

# Application of cross-species PET imaging to assess neurotransmitter release in brain

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## Abstract

**Rationale** This review attempts to summarize the current status in relation to the use of positron emission tomography (PET) imaging in the assessment of synaptic concentrations of endogenous mediators in the living brain.

**Objectives** Although PET radioligands are now available for more than 40 CNS targets, at the initiation of the Innovative Medicines Initiative (IMI) “Novel Methods leading to New Medications in Depression and Schizophrenia” (NEWMEDS) in 2009, PET radioligands sensitive to an endogenous neurotransmitter were only validated for dopamine. NEWMEDS work-package 5, “Cross-species and

neurochemical imaging (PET) methods for drug discovery”, commenced with a focus on developing methods enabling assessment of changes in extracellular concentrations of serotonin and noradrenaline in the brain.

**Results** Sharing the workload across institutions, we utilized in vitro techniques with cells and tissues, in vivo receptor binding and microdialysis techniques in rodents, and in vivo PET imaging in non-human primates and humans. Here, we discuss these efforts and review other recently published reports on the use of radioligands to assess changes in endogenous levels of dopamine, serotonin, noradrenaline,  $\gamma$ -aminobutyric acid, glutamate, acetylcholine, and opioid peptides. The emphasis is on assessment of the availability of appropriate translational tools (PET radioligands, pharmacological challenge agents) and on studies in non-human primates and human subjects, as well as current challenges and future directions.

**Conclusions** PET imaging directed at investigating changes in endogenous neurochemicals, including the work done in NEWMEDS, have highlighted an opportunity to further extend the capability and application of this technology in drug development.

**Keywords** PET imaging · Pharmacological challenge · Dopamine · Noradrenaline · Serotonin · GABA · Glutamate · Acetylcholine · Neurotransmitter · Non-human primate

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## Introduction

Brain imaging with positron emission tomography (PET) is now widely used in both academic and industry-driven research for a range of applications in neuroscience research and drug discovery. PET radioligands are available for many G-protein-coupled receptors (GPCRs) and neurotransmitter

transporters, as well as some enzymes and ion channels, and have been used to provide information about the concentration, distribution, and occupancy of specific drug targets in the central nervous system (CNS) (for reviews see Grimwood and Hartig 2009; Halldin et al. 2001; Jones et al. 2012; Lee and Farde 2006; Zimmer and Luxen 2012). Molecular imaging with PET provides high sensitivity when compared to other methods such as single photon emission computed tomography (SPECT), and with the recent development of a new generation of PET systems, also fairly high spatial resolution. The current model of the high-resolution research tomograph (ECAT HRRT; Siemens CTI, Knoxville, TN, USA) allows for spatial image resolution of 1.5 mm when employing point spread function reconstruction and allows for reliable imaging of small brain structures (Varrone et al. 2009). Moreover, the development of PET systems dedicated to the imaging of rodents has provided opportunities for both forward- and back-translation across species and increased confidence in the validity of animal models (Nagy et al. 2013).

PET imaging can be employed in the drug development process in several different ways. First, radiolabeling and administration of a microgram dose of the radiolabeled drug can confirm brain exposure using PET (Bergstrom et al. 2003). Second, target engagement can be confirmed in a receptor occupancy study in which radioligand and drug interact at the same target. Target engagement has now been demonstrated for many different drug mechanisms and has provided significant progress towards establishing a better understanding of relationships between drug exposure levels and drug target occupancy, informing dose selection for studies aiming to demonstrate therapeutic efficacy in patients (for review see Grimwood and Hartig 2009). One potential confounding factor of target occupancy determinations is that endogenous ligands may compete directly with radioligand binding. However, this potential interaction raises the opportunity for a third PET application, to investigate drug-induced changes in neurotransmitter release, providing insight on receptor function beyond occupancy as well as modes of drug action.

Although PET radioligands are now available for more than 40 CNS targets, at the initiation of the Innovative Medicines Initiative (IMI) “Novel Methods leading to New Medications in Depression and Schizophrenia” (NEWMEDS) in 2009, PET radioligands sensitive to an endogenous neurotransmitter were limited to dopamine. Spurred by the success of developing novel radioligands optimized towards assessment of neurotransmitter release, such as the dopamine D<sub>2</sub> receptor agonists [<sup>11</sup>C]NPA (Narendran et al. 2004) and [<sup>11</sup>C]MNPA (Seneca et al. 2006), NEWMEDS work-package 5, “Cross-species and neurochemical imaging (PET) methods for drug discovery”, commenced with a focus on developing methods enabling assessment of changes in extracellular concentrations of serotonin and noradrenaline

in the brain. Here, we discuss these efforts and also review other recently published reports on the use of radioligands to assess changes in endogenous levels of dopamine, serotonin, noradrenaline,  $\gamma$ -aminobutyric acid (GABA), glutamate, acetylcholine (ACh), and opioid peptides, also including detailed summary tables of the PET studies referred to (Tables 1, 2, 3, and 4). Focus has been placed on assessing the availability of appropriate tools (PET radioligand, pharmacological challenge agent) and studies in non-human primates and human subjects, as well as current challenges and future directions.

## Current state of the art: imaging neurotransmitter changes using PET

### Dopamine

The dopamine system has historically been one of the most extensively studied neurotransmitter systems of the brain. The results of these investigations have been fruitful in terms of basic science and therapeutic applications. Studies on the dopamine system have over time seen considerable advancements of new technologies and methodological developments. Dopamine was also the first neurotransmitter for which changes in extracellular concentrations could be evaluated in the living human brain using PET and SPECT (Farde et al. 1992; Laruelle et al. 1995; Volkow et al. 1994).

The effects of dopamine are mediated through five receptor subtypes, divided into two families, the D<sub>1</sub>-like receptors (D<sub>1</sub> and D<sub>5</sub>) and the D<sub>2</sub>-like receptors (D<sub>2</sub>, D<sub>3</sub>, and D<sub>4</sub>) (for review see Vallone et al. 2000). Several dopamine D<sub>2</sub>-like receptor radioligands have now been tested for sensitivity to endogenous dopamine in experiments conducted using <sup>3</sup>H-labeled radioligands for in vitro or in vivo binding measurements in rodents, and <sup>11</sup>C- or <sup>18</sup>F-labeled radioligands for in vivo PET measurements in animals or humans. In these studies, extracellular dopamine concentrations in the brain have typically been elevated using amphetamine or methylphenidate, or reduced using reserpine and/or alpha-methyl-para-tyrosine (AMPT), respectively.

Initially, the butyrophenone derivatives [<sup>3</sup>H]spiperone (Leyens et al. 1978) and [<sup>3</sup>H]- and [<sup>11</sup>C]N-methyl-spiperone ([<sup>3</sup>H]/[<sup>11</sup>C]NMSP) (Lyon et al. 1986; Wagner et al. 1983) were found not to display changes in binding consistent with competition by endogenous dopamine, possibly because these radioligands also bind to intracellular or internalized receptors (Chugani et al. 1988; for review see Laruelle 2000). The substituted benzamide derivatives [<sup>11</sup>C]raclopride (Ehrin et al. 1985) and [<sup>123</sup>I]IBZM (Kung et al. 1988) have, however, in a large number of animal and human studies, consistently been shown to display changes in binding which are consistent with dopamine competition (for review see Laruelle 2000). In

**Table 1** PET studies examining the susceptibility of extrastriatal dopamine D<sub>2</sub> receptor radioligands for manipulation by dopamine<sup>a</sup>

Receptor	Radioligand	Challenge	Species	Protocol	Outcome parameter	Effect on outcome parameter	Reference	
D <sub>2</sub> R	[ <sup>11</sup> C]FLB 457	Amphetamine (2 mg/kg i.v., 15 min and 3 h prior) vs. baseline	Monkey (n=3)	B	SBR	↓6–16 % and ↓2–16 % in neoCx and thalamus <sup>b</sup>	(Chou et al. 2000)	
	[ <sup>11</sup> C]FLB 457	Methamphetamine (1 mg/kg i.v., 15 min prior) vs. baseline	Monkey (n=3)	B	BP <sub>ND</sub>	↓3–7 % in frontal Cx, thalamus, and temporal Cx <sup>b</sup>	(Okauchi et al. 2001)	
	[ <sup>11</sup> C]FLB 457	Nicotine (32 µg/kg+0.8 µg/kg/min and 100 µg/kg+2.53 µg/kg/min i.v., 30 min prior) and saline	Monkey (n=5 and 5 and 5)	B	V <sub>T</sub>	NS	(Tsukada et al. 2005a)	
	[ <sup>11</sup> C]FLB 457	MK801 (0.03, 0.1, and 0.3 mg/kg i.v., 30 min prior) vs. saline	Monkey (n=8)	B	V <sub>T</sub>	NS, ↓~15 % and ↓35 % in prefrontal Cx	(Tsukada et al. 2005b)	
	[ <sup>11</sup> C]FLB 457	Ketamine (200 ng/ml target in plasma i.v., 15 min prior to 89 min post) and control	Human (n=8 and 8)	B	BP <sub>ND</sub>	↓11 % in posterior cingulate Cx	(Aalto et al. 2005)	
	[ <sup>11</sup> C]FLB 457	Methylphenidate (40 and 60 mg p.o., 1 h prior) vs. placebo	Human (n=5 and 5)	B	V <sub>T</sub>	↓6–7 % in frontal Cx, temporal Cx, and thalamus	(Montgomery et al. 2007)	
	[ <sup>11</sup> C]FLB 457	D-Amphetamine (0.3 mg/kg i.v., 5 min prior) and placebo	Human (n=12 and 11)	B	BP <sub>ND</sub> V <sub>T</sub> BP <sub>ND</sub>	NS NS ↓13 % in hippocampus and posterior cingulate	(Aalto et al. 2009)	
	[ <sup>11</sup> C]FLB 457	Amphetamine (0.5 mg/kg p.o., 3 h prior) vs. baseline	Human (n=12)	B	BP <sub>P</sub> BP <sub>ND</sub> BP <sub>P</sub>	↓12 % in parietal Cx ↓7–13 % in various regions NS	(Narendran et al. 2009)	
	[ <sup>11</sup> C]Fallypride							
	[ <sup>11</sup> C]FLB 457	α-Methyl-para-tyrosine (103–129 mg/kg p.o., over 2 days prior) vs. baseline	Human (n=6)	B	BP <sub>P</sub> BP <sub>ND</sub>	NS ↓9 % in temporal Cx	(Frankle et al. 2010)	
	[ <sup>11</sup> C]FLB 457	D-Amphetamine (0.3, 0.5, and 1.0 mg/kg i.v., 30 min prior) vs. baseline	Monkey (n=7, 9, and 4)	B	BP <sub>ND</sub>	↓6 %, ↓16 %, and ↓24 % in frontal Cx	(Narendran et al. 2014)	
	[ <sup>11</sup> C]FLB 457	Amphetamine (0.4–0.5 mg/kg p.o., 3 h prior) vs. baseline	Human (n=6)	B	BP <sub>ND</sub> (ZTC) BP <sub>ND</sub> (SRTM)	NS ↓8–13 % in various regions	(Sandiego et al. 2015)	
	[ <sup>18</sup> F]Fallypride	D-Amphetamine (1.0 mg/kg i.v., 5 min prior) vs. baseline	Monkey (n=3)	B	BP <sub>ND</sub>	↓25–36 % in various regions	(Slifstein et al. 2004)	
	[ <sup>18</sup> F]Fallypride	D-Amphetamine (0.3 and 0.5 mg/kg i.v., 260 min post) vs. baseline	Monkey (n=2)	BI		↓1–14 % and ↓10–34 % in various regions	(Mukherjee et al. 2005)	
	[ <sup>18</sup> F]Fallypride	Amphetamine (0.60 mg/kg i.v., 15–30 min prior) vs. baseline	Monkey (n=2)	B	BP <sub>ND</sub>	↓16–39 % in various regions <sup>b</sup>		
	Amphetamine (1.13 mg/kg i.v., 15 min prior) vs. baseline	Monkey (n=1)		BP <sub>ND</sub>	↓14–39 % in various regions <sup>b</sup>			
	Amphetamine (0.70 mg/kg i.v., 45 min post) vs. baseline	Monkey (n=1)		SBR	↓12–38 % in various regions <sup>b</sup>			
[ <sup>18</sup> F]Fallypride	D-Amphetamine (0.43 mg/kg p.o., 3 h prior) vs. baseline	Human (n=14)	B	BP <sub>ND</sub>	↓3–7 % in various regions	(Riccardi et al. 2006)		
[ <sup>18</sup> F]Fallypride	D-Amphetamine (0.5 mg/kg p.o., 3 h prior) vs. baseline	Human (n=14)	B	BP <sub>ND</sub>	↓8–13 % in various regions	(Cropley et al. 2008)		

Table 1 (continued)

Receptor	Radioligand	Challenge	Species	Protocol	Outcome parameter	Effect on outcome parameter	Reference
		$\alpha$ -Methyl- <i>para</i> -tyrosine (3 g/70 kg/day over 44 h) vs. baseline	Human (n=8)			NS	
	[ <sup>18</sup> F]Fallypride	$\alpha$ -Methyl- <i>para</i> -tyrosine (71.4 mg/kg p.o., over 26 h prior) vs. baseline	Human (n=6)	B	$BP_{ND}$	↑13 % in substantia nigra	(Riccardi et al. 2008)
	[ <sup>18</sup> F]Fallypride	D-Amphetamine (0.3 mg/kg i.v., 30 min prior) vs. baseline	Human (n=15)	B	$BP_P$ $BP_{ND}$ (SRTM)	↓11–70 % in various regions ↓4–13 % in various regions	(Slifstein et al. 2010)
	[ <sup>18</sup> F]Fallypride	S-Ketamine (0.1 mg/kg bolus+0.01 mg/kg/min i.v., 35 min prior to 30 min post) vs. placebo	Human (n=10)	B	$BP_{ND}$	NS	(Vernaleken et al. 2013)

B bolus,  $BP_{ND}$  binding potential non-displaceable,  $BP_P$  binding potential plasma, Cx cortex, NS no statistically significant effect,  $SBR$  specific binding ratio,  $V_T$  distribution volume

<sup>a</sup> This table solely includes PET studies examining dopamine changes in extra-striatal regions. The authors refer the reader to a previous review article (Laruelle 2000) for a summary on studies examining striatal dopamine release

<sup>b</sup> No statistical analysis reported

general, clinically applicable doses of amphetamine have reduced [<sup>11</sup>C]raclopride binding in the human striatum by up to 20 %, which is a sufficient effect size to be robustly measured using PET and SPECT (Breier et al. 1997; Dewey et al. 1993; for review see Laruelle 2000).

Two early observations greatly stimulated the use of the developed methodology for further research applications. First, an enhanced amphetamine effect on [<sup>11</sup>C]raclopride binding was observed in patients with schizophrenia (Breier et al. 1997; Laruelle et al. 1996). Second, videogame playing, employed as a rewarding stimulus, was shown to cause decreases in [<sup>11</sup>C]raclopride binding (Egerton et al. 2009; Koeppe et al. 1998). Several studies have now shown that dopamine release induced by pharmacological and cognitive/behavioral interventions can be investigated using dopamine D<sub>2</sub>-like receptor radioligands (for reviews see Egerton et al. 2009; Laruelle 2000), and the methodology has provided increased understanding of the role of synaptic dopamine in drug actions (Brody et al. 2004), normal neuropsychology (for review see Egerton et al. 2009), and the pathophysiology of addiction (Volkow et al. 1997), Parkinson's disease (Piccini et al. 2003), and schizophrenia (Laruelle et al. 1996).

More recently, antagonist radioligands with much higher dopamine D<sub>2</sub> receptor affinity compared to raclopride, such as [<sup>11</sup>C]FLB 457 and [<sup>11</sup>C]/[<sup>18</sup>F]fallypride, have been used to investigate extrastriatal brain regions, where the density of dopamine D<sub>2</sub> receptors is much lower than in the striatum (Hallidin et al. 1995; Mukherjee et al. 2004; Mukherjee et al. 1995). PET studies evaluating the dopamine sensitivity of these radioligands are summarized in Table 1. Explorative studies in monkeys initially indicated that relatively high intravenous doses of amphetamine reduced cortical radioligand binding to a lesser extent than striatal [<sup>11</sup>C]raclopride binding (Chou et al. 2000; Okauchi et al. 2001) (Table 1). However, follow-up investigations employing amphetamine challenge have confirmed that the high-affinity (<1 nM) antagonist radioligands may allow for monitoring of changes in cortical synaptic dopamine concentrations in monkeys and human subjects (Aalto et al. 2009; Cropley et al. 2008; Mukherjee et al. 2005; Narendran et al. 2009; Narendran et al. 2013; Narendran et al. 2014; Riccardi et al. 2006; Sandiego et al. 2015; Slifstein et al. 2010; Slifstein et al. 2004). Moreover, modulators of endogenous dopamine, including methylphenidate, ketamine, and MK801, have been shown to reduce radioligand binding (Aalto et al. 2005; Montgomery et al. 2007; Tsukada et al. 2005b). However, AMPT (Cropley et al. 2008; Frankle et al. 2010; Riccardi et al. 2008) and ketamine (Vernaleken et al. 2013) administration in humans and nicotine administration in monkeys (Tsukada et al. 2005a) have not been found to affect extra-striatal radioligand binding (Table 1). This development of methodology has provided new opportunities for assessment of extra-striatal dopamine pathways and may facilitate the understanding of the treatment

**Table 2** PET studies examining the susceptibility of 5-HT receptor radioligands for manipulation by serotonin<sup>a</sup>

Receptor	Radioligand	Challenge	Species (n=number of subjects or measurements)	Method	Outcome parameter	Effect on outcome parameter	Reference
5-HT <sub>1A</sub>	[ <sup>18</sup> F]MPPF	Citalopram (0.5 mg/kg i.p., 1 h prior) and saline	Rat (n=9 and 8)	B	BP <sub>ND</sub>	NS	(Moulin-Sallanon et al. 2009)
	[ <sup>11</sup> C]CUMI-101	Citalopram (2 and 4 mg/kg i.v., 30 min prior) vs. baseline	Monkey (n=3 and 3)	B	OC plot - V <sub>T</sub>	↓15 % and ↓30 % across regions	(Milak et al. 2011)
5-HT <sub>2A</sub>	[ <sup>18</sup> F]Alkanserlin	Fenfluramine (2.5 mg/kg i.v., 30 min prior) vs. baseline	Monkey (n=3)	B	OC plot - V <sub>T</sub>	↓24 % across regions	(Selvaraj et al. 2012)
	[ <sup>11</sup> C]CUMI-101	Citalopram (10 mg i.v., 45 min prior) vs. placebo	Human (n=13)	B	OC plot - V <sub>T</sub>	↑14 % across regions	(Selvaraj et al. 2012)
5-HT <sub>2B</sub>	[ <sup>11</sup> C]CUMI-101	Citalopram (0.15 mg/kg i.v., 30 min prior to 30 min post) vs. baseline	Human (n=6)	B	BP <sub>ND</sub>	↑5–8 % in various regions	(Pimborg et al. 2012)
	[ <sup>18</sup> F]Alkanserlin	Dexfenfluramine (40 and 60 mg p.o., 2 h prior) vs. placebo	Human (n=6 and 7)	BI	V <sub>T</sub> BP <sub>ND</sub>	NS NS	(Pimborg et al. 2012)
5-HT <sub>4</sub>	[ <sup>11</sup> C]Cimbi-36	Fenfluramine (5 mg/kg i.v., 30 min prior)	Monkey (n=2)	B	BP <sub>p</sub>	↓~17 % in various regions	(Finnema et al. 2012b)
	[ <sup>11</sup> C]SB207145	Fluoxetine (40 mg p.o., daily for 21–23 days prior) and placebo vs. baseline	Human (n=16 and 16)	B	BP <sub>ND</sub>	↓~60 % in various regions <sup>b</sup>	(Haahr et al. 2014)
5-HT <sub>1B</sub>	[ <sup>11</sup> C]AZ10419369	Fenfluramine (1.0 and 5.0 mg/kg i.v., 15 min post) vs. baseline	Monkey (n=3)	B	OC plot - BP <sub>ND</sub>	↓5 % across regions	(Finnema et al. 2010b)
	[ <sup>11</sup> C]AZ10419369	Fenfluramine (1.0 and 5.0 mg/kg i.v., 80 min post) vs. baseline	Monkey (n=3)	BI	SBR	↓27 and ↓50 % in various regions <sup>b</sup>	(Finnema et al. 2012c)
5-HT <sub>1A</sub>	[ <sup>11</sup> C]AZ10419369	Fenfluramine (5.0 mg/kg i.v., 30 min prior) vs. baseline	Monkey (n=7)	BI	BP <sub>ND</sub>	↓12 and ↓33 % in various regions <sup>b</sup>	(Nord et al. 2013)
	[ <sup>11</sup> C]AZ10419369	Escitalopram (2.0 mg/kg i.v., 45 min prior) vs. baseline	Human (n=9)	B	BP <sub>ND</sub>	↓25 % in RN and ↓11–13 % in various regions	(Nord et al. 2013)
5-HT <sub>1A</sub>	[ <sup>11</sup> C]AZ10419369	Escitalopram (20 mg p.o., 3 h prior) vs. baseline	Human (n=9)	B	BP <sub>ND</sub>	↑5 % in various Cx	(Yamanaka et al. 2014)
	[ <sup>11</sup> C]P943	Fenfluramine (5 mg/kg i.v., 15 min prior) vs. baseline	Monkey (n=4), awake	B	BP <sub>ND</sub>	↓22–41 % in various regions	(Yamanaka et al. 2014)
5-HT <sub>1A</sub>	[ <sup>11</sup> C]P943	S-(+)-fenfluramine (0.8 and 2.5 mg/kg i.v., prior) vs. baseline	Monkey (n=4), ketamine	B	OC plot - V <sub>T</sub>	↓20–38 % in various regions	(Ridler et al. 2011)
	[ <sup>11</sup> C]P943	Citalopram (4 mg/kg i.v., prior) vs. baseline	Monkey (n=2 and 2)	B	OC plot - V <sub>T</sub>	↓13 and 40 % across regions <sup>b</sup>	(Ridler et al. 2011)
5-HT <sub>1A</sub>	[ <sup>11</sup> C]P943	Amphetamine (1 mg/kg i.v., prior) vs. baseline	Monkey (n=1)	B	OC plot - V <sub>T</sub>	↓17 % across regions <sup>b</sup>	(Cosgrove et al. 2011)
	[ <sup>11</sup> C]P943	Fenfluramine (1 mg/kg i.v., 5 min prior) vs. baseline	Monkey (n=2)	BI	OC plot - V <sub>T</sub>	↓25 % across regions <sup>b</sup>	(Cosgrove et al. 2011)
5-HT <sub>1A</sub>	[ <sup>11</sup> C]P943	Fenfluramine (5 mg/kg i.v., 75 min post) vs. baseline	Monkey (n=1)	BI	OC plot - V <sub>T</sub>	↓25–29 % across regions <sup>b</sup>	(Cosgrove et al. 2011)
	[ <sup>11</sup> C]P943	Fenfluramine (5 mg/kg i.v., 75 min post) vs. baseline	Monkey (n=1)	BI	OC plot - V <sub>T</sub>	↓42 % across regions <sup>b</sup>	(Cosgrove et al. 2011)

B bolus, BI bolus+infusion, BP<sub>ND</sub> binding potential non-displaceable, BP<sub>p</sub> binding potential plasma, Cx cortex, NS no statistical significant effect, OC plot occupancy plot, RN raphe nucleus, SBR specific binding ratio, V<sub>T</sub> distribution volume

<sup>a</sup> This table is an extension to Table 2 in Paterson et al. (2010)

<sup>b</sup> No statistical analyses reported

**Table 3** PET studies examining the susceptibility of noradrenaline and GABA receptor radioligands for manipulation by their endogenous ligands

Neurotransmitter	Receptor	Radioligand	Challenge	Species ( <i>n</i> =number of subjects or measurements)	Method	Outcome parameter	Effect on outcome parameter	Reference
Noradrenaline	$\alpha_2$ -AR	[ <sup>11</sup> C]Yohimbine	D-Amphetamine (10 mg/kg i.v., 20 min prior) vs. baseline	Pig ( <i>n</i> =4)	B	$V_T$	↓14–27 % in various regions <sup>a</sup>	(Landau et al. 2012)
		[ <sup>11</sup> C]Yohimbine	Amphetamine (2 mg/kg i.v., 5–10 min prior) vs. baseline	Rat ( <i>n</i> =5)	B	$BP_{ND}$	↓35–39 % in various regions <sup>a</sup>	(Phan et al. 2015)
	$\alpha_{2C}$ -AR	[ <sup>11</sup> C]ORM-13070	D-Amphetamine (0.5 and 1.0 mg/kg i.v., 25 min prior) vs. baseline	Monkey ( <i>n</i> =2 and 2)	B	$BP_{ND}$	↓35 and ↓41 % in striatum <sup>a</sup>	(Fimmema et al. 2014b)
		[ <sup>11</sup> C]ORM-13070	Atomoxetine (0.3 mg/kg i.v., 1 h prior to 63 min post) vs. baseline	Monkey ( <i>n</i> =2)	B		↓5 % in striatum <sup>a</sup>	
	$\alpha_{2C}$ -AR	[ <sup>11</sup> C]ORM-13070	Atomoxetine (1.2 mg/kg p.o., 1 h prior) vs. placebo	Human ( <i>n</i> =4)	B	B/F	↓12 % in CN	(Lehto et al. 2015a)
GABA		[ <sup>11</sup> C]ORM-13070	Ketamine (200 ng/ml target in plasma i.v., 15 min prior to 30 min post) vs. baseline	Human ( <i>n</i> =4)			↓16 % in CN, ↓13 % in putamen	
		[ <sup>11</sup> C]ORM-13070	Insulin-induced hypoglycemia (2.5–3.0 mmol/l glucose target in plasma i.v., 45 min prior to 30 min post) vs. placebo	Human ( <i>n</i> =4)			NS	
	$\alpha_{2C}$ -AR	[ <sup>11</sup> C]ORM-13070	Modified cold pressor test vs. baseline	Human ( <i>n</i> =4)			↓12 % in putamen	
		[ <sup>11</sup> C]ORM-13070	Atomoxetine (1.2 mg/kg p.o., 1 h prior) <sup>†</sup> -modified cold pressor test vs. control condition	Human ( <i>n</i> =8)	B	B/F	↓18 % in CN, ↓12 % in putamen	(Lehto et al. 2015c)
		[ <sup>11</sup> C]ORM-13070	Ketamine (300 ng/ml target in plasma i.v., 15 min prior to 30 min post) vs. baseline	Human ( <i>n</i> =8)			↓14 % in CN, ↓10 % in putamen	
	GABA <sub>A</sub> R	[ <sup>11</sup> C]Flumazenil	Tiagabine (16 mg p.o., 30 min prior) vs. baseline	Human ( <i>n</i> =8)	B	$V_T$	↑ in various regions	(Frankle et al. 2009)
		[ <sup>11</sup> C]Flumazenil	Tiagabine (0.15 and 0.25 mg/kg p.o., 1 h prior) vs. baseline	Human ( <i>n</i> =9 and 9)	B	$BP_{ND}$ (SRTM)	↑13–18 % in various regions	
		[ <sup>11</sup> C]Flumazenil				$V_T$	NS and ↑9–11 % in various regions	(Frankle et al. 2012)
		[ <sup>11</sup> C]Flumazenil				$BP_p$	NS and ↑10–12 % in various regions	
		[ <sup>11</sup> C]Flumazenil [ <sup>11</sup> C]Ro15-4513	Tiagabine (1.0 mg/kg i.v., 30 min prior) vs. baseline	Monkey ( <i>n</i> =2) Monkey ( <i>n</i> =4)	B	$BP_{ND}$ $BP_{ND}$	↑5 % in various regions <sup>a</sup> ↓9 % in various regions <sup>a</sup>	(Fimmema et al. 2012a)
	[ <sup>11</sup> C]Ro15-4513	Tiagabine (0.18 mg/kg p.o., 1.5 h prior) vs. placebo	Human ( <i>n</i> =12)	B	$V_S$ - $\alpha_1$	↓29–61 % in various regions	(Stokes et al. 2014)	
					$V_S$ - $\alpha_5$	↑23 % in anterior cingulate		

$B$  bolus,  $B/F$  bound/free,  $BP_{ND}$  binding potential non-displaceable,  $BP_p$  binding potential plasma,  $CN$  caudate nucleus,  $DVR$  distribution volume ratio,  $NS$  no statistical significant effect,  $V_T$  distribution volume,  $V_S$  specific volume of distribution

<sup>a</sup>No statistical analyses reported

and pathophysiology of psychiatric diseases, including schizophrenia (Slifstein et al. 2015; Woodward et al. 2011) and smoking (Wing et al. 2014).

The published reports on dopamine also include important validation aspects with regard to PET measurement of neurotransmitter release in general. First, release-dependent changes in dopamine D<sub>2</sub>-like receptor radioligand binding were only observed when endogenous dopamine was available for release. Amphetamine-induced changes in radioligand binding were attenuated by pre-administration of inhibitors of dopamine transport (GBR12909), synthesis (AMPT), or storage (reserpine) (Innis et al. 1992; Laruelle et al. 1997; Villemagne et al. 1999). Second, the reductions in radioligand binding were shown to be proportional to drug-induced increases in dopamine concentrations measured in the extracellular fluid with microdialysis (Breier et al. 1997; Endres et al. 1997; Laruelle et al. 1997; Narendran et al. 2014; Tsukada et al. 1999a). Third, changes in radioligand binding were found to mainly reflect modulation of dopamine in the synaptic cleft, instead of inherently reflecting changes in the extracellular fluid dopamine concentration across drugs with different modes of action. Relative reductions in [<sup>11</sup>C]raclopride binding in the striatum of monkeys have been shown to be similar after administration of direct dopamine enhancers (GBR12909 and methamphetamine) or indirect dopamine modulators (benztropine and ketanserin), while microdialysis-measured increases in dopamine concentrations were much higher for the direct dopamine enhancers (500–1200 %) in comparison to the indirect dopamine modulators (140–160 %) (Tsukada et al. 1999a). Schiffer and colleagues demonstrated similar results when comparing methylphenidate and amphetamine in a combined microdialysis and PET study in rodents (Schiffer et al. 2006). Differences in endogenous ligand assessment might be expected between microdialysis and PET, since microdialysis provides a direct method of measurement, giving absolute values, and PET is an indirect method for displaying changes. In addition, changes in neurotransmitter levels measured with microdialysis might appear to be relatively larger than when measured using PET since some protocols include uptake blockers in the microdialysis probes. Nevertheless, together these studies provide support for the notion that this approach might be applied to other neurotransmitters.

In an attempt to increase the sensitivity of radioligand binding to endogenous dopamine, relative to that of antagonist radioligands such as [<sup>11</sup>C]raclopride, agonist radioligands were developed for dopamine D<sub>2</sub>-like receptors, such as [<sup>11</sup>C]NPA (Hwang et al. 2000), [<sup>11</sup>C]MNPA (Finnema et al. 2005), [<sup>11</sup>C]PHNO (Wilson et al. 2005), and [<sup>18</sup>F]MCL-524 (Finnema et al. 2014e; for review see Finnema et al. 2010a). Agonist radioligands preferentially bind to the high-affinity, G-protein-coupled state of receptors (Sibley et al. 1982). Agonist radioligand binding is thereby proposed to be more

susceptible to the endogenous agonist than the binding of antagonist radioligands, which do not discriminate between the low- and high-affinity states of the receptor. Indeed, these agonist radioligands were shown to be more sensitive to drug-induced changes in extracellular dopamine concentrations than [<sup>11</sup>C]raclopride, both in anesthetized animals (Gallezot et al. 2014b; Ginovart et al. 2006; Narendran et al. 2004; Seneca et al. 2006; Skinbjerg et al. 2010) and in awake human subjects (Caravaggio et al. 2014; Narendran et al. 2010; Shotbolt et al. 2012). However, there is a growing body of evidence which challenges the existence of two distinct affinity states in vivo (for reviews see Finnema et al. 2010a; Skinbjerg et al. 2012), and the exact mechanism of the increased sensitivity of agonist radioligands to dopamine therefore warrants further evaluation.

In contrast to the dopamine D<sub>2</sub>-like receptor radioligands, dopamine D<sub>1</sub>-like receptor antagonist radioligands such as [<sup>11</sup>C]SCH-23390 (DeJesus et al. 1987; Halldin et al. 1986) and [<sup>11</sup>C]NNC-112 (Halldin et al. 1998) have not appeared to be sensitive to acute changes in extracellular dopamine concentrations (Abi-Dargham et al. 1999; Chou et al. 1999; Tsukada et al. 2001). A number of hypotheses have been proposed to explain this difference, including that (1) dopamine D<sub>1</sub>-like receptors are predominantly located extrasynaptically (Hersch et al. 1995; Levey et al. 1993), (2) dopamine has significantly lower affinity to D<sub>1</sub> receptors than D<sub>2</sub> receptors (Marcellino et al. 2012), and (3) dopamine D<sub>1</sub> receptors exist predominantly in the low-affinity state (Richfield et al. 1989). Recently developed partial dopamine D<sub>1</sub> receptor agonist radioligands, such as (*R*)-(+)-[<sup>11</sup>C]SKF 82957 and (*S*)-[<sup>11</sup>C]*N*-methyl-NNC 01-0259, were also found not to be sensitive to amphetamine-induced changes in dopamine release (Finnema et al. 2013a; Palner et al. 2010).

The described studies on PET-measured dopamine release illustrate the potential of the methodology but also provide caution for the interpretation of changes in radioligand binding. The PET outcome measuring binding potential (*BP*) represents the ratio of  $B_{\max}/K_D$ , in which  $B_{\max}$  is the receptor density and  $K_D$  the equilibrium dissociation rate constant. Changes in *BP* are typically interpreted according to a competition model, e.g., reflecting a change in  $K_D$ , but several observations suggest a contribution of dopamine receptor internalization as well (see for reviews Ginovart 2005; Laruelle 2000). First, a temporal discrepancy has been observed between the amphetamine-induced dopamine pulse (~2 h) and the prolonged effect on radioligand binding (~24 h) (Cardenas et al. 2004; Ginovart et al. 2006; Houston et al. 2004; Laruelle et al. 1997; Narendran et al. 2007). Second, a change in cellular environment consequent to receptor internalization affects radioligand binding properties differently for radioligands originating from diverse chemical classes (e.g., butyrophenones vs. benzamides) (Guo et al. 2010; Sun et al. 2003). Third, amphetamine has been shown to decrease

[<sup>11</sup>C]raclopride  $B_{\max}$  values in vivo in cat and ex vivo in rat (Ginovart et al. 2004; Sun et al. 2003). These observations together suggest a contribution of receptor internalization to the observed changes in radioligand binding, possibly affecting both  $B_{\max}$  and  $K_D$ . Skinbjerg et al. (2010) indeed confirmed that the long-lasting effect of amphetamine on radioligand binding was caused by internalization of the D<sub>2</sub> receptors. Acute amphetamine administration decreased radioligand binding to the same extent in wild-type and arrestin 3 knockout mice. However, 4 h post-amphetamine administration, radioligand binding was still reduced in wild-type mice but recovered to baseline in arrestin 3 knockout mice, which lack the capacity to internalize D<sub>2</sub> receptors (Skinbjerg et al. 2010). This study supports that amphetamine-induced changes in radioligand binding mainly reflect changes in extracellular dopamine concentration initially, but after a prolonged time-interval also reflect internalization.

## Serotonin

Fourteen mammalian serotonin (5-HT) receptor subtypes (assigned to seven families, 5-HT<sub>1–7</sub>) have been characterized, and 13 of these have been identified in the human brain. Assessment of extracellular serotonin with PET imaging has the potential to provide important insights into the pathophysiology and treatment of common CNS diseases such as anxiety and mood disorders, as well as sleep and eating behaviors (for review see Millan et al. 2008). Several radioligands have been developed for PET imaging of the serotonin system, specifically for the 5-HT<sub>1A</sub>, 5-HT<sub>1B</sub>, 5-HT<sub>2A</sub>, 5-HT<sub>4</sub>, and 5-HT<sub>6</sub> receptor subtypes and the neuronal serotonin transporter (SERT) (for review see Paterson et al. 2013). Most of these radioligands have been investigated for their sensitivity to extracellular serotonin levels. Typically, extracellular concentrations of serotonin have been elevated using serotonin releasers, e.g., fenfluramine, or SERT inhibitors (SSRIs), such as fluoxetine and the racemate citalopram, or reduced using tryptophan depletion. Paterson et al. previously reviewed these studies in great detail (Paterson et al. 2010), so only more recent work has been summarized in Table 2.

The 5-HT<sub>1A</sub> receptor distribution has been extensively characterized and high levels are found in the cortex, hippocampus, and raphe nuclei (Pazos et al. 1987a). The 5-HT<sub>1A</sub> receptors function as somatodendritic autoreceptors in the raphe nuclei and as postsynaptic receptors in other brain regions (for review see Millan et al. 2008). PET studies performed with the high-affinity (~2 nM) 5-HT<sub>1A</sub> receptor antagonist [<sup>11</sup>C]WAY-100635 (Pike et al. 1995; Pike et al. 1996) have demonstrated that fenfluramine-induced increases in synaptic serotonin concentration decreased radioligand binding in the hippocampus of rats (Hume et al. 2001), although this was not reported by others (Maeda et al. 2001). Tryptophan depletion and infusion did not affect [<sup>11</sup>C]WAY-100635 binding in

human subjects (Rabiner et al. 2002). The 5-HT<sub>1A</sub> receptor antagonist [<sup>18</sup>F]MPPF (Shiue et al. 1997), which has lower affinity when compared with [<sup>11</sup>C]WAY-100635, has been evaluated in a series of studies. Fluoxetine-induced elevation of serotonin concentrations has been demonstrated to decrease [<sup>18</sup>F]MPPF binding in the raphe nuclei of cats (Aznavour et al. 2006) and humans (Sibon et al. 2008), while citalopram and fenfluramine did not affect [<sup>18</sup>F]MPPF binding in rats (Moulin-Sallanon et al. 2009) and monkeys (Udo de Haes et al. 2006), respectively. These results suggest limited sensitivity of [<sup>18</sup>F]MPPF to serotonin, which is consistent with tryptophan depletion and infusion studies also showing no effect on [<sup>18</sup>F]MPPF binding in humans (Praschak-Rieder et al. 2004; Udo de Haes et al. 2002). Interestingly, it has been demonstrated that sleep may cause an elevation in [<sup>18</sup>F]MPPF binding when compared to wakefulness, but this study was performed in narcolepsy cataplexy patients and confirmation is required in healthy subjects (Derry et al. 2006). Similar to [<sup>18</sup>F]MPPF, [<sup>18</sup>F]FPWAY is a structural analogue of [<sup>11</sup>C]WAY100635 with moderate affinity (<10 nM) to the 5-HT<sub>1A</sub> receptor (Lang et al. 1999). Administration of paroxetine in monkeys induced 8–27 % decreases in [<sup>18</sup>F]FPWAY binding in the raphe nuclei and 10–20 % increases in cortical [<sup>18</sup>F]FPWAY binding (Giovacchini et al. 2005). Although these results were encouraging, so far no follow-up human study has been reported.

In summary, serotonin-dependent decreases in 5-HT<sub>1A</sub> receptor antagonist radioligand binding have primarily been observed in the raphe nuclei and have been small or absent in serotonin projection regions. These regional differences may be partly caused by SSRI-induced increases in extracellular serotonin being larger in the raphe nuclei than in serotonin projection regions, as shown with microdialysis (Fuller 1994). In addition, it has been proposed that there may be differences in the regional responses to increased synaptic serotonin. 5-HT<sub>1A</sub> autoreceptors in the raphe nuclei have been suggested to undergo internalization, while postsynaptic receptors in the serotonin projection regions may not internalize (Aznavour et al. 2006; Riad et al. 2004). The raphe nuclei are, however, small structures of the brain stem, and reliable determination of 5-HT<sub>1A</sub> receptor binding has proven challenging because of limitations in spatial resolution. This consideration may have limited the application of the use of 5-HT<sub>1A</sub> receptor radioligands for measurement of acute changes in extracellular serotonin in patient populations.

The 5-HT<sub>2A</sub> receptor is the main excitatory GPCR for serotonin and is widely distributed in the brain, with highest density in the cerebral cortex (Pazos et al. 1987b). Several 5-HT<sub>2A</sub> receptor antagonist radioligands have been developed for PET, e.g., [<sup>18</sup>F]altanserin (Lemaire et al. 1991) and [<sup>11</sup>C]MDL 100907 (Lundkvist et al. 1996). The 5-HT<sub>2A</sub> receptor antagonist radioligands have in general shown no serotonin sensitivity in PET studies on rats (Hirani et al. 2003),



baboons (Staley et al. 2001), or humans (Matusch et al. 2007; Meyer et al. 1999; Pinborg et al. 2004), but Yatham et al. found a paradoxical decrease in cortical [ $^{18}\text{F}$ ]setoperone binding following tryptophan depletion (Yatham et al. 2001). As exceptions, clomipramine and dexfenfluramine have been found to decrease cortical [ $^{18}\text{F}$ ]altanserin binding in human subjects (Larisch et al. 2003; Quednow et al. 2012). These two studies, however, may be inconclusive as clomipramine and the main metabolite of dexfenfluramine, nordexfenfluramine, might also have binding affinity for the 5-HT<sub>2A</sub> receptor, which may have contributed to the observed reductions in binding (Paterson et al. 2010; Rothman et al. 2000).

Another approach has made use of radioligands targeting the SERT, e.g., [ $^{11}\text{C}$ ]DASB (Houle et al. 2000) and [ $^{11}\text{C}$ ]MADAM (Halldin et al. 2005), which were originally used for the determination of SERT occupancy by inhibitors of serotonin reuptake (tricyclic antidepressants and SSRI drugs). This approach to demonstrate changes in endogenous serotonin has been more challenging as most serotonin challenge studies utilize drugs that directly act on the SERT. However, several attempts have been reported using non-selective MAO inhibitors, 5-hydroxytryptophan (5-HTP), or tryptophan depletion. After elevation of extracellular serotonin levels, decreases in [ $^{11}\text{C}$ ]DASB binding have been consistently shown in the brain of rats (Lundquist et al. 2007), cats (Ginovart et al. 2003), and non-human primates (NHPs) (Lundquist et al. 2007; Yamamoto et al. 2007). Milak and colleagues, however, reported that tryptophan depletion paradoxically decreased binding potential values, possibly related to protein kinase-C-dependent SERT sequestration being less inhibited by serotonin and resulting in increased SERT internalization (Milak et al. 2005). In two reported human studies, tryptophan depletion did, however, not affect [ $^{11}\text{C}$ ]DASB binding (Praschak-Rieder et al. 2005; Talbot et al. 2005).

In conclusion, at the start of NEWMEDS, no established methodology existed for assessment of changes in extracellular serotonin concentrations in the human brain. We therefore initiated the validation of the use of the novel 5-HT<sub>1B</sub> receptor radioligand [ $^{11}\text{C}$ ]AZ10419369 for evaluation of changes in extracellular serotonin. The characterization of [ $^{11}\text{C}$ ]AZ10419369 binding in NHPs and humans was initiated at the start of NEWMEDS (Pierson et al. 2008; Varnäs et al. 2011), allowing for a quick cross-species evaluation of serotonin sensitivity. The 5-HT<sub>1B</sub> receptor was considered a promising target as serotonin binds with quite high affinity to this receptor (~1 nM) (Millan et al. 2002; for review see Paterson et al. 2010), and a relatively large proportion of the receptors may be in the high affinity state in vitro (Granás et al. 2001). In addition, 5-HT<sub>1B</sub> receptors are expressed at relatively high density in the raphe nuclei and in serotonergic projection areas (Bonaventure et al. 1997), allowing for detailed examination of regional effects.

The serotonin sensitivity of [ $^{11}\text{C}$ ]AZ10419369 binding was initially explored in three monkeys using the potent serotonin releaser ( $\pm$ )-fenfluramine. Fenfluramine reduced the specific binding of [ $^{11}\text{C}$ ]AZ10419369 in a dose-dependent manner with a regional average of 27 % after 1.0 mg/kg and 50 % after 5.0 mg/kg (Finnema et al. 2010b). This study showed for the first time a major decrease in radioligand receptor binding measured with PET in the NHP brain after administration of a serotonin releasing agent. Importantly, these observations have now been confirmed in four other studies using the 5-HT<sub>1B</sub> receptor radioligands [ $^{11}\text{C}$ ]P943 or [ $^{11}\text{C}$ ]AZ10419369 (Cosgrove et al. 2011; Finnema et al. 2012c; Ridler et al. 2011; Yamanaka et al. 2014) (Table 2). These studies indicate that 5-HT<sub>1B</sub> receptor radioligands are promising tools for the measurement of serotonin release and provide a good rationale for further development of the methodology.

It is not easily feasible to continue the fenfluramine studies in human subjects since fenfluramine products were withdrawn from the market in 1997 following reports of valvular heart disease and pulmonary hypertension in chronic users (Connolly et al. 1997). The serotonin sensitivity of [ $^{11}\text{C}$ ]AZ10419369 in humans was therefore assessed using the SSRI escitalopram which has been shown devoid of significant affinity for the 5-HT<sub>1B</sub> receptor (Maier et al. 2009; Sanchez et al. 2003). In pilot PET studies in monkeys, we confirmed that a high dose of escitalopram (2.0 mg/kg, i.v.) decreased [ $^{11}\text{C}$ ]AZ10419369 binding by 11 % in serotonin projection areas and by 25 % in the raphe nuclei (Nord et al. 2013). In healthy human subjects, administration of a single dose of escitalopram (20 mg, p.o.), a daily dose commonly used in the treatment of depression, tended to decrease [ $^{11}\text{C}$ ]AZ10419369 binding in the raphe nuclei but increased radioligand binding by 5 % in serotonergic projection areas ( $p < 0.05$ ) (Table 2). In a consecutive test-retest study in human subjects, [ $^{11}\text{C}$ ]AZ10419369 binding was found to be very reproducible with an absolute mean difference in  $BP_{\text{ND}}$  of less than 3 % in serotonergic projection areas between morning and afternoon measurements (Nord et al. 2014). These studies suggested that a single clinically relevant dose of escitalopram may decrease extracellular serotonin concentrations in serotonergic projection areas in the human brain. Since, hypothetically, desensitization of inhibitory serotonin autoreceptors will cause the serotonin concentration in projection areas to increase over time with chronic administration, these findings might aid in understanding the mechanism of the delayed onset of the clinical effects of SSRIs.

Concomitantly, other investigators also examined the effects of SSRI-induced changes in extracellular serotonin using radioligand binding. The binding of [ $^{11}\text{C}$ ]P943 was reduced by 9–30 % in serotonergic projection areas in monkeys after a large acute dose of citalopram (4 mg/kg, i.v.) (Ridler et al. 2011), but to date there have been no follow-up studies

reported in human subjects. Kumar and colleagues reported the development of [ $^{11}\text{C}$ ]CUMI-101 which was demonstrated to be an agonist to human 5-HT $_{1A}$  receptors expressed on Chinese hamster ovary cells (Kumar et al. 2007). However, more recently, [ $^{11}\text{C}$ ]CUMI-101 was shown to be a 5-HT $_{1A}$  receptor antagonist in binding assays using native rat, NHP, or human brain tissue (Hendry et al. 2011; Shrestha et al. 2014). Nevertheless, [ $^{11}\text{C}$ ]CUMI-101 was found to be sensitive to serotonin, and citalopram (4 mg/kg, i.v.) reduced its binding by 30 % in serotonergic projection areas in monkeys (Milak et al. 2011). Selvaraj and colleagues demonstrated that citalopram (10 mg, i.v.) increased the cortical binding of this radioligand by 7 % in human subjects (Selvaraj et al. 2012), but this finding was not replicated in another study (Pinborg et al. 2012). Other recent initiatives include work with the 5-HT $_4$  receptor antagonist [ $^{11}\text{C}$ ]SB207145 and the 5-HT $_{2A}$  receptor agonist [ $^{11}\text{C}$ ]Cimbi-36. Binding of [ $^{11}\text{C}$ ]SB207145 was not found to be sensitive to acute changes in extracellular serotonin following pindolol and acute citalopram administration (Marnier et al. 2010), but treatment with fluoxetine (40 mg/day) decreased [ $^{11}\text{C}$ ]SB207145 binding by 5 % in a 3-week placebo-controlled study in healthy human subjects (Haahr et al. 2014). [ $^{11}\text{C}$ ]Cimbi-36 is a recently developed agonist radioligand for the 5-HT $_{2A}$  receptor (Ettrup et al. 2014; Ettrup et al. 2011; Finnema et al. 2014d), and preliminary evaluation of the serotonin sensitivity of [ $^{11}\text{C}$ ]Cimbi-36 in monkeys demonstrated that fenfluramine (5 mg/kg) reduced cortical  $B_{P_{ND}}$  values by ~60 % (Finnema et al. 2012b) (Table 2). These results warrant further evaluation of [ $^{11}\text{C}$ ]Cimbi-36 in humans to confirm the potential of agonist radioligands for PET measurement of changes in extracellular neurotransmitter concentrations.

In conclusion, the recently developed radioligands [ $^{11}\text{C}$ ]AZ10419369, [ $^{11}\text{C}$ ]P943, and [ $^{11}\text{C}$ ]CUMI-101 were shown to be highly sensitive to SSRI-induced changes in serotonin concentrations in NHPs (Milak et al. 2011; Nord et al. 2013; Ridler et al. 2011). Studies using [ $^{11}\text{C}$ ]AZ10419369 and [ $^{11}\text{C}$ ]CUMI-101 in human subjects (Nord et al. 2013; Pinborg et al. 2012; Selvaraj et al. 2012) suggest that single doses of SSRIs do not increase extracellular serotonin concentrations in serotonergic projection regions, as assessed with PET. Of interest is that the cross-species differential effect of SSRIs on cortical extracellular serotonin concentrations may be related to the SSRI dose. In a recent preliminary study, we determined SERT occupancy after single doses of citalopram and escitalopram and concluded that the previously applied doses of citalopram and escitalopram nearly saturated SERT in the monkey brain (Finnema et al. 2014a). Similarly, brain microdialysis experiments in rodents have demonstrated that a high dose of citalopram (10 mg/kg, i.p.) significantly increased serotonin concentrations in the cortex but that a lower dose (1 mg/kg, i.p.) did not have the same effect (Invernizzi et al. 1992). Consideration of SERT occupancy is therefore of

importance when comparing changes in extracellular serotonin across species.

A second observation was that a single dose of an SSRI was associated with possible reductions in extracellular serotonin in the projection regions of the human brain (Nord et al. 2013; Selvaraj et al. 2012). These results are very similar to a previous monkey study in which paroxetine induced a decrease in [ $^{18}\text{F}$ ]FPWAY binding in the raphe nuclei while binding in the cortex was increased (Giovacchini et al. 2005). These results appear in contrast to the majority of brain microdialysis experiments in rodents. However, a number of microdialysis studies have shown that SSRI-induced increases in extracellular serotonin in the raphe nuclei may lead to activation of 5-HT $_{1A}$  autoreceptors and decreased neuronal firing and serotonin release in the projection areas (Bel and Artigas 1992; Hervas and Artigas 1998; Invernizzi et al. 1992). The effect of an SSRI on extracellular serotonin in the projection regions depends on the balance between local SERT occupancy, promoting increased extracellular serotonin concentrations, and the consequent decrease in neuronal firing and serotonin release leading to decreased extracellular serotonin concentrations. It may thus be hypothesized that overall, a single clinically relevant dose of an SSRI results in a decrease in the extracellular concentration of serotonin in the projection regions. These studies may thus provide further understanding of the mode of action of SSRIs, and the developed radioligands appear useful for applications in the clinical development of drug treatments intended to modulate endogenous serotonin. However, to date, no methodology has been sufficiently validated for assessment of acute changes in extracellular serotonin in the human brain. Other serotonin challenge paradigms than SSRI administration should be considered for combination with these new 5-HT $_{1A}$  and 5-HT $_{1B}$  receptor radioligands.

## Noradrenaline

Receptors for noradrenaline (and adrenaline) are divided into three main classes, i.e.,  $\alpha_1$ -,  $\alpha_2$ -, and  $\beta$ -adrenoceptors (ARs), each of which is comprised of three subtypes. There are three  $\alpha_2$ -AR subtypes in humans and other mammals: the  $\alpha_{2A}$ -,  $\alpha_{2B}$ -, and  $\alpha_{2C}$ -ARs. Of these, the  $\alpha_{2A}$ -AR subtype has the most widespread distribution both in the CNS and in peripheral tissues, and has wide-ranging physiological functions that include a role as a presynaptic and auto-inhibitory regulator of neurotransmitter release and many postsynaptic effects. The  $\alpha_{2B}$ -subtype does not have a significant presence in the CNS. The  $\alpha_{2C}$ -subtype is considered to have a predominantly modulatory function, at least to some extent with a presynaptic auto- and heteroreceptor localization (Ihalainen and Tanila 2002; Sallinen et al. 1997). The lack of major physiological functions in peripheral tissues, together with a neuromodulatory heteroreceptor role, makes

the  $\alpha_2$ -AR subtype an appealing potential CNS drug target (Scheinin et al. 2001).

There is no subtype-selective radioligand for PET imaging of CNS ARs available for clinical use. Most experience has been gained from attempts to develop PET radioligands for  $\alpha_2$ -ARs, whereas  $\alpha_1$ -ARs and  $\beta$ -ARs have received less attention. Two  $^{11}\text{C}$ -labeled sertindole analogues exhibited high in vitro selectivity for  $\alpha_1$ -ARs, but PET imaging in cynomolgus monkeys revealed poor brain uptake and a high degree of non-displaceable binding (Airaksinen et al. 2013). [ $^{18}\text{F}$ ](Fluoromethoxy)ethoxy)methyl)-1H-1,2,3-triazol-1-yl)propan-2-ol ([ $^{18}\text{F}$ ]FPTC) showed specific binding to  $\beta$ -ARs in vitro, but binding was non-displaceable in vivo in rats (Mirfeizi et al. 2014).

The recently developed PET radioligand candidates [ $^{11}\text{C}$ ]MBF and [ $^{11}\text{C}$ ]JP-1302 are indeed selective for the  $\alpha_2$ -AR subtype, but have poor access to their targets in the CNS due to the effect of efflux transporters, which became evident in a study performed with p-glycoprotein and breast cancer resistance protein (P-gp/Bcrp) knockout mice compared to wild-type mice (Kawamura et al. 2010). The  $\alpha_2$ -AR radioligands [ $^{11}\text{C}$ ]MK-912 (Shiue et al. 1998), [*O*-methyl- $^{11}\text{C}$ ]RS-15385-197 (Hume et al. 2000), [ $^{11}\text{C}$ ]R107474 (Van der Mey et al. 2006), [ $^{11}\text{C}$ ]yohimbine (Jakobsen et al. 2006), and [ $^{11}\text{C}$ ]mirtazapine (Marthi et al. 2002) do not differentiate between the  $\alpha_2$ -AR subtypes. Brain uptake of [*O*-methyl- $^{11}\text{C}$ ]RS-15385-197 was minimal in humans during a 90-min PET measurement (Hume et al. 2000).

In a PET study on pigs, based on a decreased volume of distribution of [ $^{11}\text{C}$ ]yohimbine, a high dose (10 mg/kg, i.v.) of amphetamine led to approximately 5–30 % binding reductions in different brain regions known to express  $\alpha_2$ -ARs (Table 3). These data suggested that [ $^{11}\text{C}$ ]yohimbine binding could be used as a surrogate marker of noradrenaline release in vivo (Landau et al. 2012). This finding has so far not been extended to alternative challenge protocols or to human subjects. The test-retest reliability of [ $^{11}\text{C}$ ]yohimbine has also yet to be confirmed. After i.v. administration of [ $^{11}\text{C}$ ]yohimbine, there were no radioactive metabolites observed in plasma (at least in pigs), which should facilitate quantification of [ $^{11}\text{C}$ ]yohimbine binding. However, because of the wide distribution of the  $\alpha_{2A}$ -AR subtype in the CNS, no suitable reference region could be identified in pigs (Jakobsen et al. 2006). Very recently, first in human studies with [ $^{11}\text{C}$ ]yohimbine were reported. For quantitative analysis in humans, a metabolite-corrected arterial input function may be required, although the corpus callosum was explored as a potential reference region (Nahimi et al. 2015). Phan et al. (2015) recently demonstrated that amphetamine also reduced [ $^{11}\text{C}$ ]yohimbine binding in rats (Phan et al. 2015).

Of the evaluated radioligand candidates for  $\alpha_2$ -AR, [ $^{11}\text{C}$ ]mirtazapine has also been tested in humans (Munk and Smith 2011). However, its non-selectivity and binding to 5-

HT<sub>2</sub>, 5-HT<sub>3</sub>, and histamine H<sub>1</sub> receptors (de Boer 1996) precludes an assessment of its suitability as a radioligand for  $\alpha_2$ -ARs and for monitoring noradrenaline release. While [ $^{11}\text{C}$ ]mirtazapine binding has been reported to be significantly reduced in non-responders to antidepressant treatments (Smith et al. 2009), the receptor and neurotransmitter implications of this finding are at most speculative because of this lack of selectivity. It can be concluded that there was no methodology for measurement of endogenous synaptic noradrenaline in vivo in the human brain before the initiation of NEWMEDS.

Labeling of the subtype-selective  $\alpha_2$ -AR antagonist ORM-13070 (1-[(*S*)-1-(2,3-dihydrobenzo[1,4]dioxin-2-yl)methyl]-4-(3-methoxymethylpyridin-2-yl)-piperazine) with  $^{11}\text{C}$  at high radiochemical purity (>99 %) and specific radioactivity (640±390 GBq/μmol) and the preliminary characterization of the radioligand candidate in rats and  $\alpha_2$ -AR subtype knockout mice (Arponen et al. 2014) paved the way for a metabolism and dosimetry study in healthy human males (Luoto et al. 2014). This study revealed the presence of two radioactive metabolites of the radioligand in human blood, in agreement with a previous investigation in rats (Arponen et al. 2014), but indicated that reference tissue methods would still be applicable for clinical imaging. The molecular identity of these metabolites has not been determined, but it was concluded that they probably represent small volatile molecules not detectable with the employed HPLC-mass spectroscopy approaches and unlikely to interfere with receptor binding (Arponen et al. 2014; Luoto et al. 2014). Also, from a radiation safety viewpoint, [ $^{11}\text{C}$ ]ORM-13070 appeared suitable for repeated measurements in humans (Luoto et al. 2014).

The test-retest reliability of [ $^{11}\text{C}$ ]ORM-13070 PET was investigated by imaging six healthy male subjects twice (Lehto et al. 2015b). The bound/free ratio of radioligand uptake relative to non-specific uptake into the cerebellum during the time interval of 5–30 min was largest in the dorsal striatum: 0.77 in the putamen and 0.58 in the caudate nucleus. Absolute test-retest variability in the bound/free ratio of the radioligand was 4.3 % in the putamen and also <10 % in the caudate nucleus and thalamus. The pattern of [ $^{11}\text{C}$ ]ORM-13070 binding, as determined by PET, was in agreement with receptor density results previously derived from postmortem autoradiography (Fagerholm et al. 2008). PET data analysis results obtained with a compartmental model fit, the simplified reference tissue model, and a graphical reference tissue analysis method were convergent with the tissue ratio method (Lehto et al. 2015b). The results of this study supported the use of [ $^{11}\text{C}$ ]ORM-13070 PET in the quantitative assessment of  $\alpha_2$ -ARs in the human brain in vivo.

The suitability of [ $^{11}\text{C}$ ]ORM-13070 for measurement of amphetamine-evoked changes in extracellular noradrenaline levels was explored ex vivo in rat brain sections and in vivo with PET imaging in monkeys; rat striatal microdialysis experiments confirmed amphetamine-induced elevations in

noradrenaline and dopamine concentrations (Finnema et al. 2014b). After injection of [ $^{11}\text{C}$ ]ORM-13070 in rats, mean striatal specific binding ratios, determined using the cerebellum as a reference region, were  $1.4 \pm 0.3$  after saline pretreatment and  $1.2 \pm 0.2$  after amphetamine administration (0.3 mg/kg, s.c.). Injection of [ $^{11}\text{C}$ ]ORM-13070 in NHPs resulted in mean striatal binding potential ( $BP_{\text{ND}}$ ) estimates of  $0.65 \pm 0.12$  at baseline. Intravenous administration of amphetamine (0.5 and 1.0 mg/kg) reduced  $BP_{\text{ND}}$  values by 31–50 % in monkeys (Table 3). Amphetamine (0.3 mg/kg, s.c.) increased extracellular noradrenaline (by 400 %) and dopamine (by 270 %) in the rat striatum. Together, these results indicated that [ $^{11}\text{C}$ ]ORM-13070 may be a useful tool for evaluation of synaptic noradrenaline concentrations in vivo (Finnema et al. 2014b).

A limitation of the use of amphetamine in the animal validation studies was the non-selective elevation of noradrenaline and dopamine. Although the relative amphetamine-evoked increase in noradrenaline concentrations was greater than the dopamine increase, dopamine also binds to  $\alpha_{2\text{C}}$ -ARs, albeit with 5-fold lower affinity than noradrenaline (Ruuskanen et al. 2005). It could thus not be ruled out that the amphetamine-induced decrease in [ $^{11}\text{C}$ ]ORM-13070 binding was partly related to increased dopamine. In an attempt to selectively elevate synaptic noradrenaline concentrations, we performed experiments with the potent noradrenaline transporter (NET) inhibitor atomoxetine. In preliminary PET studies in two monkeys, atomoxetine did not influence the striatal binding of [ $^{11}\text{C}$ ]ORM-13070 consistently (Finnema et al. 2014b). This was not surprising since atomoxetine increased extracellular noradrenaline to a smaller extent than amphetamine, as shown by microdialysis.

A subsequent study explored the use of [ $^{11}\text{C}$ ]ORM-13070 to monitor  $\alpha_{2\text{C}}$ -AR occupancy and the tracer's capacity to reflect changes in extracellular concentrations of endogenous noradrenaline in the human brain. The subtype non-selective  $\alpha_2$ -AR antagonist atipamezole was administered at different doses to eight healthy volunteer subjects. Using the bound/free ratio during 5–30 min after [ $^{11}\text{C}$ ]ORM-13070 injection, the maximal extent of inhibition of striatal [ $^{11}\text{C}$ ]ORM-13070 uptake achieved by atipamezole was 78 % (95 % CI 69–87 %) in the caudate nucleus and 65 % (53–77 %) in the putamen. The  $\text{EC}_{50}$  estimates of atipamezole (1.6 and 2.5 ng/ml, respectively) were in agreement with the drug's  $\alpha_{2\text{C}}$ -AR affinity. This provided strong support for the use of [ $^{11}\text{C}$ ]ORM-13070 to monitor drug occupancy of  $\alpha_{2\text{C}}$ -ARs in the living human brain. Three of the four noradrenaline challenges that were explored, each challenge in four subjects, were associated with small, approximately 10–16 % average reductions in [ $^{11}\text{C}$ ]ORM-13070 binding (atomoxetine, ketamine, and the cold pressor test;  $p < 0.05$  for all), but the fourth investigated challenge, insulin-induced hypoglycemia, did not have any evident effect on radioligand uptake (Table 3). This suggested

that [ $^{11}\text{C}$ ]ORM-13070 has potential as a tool for in vivo monitoring of synaptic concentrations of noradrenaline in the human brain (Lehto et al. 2015a) and provided a rationale for a larger study aimed to confirm these findings. The results of the follow-up study are featured in this issue (Lehto et al. 2015c). This study showed significant effects on [ $^{11}\text{C}$ ]ORM-13070 binding in the dorsal striatum in response to ketamine infusion (up to 24 % average reduction in B/F), and also in response to a combination of oral atomoxetine and cold stimulation (up to 23 % average reduction in B/F). These results provide evidence in favor of [ $^{11}\text{C}$ ]ORM-13070 binding in the dorsal striatum being sensitive to increased extracellular concentrations of noradrenaline in healthy human subjects.

## GABA

GABA is the principal inhibitory neurotransmitter in the mammalian CNS. Heteropentameric GABA $_A$  receptors in the CNS mainly contain two  $\alpha$ , two  $\beta$ , and a single  $\gamma$  subunit (McKernan and Whiting 1996). Compounds that bind to the GABA $_A$  benzodiazepine (BZD) binding site are allosteric ligands; they exhibit no intrinsic activity of their own, but potentiate or inhibit the effects of GABA at receptors that contain  $\alpha_1$ ,  $\alpha_2$ ,  $\alpha_3$ , or  $\alpha_5$  subunits (Dawson et al. 2005). Changes in synaptic GABA levels have been assessed in healthy control subjects with the GABA $_A$  receptor BZD site radioligands [ $^{11}\text{C}$ ]flumazenil and [ $^{11}\text{C}$ ]Ro15-4513 (Frankle et al. 2012; Frankle et al. 2009; Stokes et al. 2014). Synaptic GABA levels were reported to be increased following oral administration of tiagabine, which acts by blocking the GABA transporter 1 (GAT1). Maximal effects observed for [ $^{11}\text{C}$ ]flumazenil were a ~15 % increase in the  $BP_{\text{ND}}$  for the cortex and medial temporal lobe after administration of 0.21 mg/kg tiagabine (Frankle et al. 2009) (Table 3). Although the observed changes in [ $^{11}\text{C}$ ]flumazenil binding were relatively small, they appeared to be reproducible (Frankle et al. 2012). More recently, Stokes et al. demonstrated that oral tiagabine administration (0.18 mg/kg) resulted in significant reductions in  $\alpha_1$ -subunit specific [ $^{11}\text{C}$ ]Ro15-4513 binding in the hippocampus, parahippocampus, amygdala, and anterior cingulate (Stokes et al. 2014). The maximal effects were greater than those seen with [ $^{11}\text{C}$ ]flumazenil, with tiagabine causing a 61 % reduction in the  $\alpha_1$ -subunit specific [ $^{11}\text{C}$ ]Ro15-4513 binding ( $V_S$ ) (Stokes et al. 2014) (Table 3). The proposed underlying mechanism is that increased extracellular GABA levels evoked by tiagabine result in changes in the affinity of BZD receptor ligands produced by GABA $_A$  receptor activation, known as the “GABA shift” (Kemp et al. 1987). The observed differences in sensitivity between the two radioligands can therefore be explained by [ $^{11}\text{C}$ ]flumazenil acting as a weak partial agonist in vivo (Higgitt et al. 1986; Miller et al. 1988) and

[<sup>11</sup>C]Ro15-4513 being a partial inverse agonist at the BZD site, respectively. Within NEWMEDS, we have attempted to measure tiagabine-induced changes in <sup>3</sup>H- and <sup>11</sup>C-labeled flumazenil and Ro15-4513 binding to brains of rodents and anesthetized NHPs, but did not observe significant changes in radioligand binding (Finnema et al. 2012a). Our ongoing efforts in this area are to investigate whether a novel BZD site full agonist, [<sup>11</sup>C]RO6899880, provides a more sensitive approach for measuring changes in synaptic GABA concentrations (Finnema et al. 2013b, 2014c; Stepanov et al. 2013).

## Glutamate

L-Glutamate is an important mediator of excitatory neurotransmission in the CNS. A large and diverse array of receptors, comprising both ligand-gated ion channels (ionotropic) and GPCRs (metabotropic), mediates the cellular effects of glutamate (Kew and Kemp 2005). With growing knowledge and understanding on the physiological roles of the glutamate receptor subtypes, interest has developed in their potential as promising CNS therapeutic targets in a large number of diseases. This has provided a powerful stimulus for the development of new tools, including PET radioligands, to assist translational drug discovery and also enabling further insight into glutamatergic neurotransmission in the human brain.

Ionotropic glutamate receptors are comprised of *N*-methyl-D-aspartate (NMDA),  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainate receptors; these gate monovalent and/or divalent cations and have widespread distributions in the brain (Watkins and Jane 2006). Several NMDA receptor radioligands have been evaluated for their utility as PET imaging agents, but no suitable AMPA or kainate receptor radioligands have so far been developed. The suitability of [<sup>11</sup>C]ketamine, which binds to the intrachannel phencyclidine (PCP) site of NMDA receptors, as a PET ligand was first established in NHPs (Hartvig et al. 1994). A subsequent study in humans demonstrated that (*S*)-[*N*-methyl-<sup>11</sup>C]ketamine produced a radioactivity distribution in the brain consistent with the known NMDA receptor expression pattern, and that its binding could be displaced by unlabeled enantiomers of ketamine (Hartvig et al. 1995). In patients with medial temporal lobe epilepsy, (*S*)-[*N*-methyl-<sup>11</sup>C]ketamine demonstrated decreased binding in the temporal lobes of ictal onset, in comparison with the contralateral lobes (Kumlien et al. 1999). Since endogenous glutamate concentrations may change in relation to seizure activity (Carlson et al. 1992), these binding differences may be explained by glutamate-dependent changes to the opening state of the NMDA receptor ion channel, thereby regulating radioligand binding to the PCP binding site, but they might also be a consequence of neuronal loss or reduced regional blood flow (Kumlien et al. 1999). There is also some

evidence, albeit mixed, from <sup>1</sup>H-MRS imaging in rats (Kim et al. 2011) and humans (Rowland et al. 2005; Stone et al. 2012), indicating that ketamine can elevate brain glutamate or related metabolite levels, suggesting that ketamine may also be a useful pharmacological challenge tool for examining glutamate release.

Somewhat more promising progress has been made towards PET imaging of metabotropic glutamate receptor subtypes, particularly with some non-competitive antagonists of the mGluR5 subtype (Sobrio 2013). Application of one of these radioligands, the negative allosteric modulator [<sup>11</sup>C]ABP688, has provided initial indirect evidence to support sensitivity to changes in glutamate release. [<sup>11</sup>C]ABP688 binding appears to be sensitive to increased glutamate release, most likely via an allosteric interaction, following *N*-acetylcysteine administration (Miyake et al. 2011; Sandiego et al. 2013) and sub-anesthetic ketamine challenge (DeLorenzo et al. 2014), although *N*-acetylcysteine and MK-801 did not affect [<sup>11</sup>C]ABP688 binding in rats (Wyckhuys et al. 2013; Table 4). [<sup>18</sup>F]FPEB (Wong et al. 2013; Zhang et al. 2014) and [<sup>18</sup>F]SP203 (Kimura et al. 2012) have also been demonstrated as mGluR5 PET radioligands, but there have been no studies reported with them investigating endogenous glutamate changes.

## Acetylcholine

Muscarinic receptor (mAChR: M<sub>1</sub>–M<sub>5</sub>) GPCRs and nicotinic ligand-gated ion channel receptors (nAChRs) mediate diverse effects of ACh in the CNS (for reviews see Gotti et al. 2009; Langmead et al. 2008). There has been a strong drive to identify and develop molecules with high receptor subtype selectivity, and as a result, many receptor-selective cholinergic ligands, including putative PET radioligands, have been developed (for review see Eckelman 2006), some of which have been shown to be sensitive to endogenous ACh (Table 4).

The M<sub>2</sub> mAChR subtype selective agonist [<sup>18</sup>F]FP-TZTP has been shown sensitive to ACh in monkeys (Carson et al. 1998). Intravenous administration of physostigmine reduced radioligand binding across brain regions with significant reductions of 22–29 % in *V*<sub>T</sub> of cortical regions (Table 4). In a more recent study, it has been confirmed that physostigmine affects [<sup>18</sup>F]FP-TZTP binding in human subjects but the effect is influenced by age and APOE- $\epsilon$ 4 genotype of the subjects (Cohen et al. 2006).

(+)-*N*-[<sup>11</sup>C]methyl-3-piperidyl benzilate ([<sup>11</sup>C](+)-3-MPB) is a subtype non-selective mAChR orthosteric antagonist which has been used to demonstrate mAChR occupancy by scopolamine in conscious monkey brain (Yamamoto et al. 2011). [<sup>11</sup>C](+)-3-MPB was not sensitive to increased endogenous ACh levels following administration of an acetylcholinesterase (AChE) inhibitor, but two related compounds, [<sup>11</sup>C](+)-3-EPB and [<sup>11</sup>C](+)-3-PPB, with lower mAChR

**Table 4** PET studies examining the susceptibility of glutamate, ACh, and opioid receptor radioligands for manipulation by their endogenous ligands

Neurotransmitter	Receptor	Radioligand	Challenge	Species ( <i>n</i> =number of subjects or measurements)	Method	Outcome parameter	Effect on outcome parameter	Reference
Glutamate	mGluR5	[ <sup>11</sup> C]ABP688	<i>N</i> -acetylcysteine (50 mg/kg over 1 h i.v., 1 h prior) vs. baseline	Monkey ( <i>n</i> =3)	B	<i>BP</i> <sub>ND</sub>	↓2–21 % in various regions	(Miyake et al. 2011)
		[ <sup>11</sup> C]ABP688	<i>N</i> -acetylcysteine (50 and 100 mg/kg over 1 h i.v., 1 h prior) vs. baseline	Monkey ( <i>n</i> =3 and 3)	B and BI	<i>V</i> <sub>T</sub>	NS and ↑18–25 % in various regions	(Sandiego et al. 2013)
	[ <sup>11</sup> C]ABP688	<i>N</i> -acetylcysteine (50 mg/kg/h i.v. over 1 h, 1 h prior) vs. vehicle	Rat ( <i>n</i> =12)	B	<i>BP</i> <sub>ND</sub>	NS	(Wyckhuys et al. 2013)	
		MK-801 (0.16 mg/kg i.p., 20 min prior) vs. vehicle	Rat ( <i>n</i> =12)	B	<i>BP</i> <sub>ND</sub>	NS	(DeLorenzo et al. 2014)	
		Ketamine (0.23 mg/kg over 1 min+0.58 mg/kg over 1 h i.v., 1 min post) vs. baseline	Human ( <i>n</i> =10)	B	<i>V</i> <sub>T</sub>	↓21 % in various regions	(DeLorenzo et al. 2014)	
Acetylcholine	M <sub>2</sub> R	[ <sup>18</sup> F]FP-TZTP	Physostigmine (100–200 µg/kg/h i.v., 30 min prior) vs. baseline	Monkey ( <i>n</i> =7)	B	<i>V</i> <sub>T</sub>	↓22–29 % in various Cx	(Carson et al. 1998)
		[ <sup>18</sup> F]FP-TZTP	Physostigmine (1.93 mg/h i.v., for 10 min 3.5–4.5 min prior+0.816 mg/kg/h i.v. till end of PET) vs. saline	Human ( <i>n</i> =7) APOE-ε4– Human ( <i>n</i> =6) APOE-ε4+	B	<i>V</i> <sub>T</sub>	↓7 % in global gray matter ↑6 % in global gray matter	(Cohen et al. 2006)
	MR	[ <sup>11</sup> C](+)-3-MPB	Donepezil (50 and 250 µg/kg i.v., 45 min prior) vs. baseline	Monkey ( <i>n</i> =5)	B	<i>BP</i> <sub>ND</sub>	~0 and ~0 % <sup>a</sup>	(Nishiyama et al. 2001)
		[ <sup>11</sup> C](+)-3-EPB	Donepezil (50 and 250 µg/kg i.v., 45 min prior) vs. saline	Monkey ( <i>n</i> =5)	B	<i>BP</i> <sub>ND</sub>	↓2–8 % and ↓~12–28 % in various regions <sup>a</sup>	(Nishiyama et al. 2001)
		[ <sup>11</sup> C](+)-3-PPB	Donepezil (50 and 250 µg/kg i.v., 45 min prior) vs. saline	Monkey ( <i>n</i> =5)	B	<i>BP</i> <sub>ND</sub>	↓~4–9 % and ↓~18–52 % in various regions <sup>a</sup>	(Nishiyama et al. 2001)
M <sub>1</sub> R	[ <sup>11</sup> C]NMPYB	Donepezil (50 and 250 µg/kg i.v., 45 min prior) vs. saline	Monkey ( <i>n</i> =5)	B	<i>BP</i> <sub>ND</sub>	NS and ↓~20–55 % in various regions	(Tsukakada et al. 2004)	
	[ <sup>11</sup> C]AF150(S)	Phenserine (5 mg/kg i.p., 30 min prior) vs. saline	Rat ( <i>n</i> =5)	B	DVR	NS	(Ma et al. 2004)	
	[ <sup>11</sup> C]AF150(S)	Haloperidol (1 mg/kg s.c., 30 min prior) and baseline	Rat ( <i>n</i> =4 and 4)	B	<i>BP</i> <sub>ND</sub>	↓25 % in right striatum and ↓16 % in hippocampus	(Buitter et al. 2013)	
	[ <sup>11</sup> C]AF150(S)	AF-DX 384 (5 mg/kg i.p., 30 min prior) and baseline	Rat ( <i>n</i> =4 and 4)	B	<i>BP</i> <sub>ND</sub>	NS	(Buitter et al. 2013)	
	[ <sup>11</sup> C]AF150(S)	AF-DX 384 (5 mg/kg i.p., 30 min prior) and baseline	Rat ( <i>n</i> =4 and 4)	B	<i>BP</i> <sub>ND</sub>	↑14–26 % in right striatum, hippocampus, and frontal Cx	(Buitter et al. 2013)	
α4β2*	[ <sup>18</sup> F]2-F-A85380 ([ <sup>18</sup> F]2FA)	Physostigmine (0.0375 and 0.15 mg/kg/h i.v.) vs. baseline	Monkey ( <i>n</i> =2 and 1)	B	<i>V</i> <sub>T</sub>	↓4–41 % and ↓11–40 % in various regions	(Valette et al. 2005)	
		Galantamine (2 and 4 mg i.v., 80–100 min post) vs. baseline	Monkey ( <i>n</i> =2 and 3)	B	<i>V</i> <sub>T</sub>	NS	(Valette et al. 2005)	
	[ <sup>18</sup> F](-)-NCFHEB ([ <sup>18</sup> F](-)-flubatine)	Physostigmine (0.06–0.28 mg/kg i.v., 10 min prior to 2 h post) vs. baseline	Monkey ( <i>n</i> =4)	B	<i>V</i> <sub>T</sub> /f <sub>p</sub>	↓22–34 % in various regions <sup>a</sup>	(Gallezot et al. 2014)	
		Donepezil (0.25 mg/kg i.v., 23 min prior) vs. baseline	Monkey ( <i>n</i> =2)	B	<i>V</i> <sub>T</sub> /f <sub>p</sub>	↓10–24 % in various regions <sup>a</sup>	(Gallezot et al. 2014)	
		Physostigmine (0.03 mg/kg i.v., 20 min prior) vs. baseline	Monkey ( <i>n</i> =3)	B	DVR	↓14–25 % in various regions <sup>a</sup>	(Ding et al. 2000)	
Opioids	µ-OR	[ <sup>18</sup> F]Nifene	Physostigmine (0.1 and 0.2 mg/kg i.v., 30 min post) vs. saline	Rats ( <i>n</i> =1)	BI	<i>R</i>	↓8–9 % and NS in cortex and thalamus	(Hillmer et al. 2013)
		[ <sup>11</sup> C]Carfentanil	Galantamine (5 mg/kg i.v., 30 min post) vs. saline	Rats ( <i>n</i> =2)	B	<i>BP</i> <sub>ND</sub>	↓3–10 % in cortex and thalamus	(Hillmer et al. 2013)
	κ-OR	[ <sup>11</sup> C]Carfentanil	<i>D</i> -Amphetamine (0.017 and 0.5 mg/kg p.o., 3 h prior) vs. baseline	Human ( <i>n</i> =6 and 6)	B	<i>BP</i> <sub>ND</sub>	NS and ↓7–10 % in various regions	(Colasanti et al. 2012)

**Table 4** (continued)

Neurotransmitter	Receptor	Radioligand	Challenge	Species ( <i>n</i> =number of subjects or measurements)	Method	Outcome parameter	Effect on outcome parameter	Reference
		[ <sup>11</sup> C]Carfentamil	D-Amphetamine (0.3 mg/kg i.v., 2 min prior) vs. placebo vs. baseline	Human ( <i>n</i> =10)	B	<i>BP<sub>ND</sub></i>	NS	(Guterstam et al. 2013)
		[ <sup>11</sup> C]Carfentamil	D-Amphetamine (0.5 mg/kg p.o., 3 h prior) vs. baseline	Human ( <i>n</i> =9)	B	<i>BP<sub>ND</sub></i>	↓4–7 % in various regions	(Mick et al. 2014)

*B* bolus, *BI* bolus+infusion, *BP<sub>ND</sub>* binding potential non-displaceable, *D*/*V**R* distribution volume ratio, *f<sub>p</sub>* fraction of radioligand bound to plasma, *NS* no statistically significant effect, *R* tissue ratio, *V<sub>T</sub>* distribution volume

<sup>a</sup> No statistical analyses reported

affinity, were sensitive to elevations of endogenous ACh provoked by administration of donepezil (250 µg/kg), leading to significantly reduced binding (e.g., for [<sup>11</sup>C](+)-3-PPB ~19 % in striatum and ~52 % in frontal cortex) in monkey brain (Nishiyama et al. 2001; Tsukada et al. 2004) (Table 4). Another subtype non-selective mAChR radioligand, [<sup>11</sup>C]NMPYB, was found not to be sensitive to ACh in PET studies on rats (Ma et al. 2004).

Considerable efforts have been devoted to the development of subtype-selective mAChR PET radioligands, primarily to assist drug development, but also to provide more detailed insight into the specific roles of the different receptor subtypes in interactions between cholinergic mechanisms and other endogenous mediators in the CNS. Progress has been slow but some leads have emerged and some potential radioligands are in the early stages of evaluation and validation in animal studies, for example, the M<sub>1</sub> selective mAChR agonist radioligand [<sup>11</sup>C]AF150(S) (Buiter et al. 2013). In rat brain, regional [<sup>11</sup>C]AF150(S) binding was consistent with the reported distribution of M<sub>1</sub> receptors, although *BP<sub>ND</sub>* values were relatively low (<0.3; Buiter et al. 2013). Administration of the dopamine D<sub>2</sub> receptor antagonist haloperidol (1 mg/kg, s.c.) reduced [<sup>11</sup>C]AF150(S) binding by 27 and 15 % in the striatum and hippocampus, respectively. Since haloperidol is known to elevate extracellular ACh levels at this dose, these findings suggest that [<sup>11</sup>C]AF150(S) may be sensitive to changes in extracellular concentrations of ACh, but further investigation is required (Buiter et al. 2013).

Developments in nAChR research have also stimulated the search for subtype-selective ligands, including the development of PET radioligands, particularly for the most abundant nAChR subtypes in mammalian brain, α4β2 and α7, due to their prospective therapeutic potential (for reviews see Jasinska et al. 2014; Mo et al. 2014). Many potential PET radioligands have been identified and investigated for imaging of brain nAChRs (Mo et al. 2014; Wu et al. 2010), but only the non-selective agonist [<sup>11</sup>C]nicotine and the α4β2 agonists 2-<sup>18</sup>F-fluoro-3-(2(S)-azetidylmethoxy)pyridine (2-[<sup>18</sup>F]FA) and 6-[<sup>18</sup>F]FA have been available for studies in humans (for review see Horti et al. 2010). [<sup>11</sup>C]Nicotine has been used extensively to study brain nAChR changes in smokers and in different patient groups, but its utility is limited because of issues of cerebral blood flow dependence and relatively high non-specific binding (Nyback et al. 1994). 2-[<sup>18</sup>F]FA has also been used to show changes in α4β2 nicotinic receptor availability. For example, it has been demonstrated that cigarette smoking inhibits 2-[<sup>18</sup>F]FA binding in an exposure-related manner, such that smoking of one cigarette produced almost 90 % receptor occupancy (Brody et al. 2006). However, 2-[<sup>18</sup>F]FA has slow brain kinetics which is a limitation of a PET ligand, so new radioligands are being explored (Mo et al. 2014).

Investigations of the sensitivity of α4β2 nAChR PET radioligands for measuring ACh changes have not been

reported in humans, but there are some indications from AChE inhibitor treatment studies in rats and NHPs which suggest that this might become feasible with improved radioligands (Table 4). Physostigmine infusion was shown to result in significant reductions in distribution volumes of 2-[<sup>18</sup>F]FA (Valette et al. 2005), [<sup>18</sup>F]-(-)-flubatine (Gallezot et al. 2014), and [<sup>18</sup>F]norchloro-fluoro-epibatidine ([<sup>18</sup>F]NFEP; Ding et al. 2000) in various brain regions of NHPs, as well as reducing [<sup>18</sup>F]nifene binding in rat brain (Hillmer et al. 2013). Similarly, donepezil and galantamine were shown to decrease [<sup>18</sup>F]NFEP binding in various regions of monkey brain (Gallezot et al. 2014) and [<sup>18</sup>F]nifene binding (Hillmer et al. 2013) in regions of rat brain, respectively (Table 4). Taken together, these studies demonstrate that  $\alpha\beta 2$  nAChR radioligands may be suitable for measuring ACh changes and future application of the methodology in human subjects may be anticipated.

### Opioid peptides

The endogenous opioid systems of the brain contain four families of opioid peptides (endorphins, enkephalins, dynorphins, and endomorphins) which are thought to act through four subtypes of G-protein-coupled opioid receptors ( $\mu$ -,  $\kappa$ -,  $\delta$ -, and nociceptin-OR). Endorphins and enkephalins are thought to preferentially act on  $\mu$ - and  $\delta$ -ORs, dynorphins on  $\kappa$ -ORs, and endomorphins on  $\mu$ -ORs (Gianoulakis 2009). Evidence for PET radioligand binding being sensitive to changes in endogenous opioid release originated from clinical PET studies. Acute somatic pain (Bencherif et al. 2002; Scott et al. 2007; Zubieta et al. 2001), inflammatory pain (Jones et al. 1994), affective responses (Zubieta et al. 2003), and placebo administration (Scott et al. 2008; Zubieta et al. 2005) were shown to modulate [<sup>11</sup>C]carfentanil or [<sup>11</sup>C]diprenorphine binding in a manner consistent with changes in endogenous opioid peptide concentrations. More recently, Colasanti and colleagues reported that a pharmacologically induced elevation in endogenous opioid release reduced [<sup>11</sup>C]carfentanil binding in several regions of the human brain, including the basal ganglia, frontal cortex, and thalamus (Colasanti et al. 2012). Oral administration of D-amphetamine, 0.5 mg/kg, 3 h before [<sup>11</sup>C]carfentanil injection, reduced  $BP_{ND}$  values by 2–10 %. The results were confirmed in another group of subjects (Mick et al. 2014). However, Guterstam and colleagues observed no change in [<sup>11</sup>C]carfentanil binding when D-amphetamine, 0.3 mg/kg, was administered intravenously directly before injection of [<sup>11</sup>C]carfentanil (Guterstam et al. 2013). It has been hypothesized that this discrepancy may be related to delayed increases in extracellular opioid peptide concentrations following amphetamine-evoked monoamine release (Colasanti et al. 2012; Mick et al. 2014).

### Summary of results of NEWMEDS work-package 5

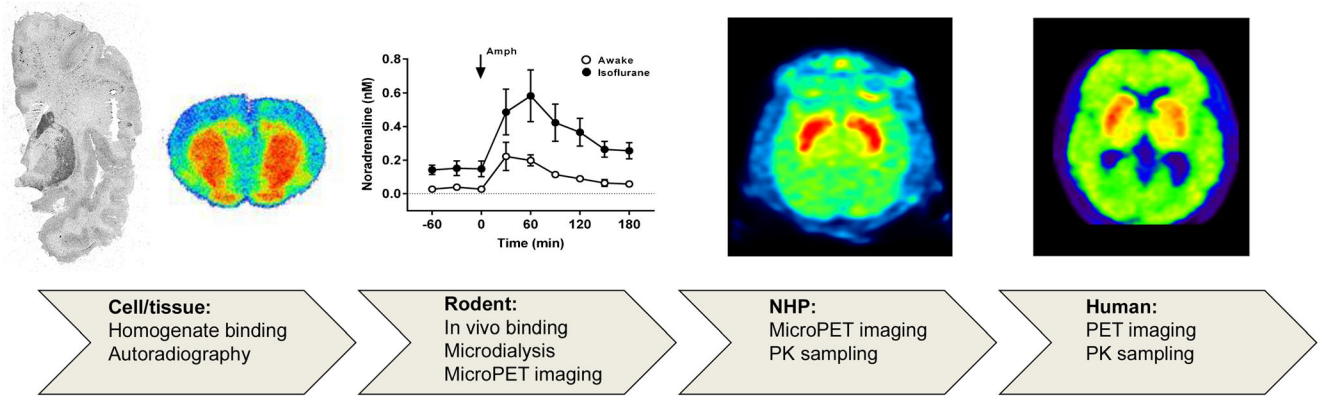
The development and validation of PET radioligands requires significant resources and expertise and often coincides with drug development. Radioligand development specifically tailored toward measurement of neurotransmitter release has so far been limited. For NEWMEDS work-package 5 we collaborated across institutions to utilize radioligands that were already available from drug development programs, such as [<sup>11</sup>C]AZ10419369, [<sup>11</sup>C]ORM-13070, and [<sup>11</sup>C]LuAE92686 (Fig. 1), and tested them for sensitivity to changes in extracellular concentrations of endogenous neurotransmitters. In addition, we initiated development of new radioligands tailored towards the measurement of neurotransmitter release, e.g., [<sup>11</sup>C]RO6899880 for GABA (Fig. 1). Sharing the workload across institutions, we utilized *in vitro* techniques in cell and tissue models, *in vivo* receptor binding and microdialysis techniques in rodents, and *in vivo* PET imaging in NHPs and humans (Fig. 2). Methods were validated for the measurement of changes in extracellular serotonin levels using the 5-HT<sub>1B</sub> receptor radioligand [<sup>11</sup>C]AZ10419369 in NHPs (Finnema et al. 2010b, 2012c; Nord et al. 2013) and humans (Nord et al. 2013, 2014). In addition, this cross-institution approach enabled us to validate measurement of endogenous noradrenaline using the  $\alpha_{2C}$ -AR antagonist [<sup>11</sup>C]ORM-13070 preclinically, using cross-species autoradiography (Arponen et al. 2014; Finnema et al. 2014b), rat microdialysis and PET imaging in NHPs (Finnema et al. 2014b), and clinically using PET (Lehto et al. 2015a, c) (Fig. 2). Work is ongoing to explore the utility of the novel GABA<sub>A</sub> agonist [<sup>11</sup>C]RO6899880 (Finnema et al. 2013b, 2014c; Stepanov et al. 2013) and the phosphodiesterase 10A (PDE10A) inhibitor [<sup>11</sup>C]LuAE92686 (Kehler et al. 2014) for the measurement of changes in concentrations of GABA and cyclic nucleotides, respectively, using cells, rodent *in vivo* receptor binding, and NHP PET imaging. Perhaps the most valuable aspect of being able to access multiple techniques was that different pharmacological challenge regimens could be explored in rodents, significantly improving the success of exploratory NHP studies, before progressing to humans.

### Considerations and current challenges

#### Development and characterization of radioligands

Since PET imaging provides an indirect approach to assess changes in extracellular neurotransmitter levels, the need to have high-quality PET radioligands and pharmacological challenge tools is paramount. Several review papers have





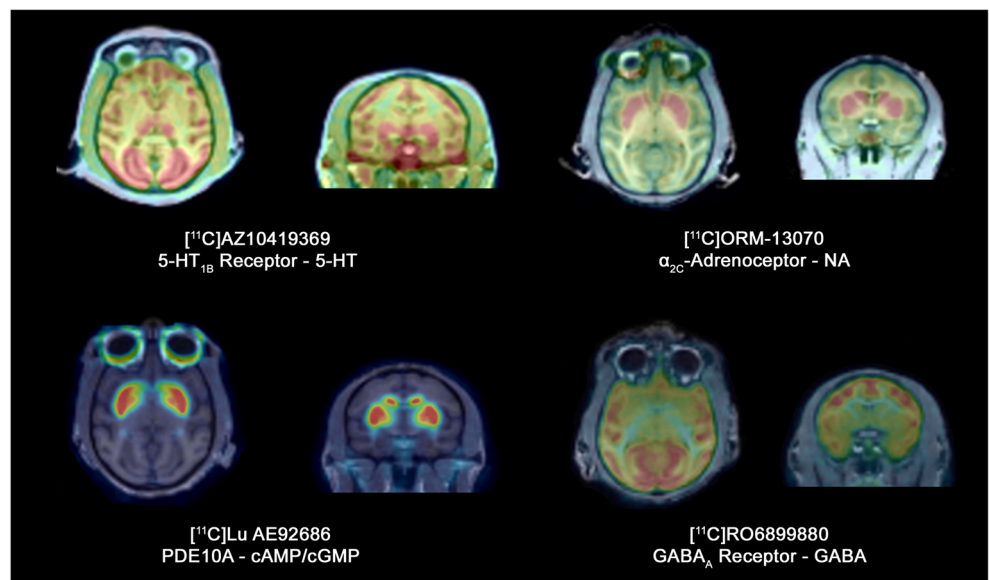
**Fig. 1** Translational techniques for measuring changes in endogenous neurotransmitter concentrations with radioligands. A series of experiments is illustrated for validation of measurement of changes in extracellular noradrenaline concentrations using the  $\alpha_2C$ -AR antagonist [ $^{11}C$ ]ORM-13070. From *left to right*: autoradiography of a coronal section of a human brain analyzed by incubation with [ $^3H$ ]ORM-13070

(unpublished results, Karolinska Institutet) and of a rat brain obtained after in vivo [ $^{11}C$ ]ORM-13070 binding (Arponen et al. 2014). Microdialysis studies demonstrated amphetamine-induced noradrenaline release in rat striatum (Finnema et al. 2014b). PET summation images after injection of [ $^{11}C$ ]ORM-13070 in a cynomolgus monkey or a human subject (Finnema et al. 2014b; Lehto et al. 2015a)

extensively discussed requirements for optimal radioligands for the CNS (Halldin et al. 2001; Pike 2009), and based on these criteria biomathematical modeling approaches have been described to predict the in vivo performance of radioligands based on in vitro and in silico assessments (Friden et al. 2014; Guo et al. 2009; Zhang et al. 2013). These approaches are promising and may possibly be further refined for radioligands tailored to the use of measuring changes in neurotransmitter concentrations. For instance, high affinity for the target ( $K_D$  in the nanomolar range) and high selectivity towards the target versus other related proteins are considered important criteria for optimal CNS radioligands (Halldin et al. 2001; Pike 2009), but may need further consideration for radioligands tailored toward measurement of neurotransmitter release.

During PET measurements for the determination of drug occupancy, the receptor binding of the radioligand is typically assessed during approximate equilibrium and at tracer dose conditions, making the receptor occupancy estimate independent of the tracer's  $K_D$ . However, monitoring of changes in extracellular neurotransmitter concentrations is typically performed under rapidly changing conditions, and it has been suggested that the tracer's  $K_D$ , or more specifically, its dissociation rate,  $K_{off}$  (corresponding to the PET rate constant  $k_4$ ), is an important parameter allowing for rapid adjustments to changes in the concentration of the neurotransmitter (Endres and Carson 1998). Also, the rate constant  $k_2$  has been proposed to be an important radioligand characteristic when the radioligand is intended to be used for measurement of neurotransmitter release during dynamic conditions (Endres and

**Fig. 2** PET radioligands used in NEWMEDS work-package 5. PET summation images of 5-HT $_{1B}$  receptor radioligand [ $^{11}C$ ]AZ10419369,  $\alpha_2C$ -AR radioligand [ $^{11}C$ ]ORM-13070, PDE10A radioligand [ $^{11}C$ ]Lu AE92686, and GABA $_A$  receptor radioligand [ $^{11}C$ ]RO6899880 in monkey brain



Carson 1998; Logan et al. 1991; Morris and Yoder 2007). The  $K_{\text{off}}$  and  $k_2$  may therefore need to be considered for tailored radioligand development. Experimental studies with high affinity dopamine  $D_2$  receptor radioligands suggest that a relative low  $K_{\text{off}}$  rate does not limit a radioligand to be suitable for measurement of neurotransmitter release. A more systematic study may need to be conducted to further understand the relative importance of these parameters.

Target selectivity is another important parameter to be optimized for radioligands to be used for diagnostic purposes. However, when the radioligand is developed for measurement of neurotransmitter release, target selectivity might not be so critical since selectivity may be achieved by use of a well-characterized pharmacological challenge with high selectivity to a particular neurotransmitter. Differential affinities should also be considered. For example, WAY-100635, which was originally considered to be highly selective for 5-HT<sub>1A</sub> receptors, was subsequently shown to be a potent  $D_4$  receptor agonist, albeit with 10-fold lower affinity than for 5-HT<sub>1A</sub> (Chemel et al. 2006). Still, relative target distributions should also be considered, as for example in the case of WAY-100635 binding, the density of  $D_4$  receptors in vivo is considerably lower than that of 5-HT<sub>1A</sub> receptors. The importance of target selectivity is also exemplified by NMSP, which is selective for  $D_2$  receptors in the striatum and for 5-HT<sub>2A</sub> receptors in the cortex. However, although PET radioligand selectivity might not be an absolute prerequisite from a biological perspective, and such characterization may have been limited for older PET radioligands, more recent and future radioligands will likely be developed with the intention of high selectivity, which will facilitate the interpretation of the binding results, and this might be predicted for radioligands targeting allosteric binding sites.

In the selection of the radioligand to study changes in neurotransmitter release, it is also important to consider the nature of the interaction between the radioligand and the endogenous mediator. The current applications are primarily based on direct competition, e.g., with dopamine, serotonin, and noradrenaline. The effects of GABA and glutamate on tracer binding involve allosteric interactions and have so far been less well validated, and the reported changes in binding require further mechanistic understanding. In addition, it is worthwhile to consider the affinity of the neurotransmitter to the receptor targeted by the radioligand. Dopamine has higher affinity for the dopamine  $D_2$  receptor than the  $D_1$  receptor (Marcellino et al. 2012) and serotonin has relatively high affinity for the 5-HT<sub>1A</sub>, 5-HT<sub>1B</sub>, and 5-HT<sub>7</sub> receptors (Paterson et al. 2010). The success of a radioligand for investigating changes in extracellular neurotransmitter levels may thus depend on the affinity of the neurotransmitter to the corresponding receptor.

Another criterion specifically related to the development of radioligands tailored towards measurement of neurotransmitter

release is its intrinsic activity towards the target receptor. Agonist radioligands have been found to be more sensitive to changes in endogenous neurotransmitter release for dopamine, e.g., using dopamine  $D_2$ -like receptor agonists, and for serotonin, using a 5-HT<sub>2A</sub> receptor agonist (see relevant sections above). Agonist radioligands preferentially bind to the high-affinity state of the receptors and may therefore be more susceptible to the endogenous agonist than antagonist radioligands, which have similar affinity for the low- and high-affinity states of the receptor. The existence of two distinct affinity states has, however, not yet been demonstrated in vivo (for reviews see Finnema et al. 2010a; Skinbjerg et al. 2012), and the exact mechanism of the increased sensitivity of agonist radioligands therefore warrants further evaluation.

Receptor internalization has been demonstrated to contribute to amphetamine-induced changes in dopamine  $D_2$  receptor radioligands. Since, for example, most GPCRs undergo internalization, it may be speculated that the internalization model also applies to other neurotransmitter systems discussed in this review. It is therefore important to understand effects of the physiological environment on the binding of a radioligand to a receptor. In vitro studies using native brain tissue or cell lines have provided understanding of the effect of cellular location on radioligand affinity to dopamine  $D_2$  receptors (Guo et al. 2010; Quelch et al. 2014b; Skinbjerg et al. 2009; Sun et al. 2003), SERT (Quelch et al. 2012), opioid receptors (Quelch et al. 2014a), and GABA<sub>A</sub> receptors (Quelch et al. 2015). Alterations in the ability to bind internalized receptors may thus be a characteristic of a radioligand which could be evaluated to understand if the radioligand will be sensitive to neurotransmitter release.

### Quantification of PET signals

Quantification of drug-induced changes in extracellular neurotransmitter concentrations in the human brain is challenging since the magnitude of the observed change in radioligand binding has usually been quite small (typically <25 %) in comparison to the effects seen in drug occupancy studies (typically >50 %). The high cost of PET further limits the number of study subjects, thereby putting high demands on the reproducibility of the procedure. The use of reference region approaches is therefore highly desirable since the corresponding outcome parameters ( $BP_P$  and  $BP_{ND}$ ) are more reproducible when compared to distribution volumes ( $V_T$ ) calculated using metabolite-corrected blood data. In particular, quantification of receptors with a broad regional brain distribution poses a methodological challenge as they lack a suitable reference region for the determination of non-specific binding. In addition, PET imaging studies are traditionally analyzed using the receptor occupancy model, but this assumes that the radioligand's volume of distribution and fractional receptor occupancy are uniform across the brain. This characteristic

makes it possible to use occupancy plots, a linear model for reliable quantification of receptor occupancy utilizing  $V_T$  values (Cunningham et al. 2010). Importantly, this approach may be confounded when endogenous neurotransmitters are not released in equal fractions across different brain regions. Further development of quantitative methods dedicated towards measurement of neurotransmitter release is required. Recent progress include the development of methods aimed to detect relatively small changes in radioligand binding consequential of neurotransmitter release (Alpert et al. 2003; Friston et al. 1997; Watabe et al. 2000) and methods to uncover the temporal pattern of neurotransmitter release during a single PET measurement (Morris et al. 2005; Normandin et al. 2012).

### Selection of pharmacological challenge

An additional important consideration for studying fluctuations in a certain neurotransmitter is to identify a pharmacological challenge which does not directly interfere with radioligand binding. Within NEWMEDS work-package 5, to allow for direct translation between methodologies and species, we aimed to evaluate the same pharmacological challenges across species. However, this was sometimes not feasible as not all compounds which were suitable for use in experimental animals were appropriate or available for human studies. Tables 1, 2, 3, and 4 include detailed information on pharmacological regimens that have been used in studies to investigate changes in extracellular neurotransmitter concentrations.

### Other factors to consider

Other factors challenging or possibly confounding our studies included whether the interaction between the radioligand and the neurotransmitter was directly competitive, allosteric, or indirect. Endogenous mediators may not be released in equal amounts across different brain regions, the cell membrane transporters of the neurotransmitters may have different brain distributions than the target protein of the radioligand, and agonist-dependent receptor internalization may complicate the interpretation of the findings. In addition, the challenge itself might affect important physiological parameters such as blood pressure, cerebral blood flow, and peripheral clearance of the tracer, which in turn may affect radioligand uptake (Laruelle 2000). The use of anesthesia can also affect the release, re-uptake, and clearance of endogenous mediators in the brain and consequently the interpretation of the results of the pharmacological challenge. Dr. Tsukada and his colleagues (in Hamamatsu, Japan) have extensively investigated the neurotransmitter effects of anesthesia in NHPs by performing PET studies in awake and anesthetized experimental animals. They have shown that isoflurane anesthesia

induced a decrease in the binding of [ $^{11}\text{C}$ ]raclopride binding to  $D_2$  receptors through increased extracellular levels of dopamine (Tsukada et al. 1999b) and that nicotine, in high tobacco-smoking related doses, did not release sufficient dopamine to displace [ $^{11}\text{C}$ ]raclopride in the striatum of awake NHPs, but did so when the animals were anesthetized with isoflurane (Tsukada et al. 2002). Similarly, in NEWMEDS, we determined using rat microdialysis that striatal extracellular dopamine and noradrenaline concentrations were significantly higher under isoflurane anesthesia compared to awake rats (Finnema et al. 2014b). Nevertheless, most of the findings of NEWMEDS work-package 5 are clearly independent of anesthesia-induced bias or changes in cerebral blood flow. As an example, the noradrenaline challenges monitored with [ $^{11}\text{C}$ ]ORM-13070 produced consistent effects across anesthetized rodents and monkeys and awake humans.

### Future work

Assessment of molecular target engagement, accompanied by compound exposure levels at the site of action, is increasingly being recognized as critical information for effective decision making in drug discovery and development (Morgan et al. 2012; Van der Graaf and Danhof 1997). High-resolution imaging technology such as PET, which is amenable to preclinical-to-clinical translation, is playing a key role in addressing the need to investigate target occupancy as well as providing insight into pharmacodynamic actions related to the drug target. Pharmacological challenge approaches have proved useful in providing insight into direct or indirect drug effects on synaptic neurotransmission. This review attempts to summarize the current situation in relation to the use of PET imaging towards investigation of the interactions of endogenous mediators with their target receptors. The focus has been placed on assessing the availability of appropriate tools (PET radioligands, pharmacological challenge agents) and studies in NHPs and human subjects. Here, we have discussed in detail the progress made for PET measurement of dopamine, serotonin, noradrenaline, GABA, glutamate, ACh, and opioid peptides. In addition to the aforementioned investigation utilizing PDE10A radioligands to measure changes in cyclic nucleotides, ongoing activity towards the development of PET radioligands for GPCR histamine receptor subtypes  $H_1$  to  $H_4$  (for review see Funke et al. 2013) may soon provide radioligand tools which are sensitive to measuring endogenous histamine levels. The  $H_1$  receptor antagonist [ $^{11}\text{C}$ ]doxepin and the  $H_3$  receptor antagonists [ $^{11}\text{C}$ ]GSK189254 (Ashworth et al. 2010) and [ $^{11}\text{C}$ ]MK8278 (Van Laere et al. 2014) have been successfully characterized in human PET studies, but so far no reports exist in which these radioligands have been utilized for

assessment of drug-induced changes in extracellular histamine levels.

Neuropsychiatric disorders such as schizophrenia and depression present significant challenges to researchers striving to deliver improved therapeutics (Rizzo et al. 2013). The development of PET radioligands to enable dynamic monitoring of neurotransmission could be employed to determine changes in target expression and distribution within subjects, to measure phenotypic differences in target expression and distribution, to explore intervention-evoked changes in endogenous ligand levels within subjects, and to investigate phenotypic differences in brain neurotransmission over time. In addition to informing on the diagnosis and monitoring of disease states and their progression, PET radioligands that are sensitive to changes in endogenous mediators can be used to observe functional effects of drugs, thereby serving as biomarkers for target engagement in drug development. In order to achieve this, there is a need to develop more tailored PET radioligands and pharmacological challenge tools. Since a significant challenge of neuropsychiatric drug discovery is the availability of animal models with translational validity (O'Donnell 2013), the approaches reviewed here could also be used to test whether animal models show the same pathophysiology as seen in human patients, e.g., whether “schizophrenia models” show increased uptake of [<sup>18</sup>F]DOPA or increased amphetamine-induced reduced binding of [<sup>11</sup>C]raclopride, as is now well established for patients (Howes et al. 2012).

Finally, with recent technological advances in multimodality imaging, enabling simultaneous PET and functional magnetic resonance imaging (fMRI) determinations (for review see Duncan et al. 2014), there is potential to gain new insights into estimating basal receptor occupancy coupled to neurotransmitter levels. This has been successfully demonstrated by measuring neurovascular responses and dopamine receptor occupancy in the basal ganglia across a wide dynamic range using different specific activities of [<sup>11</sup>C]raclopride. Distinct relative cerebrovascular response magnitudes were observed between the putamen and the caudate nucleus, consistent with higher basal extracellular dopamine levels in the putamen (Sander et al. 2013). These applications are likely to further our understanding of psychiatric disorders and pave the way to novel effective therapeutics. Substantial progress has been made in the development of novel radiotracer tools and methodology, particularly over the past two decades. This has firmly established an important role for PET imaging in CNS research and drug discovery for target engagement analysis and confirmation of modes of action. PET imaging directed at investigating changes in endogenous neurochemicals, including the work done in NEWMEDS work-package 5, has highlighted an opportunity to further extend the capability and application of this technology in new directions.

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