3 Pharmacological Interventions That Have the Potential to Alter Neurotransmitter Levels in the Human Brain

Fokko J. Bosker, Janine Doorduin, Minke E. Jongsma, Anniek K.D. Visser, Joanna Udo de Haes, Thomas I.F.H. Cremers, Kieran Rea, Robin Tyacke, David J. Nutt, Johan A. den Boer, and Ben H.C. Westerink

Contents

F.J. Bosker, PhD (✉)
University Center for Psychiatry, University Medical Center Groningen (UMCG), Hanzeplein 1, Groningen 9713 GZ, The Netherlands
e-mail: f.j.bosker@umcg.nl

J. Doorduin, PhD • A.K.D. Visser, MSc
Department of Nuclear Medicine and Molecular Imaging, University of Groningen, University Medical Center Groningen, Hanzeplein 1, Groningen 9713 GZ, The Netherlands

M.E. Jongsma, PhD • T.I.F.H. Cremers, PhD • B.H.C. Westerink, PhD
Department of Biomonitoring and Sensoring, University of Groningen, University Center for Pharmacy, Antonius Deusinglaan 1, Groningen 9713 AV, The Netherlands

J.U. de Haes, PhD
Clinical Pharmacology, Merck Sharp and Dohme, Molenstraat 110, Oss 5342 CC, The Netherlands

K. Rea, PhD
Pharmacology Department, National University of Ireland, University Road, Galway, Ireland

R. Tyacke, PhD • D.J. Nutt, MD, PhD
Neuropsychopharmacology Unit, Imperial College London, South Kensington Campus, London W12 0NN, UK

J.A. den Boer, MD, PhD
PRA International, Zuidlaren, The Netherlands

Department of Nuclear Medicine and Molecular Imaging, University Medical Center Groningen (UMCG), Hanzeplein 1, Groningen 9713 GZ, The Netherlands

R.A.J.O. Dierckx et al. (eds.), *PET and SPECT in Psychiatry*,
DOI 10.1007/978-3-642-40384-2_3, © Springer-Verlag Berlin Heidelberg 2014

Abstract

Monitoring of neuronal activity in vivo is one of the greatest challenges in neuropsychiatry. Theoretically, levels of intra and extra synaptic neurotransmitters can be estimated through competition with suitable PET ligands at their receptors. When validating candidate receptor PET ligands for competition studies it is essential to manipulate neurotransmitter levels in vivo using interventions with drugs that have negligible affinity for the receptors aimed at and are allowed to be used in humans. Neurochemical evidence for pharmacological interventions mostly originates from microdialysis studies in animals. First we will give a brief historical and methodological overview of the microdialysis technique. We will focus on serotonin and present microdialysis data of various pharmacological interventions in rats that have the potential to alter serotonin levels in humans. Our primary aim is to broaden the arsenal of pharmacological tools for PET competition studies, in particular because the type of neuronal manipulation might be a critical factor. Microdialysis of glutamate is briefly discussed, merely to illustrate some of the shortcomings of the technique.

3.1 Introduction

Monitoring of neuronal activity in vivo is one of the greatest challenges in neuropsychiatry. The brain is a very complex organ protected by a blood–brain barrier, and direct neurobiological assessment is difficult because invasive techniques are normally not allowed in humans. Measurement of neurotransmitters and their metabolites in cerebrospinal fluid, receptor binding of blood platelets or white blood cells, and so-called neuroendocrine strategies have been adopted as substitutes for assessing neurobiological function (Syvälahti 1994), but these are relatively crude and indirect approaches to extract information from the normal or pathological brain. Postmortem studies may have some merit (Stockmeier 1997), but the progressive character of many neuropsychiatric diseases and the fact that many patients

have been treated with drugs for a substantial part of their lives limit the worth of most postmortem data (not to mention the effects of dying tissue on brain physiology, such as inactivation of enzymes and an instantaneous and massive release of neurotransmitters). On the other hand, neuroimaging has matured with a still growing number of receptor-specific PET ligands becoming available. This will likely render the indirect assessment of cerebral receptor function via blood cells or neuroendocrine strategies largely obsolete in the near future. However, neuronal function is characterized not only by the number and affinity of receptors but also by the concentrations of neurotransmitters in and outside the synaptic cleft. Theoretically, levels of intra- and extrasynaptic neurotransmitters can be estimated through competition with suitable PET ligands at their receptors. For dopamine this approach seems successful, as witnessed by a significantly reduced ^{11}C-raclopride binding potential for dopamine D_2 receptors in the basal ganglia when increasing the levels of the monoamine via pharmacological (e.g., methylphenidate; Volkow et al. 1994; Udo de Haes et al. 2005a) or psychological (e.g., monetary reward task; Zald et al. 2004) challenges. Yet, in combined PET and microdialysis studies in monkeys, it was demonstrated that modification of ^{11}C-raclopride binding is not directly related to synaptic dopamine concentrations but also depends on the mechanism of the neuronal manipulation (Tsukada et al. 1999, 2000). Imaging of synaptic neurotransmission using in vivo binding competition techniques has been critically reviewed by Laruelle (2000). He concluded that the relationship between the magnitude of changes in binding potential measured with PET or SPECT and the magnitude of changes in dopamine concentration measured by microdialysis supports the use of these noninvasive techniques to measure changes in neurotransmission, but also noted that several observations remain unexplained.

For serotonin the competition approach appeared less successful (for review, see Paterson et al. 2010). For instance, competition studies in humans, monkeys, and rodents using the 5-HT_{1A} receptor ligand ^{18}F-MPPF were not conclusive, showing significant effects only when serotonin levels were massively increased (>30×) using the 5-HT releaser (and reuptake inhibitor) fenfluramine in rats (Udo de Haes et al. 2002, 2005b, 2006). It came rather unexpected that a similar fenfluramine challenge in conscious monkeys had no effect on the ^{18}F-MPPF binding potential (Udo de Haes et al. 2006). However, a combined β-probe and microdialysis study could demonstrate significantly decreased ^{18}F-MPPF binding in rat hippocampus following a fenfluramine challenge (Zimmer et al. 2002). Interestingly, the positive results with ^{18}F-MPPF were both obtained from rats, using alternative imaging techniques such as the β-probe (Zimmer et al. 2002) and ex vivo autoradiography (Udo de Haes et al. 2005b).

Lately more promising results were reported when targeting at 5-HT_{1B} receptors using ^{11}C-AZ10419369 (Finnema et al. 2010, 2012), but 5-HT_{1A} receptor agonists such as ^{11}C-CUMI-101 might also be of interest (Milak et al. 2011). One can only speculate why the PET competition approach has been more successful for dopamine than for serotonin. Maybe the answer can be found in the much higher extracellular levels of dopamine in the basal ganglia, different cellular locations of the receptors, or physicochemical properties of the PET ligands.

When validating candidate receptor PET ligands for competition studies, it is essential to manipulate neurotransmitter levels in vivo using interventions with drugs that have negligible affinity for the receptors aimed at and are allowed to be used in humans. It must also be taken into consideration that the mechanism of the neuronal manipulation can be a critical factor (Tsukada et al. 1999, 2000; Laruelle 2000).

Neurochemical evidence for pharmacological interventions mostly originates from microdialysis studies in animals. We will focus on serotonin and present microdialysis data of various pharmacological interventions in rats that have the potential to alter serotonin levels in humans. Our primary aim is to broaden the arsenal of pharmacological tools for PET competition studies, in particular because the type of neuronal manipulation might be a critical factor. We will also briefly discuss microdialysis of glutamate merely to illustrate some of the shortcomings of the technique. First we will give a brief historical and methodological overview of the microdialysis technique.

3.2 History of Microdialysis

The first reports on intracerebral microdialysis in animals stem from the early 1980s (e.g., Zetterström et al. 1982). Microdialysis was developed to circumvent the tissue damage associated with its membrane-less push-pull forebear, which could pressurize brain tissue when the push and pull pumps were not perfectly aligned. Microdialysis does not involve exchange of fluid with brain tissue, and owing to the membrane, it also provides cleaner samples, which can often be injected without purification into a high-performance liquid chromatograph (HPLC). A disadvantage of microdialysis is its modest recovery of the analyzed compounds at practicable flow rates, which in the early years challenged the analytical capabilities of many laboratories. For a long time, it was even necessary to boost serotonin and acetylcholine levels by including a serotonin reuptake inhibitor and a cholinesterase inhibitor in the perfusion fluid, respectively. It is obvious that such measures have an impact on (local) neurochemistry in the brain, for instance, by influencing local and global feedback mechanisms. It must also be noted that insertion of a microdialysis probe into the brain is an invasive procedure that will provoke cellular reactions in its direct environment (Benveniste and Diemer 1987). These effects were somewhat lessened when the relatively crude U-shaped probes from the first studies were replaced by the more sophisticated transversal and Y-shaped probes, but the transversal probes in particular could be very stressful for the animals thus trading one problem for another.

It was soon realized that solid criteria were needed to establish the neuronal origin of neurotransmitters sampled by microdialysis. These classic criteria for exocytotic release were mostly based on in vitro studies, showing that neurotransmitter release depends on K^+/Na^+ exchange (K^+ stimulation, tetrodotoxin infusion) and mobilization of Ca^{2+} ions (Ca^{2+} depletion). In addition the release of many neurotransmitters is controlled by presynaptic autoreceptors (local or systemic administration of agonists/antagonists). This approach worked satisfactorily for the monoamines including serotonin (see Fig. 3.1), but unfortunately not that well for glutamate and only

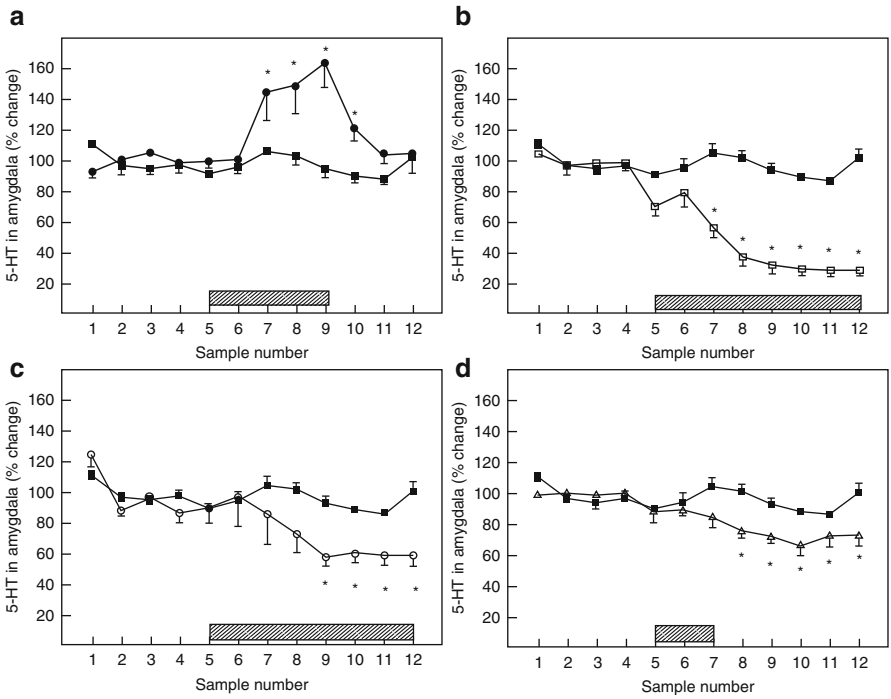

Fig. 3.1 Validation of the neuronal origin of serotonin (5-HT) sampled by a microdialysis probe (exposed tip length, 1 mM) in the central nucleus of the amygdala (CeA) of freely moving rats. (**a**) Local administration of high concentrations of potassium through the probe (60 mM for 60 min). (**b**) Administration of tetrodotoxin (1 μM for 105 min) through the probe. (**c**) Perfusion with calcium-free Ringer supplemented with EGTA (1 mM for 105 min). (**d**) Local application of the 5-HT_{1B} agonist RU 24969 (300 nM for 30 min). Measurements in the CeA were performed in the presence of 10 μM fluvoxamine (flow rate = 1.5 μl/min; sample time =15 min). *Horizontal bars* indicate perfusion with the test substance. The bars are corrected for the lag time in the microdialysis system. *significantly different from Ringer, Bonferroni contrast test following ANOVA; $p < 0.05$. Key: ■ Ringer ($n = 7$); ● potassium ($n = 7$); □ TTX ($n = 7$); ○ calcium-free Ringer ($n = 7$); Δ RU 24969 ($n = 4$) (From Bosker et al. 1997)

partly for GABA. For glutamate this could largely be attributed to an insufficient spatial and temporal resolution of the microdialysis technique making it almost impossible to discriminate the neuronal from the astroglial pool under baseline conditions (van der Zeyden et al. 2008), but for GABA, it also appeared to be a general analytical problem (Rea et al. 2005; van der Zeyden et al. 2008).

3.3 Methodology of Microdialysis

Basically a microdialysis probe consists of an inlet and an outlet tube connected by a membrane. Performance of the membrane in terms of recovery and responsiveness greatly depends on its physical (molecular weight cutoff) and chemical (hydrophobicity) properties. The most popular Y-shaped microdialysis probe is a concentric

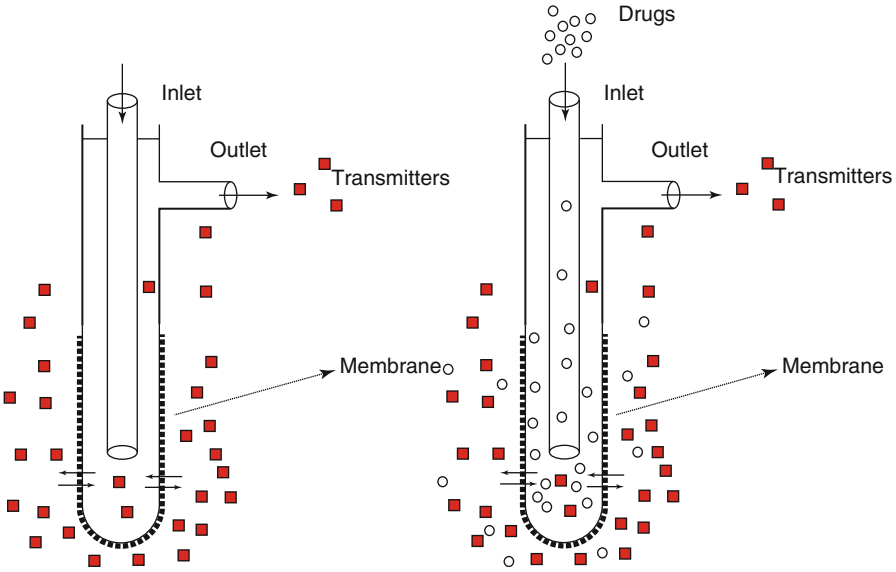

Fig. 3.2 Schematic representation of a Y-shaped microdialysis probe with both anterograde and retrograde perfusion

design with an outer diameter of approximately 300 μm (see Fig. 3.2). Probes are inserted into the brains of laboratory animals at coordinates derived from a dedicated atlas (for instance, Paxinos and Watson 1986) using a stereotaxic instrument. In rodents, surgery takes place under anesthesia, and the animals are allowed to recover from surgery for at least 24 h before the microdialysis experiments commence.

A microdialysis experiment begins by connecting the inlet of the probe via tubing to a high-performance perfusion pump carrying a syringe filled with Ringer solution (artificial CSF) to be perfused through the probe at a constant flow rate mostly in the range of 1–2 μl/min. It is important that membrane and tubing are essentially inert to minimize sticking of endogenous or exogenous compounds. It is common practice to perfuse the probe for 2 h prior to the actual microdialysis experiment to obtain a stable baseline. Samples can be collected in vials using a fraction collector for later analysis (off-line) or in an HPLC injection loop for immediate analysis (semi-online).

Theoretically, microdialysis does not involve exchange of fluid with brain tissue, but endogenous compounds (anterograde microdialysis) and exogenous compounds (retrograde microdialysis) are able to diffuse through the membrane driven by the concentration gradients between the extracellular fluid in the brain and the perfusion fluid pumped through the microdialysis probe (see Fig. 3.2). Except for glutamate, it is thus possible to directly measure the effects of most pharmacological interventions on the release of neurotransmitters. At the end of the experiments, the animals are sacrificed and the location of the probe is verified histologically. Monoamines can be analyzed with either HPLC and electrochemical detection or

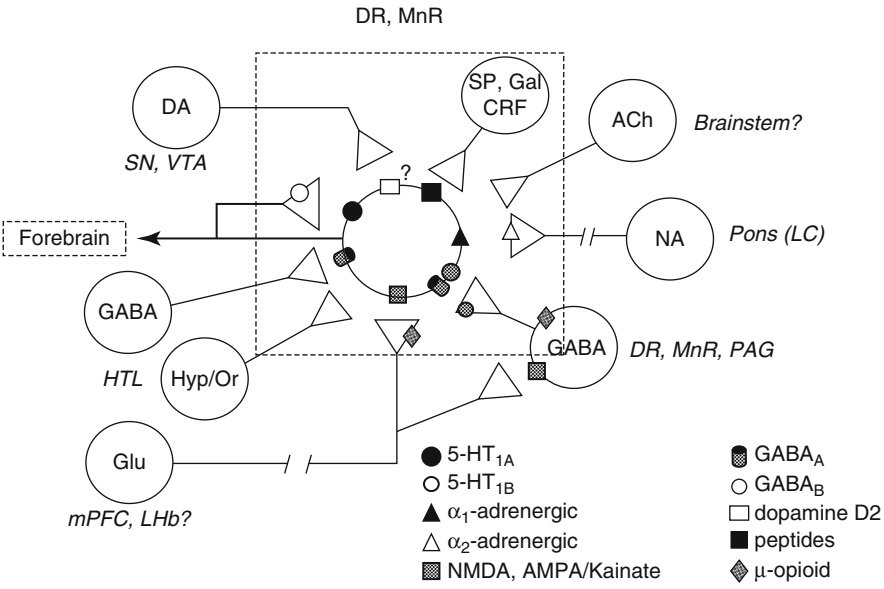

Fig. 3.3 Schematic depictions of serotonergic interactions with other neurotransmitter systems

liquid chromatography with mass spectrometry (LC-MS). The latter method is more expensive but also more accurate and sensitive, and it allows the measurement of many neurotransmitters in one run, including glutamate and GABA.

It is important to note that the here-described methodology refers to rodents. For nonhuman primates (NHP), the methodology is different in several important areas. For instance, NHP are allowed to recover for at least 2 weeks before the microdialysis studies commence, and the artificial CSF for NHP is also different from that used in rodents and not always based on a Ringer solution. In addition, the pre-experimental perfusion is usually shorter (1 h), due to restrictions on how long the NHP can be restrained during the study. Finally, NHP are usually not sacrificed following these studies, and the location of the guide cannula is often verified via MRI. For more details, we refer to the microdialysis studies in rhesus monkeys by Bradberry (2002), Wilcox et al. (2005), Howell et al. (2006), Banks et al. (2009), (Andersen et al. 2010), Murnane et al. (2010); in squirrel monkeys by Czoty et al. (2002), Bauzo et al. (2009, 2012), Manvich et al. (2012); the review of the microdialysis methodology in NHP by Bradberry (2000); and the protocols by Saunders et al. (2001).

3.4 Pharmacological Interventions for Serotonin

It is common practice to manipulate serotonin levels in PET competition studies using serotonin reuptake inhibitors or releasers, but there are other options available as outlined below. Figure 3.3 schematically depicts various interactions of the

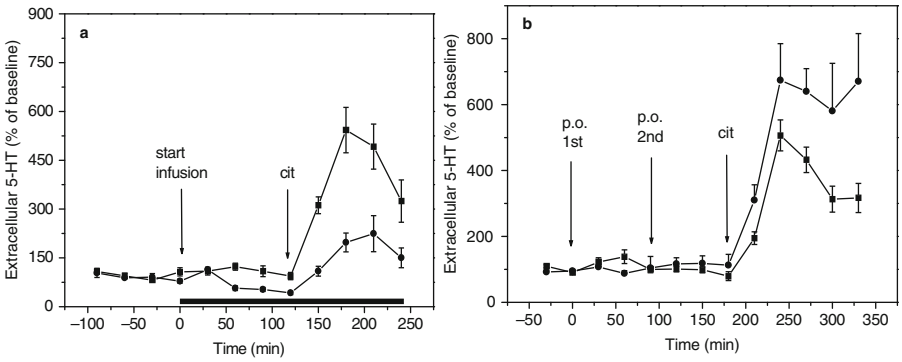

Fig. 3.4 (**a**) Microdialysis of 5-HT in hippocampus of freely moving rats (flow rate = 1.5 µl/min; sample time = 15 min). Effect of local 5-HT synthesis inhibition on the response to citalopram following retrograde infusion of the aromatic amino acid decarboxylase inhibitor NSD 1015 (3-[hydrazinomethyl] phenol dihydrochloride). ■: no synthesis inhibition, $t = 120$ citalopram 10 µmol/kg s.c.; ●: synthesis inhibition following local infusion of 10 µM of NSD 1015 at $t = 0$, $t = 120$ citalopram 10 µmol/kg s.c. (From Bosker et al. 2010). (**b**) Microdialysis of 5-HT in hippocampus of freely moving rats (flow rate = 1.5 µl/min; sample time = 15 min). Effect of oral tryptophan depletion on the response to citalopram. ■ low tryptophan, ● normal tryptophan. *First arrow* at $t = 0$: first oral administration of low tryptophan amino acid mixture; *second arrow* at $t = 90$: second oral administration; *third arrow* at $t = 180$: subcutaneous administration of citalopram 10 µmol/kg s.c. (From Bosker et al. 2010)

serotonergic system with other neurotransmitters in the cell body area. However, such interactions may vary between brain areas and also the effect of pharmacological interventions on serotonin release, in particular between cell body and axon terminal areas.

3.4.1 Manipulation of Extracellular Serotonin Levels Through Synthesis and Reuptake Inhibition

A well-known strategy to manipulate serotonin levels in the brain is through the synthesis of the monoamine. Release and synthesis of serotonin depends on the availability of its precursor molecule, the essential amino acid tryptophan. This notion has been used to demonstrate the role of serotonin in antidepressant efficacy, as witnessed by the relapse of depressive symptoms following depletion of serotonin by tryptophan depletion in patients successfully treated with antidepressants (Delgado et al. 1990).

We have investigated the effects of 5-HT synthesis inhibition on the response to citalopram following retrograde infusion of the aromatic amino acid decarboxylase inhibitor NSD 1015 (3-[hydrazinomethyl] phenol dihydrochloride) and by oral tryptophan depletion. The microdialysis experiments in rats clearly show that inhibition of serotonin synthesis by local NSD 1015 infusion as well as tryptophan depletion significantly decreases the effect of an SSRI on extracellular serotonin levels (see Fig. 3.4a, b).

However, the effect of tryptophan depletion on basal serotonin levels was not significant. This indicates that synthesis might not be a limiting factor under normal conditions, but only when serotonin reuptake is inhibited (see also Bosker et al. 2010). The latter would be in agreement with a competition PET study using ^{18}F-MPPF, which could not demonstrate increased 5-HT$_{1A}$ receptor binding following tryptophan depletion in healthy volunteers (Udo de Haes et al. 2002).

Inversely, serotonin levels are only moderately increased following intraperitoneal administration of tryptophan in rats (Fig. 3.5a). However, when given prior to an SSRI, a strong dose-dependent augmentation of its effect on serotonin levels was observed (Fig. 3.5a). This also indicates that under normal conditions, synthesis is capable of maintaining serotonin levels but that reuptake inhibition puts an extra demand on synthesis.

3.4.2 Manipulation of Extracellular Serotonin Levels Through Synthesis, Reuptake Inhibition, and Receptors Involved in the Regulation of Release and/or Synthesis

Synthesis and release of serotonin are controlled by somatodendritic 5-HT$_{1A}$ and presynaptic 5-HT$_{1B}$ autoreceptors. Blocking the 5-HT$_{1A}$ and 5-HT$_{1B}$ autoreceptors by, respectively, WAY 100.635 and GR 129735 augments the effect of the selective serotonin reuptake inhibitor citalopram on extracellular serotonin levels (Cremers et al. 2000a). Another form of negative feedback control of serotonin release is mediated by 5-HT$_{2C}$ receptors on GABA-B-ergic neurons (Cremers et al. 2007). Blocking the 5-HT$_{2C}$ receptors by SB 242084 augments the effect of the selective serotonin reuptake inhibitor citalopram on extracellular serotonin levels (Cremers et al. 2004). We have combined these SSRI augmentation strategies with tryptophan supplementation and monitored the effects on extracellular serotonin using microdialysis in hippocampus of freely moving rats (Fig. 3.5b, c, d).

SSRI augmentation with tryptophan in combination with inhibition of receptor-mediated feedback mechanisms seems a promising pharmacological intervention to increase extracellular serotonin levels in humans. The critical role of serotonin synthesis following reuptake inhibition and tryptophan supplementation is emphasized when serotonin levels are further increased using an augmentation strategy based on antagonism of 5-HT$_{1B}$ receptors involved in feedback control of serotonin synthesis and release (Fig. 3.5d).

It is clear that the very potent combination of citalopram, tryptophan, and 5-HT$_{1B}$ antagonist GR 129735 cannot be used in competition studies with 5-HT$_{1B}$ receptor PET tracers. It is also important to note that the augmentation strategies in these microdialysis studies were performed with experimental drugs, which are not currently available for use in humans. The antihypertensive drug pindolol has been used to antagonize 5-HT$_{1A}$ receptors in clinical SSRI augmentation studies. Eventually pindolol appeared to have partial agonist properties, and its dose was probably too low to bind sufficient 5-HT$_{1A}$ receptors (Artigas et al. 2001; Cremers et al. 2001).

For the 5-HT$_{2C}$ receptor augmentation strategy, a feasible alternative exists in the form of the antihypertensive drug ketanserin (Cremers et al. 2004; Udo de Haes

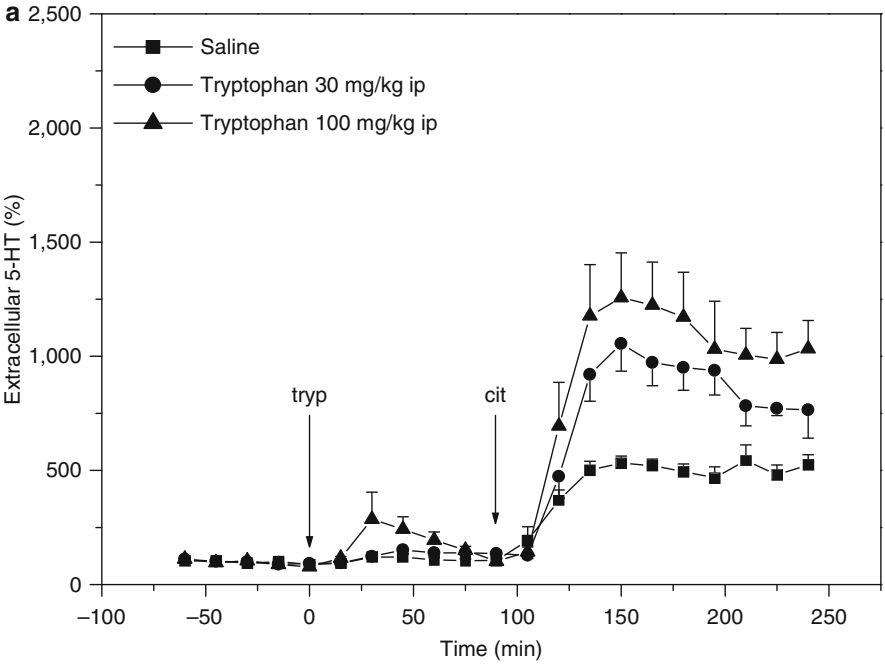

Fig. 3.5 (**a**) Microdialysis of serotonin (5-HT) in hippocampus of freely moving rats (flow rate = 1.5 µl/min; sample time = 15 min). Effect of tryptophan administration on the response to citalopram. ■ $t = 0$ saline i.p., $t = 90$ citalopram 10 µmol/kg s.c., ● $t = 0$ tryptophan 30 mg/kg i.p., $t = 90$ citalopram 10 µmol/kg s.c., ▲ $t = 0$ tryptophan 100 mg/kg i.p., $t = 90$ citalopram 10 µmol/kg s.c. (From Bosker et al. 2010). (**b**) Microdialysis of serotonin (5-HT) in hippocampus of freely moving rats (flow rate = 1.5 µl/min; sample time = 15 min). Citalopram and tryptophan administration synergistically increase 5-HT levels. Theoretically the effect is counteracted by an increased activation of somatodendritic 5-HT_{1A} autoreceptors involved in feedback control of 5-HT synthesis and release (Cremers et al. 2000a, b and references therein), yet the effect of 5-HT_{1A} antagonist WAY 100635 is only marginal. $t = 90$ citalopram (10 µmol/kg s.c.) and WAY 100.635 (1 µmol/kg s.c.). ■ $t = 0$ saline i.p., ● $t = 0$ tryptophan 30 mg/kg i.p., ▲ $t = 0$ tryptophan 100 mg/kg i.p. (From Bosker et al. 2010). (**c**) Microdialysis of serotonin (5-HT) in hippocampus of freely moving rats (flow rate = 1.5 µl/min; sample time = 15 min). Citalopram and tryptophan administration synergistically increase 5-HT levels. Theoretically the effect is counteracted by an increased activation of 5-HT_{2C} receptors on GABA-B-ergic neurons involved in feedback control of 5-HT release (Cremers et al. 2007), yet the effect of 5-HT_{2C} antagonist SB 242084 appears only marginal. $t = 90$ citalopram 10 µmol/kg s.c. and SB 242084 1 µmol/kg s.c. ■ $t = 0$ saline i.p., ● $t = 0$ tryptophan 30 mg/kg i.p., ▲ $t = 0$ tryptophan 100 mg/kg i.p. (From Bosker et al. 2010). (**d**) Microdialysis of serotonin (5-HT) in hippocampus of freely moving rats (flow rate = 1.5 µl/min; sample time = 15 min). Citalopram and tryptophan administration synergistically increase 5-HT levels. Theoretically the effect is counteracted by an increased activation of presynaptic 5-HT_{1B} autoreceptors involved in feedback control of 5-HT synthesis and release (Cremers et al. 2000a, b and references therein), which is indeed supported by the marked effect of 5-HT_{1B} antagonist GR 129735. $t = 90$ citalopram 10 µmol/kg s.c. and GR 129735 1 µmol/kg s.c. ■ $t = 0$ saline i.p., ● $t = 0$ tryptophan 30 mg/kg i.p., ▲ $t = 0$ tryptophan 100 mg/kg i.p. (From Bosker et al. 2010)

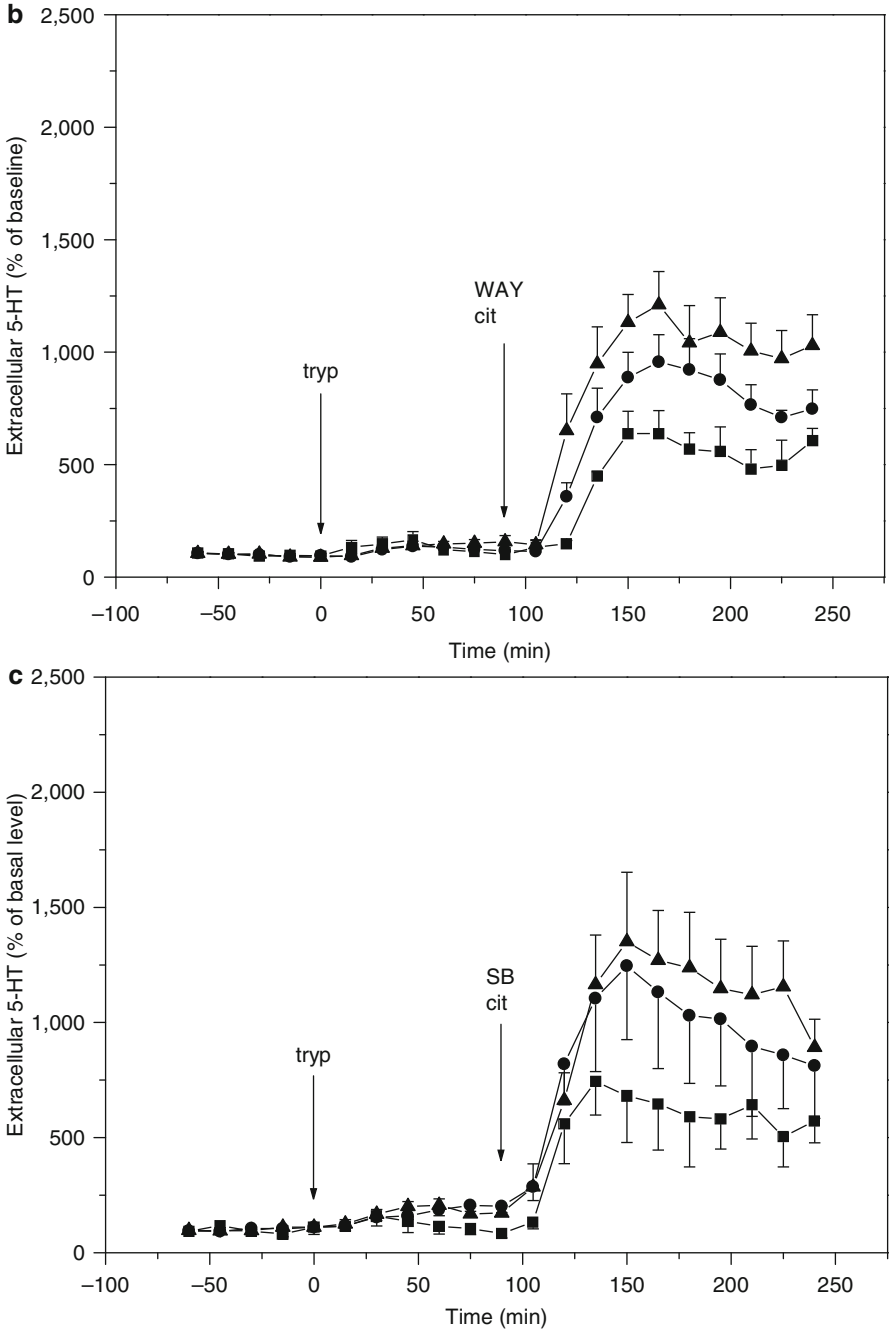

Fig. 3.5 (continued)

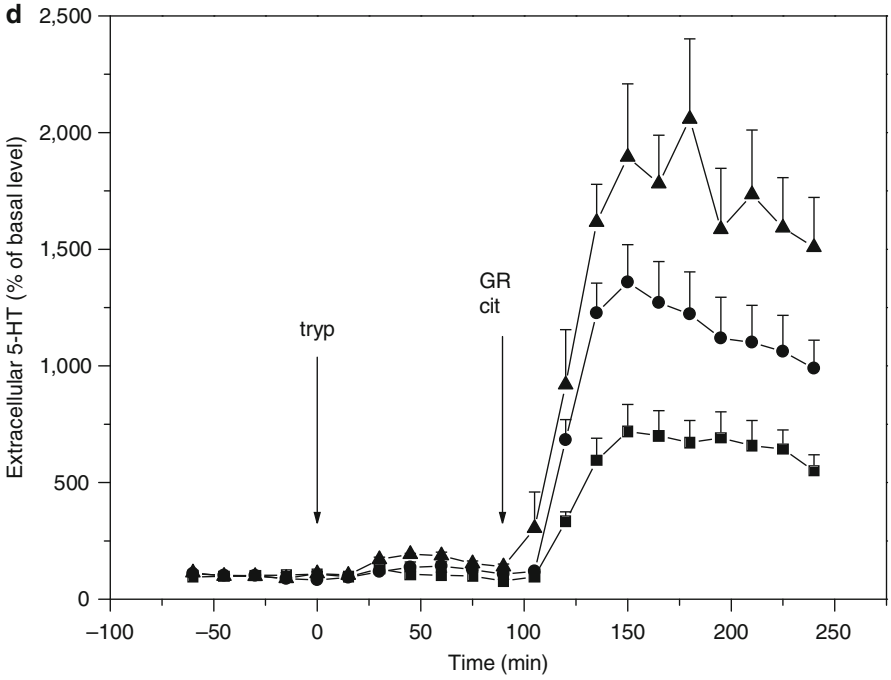

Fig. 3.5 (continued)

et al. 2005b). Compared to 5-HT_{1B} receptor antagonism both 5-HT_{1A} and 5-HT_{2C} antagonism seem to have little added value to the coadministration of citalopram and tryptophan, but an advantage of ketanserin could be its potential in minimizing the risk of serotonin syndrome through its blockade of $5HT_2$ receptors (Bosker et al. 2004).

3.4.3 Manipulation of Extracellular Serotonin Levels with Releasing Agents

Several serotonin-releasing agents are known, including parachloroamphetamine, fenfluramine, and dexfenfluramine. At higher doses, serotonin releasers might also display reuptake-inhibiting properties. Fenfluramine and dexfenfluramine have been registered as anorectics, but were withdrawn from the European market because long-term administration was associated with heart problems. It is not inconceivable, however, that for study purposes a single dose of fenfluramine in humans will be permitted by medical ethical committees in European countries (see, e.g., Finnema et al. 2010, 2012). The effect of fenfluramine on extracellular serotonin levels is very profound (see Fig. 3.6), and significant reductions of

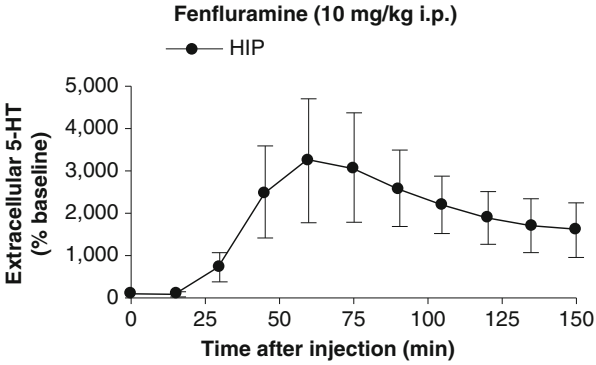

Fig. 3.6 Effect of fenfluramine (10 mg/kg i.p.) on serotonin (5-HT) levels in ventral hippocampus of freely moving rats (flow rate = 1.5 µl/min; sample time = 15 min) (From Udo de Haes et al. 2005b)

^{18}F-MPPF binding at 5-HT$_{1A}$ receptors in various brain areas could be demonstrated following administration in rats (Udo de Haes et al. 2005b).

3.4.4 Manipulation of Extracellular Serotonin Levels Through Interaction with the Noradrenergic System

The noradrenergic system interacts with the serotonergic system via α_1 and α_2 adrenoceptors (Rea et al. 2010). The specific noradrenaline reuptake inhibitor reboxetine had no effect on basal serotonin levels but significantly augmented the effect of citalopram on serotonin levels (see Fig. 3.7), preferentially through α_1 adrenoceptors in axon terminal areas (Rea et al. 2010). SSRI augmentation with reboxetine or administration of the combined serotonin and noradrenaline reuptake inhibitor venlafaxine seems a potent and safe pharmacological intervention to increase serotonin levels in PET competition studies.

3.4.5 Manipulation of Extracellular Serotonin Levels Through Interaction with the GABA-A System

Benzodiazepines are often used in combination with an antidepressant to diminish symptoms of anxiety, which can manifest in the early phase of treatment. Benzodiazepines are functional agonists of GABA-A receptors in potentiating the effects of GABA through binding at an allosteric site of the GABA-A receptor/chlorine channel complex. Figure 3.8a, b show that the benzodiazepines oxazepam and temazepam significantly reduce the effect of an SSRI on serotonin levels. Clearly, benzodiazepines have the potential to modulate the effects of SSRI-based interventions in PET competition studies. It is to note that these microdialysis data are consistent with a study in humans, showing that acute diazepam administration decreases 5-HT function (Nutt and Cowen 1987).

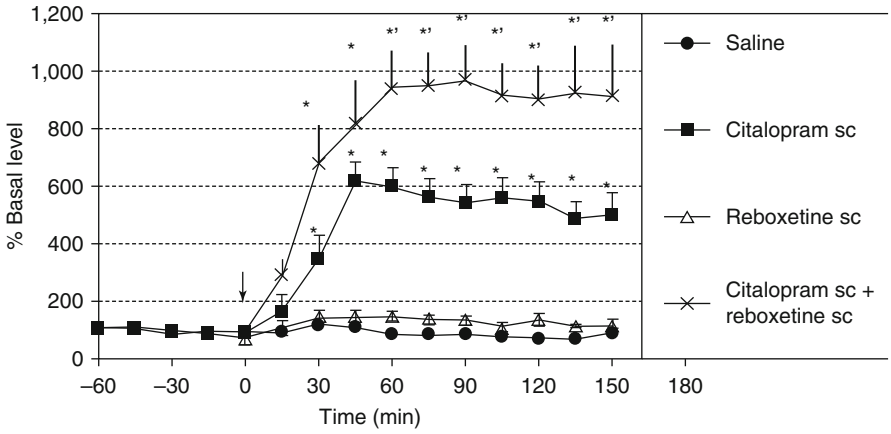

Fig. 3.7 Time course of the effect of citalopram (3 mg/kg s.c.), reboxetine (5.0 mg/kg s.c.), and the combination of the two on serotonin levels in ventral hippocampus (flow rate = 1.5 μl/min; sample time = 15 min). Results are expressed as mean ± s.e. mean % change from predrug baseline levels. Systemic administration of compounds occurred at $t = 0$ as indicated by the arrow. The observed serotonin levels with the combination ($p < 0.01$*') were significantly different from results observed with citalopram administration alone ($p < 0.01$*) (From Rea et al. 2010)

3.5 Discussion

We have shown several pharmacological interventions that have the potential to alter serotonin levels in the human brain. It is important to note that all the microdialysis experiments were performed in hippocampus and that the effects might be different in other brain regions, especially in cell body areas such as the dorsal and median raphe nuclei. Moreover, microdialysis estimates extracellular neurotransmitter levels, and we can only speculate what the effects of these pharmacological interventions will be on the synaptic serotonin concentrations. Another point of concern is the translation of animal data to the human condition as outlined below.

3.5.1 General Translational Aspects

When translating animal data to the human condition, it is important to realize that pharmacodynamics as well as pharmacokinetics might exhibit differences between species. A well-known example with respect to pharmacodynamics is the presynaptic 5-HT_{1B} autoreceptor, which displays different properties in humans compared with rodents (Hoyer et al. 1988; Adham et al. 1992). When developing antiaggression medication (*serenics*) for humans, Solvay Pharmaceuticals made a crucial and expensive misjudgment by initially performing the preclinical studies in rats instead of (guinea) pigs, which do possess the human receptor homologue.

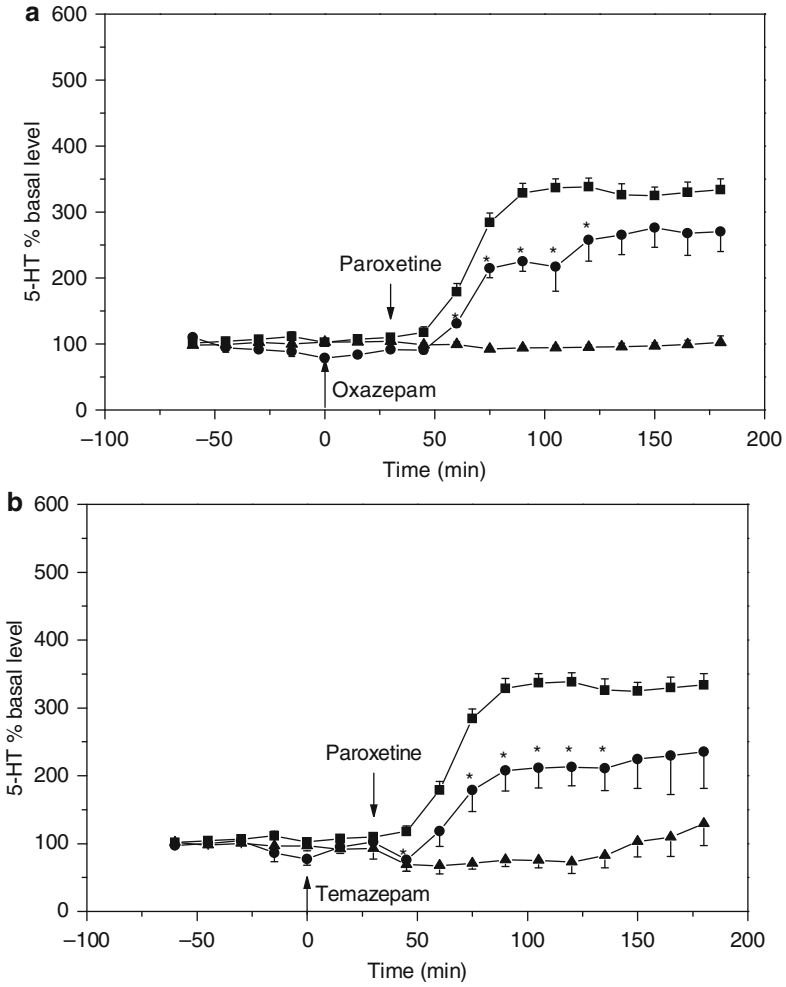

Fig. 3.8 (**a**) Effects of administration of paroxetine (5 mg/kg s.c.) (■, $n=10$, vehicle $t=0$, paroxetine $t=30$), oxazepam (1 μmol/kg s.c.) (▲, $n=5$, oxazepam $t=0$, vehicle $t=30$), and paroxetine (5 mg/kg s.c.) together with oxazepam (1 μmol/kg s.c.) (●, $n=4$, oxazepam $t=0$, paroxetine $t=30$) on serotonin (5-HT) levels in hippocampus (flow rate = 1.5 μl/min; sample time = 15 min). * denotes significant vs. paroxetine alone (From Cremers et al. 2010). (**b**) Effect of administration of paroxetine (5 mg/kg s.c.) (■, $n=10$, vehicle $t=0$, paroxetine $t=30$), temazepam (1 μmol/kg s.c.) (▲, $n=4$, temazepam $t=0$, vehicle $t=30$), and paroxetine 5 mg/kg together with temazepam (1 μmol/kg s.c.) (●, $n=4$, temazepam $t=0$, paroxetine $t=30$) on serotonin (5-HT) levels in hippocampus (flow rate = 1.5 μl/min; sample time = 15 min). * denotes significant vs. paroxetine alone (From Cremers et al. 2010)

Pharmacokinetic differences are also evident, as witnessed by the generally far more rapid elimination of drugs in rodents. For instance, SSRIs can have a ten times shorter half-life time in rodents compared with humans. This necessitates multiple injections (stressful) or the use of osmotic mini-pumps (expensive) in rodents to

mimic steady-state conditions with long-term SSRI treatment in humans (Cremers et al. 2000b).

Other confounding factors may be the disease process in humans and the invasive microdialysis procedure in animals which both may influence pharmacodynamics as well as pharmacokinetics.

3.5.2 Specific Translational Aspects Related to Microdialysis

Microdialysis enables measuring extracellular neurotransmitter levels in vivo, at least when the criteria of neuronal origin are fulfilled. For many classical neurotransmitters such as the monoamines and possibly also GABA, this might work, but not for extracellular glutamate under basal conditions (Timmerman and Westerink 1997). Attempts have been made to assess neuronal glutamate via an indirect approach based on glutamatergic interaction with other neurotransmitter systems that do fulfill the criteria of exocytotic release. For instance, using dual-probe microdialysis, the well-defined interaction between glutamate and dopaminergic projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) has been studied. Infusion of the glutamate reuptake inhibitor TBOA into the VTA (via retrograde microdialysis) significantly increased local glutamate levels and also dopamine levels in the NAc (Evering – van der Zeyden 2011). It is important to note that the corresponding dopamine response in the NAc was somewhat blurred in comparison with the TBOA-induced glutamate increase in the VTA. Moreover, the TBOA-induced glutamate increase in the VTA appeared to be largely tetrodotoxin independent, which could indicate that the effect on dopamine in the NAc was the result of either a non-synaptic event or diffusion of TBOA into the NAc.

Conclusion

The authors believe that the here-presented microdialysis data can be used as basis for pharmacological interventions in PET competition studies but also want to emphasize that microdialysis has its limitations and that one must be cautious when interpreting the data, in particular with glutamate.

References

Adham N, Romanienko P, Hartig P, Weinshank RL, Branchek T (1992) The rat 5-hydroxytryptamine1B receptor is the species homologue of the human 5-hydroxytryptamine1D beta receptor. Mol Pharmacol 41:1–7

Andersen ML, Kessler E, Murnane KS, McClung JC, Tufik S, Howell LL (2010) Dopamine transporter-related effects of modafinil in rhesus monkeys. Psychopharmacology (Berl) 210: 439–448

Artigas F, Celada P, Laruelle M, Adell A (2001) How does pindolol improve antidepressant action? Trends Pharmacol Sci 22:224–228, Review

Banks ML, Andersen ML, Murnane KS, Meyer RC, Howell LL (2009) Behavioral and neurochemical effects of cocaine and diphenhydramine combinations in rhesus monkeys. Psychopharmacology (Berl) 205:467–474

Bauzo RM, Kimmel HL, Howell LL (2009) Interactions between the mGluR2/3 agonist, LY379268, and cocaine on in vivo neurochemistry and behavior in squirrel monkeys. Pharmacol Biochem Behav 94:204–210

Bauzo RM, Kimmel HL, Howell LL (2012) The cystine-glutamate transporter enhancer N-acetyl-L-cysteine attenuates cocaine-induced changes in striatal dopamine but not self-administration in squirrel monkeys. Pharmacol Biochem Behav 101:288–296

Benveniste H, Diemer NH (1987) Cellular reactions to implantation of a microdialysis tube in the rat hippocampus. Acta Neuropathol 74:234–238

Bosker F, Vrinten D, Klompmakers A, Westenberg H (1997) The effects of a 5-HT1A receptor agonist and antagonist on the 5-hydroxytryptamine release in the central nucleus of the amygdala: a microdialysis study with flesinoxan and WAY 100635. Naunyn Schmiedebergs Arch Pharmacol 355:347–353

Bosker FJ, Westerink BH, Cremers TI, Gerrits M, van der Hart MG, Kuipers SD, van der Pompe G, ter Horst GJ, den Boer JA, Korf J (2004) Future antidepressants: what is in the pipeline and what is missing? CNS Drugs 18:705–732, Review

Bosker FJ, Tanke MA, Jongsma ME, Cremers TI, Jagtman E, Pietersen CY, van der Hart MG, Gladkevich AV, Kema IP, Westerink BH, Korf J, den Boer JA (2010) Biochemical and behavioral effects of long-term citalopram administration and discontinuation in rats: role of serotonin synthesis. Neurochem Int 57:948–957

Bradberry CW (2000) Applications of microdialysis methodology in nonhuman primates: practice and rationale. Crit Rev Neurobiol 14:143–163, Review

Bradberry CW (2002) Dose-dependent effect of ethanol on extracellular dopamine in mesolimbic striatum of awake rhesus monkeys: comparison with cocaine across individuals. Psychopharmacology (Berl) 165:67–76

Cremers TI, de Boer P, Liao Y, Bosker FJ, den Boer JA, Westerink BH, Wikström HV (2000a) Augmentation with a 5-HT(1A), but not a 5-HT(1B) receptor antagonist critically depends on the dose of citalopram. Eur J Pharmacol 397:63–74

Cremers TI, Spoelstra EN, de Boer P, Bosker FJ, Mørk A, den Boer JA, Westerink BH, Wikström HV (2000b) Desensitisation of 5-HT autoreceptors upon pharmacokinetically monitored chronic treatment with citalopram. Eur J Pharmacol 397:351–357

Cremers TI, Wiersma LJ, Bosker FJ, den Boer JA, Westerink BH, Wikström HV (2001) Is the beneficial antidepressant effect of coadministration of pindolol really due to somatodendritic autoreceptor antagonism? Biol Psychiatry 50:13–21

Cremers TI, Giorgetti M, Bosker FJ, Hogg S, Arnt J, Mørk A, Honig G, Bøgesø KP, Westerink BH, den Boer H, Wikstrom HV, Tecott LH (2004) Inactivation of 5-HT(2C) receptors potentiates consequences of serotonin reuptake blockade. Neuropsychopharmacology 29:1782–1789

Cremers TI, Rea K, Bosker FJ, Wikström HV, Hogg S, Mørk A, Westerink BH (2007) Augmentation of SSRI effects on serotonin by 5-HT2C antagonists: mechanistic studies. Neuropsychopharmacology 32:1550–1557

Cremers TI, Dremencov E, Bosker FJ, Westerink BH (2010) Oxazepam and temazepam attenuate paroxetine-induced elevation of serotonin levels in guinea-pig hippocampus. Int J Neuropsychopharmacol 13:807–811

Czoty PW, Ginsburg BC, Howell LL (2002) Serotonergic attenuation of the reinforcing and neurochemical effects of cocaine in squirrel monkeys. J Pharmacol Exp Ther 300:831–837

Delgado PL, Charney DS, Price LH, Aghajanian GK, Landis H, Heninger GR (1990) Serotonin function and the mechanism of antidepressant action. Reversal of antidepressant-induced remission by rapid depletion of plasma tryptophan. Arch Gen Psychiatry 47:411–418

Evering – van der Zeyden M (2011) Monitoring extracellular glutamate in the rat brain by microdialysis and microsensors: pharmacological applications. Thesis, University of Groningen

Finnema SJ, Varrone A, Hwang TJ, Gulyás B, Pierson ME, Halldin C, Farde L (2010) Fenfluramine-induced serotonin release decreases [11C]AZ10419369 binding to 5-HT1B-receptors in the primate brain. Synapse 64:573–577

Finnema SJ, Varrone A, Hwang TJ, Halldin C, Farde L (2012) Confirmation of fenfluramine effect on 5-HT(1B) receptor binding of [(11)C]AZ10419369 using an equilibrium approach. J Cereb Blood Flow Metab 32:685–695

Howell LL, Wilcox KM, Lindsey KP, Kimmel HL (2006) Olanzapine-induced suppression of cocaine self-administration in rhesus monkeys. Neuropsychopharmacology 31:585–593

Hoyer D, Waeber C, Pazos A, Probst A, Palacios JM (1988) Identification of a 5-HT1 recognition site in human brain membranes different from 5-HT1A, 5-HT1B and 5-HT1C sites. Neurosci Lett 85:357–362

Laruelle M (2000) Imaging synaptic neurotransmission with in vivo binding competition techniques: a critical review. J Cereb Blood Flow Metab 20:423–451, Review

Manvich DF, Kimmel HL, Howell LL (2012) Effects of serotonin 2C receptor agonists on the behavioral and neurochemical effects of cocaine in squirrel monkeys. J Pharmacol Exp Ther 341:424–434

Milak MS, Severance AJ, Prabhakaran J, Kumar JS, Majo VJ, Ogden RT, Mann JJ, Parsey RV (2011) In vivo serotonin-sensitive binding of [11C]CUMI-101: a serotonin 1A receptor agonist positron emission tomography radiotracer. J Cereb Blood Flow Metab 31:243–249

Murnane KS, Fantegrossi WE, Godfrey JR, Banks ML, Howell LL (2010) Endocrine and neurochemical effects of 3,4-methylenedioxymethamphetamine and its stereoisomers in rhesus monkeys. J Pharmacol Exp Ther 334:642–650

Nutt DJ, Cowen PJ (1987) Diazepam alters brain 5-HT function in man: implications for the acute and chronic effects of benzodiazepines. Psychol Med 17:601–607

Paterson LM, Tyacke RJ, Nutt DJ, Knudsen GM (2010) Measuring endogenous 5-HT release by emission tomography: promises and pitfalls. J Cereb Blood Flow Metab 30:1682–1706, Review

Paxinos G, Watson C (1986) The rat brain in stereotactic coordinates. Academic, London

Rea K, Cremers TI, Westerink BH (2005) HPLC conditions are critical for the detection of GABA by microdialysis. J Neurochem 94:672–679

Rea K, Folgering J, Westerink BH, Cremers TI (2010) Alpha1-adrenoceptors modulate citalopram-induced serotonin release. Neuropharmacology 58:962–971

Saunders RC, Kolachana BS, Weinberger DR (2001) Microdialysis in nonhuman primates. Curr Protoc Neurosci Chapter 7: Unit7.3

Stockmeier CA (1997) Neurobiology of serotonin in depression and suicide. Ann N Y Acad Sci 836:220–232, Review

Syvälahti EK (1994) Biological aspects of depression. Acta Psychiatr Scand Suppl 377: 11–15, Review

Timmerman W, Westerink BH (1997) Brain microdialysis of GABA and glutamate: what does it signify? Synapse 27:242–261

Tsukada H, Nishiyama S, Kakiuchi T, Ohba H, Sato K, Harada N (1999) Is synaptic dopamine concentration the exclusive factor which alters the in vivo binding of [11C]raclopride? PET studies combined with microdialysis in conscious monkeys. Brain Res 841:160–169

Tsukada H, Harada N, Nishiyama S, Ohba H, Sato K, Fukumoto D, Kakiuchi T (2000) Ketamine decreased striatal [(11)C]raclopride binding with no alterations in static dopamine concentrations in the striatal extracellular fluid in the monkey brain: multiparametric PET studies combined with microdialysis analysis. Synapse 37:95–103

Udo de Haes JI, Bosker FJ, Van Waarde A, Pruim J, Willemsen AT, Vaalburg W, Den Boer JA (2002) 5-HT(1A) receptor imaging in the human brain: effect of tryptophan depletion and infusion on [(18)F]MPPF binding. Synapse 46:108–115

Udo de Haes JI, Kortekaas R, Van Waarde A, Maguire RP, Pruim J, den Boer JA (2005a) Assessment of methylphenidate-induced changes in binding of continuously infused [(11) C]-raclopride in healthy human subjects: correlation with subjective effects. Psychopharmacology (Berl) 183:322–330

Udo de Haes JI, Cremers TI, Bosker FJ, Postema F, Tiemersma-Wegman TD, den Boer JA (2005b) Effect of increased serotonin levels on [18F]MPPF binding in rat brain: fenfluramine vs. the combination of citalopram and ketanserin. Neuropsychopharmacology 30:1624–1631

Udo de Haes JI, Harada N, Elsinga PH, Maguire RP, Tsukada H (2006) Effect of fenfluramine-induced increases in serotonin release on [18F]MPPF binding: a continuous infusion PET study in conscious monkeys. Synapse 59:18–26

van der Zeyden M, Oldenziel WH, Rea K, Cremers TI, Westerink BH (2008) Microdialysis of GABA and glutamate: analysis, interpretation and comparison with microsensors. Pharmacol Biochem Behav 90:135–147

Volkow ND, Wang GJ, Fowler JS, Logan J, Schlyer D, Hitzemann R, Lieberman J, Angrist B, Pappas N, MacGregor R et al (1994) Imaging endogenous dopamine competition with [11C] raclopride in the human brain. Synapse 16:255–262

Wilcox KM, Kimmel HL, Lindsey KP, Votaw JR, Goodman MM, Howell LL (2005) In vivo comparison of the reinforcing and dopamine transporter effects of local anesthetics in rhesus monkeys. Synapse 58:220–228

Zald DH, Boileau I, El-Dearedy W, Gunn R, McGlone F, Dichter GS, Dagher A (2004) Dopamine transmission in the human striatum during monetary reward tasks. J Neurosci 24:4105–4112

Zetterström T, Vernet L, Ungerstedt U, Tossman U, Jonzon B, Fredholm BB (1982) Purine levels in the intact rat brain. Studies with an implanted perfused hollow fibre. Neurosci Lett 29: 111–115

Zimmer L, Mauger G, Le Bars D, Bonmarchand G, Luxen A, Pujol JF (2002) Effect of endogenous serotonin on the binding of the 5-hT1A PET ligand 18F-MPPF in the rat hippocampus: kinetic beta measurements combined with microdialysis. J Neurochem 80:278–286