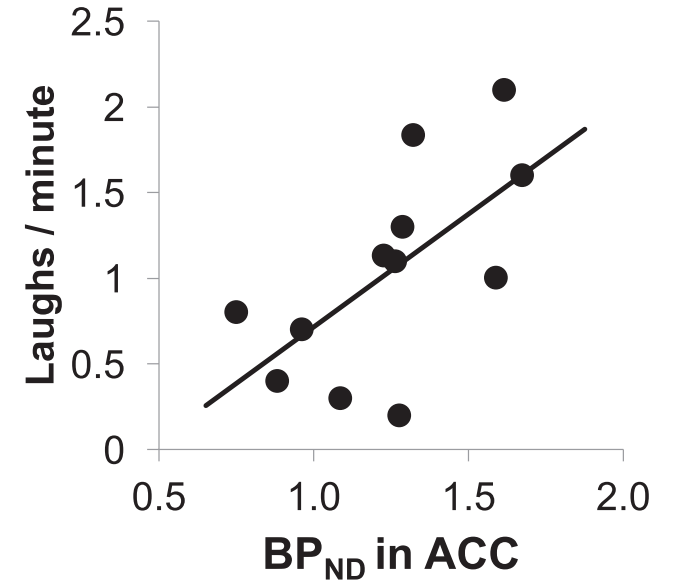
Patrick Jem¹, Jinglu Chen^{2,3}, Jouni Tuisku^{2,3}, Tiina Saanijoki^{2,7}, Jussi Hirvonen^{2,4}, Lasse Lukkarinen^{2,3}, Sandra Manninen^{2,3}, Semi Helin², Vesa Putkinen^{2,3,5}, and Lauri Nummenmaa^{2,3,6}



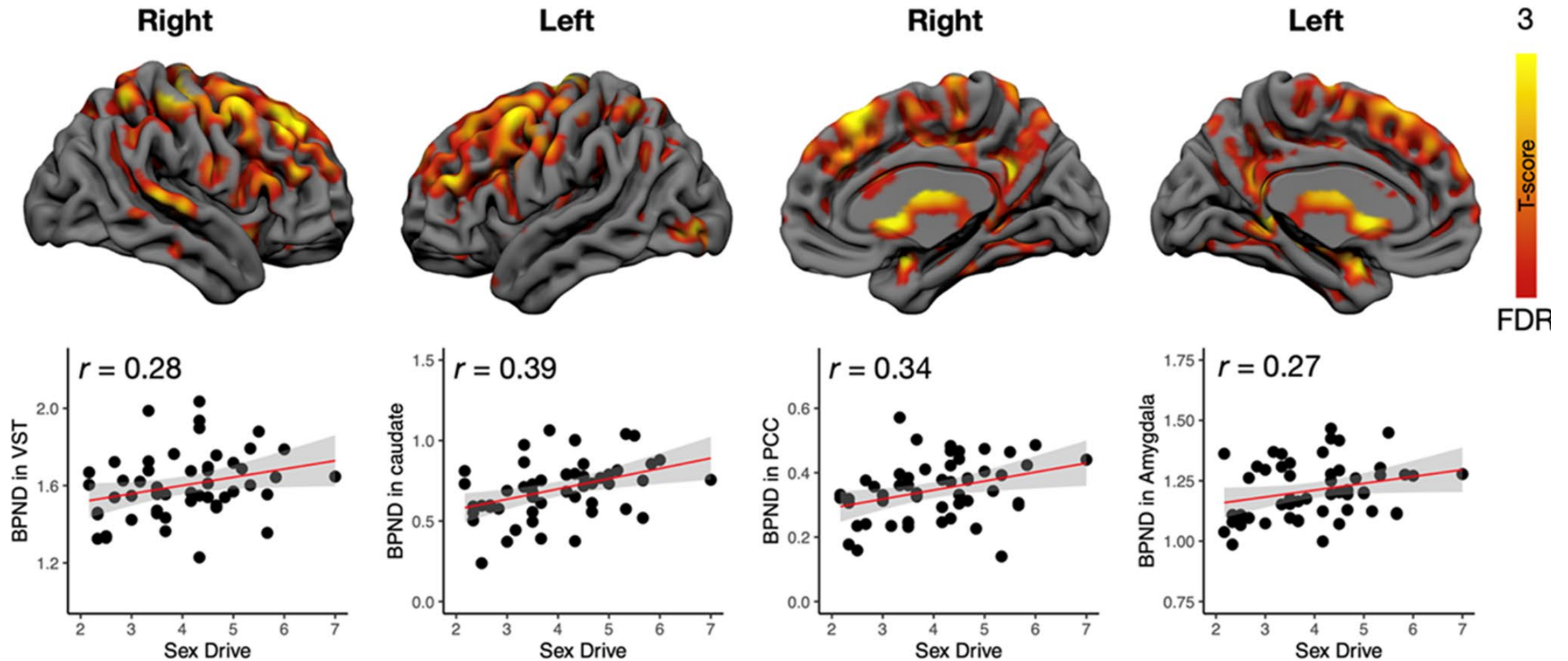
Social Laughter Triggers Endogenous Opioid Release in Humans

Sandra Manninen,¹ Lauri Tuominen,¹ Robin I. Dunbar,^{2,3} Tomi Karjalainen,¹ Jussi Hirvonen,¹ Eveliina Arponen,¹ Riitta Hari,^{2,4} Iiro P. Jääskeläinen,² Mikko Sams,² and  Lauri Nummenmaa^{1,5}

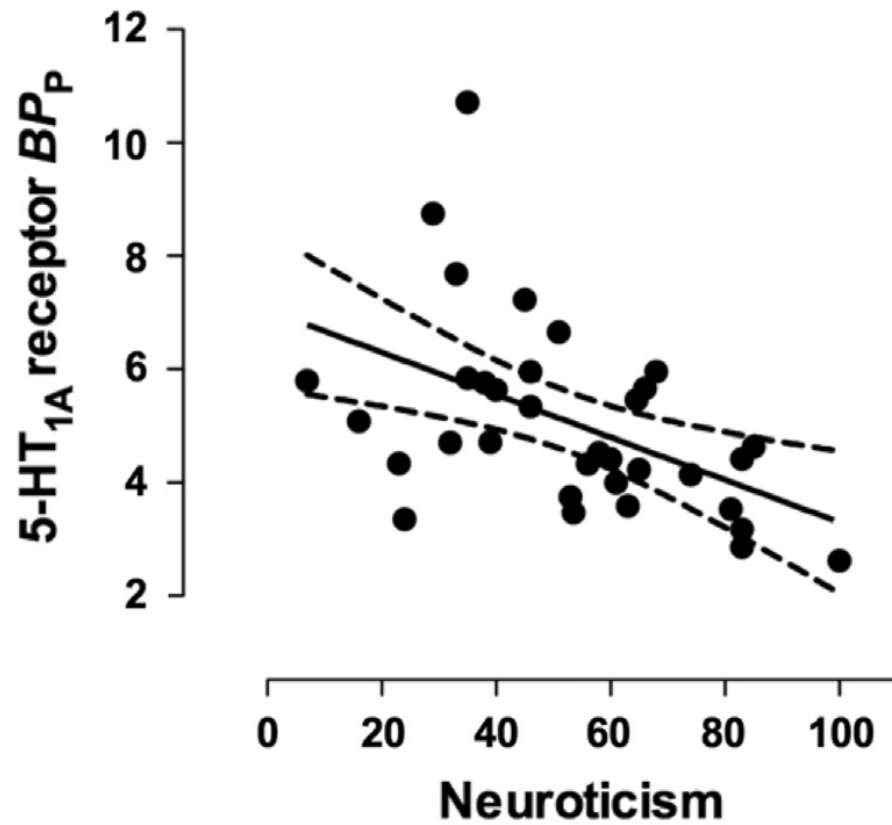
¹Turku PET Centre, University of Turku, 20520 Turku, Finland, ²Department of Neuroscience and Biomedical Engineering, School of Science, Aalto University, 00076 Aalto, Finland, ³Department of Experimental Psychology, University of Oxford, OX1 3UD Oxford, United Kingdom, ⁴Department of Art, School of Arts, Design and Architecture, Aalto University, 00076 Aalto, Finland, and ⁵Department of Psychology, University of Turku, 20014 Turku, Finland



Study of human behavior: μ opioid receptors correlate with sex drive in males

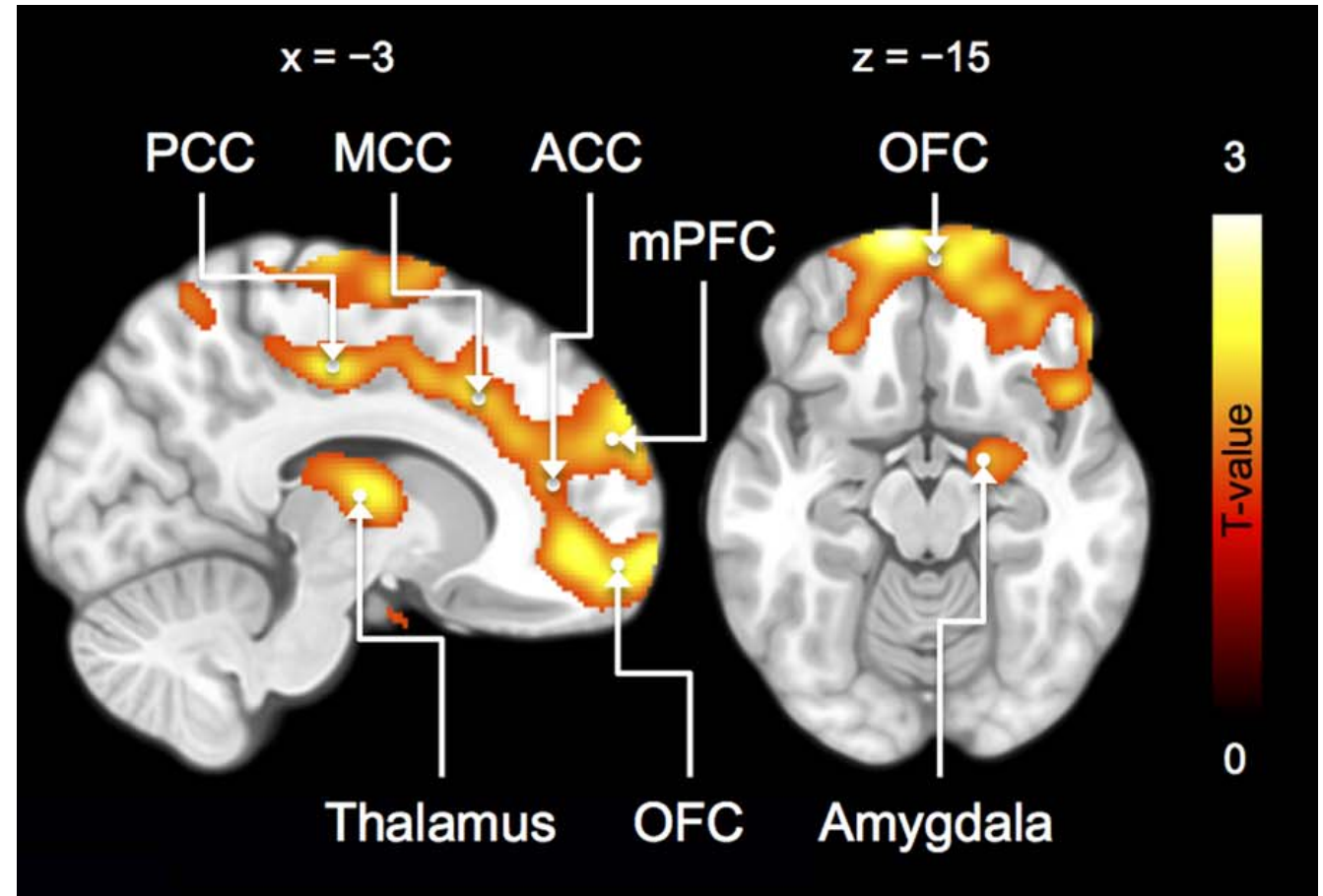
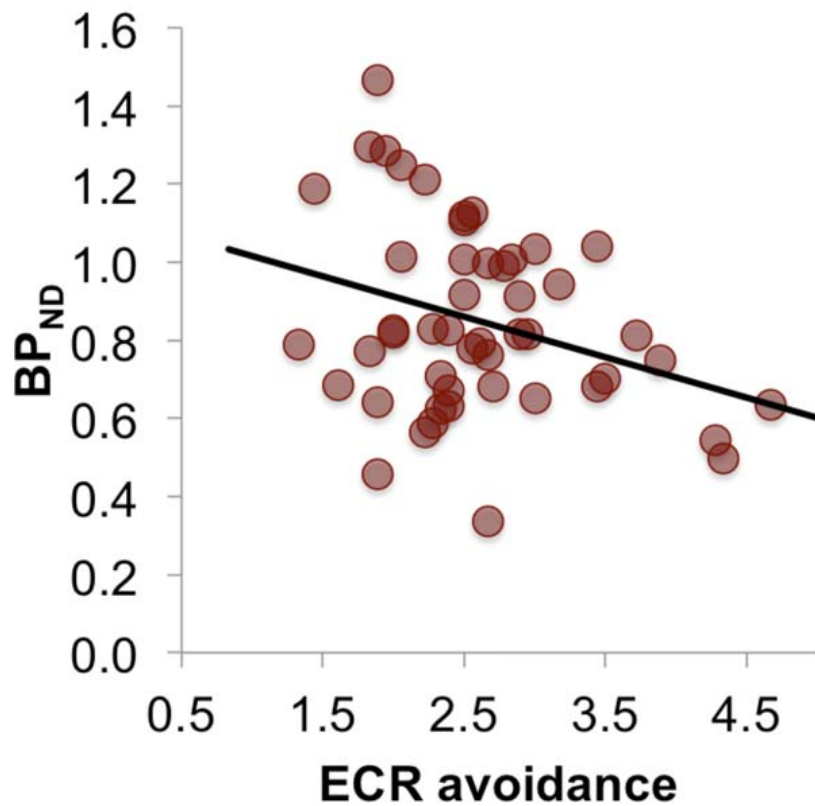


Study of vulnerability traits: Serotonin 5-HT_{1A} receptors correlate with neuroticism in healthy subjects



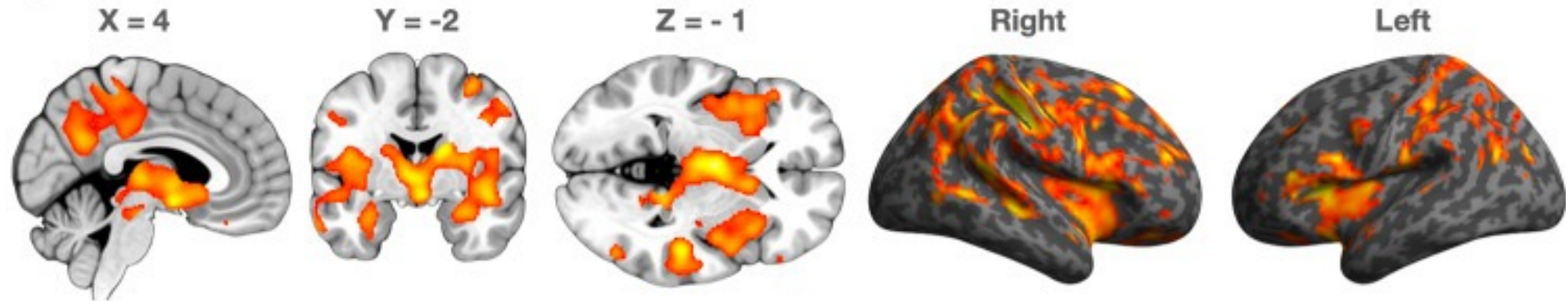
Study of vulnerability traits: μ opioid receptors correlate with avoidant attachment in healthy subjects

A) Orbitofrontal cortex

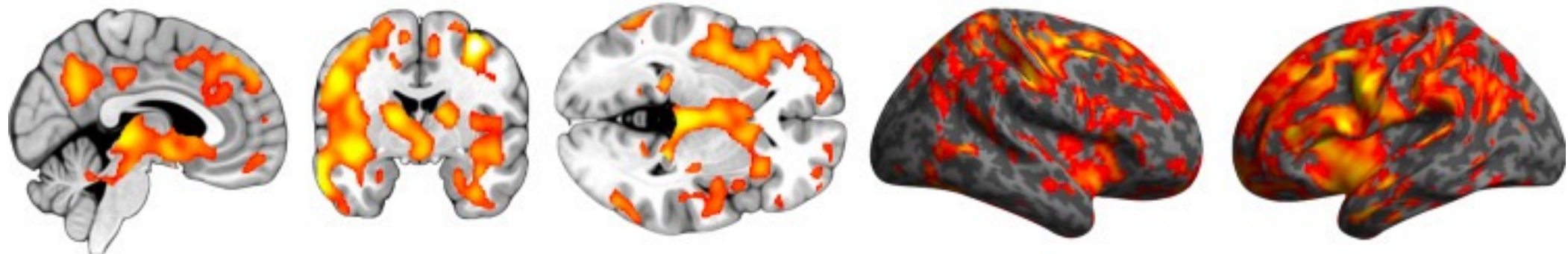


Study of vulnerability traits: μ opioid receptors correlate with subclinical depression and anxiety in healthy subjects (N=135)

A) Negative association with BDI-II scores

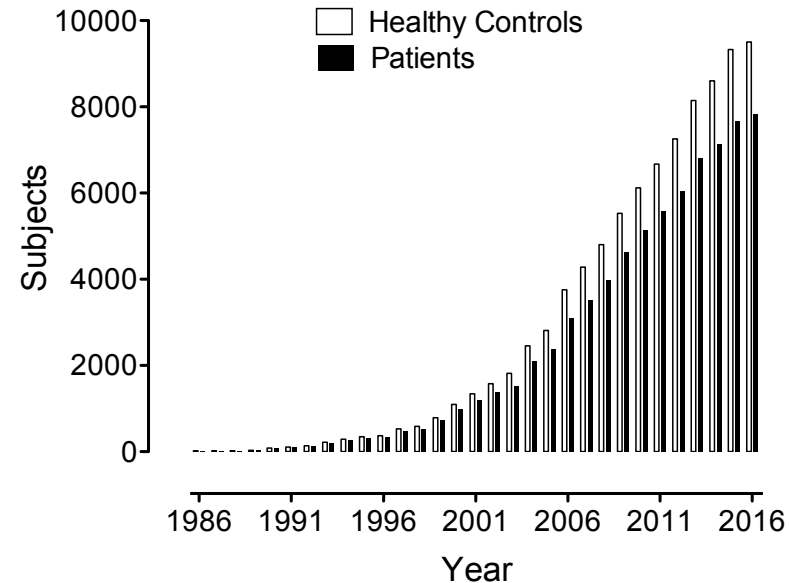
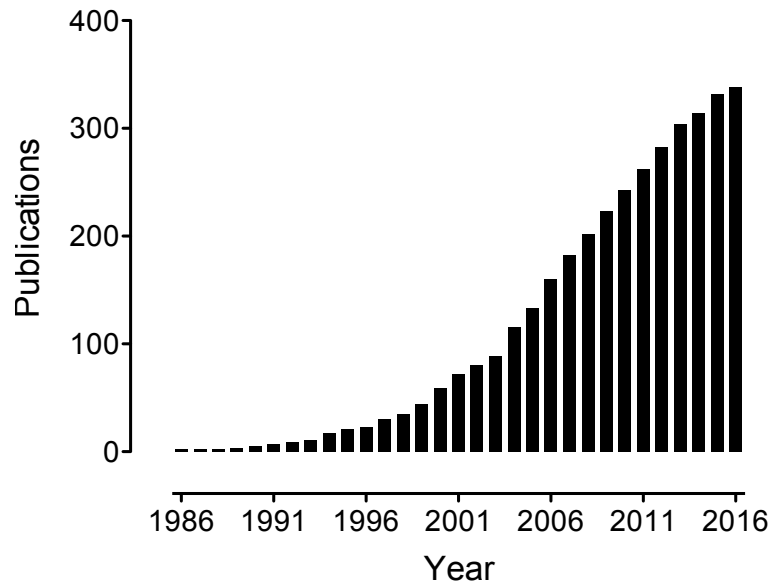
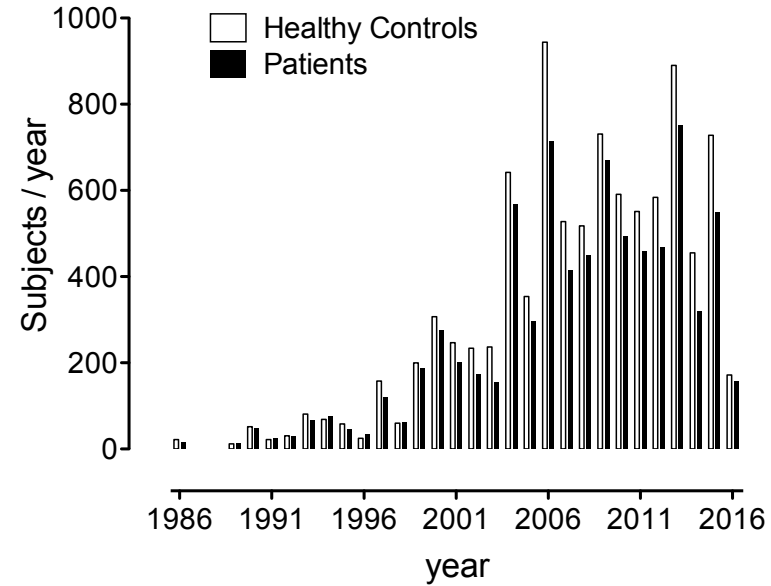
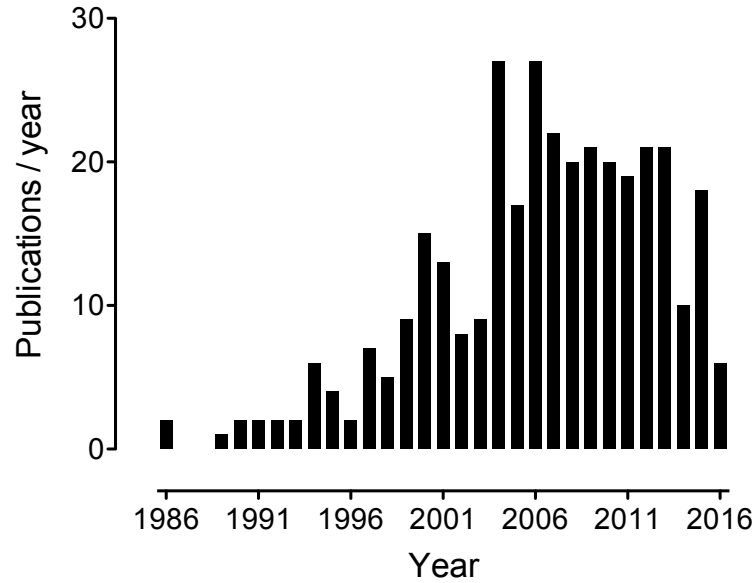


B) Negative association with STAI-X scores



FDR T-score 4

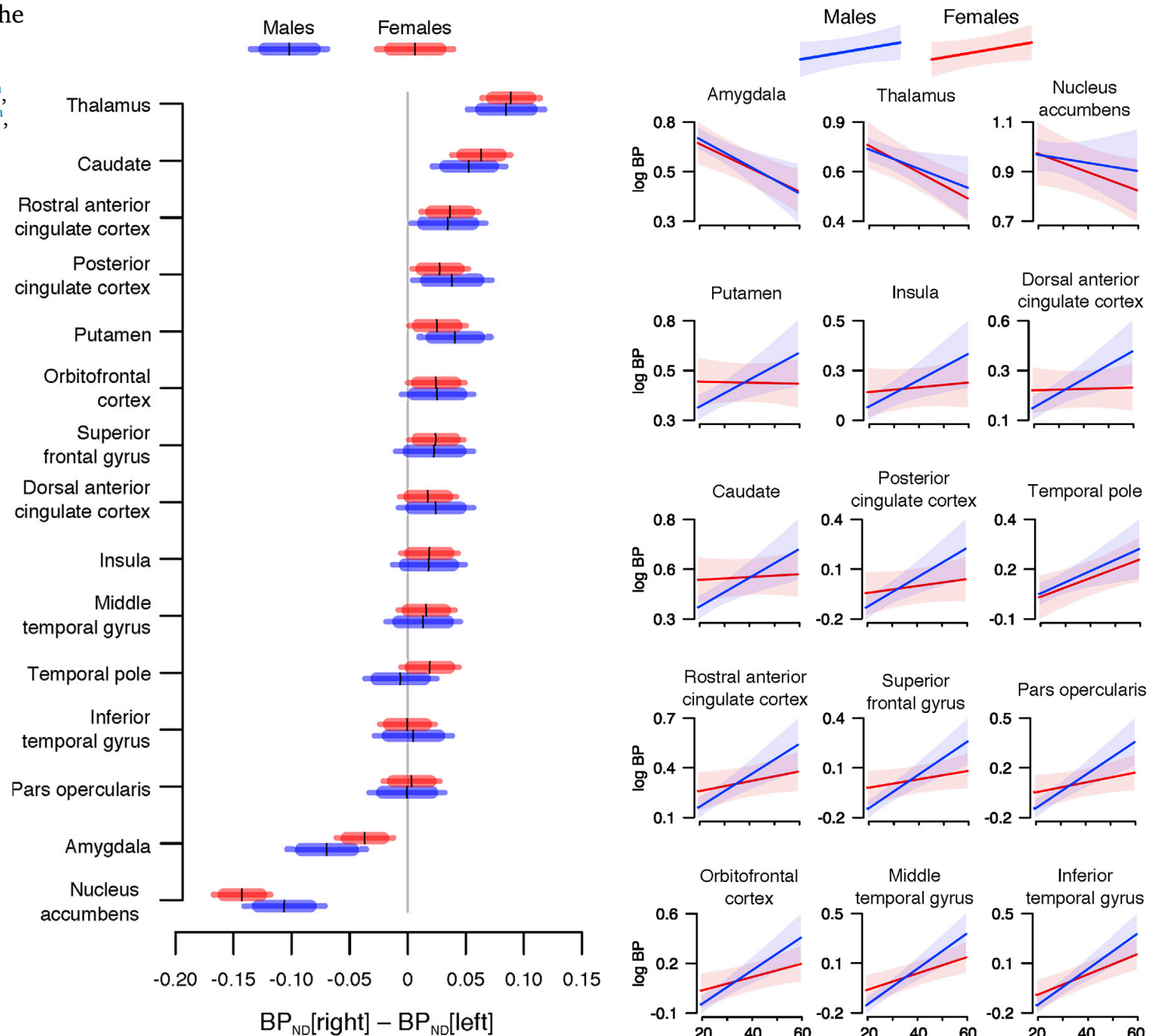
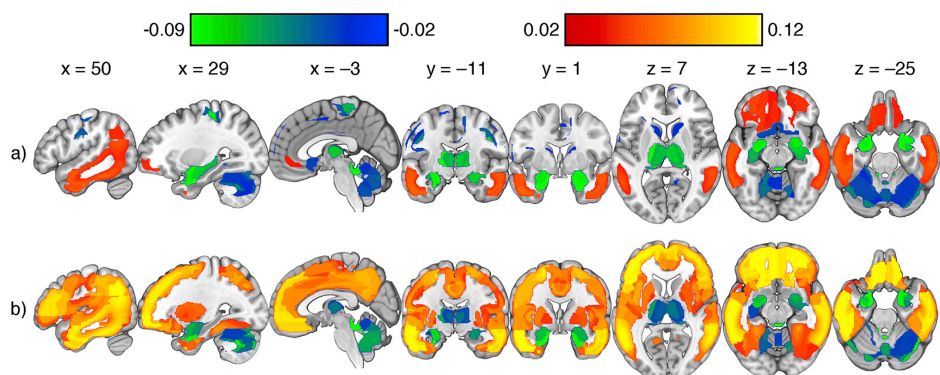
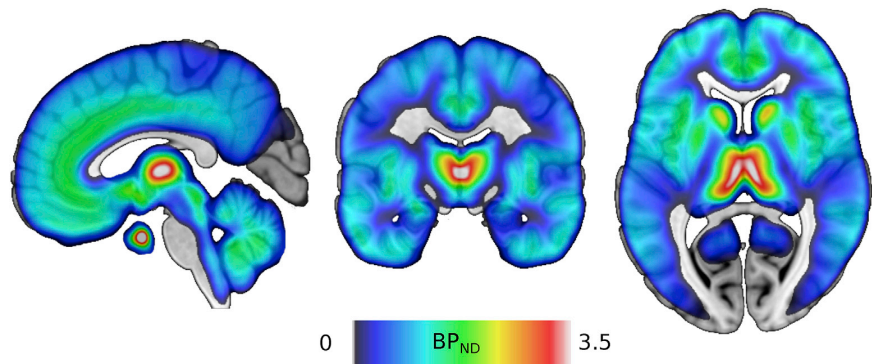
Neurotransmitter PET/SPECT studies in psychiatric disorders: 30 years of progress



Interindividual variability and lateralization of μ -opioid receptors in the human brain

Tatu Kantonen ^{a,b,*}, Tomi Karjalainen ^{a,c}, Janne Isojärvi ^a, Pirjo Nuutila ^{a,d}, Jouni Tuisku ^a, Juha Rinne ^{a,b}, Jarmo Hietala ^{a,e}, Valtteri Kaasinen ^{a,b}, Kari Kalliokoski ^a, Harry Scheinin ^a, Jussi Hirvonen ^a, Aki Vehtari ^f, Lauri Nummenmaa ^{a,g}

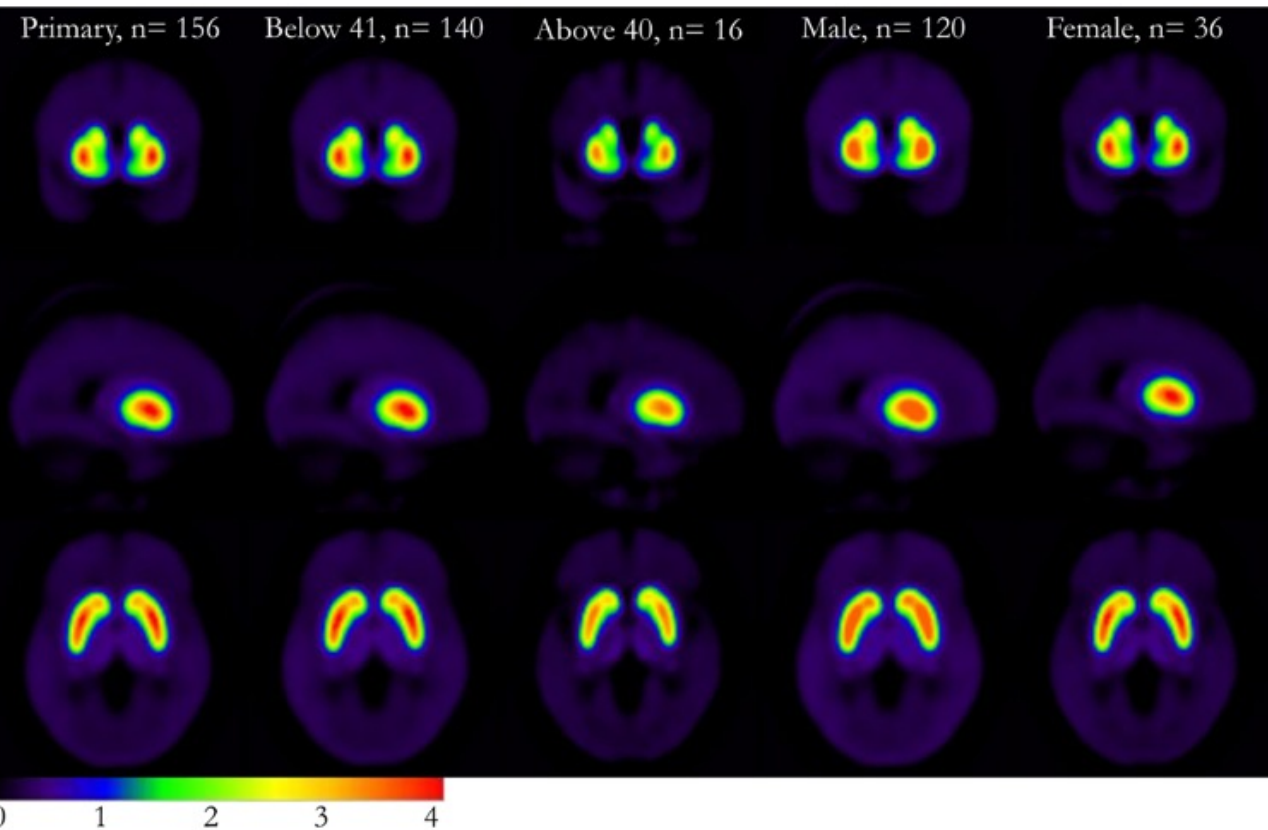
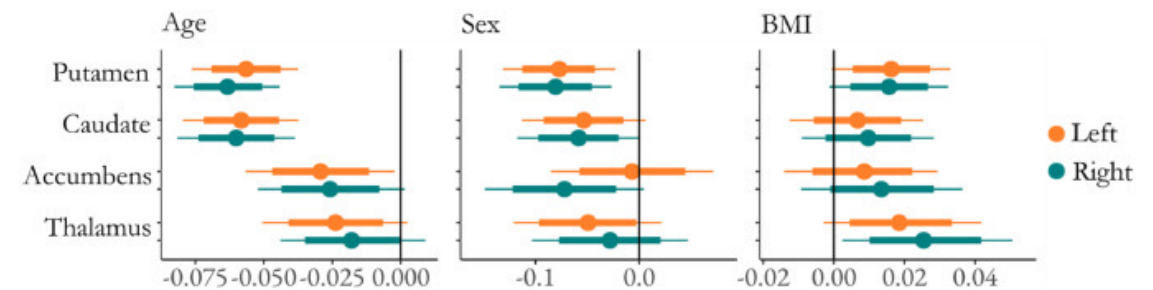
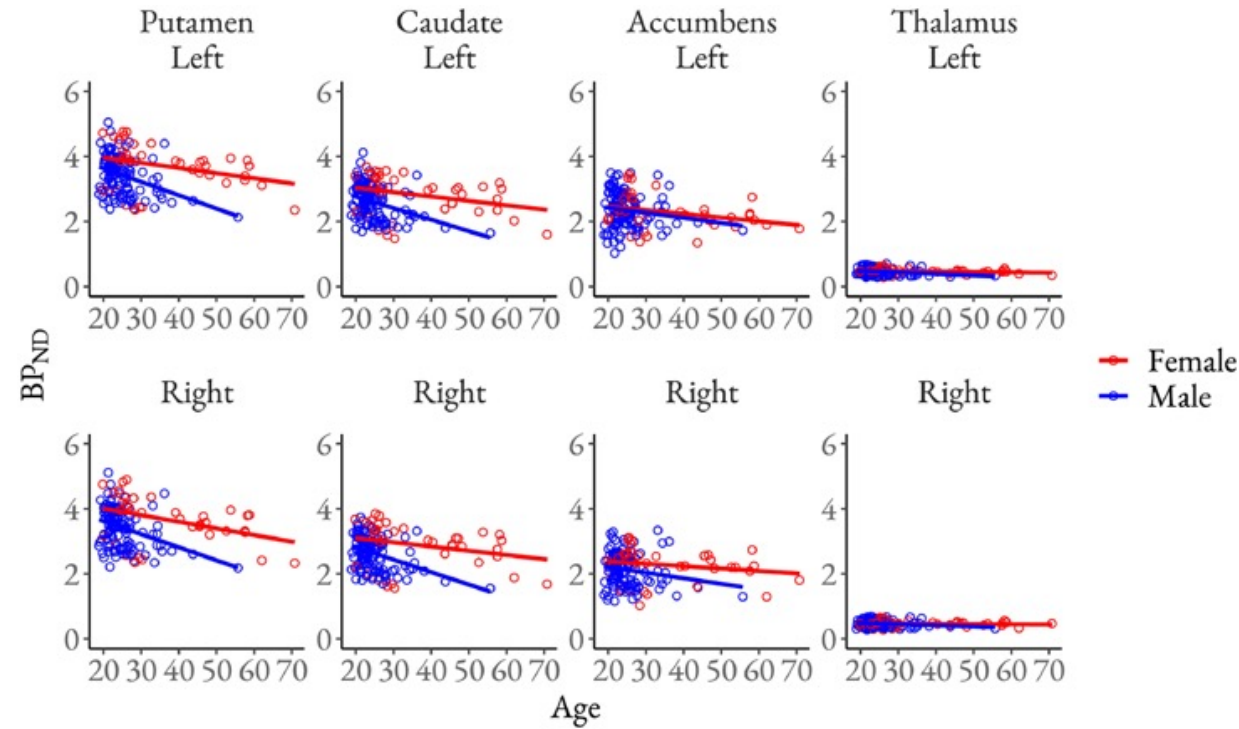
NeuroImage 217 (2020) 116922



Atlas of type 2 dopamine receptors in the human brain: Age and sex dependent variability in a large PET cohort

Tuulia Malén , Tomi Karjalainen , Janne Isojärvi , Aki Vehtari , Paul-Christian Bürkner , Vesa Putkinen , Valtteri Kaasinen , Jarmo Hietala , Pirjo Nuutila , Juha Rinne , Lauri Nummenmaa

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 DOI: <https://doi.org/10.1016/j.neuroimage.2022.119149>
 Reference: YNIMG 119149



Talk summary

- Neurotransmission – what is it?
- The Tool Kit: molecular and multimodal brain imaging
 - Magnetic Resonance Imaging (MRI)
 - Structural MRI
 - Functional MRI
 - Positron Emission Tomography (PET)
- What makes a good radioligand for brain?
- Typical study designs
 - Studies of pathophysiology
 - Group comparison: cross-sectional vs. longitudinal
 - Imaging genetics: vulnerability vs. individual genes
 - Studies of vulnerability traits in healthy subjects
 - Studies of neurotransmission dynamics
 - Large-scale studies



jussi.hirvonen@utu.fi



@jussihirvonen