Current Topics in Pharmacology
Vol. 20, 2016

Review

Differences between human and animal neocortical tissues in transmitter release studies

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ABSTRACT

Transmitter release studies represent a common pharmacological technique, in which a prepared form of neural tissue is stimulated electrically or chemically to induce the release of a given neurotransmitter. This technique has been widely used to ascertain pharmacological properties of drugs and other substances. Due to the lack of availability of human tissue, animal models are widely used as a substitute. Unfortunately, in studies where significant differences in neurochemical transmission between humans and animals are detected, an animal model may prove to be inadequate as a substitute. In this review, limited to studies performed exclusively on neocortical tissue, more than 30 cases of relevant species differences were found. This included quantitative differences such as nerve terminal density of a specific transmitter, or qualitative differences, e.g. different subtypes of receptors in the species investigated. Only original studies were included in this review. There were no restrictions as to the nature of the transmitter, date of publication or measurement of transmitter for the study to be included. The validity of the observed species differences highly depended on the comparability of the experimental conditions, e.g. preparation and origin of the tissue as well as choice of the stimuli for induction of transmitter release.

KEYWORDS: transmitter release, species difference, neocortex, synaptosomes

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ABBREVIATIONS

ACh	:	acetylcholine
CB_1 receptor	:	cannabinoid 1 receptor
^3H	:	tritium
5-HT	:	serotonin
mGluR	:	metabotropic glutamate receptor(s)
nAChR	:	nicotinic acetylcholine receptor(s)
NMDA	:	N-Methyl-D-aspartic acid
N/OFQ	:	nociceptin/orphanin
ORL1 receptor	:	opioid receptor like 1 receptor

INTRODUCTION

Superfusion of tissue or cellular elements and other forms of *in vitro* transmitter release studies are commonly used investigative pharmacological methods for more than 70 years [1]. The experimental setup of transmitter release studies allows the comparison of tissues of different species simultaneously and is widely used for pharmacological comparison between species. Of special interest are deviations of animal studies from the pharmacology of human tissues, since animal models serve as widely used substitutes, which then may prove to be unfit. This manuscript aims at reviewing intra species differences between human and animal tissues in transmitter release studies, limited to neocortical tissue.

The following aspects regarding receptor theory makes superfusion studies favorable: The known concentration of an exogenous ligand in the quasi-exhaustless superfusate, which is not diminished by the receptor binding within the tissue that is being superfused (Zone A phenomenon [2]). This

contrasts with incubation, which has a finite incubation volume, where, a negligible fraction of the total drug is combined with receptor sites. The quasi-exhaustless superfusion fluid practically allows to equate the known concentration of an exogenous ligand with the uncombined ligand [3-12].

Search strategy

In transmitter release studies, a specifically prepared form of tissue is stimulated either chemically or electrically to evoke the release of a neurotransmitter consisting of nerve ending vesicles. Depending on the preparation of the tissue, the stimulus can either be chemical or electrical in nature. The latter induces transmitter release physiologically, mainly *via* exocytosis. The most commonly used chemical stimuli is an increasing concentration of potassium ions (K^+) in the extracellular fluid or an alkaloid such as veratridine.

When measured, the gradient of K^+ over the plasma membrane is found to be diminished, which leads to the depolarization of the nerve terminal. Veratridine increases the concentration of sodium ions (Na^+) in the terminal by holding the voltage-gated Na^+-channels open, which leads to depolarization of the nerve ending. Only slices of prepared tissue can be stimulated chemically and electrically, whereas synaptosomes (resealed broken-off nerve terminals obtained by homogenization of brain tissue) are electrically inaccessible [13]. Transmitter release studies are used to test the influence of added substances on the release of a specific transmitter or to compare various forms of stimulation [14-16]. Measurement of transmitter release (e.g. by high-performance liquid chromatography) can be a direct quantification of the endogenous transmitter. Measurements can also be carried out indirectly, using radiolabeled compounds, from nerve terminals preloaded with radioactive neurotransmitter or its precursor, or detection of neuropeptides by radioimmunoassay or enzyme-linked immunosorbent assay [13]. For comprehension, only transmitter release upon stimulation will be referred to as 'transmitter release' in the following work, whereas the release of neurotransmitters from the tissue without any stimulation will be labelled as 'basal outflow'.

Transmitter release studies require vital tissue, which in the case of human tissue, can be challenging to

Table 1. Search strategy for studies with human tissue.

MeSH	Text keywords
Humans AND cerebral cortex	Transmitter release OR neurotransmitter OR transmitter OR neurotransmitter release OR neurotransmitter efflux OR neurotransmitter discharge OR neurotransmitter overflow OR neurotransmitter liberation OR glutamate release OR glutamate efflux OR glutamate discharge OR glutamate overflow OR GABA release OR GABA efflux OR GABA discharge OR GABA overflow OR GABA liberation OR glycine release OR glycine efflux OR glycine discharge OR glycine overflow OR glycine liberation OR acetylcholine release OR acetylcholine efflux OR acetylcholine discharge OR acetylcholine overflow OR acetylcholine liberation OR dopamine release OR dopamine efflux OR dopamine discharge OR dopamine overflow OR dopamine liberation OR noradrenaline release OR noradrenaline efflux OR noradrenaline discharge OR noradrenaline overflow OR noradrenaline liberation OR adrenaline release OR adrenaline efflux OR adrenaline discharge OR adrenaline overflow OR adrenaline liberation OR 5-HT release OR 5-HT efflux OR 5-HT discharge OR 5-HT overflow OR 5-HT liberation OR histamine release OR histamine efflux OR histamine discharge OR histamine overflow OR histamine liberation OR cannabinoid release OR cannabinoid efflux OR cannabinoid discharge OR cannabinoid overflow OR cannabinoid liberation OR peptide release OR peptide efflux OR peptide discharge OR peptide overflow OR peptide liberation OR opioid release OR opioid efflux OR opioid discharge OR opioid overflow OR opioid liberation OR kinin release OR kinin efflux OR kinin discharge OR kinin overflow OR kinin liberation OR nucleotide release OR nucleotide efflux OR nucleotide discharge OR nucleotide overflow OR nucleotide liberation

obtain. In most human studies freshly obtained tissue was utilized, while in a few cases, previously harvested and preserved frozen tissue was used [17]. Studies with human tissue are by far less frequent than animal studies, since only laboratories cooperating with a neurosurgical or neuropathological facility have frequent access to freshly resected human cortical tissue. Thus, initially only studies with human tissue were queried. To find all eligible studies in the MEDLINE database, various text keywords and MeSH (Medical Subject Heading) terms were combined (see Table 1).

Selection of studies

The following criteria had to be met by identified studies for inclusion in this review.

1) Method : *in vitro* transmitter release study
2) Type of work : Original paper
3) Tissue : Neocortex (Human)

The analysis of studies done with human tissue was followed by the identification of the corresponding animal studies, only if a comparison with an animal model was not carried out in the same manuscript. In this review, the studies that were included had an apparent quantitative or qualitative difference between human tissue and the corresponding animal model (see Fig. 1). This methodological limitation implied that transmitter binding, *in-vivo* microdialysis, and electrophysiological studies were excluded. There were no restrictions with respect to date of

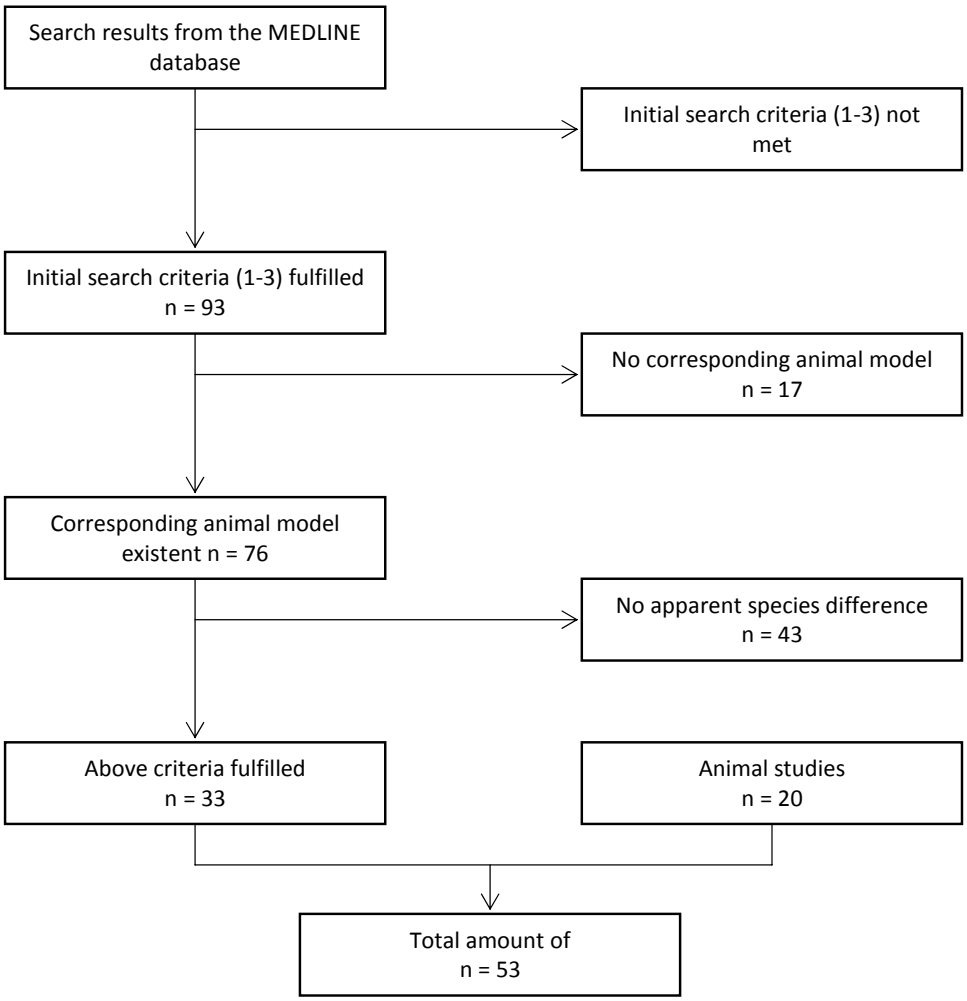

Fig. 1. Studies included with number of studies (**n**) after each step of the screening process.

publication, measurement of neurotransmitter or to any specific class of transmitter. The differences comprised of not just the apparent species difference regarding the sources, but also the form of tissue preparation and stimulation method, thereby increasing the comparability of the included studies.

RESULTS AND DISCUSSION

Species differences found in the respective studies are presented in tabular form; additionally, the form of preparation (brain slices, synaptosomes) and stimulation method is mentioned. Most studies in the following used radioactive labelling of the transmitter of interest (e.f. ^{3}H), otherwise endogenous transmitter was measured. Results are given with 95% confidence intervals, expressed in curly brackets '{}', if stated in the particular studies.

Glutamate

Aspartate release

In some studies, aspartate is used as an analogue to glutamate. Aspartate is shown to reflect the release of glutamate through membrane transport mechanisms, such as transporter reversal, and therefore offers the possibility to investigate the non-exocytotic release of glutamate [18].

Human [19]	**Guinea pig [19]**
Brain slices; K^{+} (35 mM), ouabain (5 µM, 10 µM)	
basal ^{3}H-aspartate outflow: 6.41; 8.81; 8.76 pmol/g/min	basal ^{3}H-aspartate outflow: 5.82; 5.03; 4.09 pmol/g/min
maximal stimulation (ouabain 10 µM)-evoked ^{3}H-aspartate release 36.6 {30.2, 43.0} pmol/g/min	maximal stimulation (ouabain 10 µM)-evoked ^{3}H-aspartate release 22.8 {20.9, 24.7} pmol/g/min

Basal outflow and stimulation-evoked release in human tissue exceed the corresponding values in the guinea pig. The highest values are reached upon stimulation with 10 µM ouabain. If stimulation with 5 µM ouabain is compared between both models, release in human tissue exceeds release in the guinea pig by 240%. The authors suggest that the glutamatergic neurons are more active and in larger number in the human than in the guinea pig cortex.

Human [20]	**Guinea pig [21]**	**Rat [22]**
Synaptosomes; 4-aminopyridine (1 mM)	Synaptosomes; 4-aminopyridine (1 mM)	Synaptosomes; 4-aminopyridine (1 mM)
Increase in endogenous glutamate release from 0 to 5.4 nmol/mg protein	Increase in endogenous glutamate release from 0 to 2 nmol/mg protein	Increase in endogenous glutamate release from 0.20 to 2.45 nmol/mg protein

Ca^{2+}-dependent glutamate release was investigated in the studies referenced above and we see that the release of glutamate from human synaptosomes is considerably higher than in animal models. Vinje *et al*. measured a higher basal intracellular Ca^{2+} level in human tissue compared to other animal species which increased to much higher levels after depolarization. A higher level of Ca^{2+} leads to exocytosis from an increased number of synaptic vesicles, which could account for the higher release [20]. The reason for such interspecies differences remains to be investigated.

Human	Rat
Synaptosomes; K^+ (30 mM) [23, 24]	
Total content of 22.9 {20.7, 25.1} nM glutamate/mg synaptosomal protein	Total content of 47.2 {39.6, 54.8} nM glutamate/mg synaptosomal protein
Basal glutamate outflow (in % of total content): 16.4 {15, 17.8}	Basal glutamate outflow (in % of total content): 11.5 {10.2, 12.8}
K^+-evoked release (in % of total content): 20.3 {19.1, 21.5}	K^+-evoked release (in % of total content): 33.4 {31.8, 35.0}
No correlation between resection time and transmitter release.	Reduced total content of glutamate, basal outflow and evoked glutamate release after hypoxia (15 min, 30 min) compared to controls.

There are two possible explanations for the differences in total content and basal outflow of glutamate: A possible species difference or the pathological changes in the investigated human tissue (temporal lobe epilepsy) [23, 24]. With regard to different responses to hypoxic stimuli, it seems questionable if the surgical resection time in human tissue can be compared to the experimentally evoked hypoxia in rats.

Human [15]	Rat [16]
Brain slices; ischemic medium (no glucose, no O_2, saturated with 95% N_2, 5% CO_2)	
Release of endogenous glutamate after 18 min	Release of endogenous glutamate after 12 min

In ischemic strokes, the accumulation of extracellular glutamate appears to have a cytotoxic effect [15]. The cited studies investigated glutamate release of neocortical neurons under ischemic conditions. Marcoli *et al*. found that the properties of glutamate release are highly similar in both human and rat neurons [16]. An apparent difference is that human neurons released endogenous glutamate later than rat neurons with the same experimental protocol. The authors claim that this reflects the higher resistance of human neurons against ischemia [25].

Human	Rat
Synaptosomes; K^+ (15 mM)	Brain slices; K^+ (50 mM)
Gabapentin and Pregabalin (each 100 µM) did not influence the evoked release of ^{3}H-glutamate [14]	Gabapentin und Pregabalin (each 100 µM) reduced the evoked release of endogenous glutamate by 21% and 26%, respectively [26].
Apart from possible species differences, the diverse experimental protocols (stimulation with 15 mM K^+ vs. 50 mM K^+, measurement of ^{3}H-glutamate vs. endogenous glutamate, synaptosomes vs. brain slices) could be accountable for the conflicting results from the two studies [14]. Human [27]	Rat [27]
Synaptosomes; K^+ (15 mM), veratridine (1, 3.2, 10 µM)	
K^+-evoked ^{3}H-glutamate release *via* exocytosis and transporter reversal	K^+- evoked ^{3}H-glutamate release *via* exocytosis, not *via* transporter reversal
Veratridine-evoked ^{3}H-glutamate release *via* transporter reversal and exocytosis	Veratridine-evoked ^{3}H-glutamate release *via* transporter reversal, not by exocytosis
Carbamazepine, lamotrigine, phenytoin and levetiracetam (each 100 µM) without effect on K^+-evoked ^{3}H-glutamate release.	Reduction of K^+-evoked ^{3}H-glutamate release by carbamazepine, lamotrigine and phenytoin (each 100 µM); increase by levetiracetam (100 µM).

The effects of carbamazepine, lamotrigine and phenytoin in the rat were probably based on interaction with voltage-gated Ca^{2+}-channels that differ from the ones in humans [27]. By which mechanism levetiracetam increased the evoked release is unknown.

Human [17]	Rat [28, 29]
Synaptosomes; electrical	Brain slices, synaptosomes; K^+ (15, 50 mM)
Compared to control patients ^{3}H-glutamate-release in alcohol-dependant patients increased.	Compared to control rats ^{3}H-glutamate-release in alcohol-dependant rats decreased.

The differences in presynaptic glutamatergic neurotransmission after chronic alcohol consumption can be explained by the use of diverging stimuli (electrical vs K^+-stimulation), differences in the experimental setup, but a species difference has not been ruled out by the authors [17]. In contrast to most transmitter release studies with human tissue, Kuo and Dodd did not execute their study on vital tissue but from tissue obtained at autopsies [17]. Although few studies apparently found that unfixed frozen tissue can be used for synaptosomal preparations [30, 31, 32, 33], none of them can rule out alterations due to the time of death or the freezing process. Therefore, in this case, the source of tissue presents a confounder possibly only imitating a species difference.

GABA

Human [34]	Rabbit [34]
Brain slices; electrical	
Activated 5-HT_{1D}-heteroreceptors reduced release of endogenous ^{3}H-GABA.	Activated 5-HT_{1D}-heteroreceptors did not affect release of endogenous ^{3}H-GABA.

Expression and function of 5-HT-receptors show species differences [35, 36]. Obviously, findings from a rabbit model in connection with 5-HT-heteroreceptors cannot be transferred to the conditions in humans.

Human [23, 24]	Rat [23, 24]
Synaptosomes; K^+ (30 mM)	
Total content: 4.6 {4.2, 5.0} nM GABA/mg synaptosomal protein	Total content: 18.8 {15.9, 21.7} nM GABA/mg synaptosomal protein
Basal GABA outflow (% of total content): 17.3 {15.6, 19.0}	Basal GABA outflow (% of total content): 9.9 {9.0, 10.8}

The basal outflow of endogenous GABA differs in the two compared species. However, in contrast to evoked glutamate release (see above), evoked GABA release is not so different between humans and rats (human: 30.3 {28.6, 32.0}, rat: 34.9 {31.7, 38.1}, in % of total content) [23]. Either a species difference or pathological changes in epileptic human tissue utilized can explain the different results in basal outflow [24].

Human [14]	Rat [37]
Synaptosomes; K^+ (15 mM)	Brain slices; electrical
Gabapentin reduced evoked ^{3}H-GABA release by 39 %.	Gabapentin reduced evoked ^{3}H-GABA release only minimally (-12 %).

Gabapentin obviously has only minimal effects in rat neocortical tissue. It is difficult to compare both studies due to variations in the experimental protocols (tissue preparation, stimuli). In any case, a rat animal model does not seem preferable to investigate the mode of action of gabapentin in human tissue.

In the case of lamotrigine and valproate, a species difference is evident. In the human neocortex, lamotrigine presumably affects presynaptic Ca^{2+} channels rather than presynaptic Na^+ channels and seems to be more active on Ca^{2+} channels in humans than in rats [38].

When comparing these two studies, the experimental setup plays a crucial role. Russo *et al.* prepared synaptosomes from neocortical tissue [39]. They postulated an effect of the muscarinic receptor antagonists applied through receptors localized directly at glycine releasing terminals.

Human [38]	Rat [38]
Synaptosomes; K^+ (15 mM), veratridine (1, 3.2, 10 μM)	
K^+-evoked ^{3}H-GABA release mainly *via* exocytosis, partially *via* transporter reversal.	K^+-evoked ^{3}H-GABA release only *via* exocytosis.
Veratridine-evoked ^{3}H-GABA release *via* transporter reversal, withdrawal of extracellular Ca^{2+} (Ca^{2+}_e) leads to increased release of ^{3}H-GABA.	Veratridine-evoked ^{3}H-GABA release *via* transporter reversal and exocytosis, withdrawal of extracellular Ca^{2+} (Ca^{2+}_e) does not change release of ^{3}H-GABA.
Carbamazepine, lamotrigine, phenytoin (each 100 μM) and valproate (320 μM) reduced K^+-evoked ^{3}H-GABA release.	Only carbamazepine and phenytoin (each 100 μM reduced K^+-evoked ^{3}H-GABA release.
Carbamazepine, phenytoin, but not lamotrigine reduced veratridine-evoked ^{3}H-GABA release.	Carbamazepine, phenytoin and lamotrigine reduced veratridine-evoked ^{3}H-GABA release.

Glycine

Human [39]	Rat [40]
Synaptosomes; K^+ (15 mM)	Brain slices; K^+ (105 mM)
ACh increased ^{3}H-glycine release by activating M_4-receptors.	ACh reduced evoked ^{14}C-glycine release.

The endogenous ACh (Acetyl-Choline) may be released to activate muscarinic hetero-receptors as the precondition of a disinhibition by muscarinic antagonists of glycinergic nerve terminals exists. Unfortunately, no answer to this question is given by Russo *et al*. [39]. Benjamin and Quastel prepared brain slices [40]. As per these authors, the reduced ^{14}C-glycine release after stimulation is due to less uptake of ACh after adding ACh. This applies to neurons as well as to glia cells [40]. Synaptosomes are isolated nerve terminals [41], not embedded within a neuronal network. Effects of glial cells and interactions with other neurons cannot be detected using this form of tissue preparation. Therefore, the diverging effects might result from an existing species difference, but also the different forms of tissue preparation provide a possible explanation. Additionally, a sevenfold higher concentration of K^+-ions has been used for stimulation by Benjamin *et al*. compared to Russo *et al*. (see above), which also compromises the comparability of the two studies.

Acetylcholine (ACh)

Besides radioactive-labeled Ach, in some studies equally labeled choline was utilized. Choline reflects the action potential-evoked exocytotic release of ACh based on the experimentally determined release modalities [42]. In the following text, the term outflow will be used describing both basal and evoked exiting of the transmitter.

Human [6]	Rat [43]
Brain slices; electrical (different protocols)	
No evidence of presynaptic nicotinic auto-receptors	Evidence of presynaptic nicotinic auto-receptors

Feuerstein *et al*. found no evidence of presynaptic nicotinic autoreceptors at cholinergic terminals in humans [6], whereas Loiacono and Mitchelson apparently detected presynaptic nACh receptors in rat cortex [43]. There appeared to be an obvious species difference. However, a more recent study of Amtage *et al*. showed no evidence of presynaptic nicotinic autoreceptors in rat neocortex [44], which challenges the findings of Loiacono and Mitchelson [43].

Specifically, the substantially higher evoked outflow of ^{3}H-choline in the animal model suggests that the density of cholinergic projections in the guinea pig cortex is considerably higher than in human

Human [19]	Guinea pig [19]
Brain slices; electrical	
Basal ^{3}H-choline outflow (in pmol/g/min): 0.170 {0.155, 0.185}	Basal ^{3}H-choline outflow (in pmol/g/min): 0.228 {0.203, 0.253}
Evoked ^{3}H-choline outflow (in pmol/g/min): 0.186 {0.162, 0.210}	Evoked ^{3}H-choline outflow (in pmol/g/min): 3.11 {2.70, 3.52}

neocortex [19]. Unfortunately, the authors did not discuss the possible influence of different subtypes of intra-terminal enzymes between species. The choline-acetyltransferase (ChAT) (see below) might considerably affect the concentration of choline in the nerve terminals, leading to the observed differences.

Human [7, 45]	Mouse [42]
Brain slices, synaptosomes; electrical, K^+ (15 mM)	Brain slices; electrical
5-$HT_{1F/-3}$-heteroreceptors reduced the release of ACh.	5-$HT_{1B/2A}$-heteroreceptors increased the release of ACh.

5 HT-receptors on cholinergic terminals are discussed to play a role in cognition [42]. Still, classification of those receptors remains a difficult task. In two studies with human neocortical tissue, different subtypes of 5-HT-heteroreceptors were identified, namely 5-HT_{1F} [7] and 5-HT_3 [45]. In both cases release of ACh was reduced. Maura *et al.* postulated that the inhibitory effect of the receptor might be due to an existing, so far unknown metabotropic subtype of the receptor [45], which as per a new study does not exist [cf. 46]. Because of the lack of specifity of the substances used in those studies, a definite classification of the mentioned receptors seems premature [47]. In an older study in rat tissue, it was shown that 5-HT apparently increased the release of ACh by activating 5-HT_{1B}-rezeptors [48]. Rutz *et al.* attempted to confirm those findings in wildtype and 5-HT_{1B}-knock out mice, but succeeded only partially [42]. Indeed, release of ACh in 5-HT_{1B}-receptor knock out mice was significantly lower than in wildtype mice, but a 5-HT_{1B}-agonist showed no effect in wild type mice. The authors concluded that facilitation of release *via* 5-HT_{1B}-receptors might be due to an indirect effect, i.e. by activating other neurons [42]. Using receptor antagonists, it was determined that 5-HT_1- and 5-HT_{2A}-receptors increase the release of ACh with 5-HT itself having a minor effect [42]. This is due to its endogenous tone, which diminishes its own effect. Before specific ligands may be applied therapeutically, further studies with human tissue are needed.

Human [3]	Rat [3]
Brain slices; electrical	
Autoinhibitory feedback control of evoked ^{3}H-ACh release operative at stimulation frequencies ≥ 6 Hz	Autoinhibitory feedback control of evoked ^{3}H-ACh release operative at stimulation frequencies ≥ 3 Hz

According to the authors, the marked differences in auto-inhibition might be a result of the activity of the ACh esterase in the respective species. Higher activity of the enzyme in humans compared to the rat leads to lower levels of ACh at the auto-receptor, which is why auto-inhibition is activated only at higher stimulation frequencies [3].

Human [49]	Mouse [49]
Brain slices; electrical, K^+ (20 mM)	
In K^+-evoked ^{3}H-ACh release, a second stimulation (S_2) leads to considerably less transmitter release compared to the first stimulation (S_1) (S_2/S_1=0.49 {0.35, 0.62}); significantly less than in electrically evoked release (S_2/S_1=1.08 {0.96, 1.20})	In K^+-evoked ^{3}H-ACh release, a second stimulation (S_2) leads to only slightly less transmitter release compared to the first stimulation (S_1) (S_2/S_1=0.81 {0.68, 0.94}); ratio equivalent in electrically evoked release (S_2/S_1=0.84 {0.73,0.96})

How can the differences in human and mouse be explained? Sigle *et al.* found a species difference in the activity of the choline-acetyltransferase (ChAT) upon K^+-stimulation [49]. Whilst the activity increased after stimulation in human tissue, it did not

change in the animal model. ChAT catalyzes the synthesis of ACh from its precursor choline [50]. As per Sigle *et al*. the increase in ChAT activity leads to an increased demand for choline, which must be covered at least, in part, by membrane-derived choline – a condition found in neurodegenerative diseases such as Alzheimer's [49]. Wurtman proposed the theory of "autocannibalism" of cholinergic neurons, in which the depletion of intracellular ACh leads to synthesis of the transmitter from membrane-bound phosphatidylcholine, which ultimately proves lethal for the neuron [50]. Depletion of intracellular stores of ACh can be caused by prolonged depolarization, by continuous K^+-stimulation, as per experiments performed by Sigle *et al*., [49]. Clinically this may occur detrimentally in Alzheimer's disease, for instance, through hypoxia [49] or β-amyloid [51].

Human [52]	**Rat [52]**
Synaptosomes; Tat protein (1 nM, 3 nM)	
Maximum exocytotic ^{3}H-ACh release by Tat (1 nM): 88.2 {72.2, 104.2} pmol/mg protein.	Maximum exocytotic ^{3}H-ACh release by Tat (3 nM): 36.2 {28.3, 44.1} pmol/mg protein.
Evoked exocytotic ^{3}H-ACh release dependent on mobilization of both intracellular and extracellular Ca^{2+} as well as activation of voltage-sensitive Ca^{2+} channels.	Evoked exocytotic ^{3}H-ACh release only dependent on mobilization of intracellular Ca^{2+}.
mGluR (group I) on cholinergic nerve terminals mobilize intracellular Ca^{2+} *via* IP_3.	No mGluR (group I) on cholinergic nerve terminals; mobilization of intracellular Ca^{2+} *via* activation of ryanodine receptors.
Peptide fragments Tat 32-62, Tat 49-86 and Tat 41-60 responsible for protein-specific release of ACh.	Only peptide fragments Tat 49-86 und Tat 41-60 responsible for protein-specific release of ACh.

In human immunodeficiency virus (HIV-1) infection, 50% of the patients develop motor, cognitive, and behavioral impairments, whose origins are poorly understood [52]. Tat is a nonstructural viral protein known to be synthesized and actively released by HIV-1-infected cells [53], which was also detected in patients with progressive multifocal leukoencephalopathy [54]. To determine whether and how this protein influences cholinergic neurotransmission was the primary target of the study mentioned above [52]. Tat evokes release of ^{3}H-ACh in a Ca^{2+} dependent manner and by binding to different sites in the respective species [52]. These differences might become relevant once evidence emerges that Tat is indeed responsible for the neuropsychiatric symptoms of HIV-infected patients.

Human [11]	**Mouse [11]**
Brain slices; electrical	
High endocannabinoid tone on CB_1 receptors, which strongly reduced the release of ^{3}H-ACh	Low endocannabinoid tone on CB_1 receptors, which only slightly reduced the release of ^{3}H-ACh

Blockade of CB_1 receptors should show different effects in humans and mice for the following reason: Because of the high endocannabinoid tone at CB_1 receptors on cholinergic nerve terminals in humans cannabinoid antagonists might be possible therapeutic agents in the treatment of cholinergic degenerative diseases such as Alzheimer's disease [11].

Human [55]	**Rat [55]**
Brain slices; electrical	
Basal ^{3}H-ACh outflow (in % of tissue ^{3}H): 1.23 {1.21, 1.25}	Basal ^{3}H-ACh outflow (in % of tissue ^{3}H): 1.02 {0.99, 1.05}
Evoked ^{3}H-ACh release (in % of tissue ^{3}H): 0.99 {0.96, 1.02}	Evoked ^{3}H-ACh release (in % of tissue ^{3}H): 2.34 {2.29, 2.39}
Inhibition of evoked ^{3}H-ACh release by ACh esterase inhibitors (e.g. physostigmine) max. 26.2 % {19.0, 33.5}	Inhibition of evoked ^{3}H-ACh release by ACh esterase inhibitors (e.g. physostigmine) max. 68.9 % {61.9, 75.9}

Compared to human tissue, evoked release of ^{3}H-ACh in rat tissue is markedly higher. According to the authors, this higher release reflects the higher specific activity of the ACh esterase and a higher density of cholinergic neurons in the rat neocortex compared to the human neocortex. Inhibition of the enzyme in rat neocortex would lead to higher concentrations of endogenous ACh on inhibitory muscarinergic autoreceptors and a more pronounced inhibitory effect on transmitter release [55].

Dopamine (DA)

Human [56]	**Rat [56]**
Brain slices; electrical (different protocols)	
Maximum reduction in ^{3}H-dopamine release by quinpirole (1 μM): 75%	Maximum reduction in ^{3}H-dopamine release by quinpirole (10 μM): 45%

Although D_2 autoreceptors prevail in humans and rat, the D_2/D_3 agonist quinpirole appears to be more effective in human neocortex compared to rat tissue. As per the authors, brain slices of the rat cortex are therefore not an adequate model for the development of selective human D_2 antagonists, because they assumed different subtypes in rat and humans [56]. Also, the differences in transmitter release changes could be related to a different endogenous dopaminergic tone at the autoreceptor.

Human [10]	**Rat [10]**
Brain slices; electrical, K^+ (30 mM)	
Evidence of tonically activated CB_1 receptors modulating DA release	No evidence of an endocannabinoid tone on CB_1 receptors modulating DA release

Using the CB_1 agonist CP 55.940 and the CB_1 antagonist AM 251, tonically activated CB_1 receptors in human tissue could be determined, inhibiting the release of dopamine. Therefore, pharmacological blockade of CB_1-receptors for enhancing cognition seems promising in humans, but not in the rat [10].

Human [8]	**Mouse [8]**
Brain slices; electrical	
Inhibitory D_2 autoreceptors	No inhibitory D_2 autoreceptors
Human [8]	**Rat [8]**
Brain slices; electrical	
nAChR on dopaminergic nerve terminals	no evidence of nAChR on dopaminergic nerve terminals

Löffler *et al*. confirmed the existence of D_2 autoreceptors in human cortex first described by Fedele *et al*., whereas no evidence of such receptors was found in mice [8, 56]. From the differences between human, rat and mouse, experiments with an animal model as substitute do not seem useful when investigating properties of human D_2 autoreceptors.

Löffler *et al*. also showed the existence of nAChR in human and rat cortex [8]. While there was evidence of these receptors in human tissue, the experiments in rat tissue showed no proof of nAChR. In this case too, a rat animal model would not serve as a substitute for experiments investigating human nicotinic receptors.

Noradrenaline (NA)

Human [57]	**Rat [58]**
Brain slices; NMDA (different concentrations)	
Maximum release of ^{3}H-NA caused by NMDA (3 mM): 1.87 {1.84, 1,90} % of tissue tritium	Maximum release of ^{3}H-NA caused by NMDA (3 mM): 13.9 {11.67, 16.13} % of tissue tritium

The differences in transmitter release caused by NMDA are primarily quantitative. This discrepancy might be explained by different receptor densities or epileptic pathologies in the case of the human tissue used by Fink *et al*. [57].

Human [59]	**Rat [58]**
Brain slices; veratridine (10 μM)	
Veratridine-evoked ^{3}H-NA release of 8.54% {7.64, 9.44} of total tissue tritium.	Veratridine-evoked ^{3}H-NA release of 63.30% {58.8, 67.8} of total tissue tritium.

In contrast to K^+-stimulation, where the authors found similar effects in both species, veratridine appeared to be a far more potent stimulus in the rat neocortex compared to human tissue.

Human [60]	**Mouse [60]**
Brain slices; electrical	
Maximum reduction in evoked ^{3}H-NA release by histamine (10 μM): 30%	Maximum reduction in evoked ^{3}H-NA release by histamine (10 μM): 60%

Even though both species show that the histamine receptors present on noradrenergic terminals could be classified as H_3 receptors [60], they obviously differ functionally. From the transmitter release studies, no satisfactory explanation for the differing results above was found.

Human [5]	**Rat [61]**
Brain slices; electrical	
Presynaptic α_{2A}-autoreceptors	Presynaptic α_{2D}-autoreceptors
Rabbit [61]	**Mouse [62]**
Brain slices; electrical	
Presynaptic α_{2A}-autoreceptors	Presynaptic α_{2D}-autoreceptors
Guinea pig [63]	**Monkey (Cercopithecus aethiops) [64]**
Brain slices; electrical	
Presynaptic α_{2D}-autoreceptors	Presynaptic $\alpha_{2A/D}$-autoreceptors

Adrenergic presynaptic autoreceptors were classified in human neocortex and in five other species by the use of transmitter release studies. Surprisingly, the receptor subtype in the monkey was not identical to the subtype in humans, although of all species tested, monkeys are phylogenetically most similar to humans.

N/OFQ shows similar potency in humans (pEC_{50} = 7.74), rats (pEC_{50} = 7.64) and mice (pEC_{50} = 7.50), but its maximum effect (at 1 μM) is considerably higher in rats and mice than in humans and guinea

Human [9]	**Rat [9, 65]**
Brain slices; electrical (different protocols)	
Maximum reduction in evoked ^{3}H-NA release by N/OFQ: 36.9% {32.4, 41.8}	Maximum reduction in evoked ^{3}H-NA release by N/OFQ: 66% {61.7, 72.7} / 71 {69, 73}
Guinea pig [65]	**Mouse [65]**
Brain slices; electrical	
Maximum reduction by N/OFQ of evoked ^{3}H-NA release: 36 % {29, 43}	Maximum reduction by N/OFQ of evoked ^{3}H-NA release: 80 % {79, 81}

pigs [9, 65]. Rominger *et al*. concluded that in each species investigated, ORL1-receptors are evident, but possible species differences in receptor density, mechanisms of signal transduction or degradation of transmitter may prevail [9].

Human [44, 66]	**Rat [44]**
Brain slices; nicotine, DMPP, electrical	Brain slices; nicotine, nicotinic agonists, electrical
High density of nAChR on the presynaptic noradrenergic nerve terminals	Low density/no nAChR on the presynaptic noradrenergic nerve terminals

In both studies, a high density of nAChR in human neocortex was observed [44, 66]. Amtage *et al*. remarked that some nAChR facilitating ^{3}H-NA release is likely localized at the glutamatergic nerve terminals and ruled out the presence of nAChR on the noradrenergic nerve terminals in rat cortex, for the most part [44]. This is in opposition to the results of Anderson *et al*. who found evidence of such receptors [44], but never tested sensitivity to tetrodotoxin (TTX) as a functional proof for a presynaptic location. Release of NA is likely a result of the activation of TTX-sensitive Na^+ channels upon nicotinic depolarization leading to exocytosis [44]. Only high concentrations of nicotine induced the release of NA in rat neocortex; however this effect was unspecific (no sensitivity to TTX) and unrelated to exocytosis (no sensitivity to extracellular Ca^{2+}) [44], which also questions the findings of Anderson *et al*. [67]. Regardless, the

results in human tissue seem verified. As per Amtage *et al*. ligands of nAChR are promising pharmacological targets in the treatment of nicotine addiction [44], since the receptor density of nicotinic receptors in smokers are markedly increased [68], and may be diminished by treatment with appropriate nAChR agonists.

Human [4]	Rat [4, 69]
Brain slices; electrical	Brain slices; K^+ (20 mM), electrical
δ-opioid receptors and ORL1-receptors present on noradrenergic nerve terminals	μ-opioid receptors and ORL1- receptors present on noradrenergic nerve terminals

Based on the apparent species differences, effects of subtype-specific opioid drugs should not be transferred from rat to the conditions in humans [4]. Apart from their opioid receptor differences, noradrenergic nerve endings in both species seem to be equipped with ORL1- receptors.

Serotonin (5-HT)

Human [4]	Rat [4, 70]
Brain slices; electrical	
κ-opioid receptors and ORL1-receptors on 5-HT nerve terminals	μ- and κ-opioid as well as ORL1-receptors on 5-HT nerve terminals

Both noradrenergic and serotonergic presynaptic nerve terminals in the neocortex of humans do not show evidence for μ-opioid receptors. Because of the differences, rats are not a suitable animal model with respect to opioid receptors.

Cannabinoids

Human [71]	Rat [71]
Brain slices; K^+ (50 mM)	
Basal anandamide level: 21.1 pmol/g tissue	Basal anandamide level: 3.2 pmol/g tissue
anandamide level after stimulation: 70.5 pmol/g tissue	anandamide level after stimulation: 14.3 pmol/g tissue

Upon K^+ stimulation, anandamide is released from both human and rat tissue. The basal levels and post stimulation of anandamide is considerably higher in human tissue, a fact that should be remembered when transferring results from a rat model to humans.

Histamine

Human [72]	Rat [73]
Brain slices; K^+ (30 mM)	
Evoked release: 4.2% {3.2, 5.2} (of total ^{3}H-histamine released over spontaneous efflux)	Evoked release: 12.9% {12.1, 13.7} (of total ^{3}H-histamine released over spontaneous efflux)

Apart from a possible species differences, the authors could not rule out the influence of anesthesia on the human tissue results [72].

Peptides

Human [74]	Rat [75]
Synaptosomes; K^+ (15 mM)	
CGP 47656 as a ligand for $GABA_B$ heteroreceptors reduced the release of cholecystokinin	no effect of CGP 47656 on the release of cholecystokinin

Even in concentrations up to 300 μM, CGP 47656 had no effect on $GABA_B$ receptor-mediated release; therefore, the authors postulated that $GABA_B$ heteroreceptors differ pharmacologically in the respective species [74].

CONCLUSION

For decades, transmitter release studies have shaped pharmacological research, and functional characterization has been carried out on numerous substances and receptors. Based on the experimental setup and tissue preparation, it is possible to measure presynaptic transmitter release from isolated nerve terminals or from cells embedded in a neuronal network [see 49, 52]. Ideally, a quantifiable effect can be allocated to a presynaptic structure. This isolated view is of course far from the actual conditions in *in vivo* tissue, limiting the general validity of the technique. Compared to other

electrophysiological methods (e.g. patch clamp) release studies are resource and labor effective. However, they lack the sensitivity and temporal resolution of electrophysiological and electrochemical approaches [13], where the function of individual ion channels of single physiological or pathological cells can be studied [76]. A singular advantage of transmitter release studies is the ability to test many samples simultaneously [96, 67]. Thus, the released transmitter can be averaged over many nerve terminal preparations, which can eliminate inter-synaptic variability [13]. Due to the lack of sensitivity, experimentally determined differences cannot always be isolated to be actual inter-species differences. Detecting a possible difference is easier if the experimental conditions are as identical as possible. This concerns the selection of tissue, form of preparation and stimulation technique. For example, if a species difference is detected, but a comparison between brain slices and synaptosomes a re carried out, the possibility remains that the differences can be due to confounders (e.g. glial cells) [see 39, 40]. Use of (possibly) pathologically transformed tissue is a confounding variable, which affects all techniques using human tissue. Since it would be highly unethical, i.e. impossible, to remove tissue from healthy subjects, the use of altered tissue is a necessity that needs to be coped with. Another significant possible confounder limiting the sensitivity of the transmitter release technique is the stimulation method. Equal stimuli usually lead to transmitter release *via* the same mechanism in every species. Electrical stimulation, for example, mostly leads to excocytosis regardless of the species investigated. Exceptions exist for the release modalities of glutamatergic and GABAergic nerve terminals [27, 38]. Future release studies, which include a comparison of species in their protocol, should consider the respective release modalities to eliminate or reduce this confounder.

Apart from other pharmacological techniques such as patch clamp studies etc., all of which provide a substitute for experiments with *in vivo* structures, the significance of transmitter release is also challenged by actual studies on living patients such as functional MRI studies etc [77].

CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

REFERENCES

1. Mann, P. J., Tennenbaum, M. and Quastel, J. H. 1939, Biochem. J., 33(5), 822-835.
2. Straus, O. H. and Goldstein, A. 1943, J. Gen. Physiol., 26(6), 559-579.
3. Albrecht, C., Bloss, H. G., Jackisch, R. and Feuerstein, T. J. 1999, Exp. Brain Res., 128(3), 383-389.
4. Berger, B., Rothmaier, A. K., Wedekind, F., Zentner, J., Feuerstein, T. J. and Jackisch, R. 2006, Br. J. Pharmacol., 148(6), 795-806.
5. Feuerstein, T. J., Huber, B., Vetter, J., Aranda, H., van Velthoven, V. and Limberger, N. 2000, J. Pharmacol. Exp. Ther., 294(1), 356-362.
6. Feuerstein, T. J., Lehmann, J., Sauermann, W., van Velthoven, V. and Jackisch, R. 1992, Brain Res., 572(1-2), 64-71.
7. Feuerstein, T. J. and Seeger, W. 1997, Pharmacol. Ther., 74(3), 333-347.
8. Löffler, M., Bubl, B., Huethe, F., Hubbe, U., McIntosh, J. M., Jackisch, R. and Feuerstein, T. J. 2006, Brain Res. Bull., 68(5), 361-373.
9. Rominger, A., Forster, S., Zentner, J., Dooley, D. J., McKnight, A. T., Feuerstein, T. J. and Vlaskovska, M. 2002, Br. J. Pharmacol., 135(3), 800-806.
10. Steffens, M., Engler, C., Zentner, J. and Feuerstein, T. J. 2004, Br. J. Pharmacol., 141(7), 1193-1203.
11. Steffens, M., Szabo, B., Klar, M., Rominger, A., Zentner, J. and Feuerstein, T. J. 2003, Neuroscience, 120(2), 455-465.
12. Feuerstein, T. J., Sauermann, W., Allgaier, C., Agneter, E. and Singer, E. A. 1994, Naunyn-Schmiedeberg's Arch Pharmacol., 350(1), 1-9.
13. Khvotchev, M. and Kavalali, E. T. 2008, Measuring Exocytosis. Biochemical Approaches to Measuring Neurotransmitter Release. In. T. C. Südhof, K. Starke and S. Boehm (Eds.): Pharmacology of neurotransmitter release, Springer (Handbook of experimental pharmacology, 184), Berlin.
14. Brawek, B., Löffler, M., Weyerbrock, A. and Feuerstein, T. J. 2009, Naunyn Schmiedebergs Arch. Pharmacol., 379(4), 361-369.
15. Marcoli, M., Bonfanti, A., Roccatagliata, P., Chiaramonte, G., Ongini, E., Raiteri, M. and Maura, G. 2004, Neuropharmacology, 47(6), 884-891.

16. Marcoli, M., Raiteri, L., Bonfanti, A., Monopoli, A., Ongini, E., Raiteri, M. and Maura, G. 2003, Neuropharmacology, 45(2), 201-210.
17. Kuo, S. and Dodd, P. R. 2011, Neurosignals, 19(3), 117-127.
18. Hegstad, E., Berg-Johnsen, J., Haugstad, T. S., Hauglie-Hanssen, E. and Langmoen, I. A. 1996, Acta Neurochir (Wien), 138(2), 234-241.
19. Beani, L., Bianchi, C., Antonelli, T., Calo, G., Morari, M., Ferioli, V. and Gaist, G. 1992, J. Neurochem., 58(4), 1454-1459.
20. Vinje, M. L., Valo, E. T., Roste, G. K. and Berg-Johnsen, J. 1999, Brain Res., 843(1-2), 199-201.
21. Tibbs, G. R., Barrie, A. P., van Mieghem, F. J., McMahon, H. T. and Nicholls, D. G. 1989, J. Neurochem., 53(6), 1693-1699.
22. Larsen, M., Valo, E. T., Berg-Johnsen, J. and Langmoen, I. A. 1998, Eur. J. Anaesthesiol., 15(2), 224-229.
23. Hoogland, G., Blomenrohr, M., Dijstelbloem, H., Wit, M., de Spierenburg, H. A., van Veelen, C. W., van Rijen, P. C. van Huffelen, A. C., Gispen, W. H. and de Graan, P. N. 1999, Brain Res., 837(1-2), 55-66.
24. Hoogland, G., Hens, J. J., Wit, M. de, van Veelen, C. W., van Huffelen, A. C., Gispen, W. H. and de Graan, P. N. 2000, J. Neurosci. Res., 60(5), 686-695.
25. Jiang, C. and Haddad, G. G. 1992, J. Neurophysiol. 68(6), 2165-2173.
26. Dooley, D. J., Mieske, C. A. and Borosky, S. A. 2000, Neurosci. Lett., 280(2), 107-110.
27. Kammerer, M., Brawek, B., Freiman, T. M., Jackisch, R. and Feuerstein, T. J. 2011, Naunyn-Schmiedeberg's Arch Pharmacol., 383(5), 531-542.
28. Keller, E., Cummins, J. T. and von Hungen, K. 1983, Substance and Alcohol Actions Misuse, 4(6), 383-392.
29. Sabria, J., Torres, D., Pasto, M., Peralba, J. M., Allali-Hassani, A. and Pares, X. 2003, Addict Biol., 8(3), 287-294.
30. Deutsch, C., Drown, C., Rafalowska, U. and Silver, I. A. 1981, J. Neurochem., 36(6), 2063-2072.
31. Dodd, P. R., Hardy, J. A., Baig, E. B., Kidd, A. M., Bird, E. D., Watson, W. E. J. and Johnston, G. A. R. 1986, Neurochem. Pathol., 4(3), 177-198.
32. Hardy, J. A., Dodd, P. R., Oakley, A. E., Perry, R. H., Edwardson, J. A. and Kidd, A. M. 1983, J. Neurochem., 40(3), 608-614.
33. Tibbs, G. R., Dolly, J. O. and Nicholls, D. G. 1996, J. Neurochem., 67(1), 389-397.
34. Feuerstein, T. J., Huring, H., van Velthoven, V., Lucking, C. H. and Landwehrmeyer, G. B. 1996, Neurosci. Lett., 209(3), 210-214.
35. Waeber, C. and Palacios, J. M. 1992, Synapse, 12(4), 261-270.
36. Waeber, C., Schoeffter, P., Palacios, J. M. and Hoyer, D. 1989, Naunyn Schmiedebergs Arch. Pharmacol., 340(5), 479-485.
37. Parker, D. A. S., Ong, J., Marino, V., Kerr, D. I. B. 2004, Eur. J. Pharmacol., 495(2-3), 137-143.
38. Kammerer, M., Rassner, M. P., Freiman, T. M. and Feuerstein, T. J. 2011, Naunyn Schmiedebergs Arch. Pharmacol., 384(1), 47-57.
39. Russo, C., Marchi, M., Andrioli, G. C., Cavazzani, P. and Raiteri, M. 1993, J. Pharmacol. Exp. Ther., 266(1), 142-146.
40. Benjamin, A. M. and Quastel, J. H. 1977, Can. J. Physiol. Pharmacol., 55(3), 347-355.
41. Nicholls, D. G. 2003, Neurochem. Res., 28(10), 1433-1441.
42. Rutz, S., Riegert, C., Rothmaier, A. K., Buhot, M-C., Cassel, J-C. and Jackisch, R. 2006, Brain Res. Bull., 70(1), 81-93.
43. Loiacono, R. E. and Mitchelson, F. J. 1990, Naunyn Schmiedebergs Arch. Pharmacol., 342(1), 31-35.
44. Amtage, F., Neughebauer, B., McIntosh, J. M., Freiman, T., Zentner, J., Feuerstein, T. J. and Jackisch, R. 2004, Brain Res. Bull., 62(5), 413-423.
45. Maura, G., Andrioli, G. C., Cavazzani, P. and Raiteri, M. 1992, J. Neurochem., 58(6), 2334-2337.
46. Lochner, M. and Thompson, A. J. 2015, A review of fluorescent ligands for studying 5-HT receptors. In: Neuropharmacology, 98, 31-40.
47. Fink, K. B. and Gothert, M. 2007, Pharmacol. Rev., 59(4), 360-417.
48. Consolo, S., Arnaboldi, S., Ramponi, S., Nannini, L., Ladinsky, H. and Baldi, G. 1996, J. Pharmacol. Exp. Ther., 277(2), 823-830.

49. Sigle, J-P, Zander, J., Ehret, A., Honegger, J., Jackisch, R. and Feuerstein, T. J. 2003, Brain Res. Bull., 60(3), 255-262.
50. Wurtman, R. J. 1992, Trends Neurosci., 15(4), 117-122.
51. Good, T. A., Smith, D. O. and Murphy, R. M. 1996, Biophys. J., 70(1), 296-304.
52. Feligioni, M., Raiteri, L., Pattarini, R., Grilli, M., Bruzzone, S., Cavazzani, P., Raiteri, M., Pittaluga, A. 2003, J. Neurosci., 23(17), 6810-6818.
53. Ensoli, B., Buonaguro, L., Barillari, G., Fiorelli, V., Gendelman, R., Morgan, R. A., Wingfield, P. and Gallo, R. C. 1993, J. Virol., 67(1), 277-287.
54. Del Valle, L., Croul, S., Morgello, S., Amini, S., Rappaport, J. and Khalili, K. 2000, J. Neurovirol., 6(3), 221-228.
55. Jackisch, R., Forster, S., Kammerer, M., Rothmaier, A. K. Ehret, A., Zentner, J. and Feuerstein, T. J. 2009, J. Alzheimers Dis., 16(3), 635-647.
56. Fedele, E., Fontana, G., Munari, C., Cossu, M. and Raiteri, M. 1999, Eur. J. Neurosci., 11(7), 2351-2358.
57. Fink, K., Schultheiss, R. and Gothert, M. 1992, Br. J. Pharmacol., 106(1), 67-72.
58. Fink, K., Gothert, M., Molderings, G. and Schlicker, E. 1989, Naunyn Schmiedebergs Arch. Pharmacol, 339(5), 514-521.
59. Fink, K, Schultheiss, R. and Gothert, M. 1992, Naunyn Schmiedebergs Arch. Pharmacol. 345(6), 700-703.
60. Schlicker, E., Werthwein, S. and Zentner, J. 1999, Fundam Clin. Pharmacol., 13(1), 120-122.
61. Trendelenburg, A. U., Limberger, N. and Starke, K. 1993, Naunyn Schmiedebergs Arch. Pharmacol., 348(1), 35-45.
62. Limberger, N., Trendelenburg, A. U. and Starke, K. 1995, Naunyn Schmiedebergs Arch. Pharmacol., 352(1), 43-48.
63. Trendelenburg, A. U., Limberger, N. and Starke, K. 1995, Naunyn Schmiedebergs Arch. Pharmacol., 352(1), 49-57.
64. Trendelenburg, A. U., Sutej, I. and Starke, K. 1997, Naunyn Schmiedebergs Arch. Pharmacol., 355(3), 341-346.
65. Schlicker, E., Werthwein, S., Kathmann, M. and Bauer, U. 1998, Naunyn Schmiedebergs Arch. Pharmacol., 358(4), 418-422.
66. Woo, R. S., Park, E. Y., Shin, M. S., Jeong, M. S., Zhao, R. J., Shin, B. S., Kim C. J., Park, J. W. and Kim, K. W. 2002, Br. J. Pharmacol., 137(7), 1063-1070.
67. Anderson, D. J., Puttfarcken, P. S., Jacobs, I. and Faltynek, C. 2000, Neuropharmacology, 39(13), 2663-2672.
68. Perry, D. C., Davila-Garcia, M. I., Stockmeier, C. A. and Kellar, K. J. 1999, J. Pharmacol. Exp. Ther., 289(3), 1545-1552.
69. Werling, L. L., Brown, S. R. and Cox, B. M. 1987, Neuropharmacology, 26(7B), 987-996.
70. Siniscalchi, A., Rodi, D., Beani, L. and Bianchi, C. 1999, Br. J. Pharmacol., 128(1), 119-123.
71. Steffens, M., Feuerstein, T. J., van Velthoven, V., Schnierle, P. and Knorle, R. 2003, Naunyn Schmiedebergs Arch. Pharmacol., 368(5), 432-436.
72. Arrang, J. M., Devaux, B., Chodkiewicz, J. P. and Schwartz, J. C. 1988, J. Neurochem., 51(1), 105-108.
73. Arrang, J. M., Garbarg, M. and Schwartz, J. C. 1983, Nature, 302(5911), 832-837.
74. Raiteri, M., Bonanno, G., Paudice, P., Cavazzani, P. and Schmid, G. 1996, J. Pharmacol. Exp. Ther., 278(2), 747-751.
75. Gemignani, A., Paudice, P., Bonanno, G. and Raiteri, M. 1994, Mol. Pharmacol., 46(3), 558-562.
76. Liem, L. K., Simard, J. M., Song, Y. and Tewari, K. 1995, Neurosurgery, 36(2), 382-392.
77. Cai, K., Haris, M., Singh, A., Kogan, F., Greenberg, J. H., Hariharan, H., Detre, J. A. and Reddy, R. 2012, Nat. Med., 18(2), 302-306.