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BRAIN DOPAMINE AND SEROTONIN RECEPTORS IN THE PERCEPTION OF PAIN

Positron Emission Tomography Studies in Healthy Subjects

by

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ABSTRACT

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Department of Physiology, Institute of Biomedicine; Turku PET Centre, and Department of Psychiatry, University of Turku, Turku, Finland.
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The role of dopamine and serotonin in spinal pain regulation is well established. However, little is known concerning the role of brain dopamine and serotonin in the perception of pain in humans.

The aim of this study was to assess the potential role of brain dopamine and serotonin in determining experimental pain sensitivity in humans using positron emission tomography (PET) and psychophysical methods. A total of 39 healthy subjects participated in the study, and PET imaging was performed to assess brain dopamine D2/D3 and serotonin 5-HT_{1A} receptor availability. In a separate session, sensitivity to pain and touch was assessed with traditional psychophysical methods, allowing the evaluation of potential associations between D2/D3 and 5-HT_{1A} binding and psychophysical responses. The subjects' responses were also analyzed according to Signal Detection Theory, which enables separate assessment of the subject's discriminative capacity (sensory factor) and response criterion (non-sensory factor).

The study found that the D2/D3 receptor binding in the right putamen was inversely correlated with pain threshold and response criterion. 5-HT_{1A} binding in cingulate cortex, inferior temporal gyrus and medial prefrontal cortex was inversely correlated with discriminative capacity for touch. Additionally, the response criterion for pain and intensity rating of suprathreshold pain were inversely correlated with 5-HT_{1A} binding in multiple brain areas.

The results suggest that brain D2/D3 receptors and 5-HT_{1A} receptors modulate sensitivity to pain and that the pain modulatory effects may, at least partly, be attributed to influences on the response criterion. 5-HT_{1A} receptors are also involved in the regulation of touch by having an effect on discriminative capacity.

Key words: Dopamine, D2/D3 receptor, 5-HT_{1A} receptor, pain, positron emission tomography, serotonin, touch.

TIIVISTELMÄ

Ilkka K. Martikainen

AIVOJEN DOPAMIINI- JA SEROTONIINIRESEPTORIT KIVUN AISTIMISESSA

Positroniemissiotomografiatutkimuksia terveillä koehenkilöillä

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Monoamiinit dopamiini ja serotoniini ovat keskeisiä välittäjäaineita ihmisaivoissa. Selkäydintason kivunsäätelyssä dopamiinin ja serotoniinin merkitys tunnetaan jo verrattain hyvin, mutta aivojen dopamiini- ja serotoniinireseptorien merkityksestä ihmisen kivun aistimisessa tiedetään vähän. Tutkimuksen tarkoituksena oli selvittää aivojen kahden tärkeän välittäjäaineen, dopamiinin ja serotoniinin, merkitystä ihmisen herkkyydessä kokeelliselle kivulle käyttämällä positroniemissiotomografiaa (PET) ja psykofyysisiä menetelmiä. Yhteensä 39 tervettä koehenkilöä otettiin mukaan tutkimukseen. PET-kuvauksella mitattiin aivojen dopamiini D2/D3- ja serotoniini 5-HT_{1A}-reseptorisitoutumista. Erillisellä käynnillä mitattiin koehenkilöiden herkkyyttä kivulle ja kosketukselle perinteisillä psykofyysisillä menetelmillä, jotta voitaisiin selvittää mahdolliset yhteydet aivojen D2/D3- ja 5-HT_{1A}-reseptorisitoutumisen sekä kipu- ja kosketusvasteiden välillä. Perinteisten psykofyysisten menetelmien lisäksi koehenkilöiden vasteet ärsykkeille analysoitiin käyttäen signaalindetektioteoriaa, jonka avulla koehenkilön vasteista voidaan erikseen analysoida signaalindetektioteorian mukainen erottelukyky (sensorinen tekijä) ja kriteeritaso (muut kuin sensoriset tekijät).

Tutkimus osoitti, että D2/D3-reseptorisitoutuminen oikeassa putamenissa oli kääntäen verrannollista kipukynnykseen ja kriteeritasoon, mutta kosketusvasteet eivät olleet verrannollisia D2/D3-reseptorisitoutumiseen millään tutkitulla alueella. Sitä vastoin 5-HT_{1A}-reseptorisitoutuminen cingulumissa, alemmassa ohimopoimussa ja sisemmässä etuotsalohkossa olivat kääntäen verrannollisia koehenkilön kosketusärsykkeen erottelukykyyn. Lisäksi kuumakivun kriteeritaso ja voimakkuusarvio ylikynnykselliselle kipuärsykkeelle olivat kääntäen verrannollisia 5-HT_{1A}-reseptorisitoutumiseen useilla aivokuoren ja aivokuorenalaisilla aivoalueilla. Tutkimuksen tulokset osoittavat, että aivojen välittäjäaineet dopamiini ja serotoniini liittyvät ihmisen kivun aistimiseen. Aivojen dopamiini D2/D3- ja serotoniini 5-HT_{1A}-reseptorit säätelevät kipuherkkyyttä, ja tämä kipua säätelevä vaikutus perustuu ainakin osittain vaikutuksilla kriteeritasoon. Aivojen serotoniini 5-HT_{1A}-reseptorit liittyvät myös kosketustunnon säätelyyn vaikuttamalla kosketusärsykkeen erottelukykyyn.

Avainsanat: Dopamiini, D2/D3-reseptori, 5-HT_{1A}-reseptori, kipu, kosketus, positroniemissio-tomografia, serotoniini.

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ABBREVIATIONS

ACC	anterior cingulate cortex
ANOVA	analysis of variance
B_{Avail}	concentration of receptors available for binding
BA	Brodmann area
BP	binding potential
CNS	central nervous system
COMT	catechol-*O*-methyltransferase
CPP	cold pressor pain
CPT	cold pressor test
DAT	dopamine transporter
DOPAC	dihydroxyphenylacetic acid
DRN	dorsal raphe nucleus
FDOPA	fluoro-L-DOPA
FLB 457	(S)-*N*-((1-ethyl-2-pyrrolidinyl)methyl)-5-bromo-2,3-dimethoxybenzamide
fMRI	functional magnetic resonance imaging
GABA	γ-aminobutyric acid
GPe	globus pallidus, pars externa
GPi	globus pallidus, pars interna
5-HT	5-hydroxytryptamine, serotonin
HVA	homovanillinic acid
K_D	equilibrium dissociation constant
L-DOPA	L-3,4-dihydroxyphenylalanine
MAO	monoamine oxidase
MPQ	McGill Pain Questionnaire
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
NAcc	nucleus accumbens
NRM	nucleus raphe magnus
8-OH-DPAT	8-hydroxy-2-(di-*n*-propylamino)tetralin
PANAS	Positive and Negative Affectivity Scale
PD	Parkinson's disease
PET	positron emission tomography
Raclopride	(S)-(-)-3,5-dichloro-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-2-hydroxy-6-methoxy-benzamide
RLS	restless legs syndrome
ROC	receiver operating characteristic
ROI	region of interest
RVM	rostroventromedial medulla
S1	primary somatosensory cortex
SD	standard deviation
SDT	Signal Detection Theory (or: Sensory Decision Theory)

SNc	substantia nigra, pars compacta
SNr	substantia nigra, pars reticulata
SPA	stimulation-produced analgesia
SPM	statistical parametric mapping
STN	subthalamic nucleus
V_T	distribution volume
VTA	ventral tegmental area
WAY-100635	*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridyl)cyclohexanecarboxamide trihydrochloride

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by the Roman numerals **I-IV**:

I Pertovaara A, Martikainen IK, Hagelberg N, Mansikka H, Någren K, Hietala J, Scheinin H. Striatal dopamine D2/D3 receptor availability correlates with individual response characteristics to pain. *European Journal of Neuroscience* 2004; 20: 1587-1592.

II Martikainen IK, Hagelberg N, Mansikka H, Hietala J, Någren K, Scheinin H, Pertovaara A. Association of striatal dopamine D2/D3 receptor binding potential with pain but not tactile sensitivity or placebo analgesia. *Neuroscience Letters* 2005; 376: 149-153.

III Martikainen IK, Hirvonen J, Kajander J, Hagelberg N, Mansikka H, Någren K, Hietala J, Pertovaara A. Correlation of human cold pressor pain responses with 5-HT_{1A} receptor binding in the brain. *Brain Research* 2007; 1172: 21-31.

IV Martikainen IK, Hirvonen J, Pesonen U, Hagelberg N, Laurikainen H, Tuikkala H, Kajander J, Någren K, Hietala J, Pertovaara A. Differential associations between brain 5-HT_{1A} receptor binding and response to pain versus touch. *Journal of Neural Transmission* 2009; 116: 821-830.

1. INTRODUCTION

The sensation of pain alerts us of real or potential tissue injury and initiates necessary protective behaviour that permits healing. The International Association for the Study of Pain (IASP) classifies pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (IASP Task Force on Taxonomy 1994). The perception of pain, however, is not an invariant consequence of activation of a peripheral nociceptor by a tissue-damaging stimulus: the response to pain also depends on the net effect of pain-modulating circuits on the nociceptive signal, and eventually, non-sensory factors influencing the evaluation and reporting of the sensation. The differences in top-down modulation of the nociceptive signal as well as sensory decision-making are assumed to be significant factors underpinning differences in pain sensitivity between individuals. Furthermore, dysfunction of pain-modulating circuits or an altered criterion to report pain may explain some characteristics of clinical pain. Little is known of brain receptors that determine pain sensitivity in humans, and very little is known of receptors that regulate the decision-making process in response to pain. An understanding of brain receptors and circuitry determining pain sensitivity in healthy humans could potentially help to understand physiological and pathological pain, and provide a rational basis for the development of pain therapy.

The monoamine neurotransmitters dopamine and serotonin are important in regulating human behavior. Dopamine and serotonin exert their effects in the brain by binding to specific receptors. Dopamine acts on two receptor classes, the D1- and D2-like receptors. Serotonin acts on a total of 14 receptor subtypes, receptor subtype 1A being to date one of the best characterized. The serotonin 5-HT_{1A} receptor is expressed as an autoreceptor in raphe nuclei, and thus is a key regulator of brain serotonergic neurotransmission. Experiments with animals suggest that dopamine D2/D3 and serotonin 5-HT_{1A} receptors control pain both in the spinal cord and in the brain. Furthermore, there is some evidence from human studies suggesting that brain D2/D3 receptors are involved in the perception of pain. The studies presented in this thesis aimed to study the potential associations between the binding potential of two major neurotransmitter receptors expressed in the human brain, the dopamine D2/D3 receptor and the serotonin 5-HT_{1A} receptor, and response to pain in healthy subjects.

2. REVIEW OF THE LITERATURE

2.1 Dopaminergic system

2.1.1 Dopamine

Dopamine is a biogenic amine neurotransmitter, which with norepinephrine and epinephrine belongs to a subgroup of catecholamines. The structural features of catecholamines are the single amino group, a catechol nucleus, which is a benzene ring with two adjacent hydroxyl groups, and an ethylamine (or a derivative) side chain attached in its 1 position. Dopamine is abundant in the mammalian central nervous system (CNS) and particularly in the striatum, constituting 80 % of the catecholamine content in the brain (Cooper et al. 2003), and was first proposed to serve as a neurotransmitter in 1950s (Carlsson 1959). Dopamine is synthesized from essential amino acid L-tyrosine in a biosynthetic pathway containing two enzymes: tyrosine hydroxylase (TH) and aromatic L-amino acid decarboxylase (AADC). The first enzyme, TH, converts tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA). The oxidation of tyrosine to L-DOPA is the rate-limiting factor in the synthesis of dopamine, and modulation of TH activity is the primary endogenous regulatory mechanism of dopamine synthesis. L-DOPA is decarboxylated to dopamine by AADC.

The dopamine level in the synapse is mainly regulated by diffusion of dopamine from the synaptic cleft and reuptake to the presynaptic cell by the dopamine transporter (DAT). Dopamine is also catabolized enzymatically. The enzyme monoamine oxidase (MAO), which is located both in intracellular and extracellular space, oxidates dopamine to 3,4-dihydroxyphenylacetic acid (DOPAC). MAO exists in two forms that have similar affinities for dopamine: MAO_A and MAO_B. Some of the dopamine diffuses from the synaptic cleft and is methylated to 3-methoxytyramine (3-MT) by enzyme catechol-*O*-methyltransferase (COMT), which also degrades catecholamines epinephrine and norepinephrine (Männistö & Kaakkola 1999). COMT influences synaptic dopamine levels substantially; in particular this applies to dopamine in the prefrontal cortex, where DAT is not abundant (Scatton et al. 1985, Yavich et al. 2007). The sequential action of these enzymes leads to the metabolic end-products homovanillic acid (HVA), 3-methoxy-4-hydroxyphenylglycol (VMA), and 3-methoxy-4-hydroxy-phenylglycoaldehyde (MHPG) (Cooper et al. 2003, Nestler et al. 2001). In addition to synaptic neurotransmission, dopamine may also act via volume transmission (e.g. Kreitzer 2009).

2.1.2 Dopamine receptors

Dopamine exerts its action by binding to specific receptors, which belong to the family of 7 transmembrane domain, G-protein-coupled receptors (Civelli et al. 1993, Vallone et al. 2000). The dopamine receptors, which are subdivided into five different receptor types, are categorized into either D1-like receptors, which are $G_{\alpha s}$-coupled and activate adenylyl cyclase, or D2-like receptors, which are $G_{\alpha i/o}$-coupled and inhibit adenylyl cyclase (Bibb 2005, Civelli et al. 1993, Garau et al. 1978, Jaber et al. 1996, Kebabian & Calne 1979, Vallone et al. 2000). In addition to the D1 and D2 receptors, the other identified receptors are the D3 and D4 receptors, which are D2-like receptors, and the D5 receptor, which is a D1-like receptor (Jaber et al. 1996, Lachowicz & Sibley 1997). Dopaminergic ligands discriminate between the D1- and D2-like

receptor subfamilies but not between members of the same subfamily (Vallone et al. 2000). Dopamine receptors activate many signal transduction pathways: commonly, dopamine receptor stimulation activates (D1-like receptors) or inhibits (D2-like receptors) adenylyl cyclase, but dopamine receptor activation also regulates calcium and potassium channel currents as well as many other downstream effectors (Bibb 2005, Civelli et al. 1993, Jaber et al. 1996, Lachowicz & Sibley 1997). In general, activation of D1 receptors augments neuronal activity, whereas activation of D2 receptors leads to inhibition of neuronal activity (Bonci et al. 2005, Vallone et al. 2000). There are two splice variants of the D2 gene: D2L (long) and D2S (short). Of the two isoforms, D2L is the most abundantly expressed. Splice variants of the D3 receptor gene have also been found (Jackson & Westlind-Danielsson 1994, Vallone et al. 2000). In addition, several polymorphisms in dopamine receptors have been described, but their clinical significance is still unclear (Wong et al. 2000).

In the human brain, the highest concentrations of dopamine and dopamine metabolites, such as HVA and DOPAC, are found in the basal ganglia and substantia nigra (SN) (Hall et al. 1994). In humans, the highest densities of D1 and D2 receptors as well as mRNA of the D1 and D2 receptors, which have the highest rate of expression among the dopamine receptors, are seen in the striatum (Hall et al. 1994, Hurd et al. 2001, Meador-Woodruff et al. 1996). D1 receptor density is highest in the caput and corpus of the caudate nucleus and lateral putamen, followed by the medial putamen and nucleus accumbens (NAcc) (Cortés 1989, Hall et al. 1994, Meador-Woodruff et al. 1996). D1 receptors are also found in the SN, olfactory tubercle, cerebral cortex and amygdala (Hall et al. 1994). In addition to high expression in striatum, D1 receptor mRNA is also expressed widely in the cortex, with the highest expression in the medial orbital frontal area (Brodmann areas (BAs) 11, 14), paraterminal gyrus (BA 32) and insula (BAs 13-16) (Hurd et al. 2001). D1 receptor mRNA is also highly expressed in the island of Calleja and the bed nucleus of the stria terminalis (Hurd et al. 2001). The D5 receptor is expressed in the cerebral cortex, hippocampus, hypothalamus, lateral mamillary nucleus, NAcc, olfactory tubercle, parafascicular nucleus of the thalamus, striatum and possibly also in the substantia nigra, pars compacta (SNc) (Hall et al. 1994, Khan et al. 2000, Meador-Woodruff et al. 1996). The D2 receptor is highly expressed in the striatum, particularly in the lateral putamen and caput of the caudate nucleus, followed by the medial putamen, NAcc and corpus of caudate nucleus (Camps et al. 1989, Hall et al. 1994, Khan et al. 1998, Murray 1994). There are few D2 receptors in cortical areas, mainly in non-pyramidal interneurons (Khan et al. 1998), whereas the cerebellum has virtually no D2 receptors (Hall et al. 1994), making the cerebellum an appropriate reference region for D2 receptor binding studies. As with D1 mRNA expression, D2 mRNA expression is very high in the striatum and bed nucleus of the stria terminalis (Hurd et al. 2001, Meador-Woodruff et al. 1996). D2 mRNA expression is very low in the cortex, but high in the hippocampal formation, parafascicular and paraventricular thalamic nuclei, geniculate bodies, subthalamic nucleus and the pineal gland (Hurd et al. 2001). D2 mRNA is also abundant in brainstem regions, such as the SN, red nucleus, inferior colliculus, medial lemniscus and pontine nuclei. The neuro-anatomic distribution of D3 receptors and D3 receptor mRNA is restricted to a few brain regions such as the NAcc, ventral putamen, the islands of Calleja, a few septal nuclei, the hypothalamus, dentate gyrus and distinct regions of the thalamus and cerebellum (Hurd et al. 2001, Landwehrmeyer et al. 1993, Meador-Woodruff et al. 1996, Murray 1994, Suzuki et al. 1998). In addition, the D3 receptor is also localized in the SNc

indicating a presynaptic location (Hall et al. 1994). The D4 receptor is mainly expressed in the frontal cortex, amygdala, olfactory bulb, hippocampus, hypothalamus and mesenchephalon (Hall et al. 1994, Hurd et al. 2001).

Although both D1 and D2 receptors are highly expressed in the striatum, the cellular locations of the dopamine receptors and projections are somewhat different (Kreitzer 2009). D2-like receptors have a predominantly presynaptic location, while D1-like receptors are exclusively postsynapatic (Civelli et al. 1991). In the striatum, D1 receptors are predominantly located in striatonigral cells, which are GABAergic medium spiny neurons containing dynorphin and substance P, whereas D2 receptors are expressed in striatopallidal cells, which are GABAergic medium spiny neurons that project to the globus pallidus (GP) and contain enkephalin (Gerfen et al. 1990).

2.1.3. Dopaminergic neurons and pathways

In the human brain, the total number of dopaminergic neurons is estimated to be between 300 000 and 400 000 (Cooper et al. 2003). In spite of the relatively small amount of dopaminergic neurons in comparison to the total amount of neurons in the brain (approximately 100 billion), dopaminergic neurons are central regulators of many important brain functions, such as regulation of movement, cognition, psychological processes and neuroendocrine secretion (Jaber et al. 1996, Nestler et al. 2001, Vallone et al. 2000). The brain dopamine-containing cells form several important nuclei, from which the major dopaminergic systems originate (Fallon 1988, Moore & Bloom 1978). The brain dopaminergic systems can be divided into three major categories based on the length of the efferent fibers (Cooper et al. 2003):

(1) *Ultrashort systems*. The ultrashort systems make extremely local connections; among these systems are dopaminergic fibers in the retina (interplexiform amacrine-like neurons linking the inner and outer plexiform layers of the retina) and the olfactory bulb (periglomerular dopamine cells linking mitral cell dendrites in separated adjacent glomeruli).

(2) *Intermediate-length systems*. The intermediate-length systems include the tubero-infundibular system (projections from arcuate and periventricular nuclei into the intermediate lobe of the pituitary gland and median eminence) and connections formed by incertohypothalamic neurons and neurons of the medullary periventricular group.

(3) *Long systems*. The long systems are long projections linking the ventral tegmental area (VTA; A8 and A10 according to Dahlström & Fuxe 1965) and SN (A9) with the neostriatum (caudate nucleus and putamen), limbic cortex (mesocortical system: entorhinal, medial prefrontal and cingulate cortices) and other limbic structures (mesolimbic system: septum, olfactory tubercle, nucleus accumbens septi, amygdaloid complex, piriform cortex).

Of the dopaminergic systems, the well-characterized long systems arising from the VTA and SN are critical in the regulation of behavior. The nigrostriatal pathway arises from the midbrain SNc and innervates the dorsal striatum (caudate and putamen) (Moore & Bloom 1978), and is mainly involved in the regulation of movement. The mesocortical pathway arises from cell bodies in VTA in the midbrain and innervates different regions in the frontal and cingulate

cortices (Moore & Bloom 1978), regulating learning and memory. The mesolimbic pathway also originates in the VTA, but projects primarily to the ventral striatum/NAcc, and also to the olfactory tubercle and limbic system (Moore & Bloom 1978). The mesolimbic system is an important regulator of reward and motivated behavior (Kupfermann et al. 2000, Schultz 1997). The neurons giving rise to mesocortical and mesolimbic pathways are overlapping, and these pathways are often collectively referred to as the mesocorticolimbic pathway (Fallon 1988). It should be borne in mind that although the classification of dopaminergic systems into nigrostriatal and mesocorticolimbic systems forms an important anatomical framework for many studies, it clearly is an oversimplification (Björklund & Dunnett 2007). The tubero-infundibular pathway arises from cells of the periventricular and arcuate nuclei of the hypothalamus and projects to the median eminence of the hypothalamus. In the hypothalamus, dopamine is released into the perivascular spaces of the capillary plexus of the hypothalamic–hypophyseal portal system. Stimulation of dopamine receptors in the anterior pituitary leads to the inhibition of the release of prolactin. Additionally, many other neurons utilize dopamine in CNS, including periglomerular cells in the olfactory bulb and amacrine cells in the retina (Nestler et al. 2001). Recently, the thalamus has been shown to receive dense dopaminergic input from a variety of areas in the diencephalon and mesencephalon, and the innervation of thalamus has been suggested to form a novel dopaminergic system (García-Cabezas 2007, Sánchez-González 2005).

There are few dopaminergic neurons in the spinal cord, and the dopaminergic innervation of spinal cord is almost exclusively derived from supraspinal sites, mainly from the A11 cell group of the hypothalamus, but to some extent also from the paraventricular nucleus of the hypothalamus and SNc (Skagerberg et al. 1982, Skagerberg & Lindvall 1985). Dopaminergic projections arising from the A11 cell group play a role in pain regulation, through action on spinal dopamine D2 receptors (Fleetwood-Walker et al. 1988). Dopaminergic fibers are detected extensively in dorsal horn, lamina X, but fibers are also detected in the intermediolateral cell column and ventral horn (Barasi & Duggal 1985, Millan 2002, Skagerberg et al. 1982).

2.1.4 Basal ganglia

The basal ganglia are a group of bilateral subcortical interconnected nuclei in the basal forebrain, which consists of four major nuclei: the striatum, the globus pallidus/pallidum (GP), the substantia nigra, which consists of the pars compacta (SNc) and pars reticulata (SNr), and the subthalamic nucleus (STN). The striatum consists of three nuclei: the caudate nucleus, putamen, and ventral striatum, which includes the NAcc and olfactory tubercle (DeLong 2000). In humans, the caudate nucleus and putamen are collectively known as the neostriatum. In rodents, the caudate nucleus and putamen are indistinguishable and are thus referred to as the caudate-putamen (CPu) whereas in humans, the caudate and putamen are separated by fiber tracts of the internal capsule running between the neocortex and thalamus. The striatum receives input to the basal ganglia from the cerebral cortex, thalamus and brain stem, and projects to the GP and SN; these two nuclei, in turn, send major output projections from the basal ganglia. The GP receives major GABAergic inputs from the striatum and STN, and is divided into two segments, external (GPe) and internal (GPi). The GPe projects to the STN, which projects back to the GPe, and GPi/SNr (Alexander & Crutcher 1990). The GPi is

functionally related to the SNr, and together these nuclei form the main output from the basal ganglia (Alexander & Crutcher 1990, Parent & Hazrati 1993 & 1995). The output is directed to the cortex through the thalamus (Parent & Hazrati 1995), which is important in not just relaying information to cortex, but also in integrating basal ganglia inputs (Haber & Calzavara 2009, Parent & Hazrati 1995). The cells in the SNc and its medial extension, VTA, are dopaminergic and project mainly to the striatum (Joel & Weiner 2000). The STN receives a topographic excitatory glutamatergic projection from the cortex and a GABAergic projection from the GPe (DeLong 2000, Parent 1990). The cells in the STN are glutamatergic, and the excitatory projections of the STN are the only excitatory projections from the basal ganglia (Parent & Hazrati 1993, Wichmann & DeLong 1996). Due to the powerful excitatory projections, the STN is believed to be one of the driving forces in the basal ganglia (Parent 1990).

The striatum is the main receptive component of the basal ganglia (Joel & Weiner 2000, Parent 1990). The striatum receives massive input from the intralaminar nuclei of the thalamus and virtually all areas of the cerebral cortex, dopaminergic projections from the midbrain (SNc), and less prominent input from the GP, STN, pedunculopontine tegmental nucleus and raphe nuclei (DeLong 2000, Parent 1990). The neocortex has topographic projections to the dorsal striatum: the sensorimotor cortex projects mostly to the putamen whereas the associative areas of the temporal, parietal and cingulate cortices project to the caudate nucleus (Parent 1990). Other regions, including the limbic and paralimbic cortical regions as well as the amygdala and hippocampus, project to the ventral striatum (Parent 1990). The striatum consists of two anatomically and functionally separate units, the matrix and the striosome (patch) compartments (DeLong 2000). The matrix compartment is defined by rich acetylcholinesterase and choline acetyltransferase staining, receives inputs related to sensorimotor processing, and projects to the SNr and GP (Graybiel 1990, Kreitzer 2009). The striosome compartment has high μ-opioid receptor binding, receives input from the limbic and frontal regions and projects primarily to the SNc (DeLong 2000, Graybiel 1990, Kreitzer 2009). Most of the striatal neurons (90-95 %) are GABAergic medium spiny projection neurons, which are both the major target of input and the major source of output (DeLong 2000, Kreitzer 2009). The aspiny interneurons are far fewer, and their output is directed to medium spiny neurons (Kreitzer 2009).

Two parallel pathways modulate the inhibitory output of the basal ganglia from the GPi and SNr: the direct and the indirect pathway (DeLong 2000). Striatal neurons projecting directly to the output nuclei (direct pathway) express dopamine D1 receptors, and activation of this pathway inhibits the tonic inhibitory drive of GPi neurons on the thalamus. The indirect pathway, in turn, has projection neurons expressing dopamine D2 receptors, and activation of the indirect pathway leads to disinhibition of GPi neurons. The striatonigral cells forming the direct pathway coexpress muscarinic M4 receptors, substance P and dynorphin, while the striatopallidal cells forming the indirect pathway coexpress adenosine A2A receptors and enkephalin (Gerfen et al. 1990, Kreitzer 2009). Due to the difference in expression of the two dopamine receptor subtypes, the dopaminergic input to the pathways has the same effect: an increase in thalamocortical activity and facilitation of movement initiated in the cortex (DeLong 2000). However, the effects of dopamine in the striatum may be more complex, as the effects vary, e.g. depending on the physiologic state of the medium spiny neuron (Kreitzer 2009). The medium spiny neurons from both pathways, as well as the striatal interneurons, are innervated

by dopaminergic fibers from the SNc (Joel & Weiner 2000). Although the dopamine receptor expressing projection neurons in the putamen are critical in mediating the effects of dopamine in the basal ganglia, dopamine has effects on other targets as well, as the GP, SN and STN also have dopaminergic synapses (DeLong 2000). The ventral striatum receives dopaminergic input from the VTA, which also innervates the amygdala, hippocampus and several cortical areas. Neurons in the ventral striatum are GABAergic medium spiny neurons, which mainly project either to the ventral pallidum or back to VTA, but also to the SNc and SNr (Joel & Weiner 2000).

2.1.5. Basal ganglia circuitry and functions

The basal ganglia receive primary input from the cerebral cortex and send output to the brain stem and, via ventrolateral thalamus, back to the cortex (DeLong 2000). In this way, the basal ganglia are part of the important subcortical reentrant circuits linking the cortex and thalamus (Alexander et al. 1986, Alexander & Crutcher 1990, DeLong 2000). Previously, the basal ganglia were assumed to function as an "information funnel", which receives a large amount of converging projections from distinct cortical areas, and after integrative processing within the basal ganglia, sends functionally mixed projections back to the cortex (e.g. Parent & Hazrati 1995). According to the current hypothesis, the functional arrangement of basal ganglia circuits is essentially parallel in nature, and five structurally and functionally segregated basal ganglia circuits have been described (Alexander et al. 1986, Alexander & Crutcher 1990).

The circuitry mediating the effects of the basal ganglia on skeletomotor control (circuit 1) begins in precentral motor fields (premotor cortex, supplementary motor area, motor cortex) and cortical somatosensory areas, and projects to the putamen (Alexander et al. 1986, DeLong 2000). From the putamen, the circuit projects to the ventrolateral thalamus via the caudoventral GP and SNr, and back to the precentral motor fields and somatosensory areas (Alexander et al. 1986, DeLong 2000). Saccadic eye movements are controlled by the oculomotor circuit (circuit 2), which originates in the frontal and supplementary motor eye fields and projects to the caudate nucleus, and from the caudate nucleus to the GPi and SNr. The SNr sends projections back to the frontal eye fields via thalamus, but also to the superior colliculus (Alexander et al. 1986, DeLong 2000). In addition to the circuits involved in motor control (circuits 1 and 2), three distinct cortico-basal ganglia-thalamocortical circuits with a proposed role in the non-motor aspects of basal ganglia function have been described. The dorsolateral prefrontal circuit (circuit 3) arises from the BAs 9 and 10 and projects via the head of the caudate nucleus to the dorsomedial GPi and rostral SNr, which project to the ventral anterior and medial dorsal thalamus, which in turn project back to the dorsolateral prefrontal cortex (Alexander et al. 1986, DeLong 2000). The dorsolateral prefrontal circuit regulates executive functions, such as organization of behavioral responses (DeLong 2000). The lateral orbitofrontal circuit (circuit 4) arises from the lateral frontal cortex and projects to the ventromedial caudate nucleus. From the caudate nucleus, the circuit follows the pathway of the dorsolateral prefrontal circuit, and returns to the orbitofrontal cortex (Alexander et al. 1986, DeLong 2000). This pathway is associated with selection of socially appropriate responses (DeLong 2000). The anterior cingulate circuit (circuit 5) arises in the anterior cingulate cortex (ACC) and projects to the ventral striatum, which also receives input from other structures of the limbic system (Alexander et al. 1986, DeLong 2000). The ventral striatum projects to the ventral and

rostromedial pallidum and the SNr, which send projections to the paramedian medial dorsal nucleus of the thalamus, which in turn projects back to ACC. The anterior cingulate circuit has been suggested to play a role in procedural learning (DeLong 2000).

In addition to the five main basal ganglia circuits, the basal ganglia are also involved in several subsidiary circuits, and it is likely that there are additional basal ganglia-thalamocortical circuits yet to be described (Alexander et al. 1986, DeLong 2000). One of the questions raised by the concept of closed, segregated circuits is how these circuits might interact, as interactions between the circuits may be assumed to be important in developing and modifying coherent behavior (Haber 2003, Haber & Calzavara 2009, Joel & Weiner 1994 & 2000). Indeed, despite the ample anatomical evidence supporting the concept of parallel processing in the basal ganglia, it has been suggested that the basal ganglia-thalamocortical circuits are not fully closed and segregated (Haber 2003, Joel & Weiner 1994). Due to the central position in regulating neural activity in many brain areas, the basal ganglia are associated with a number of separate functions: movement, response selection to stimuli, cognition, emotion and learning (DeLong 2000, Wichmann & DeLong 1996). Correspondingly, disorders of the basal ganglia are well known to result in abnormalities in movement as well as complex cognitive, behavioral and mood disturbances (Albin et al. 1989).

2.2. Dopamine and pain

2.2.1. Animal studies

Experimental studies on animals suggest an important pain-modulatory role for the basal ganglia (Chudler & Dong 1995, Neugebauer 2006). Both CPu (Chudler 1998) and GP (Bernard et al. 1992, Chudler et al. 1993, Chudler 1998) neurons have been shown to respond to noxious stimuli and encode the intensity of noxious stimulation. The SN has been consistently shown to respond to noxious stimulation in animal studies (Barasi 1979, Gao et al. 1990, Pay & Barasi 1982, Schultz & Romo 1987); up to 50 % of spontaneously active neurons in the SN were found to be nociceptive (Pay & Barasi 1982). Activation of the caudate nucleus (Lineberry & Vierck 1975), SN (Barnes et al. 1979) or the mesolimbic system by stimulation of the VTA produces analgesia (Sotres-Bayon et al. 2001). Correspondingly, lesioning of the VTA, SN or striatum with kainate or 6-hydroxydopamine leads to an increase in nociceptive responses (Carey 1986, Chudler & Lu 2008, Lin et al. 1984, Saadé et al. 1997, Sotres-Bayon et al. 2001).

Although the main interest in catecholamines in pain has been in the role of noradrenergic and adrenergic projections, a large number of animal studies indicate a specific role for dopamine in the modulation of pain (Millan 2002, Yaksh 2005). At the spinal level, intrathecal administration of the non-selective dopamine receptor agonist apomorphine leads to analgesia (Barasi et al. 1987, Jensen & Yaksh 1984). Supraspinally, dopamine receptor activation in the striatum by apomorphine or SN stimulation leads to analgesia; conversely, pain sensitivity is increased with striatal haloperidol administration or when the SN is destroyed and dopaminergic innervation of striatum is abolished (Lin et al. 1981). Further, descending pain modulation of stimulation-produced analgesia (SPA) has been shown to employ dopaminergic mechanisms: dopamine receptor blockade decreases SPA, whereas dopamine agonist treatment leads to an increase in SPA (Akil & Liebeskind 1975). Several studies have shown VTA-NAcc dopaminergic neurons to be involved in pain. Mesolimbic dopamine release has been shown to

mediate and suppress prolonged pain and be involved in opioid-analgesia and analgesia induced by stress and noxious stimulation (Louilot et al. 1986, Schmidt et al. 2002, Weizman et al. 2003; for reviews, see Altier & Stewart 1999 and Wood 2006). Specifically, several lines of evidence from animal studies suggest a critical role for D2 receptors in the modulation of pain (for a review, see Hagelberg et al. 2004). D2 agonists show antinociceptive properties in models of phasic pain in rodents (Barasi & Duggal 1985, Barasi et al. 1987, Ben-Sreti et al. 1983, Fleetwood-Walker et al. 1988, Michael-Titus et al. 1990). Application of D2 agonists has also been found to be antinociceptive in models of tonic pain, such as nerve ligation (Ansah et al. 2007), formalin-induced nociception (Magnusson & Fisher 2000, Morgan & Franklin 1991) and inflammatory hyperalgesia (Gao et al. 2000 & 2001, Taylor et al. 2003). Moreover, cocaine-induced analgesia has been shown to be dependent on D2-receptor activation (Kiritsy-Roy et al. 1994). Conversely, dopamine D2 receptor knockout mice exhibit enhanced mechanical and capsaicin-induced referred hypersensitivity (Mansikka et al. 2005). While D2 receptors are consistently found to modulate responses to noxious stimulation, brain D1 receptors do not seem to be involved in the modulation of pain (Altier & Stewart 1999, Barasi et al. 1987, Ben-Sreti et al. 1983, Fleetwood-Walker et al. 1988, Magnusson & Fisher 2000, Morgan & Franklin 1991, Taylor et al. 2003).

Experimental animal studies have shown that dopamine-opioid interactions may be involved in both analgesia mediated through dopamine receptor stimulation (Michael-Titus et al. 1990, Weizman et al. 2003) and opioid analgesia (Altier and Stewart 1999, Morgan & Franklin 1991). Dopamine agonists facilitate opioid analgesia (Dennis & Melzack 1983), and, correspondingly, morphine analgesia is attenuated by lesioning of midbrain dopaminergic neurons (Flores et al. 2004, Morgan & Franklin 1990) and treatment with dopamine antagonists (Altier & Stewart 1999, Morgan & Franklin 1991). However, some authors have presented conflicting findings: e.g. Kiritsy-Roy et al. (1989) showed that D1 and D2 receptor antagonists did not attenuate but enhanced morphine analgesia, and King et al. (2001) showed that D2 receptor activation may oppose opioid analgesia. The somewhat conflicting results may be related to differences in the route of drug administration, receptor subtype selectivity and differences in the experimental pain model. Dopamine receptors may also be involved in the analgesic effects of other receptors, such as spinal and striatal α-adrenoceptors (Liu et al. 1992, Pertovaara & Wei 2008).

2.2.2. Experimental human studies on healthy subjects

Brain imaging studies in humans frequently show activation of the striatum (as assessed by increased regional cerebral blood flow) during painful stimulation (Casey et al. 1996, Coghill et al. 1999 & 2001, Derbyshire et al. 1997, Iadarola et al. 1998, Jones et al. 1991, Svensson et al. 1997). A positron emission tomography (PET) imaging study by Hagelberg et al. (2002b) with a D2/D3 selective radioligand [^{11}C]raclopride has addressed the possible association between D2/D3 binding potential (BP_{ND}) and experimental pain and provided initial evidence suggesting that striatal D2/D3 receptors may be involved in the modulation of pain perception in healthy human subjects. In this study, baseline (resting) dopamine D2/D3 receptor BP_{ND} in the putamen in healthy humans was negatively correlated with sensitivity to cold pressor pain (CPP) and positively correlated with capacity to modulate pain by conditioning painful stimulation. In other words, high dopamine D2/D3 receptor BP_{ND} in striatum was associated with a low pain threshold, but a high capacity to modulate pain by a concurrent painful stimulus (Hagelberg et

al. 2002b). As differences in D2/D3 BP_{ND} reflect not only differences in receptor density, but also individual differences in endogenous striatal dopamine release (Laruelle 2000), it is possible that subjects with high D2/D3 BP_{ND} either have a high density of D2/D3 receptors or a low baseline endogenous dopaminergic tone. Earlier, the activation of the striatum in brain imaging studies of pain has often been attributed to inhibition or preparation of motor activity (e.g. Peyron et al. 2000). The association of baseline D2/D3 BP_{ND} with pain suggests that the striatal activation often found in brain imaging studies is not, at least entirely, due to motor activation but may represent the activation of an endogenous supraspinal pain-inhibiting circuitry. In addition to striatal, extrastriatal D2/D3 receptors were also associated with pain in the study by Hagelberg et al. (2002b). Tolerance of CPP, but not the CPP threshold, was inversely correlated with baseline D2/D3 receptor BP_{ND} in the right medial temporal cortex (Hagelberg et al. 2002b), possibly reflecting a D2/D3 receptor-mediated modulation of the medial pain system and the affective qualities of pain (Treede et al. 1999). Despite the fact that D2/D3 receptor binding occurs in also other extrastriatal brain areas important in pain, such as the thalamus, frontal cortex and ACC (Hall et al. 1994, Rieck et al. 2004), D2/D3 BP_{ND} in these brain areas was not correlated with sensitivity to pain (Hagelberg et al. 2002b). In a recent study by Scott et al. (2006), healthy volunteers were shown to release dopamine in the ventral and dorsal basal ganglia during painful stimulation (as indicated by a decrease in D2/D3 BP_{ND}), and furthermore, the dopamine release in the ventral and dorsal basal ganglia was differentially associated with individual variations in subjective reports of sensory and affective qualities of the pain. This study strongly supports the hypothesis that the basal ganglia, and particularly the striatal D2/D3 receptors are involved in the regulation of pain in humans.

Experimental human studies have also provided important insights into the dopamine-opioid interactions, which may be critical in the modulation of pain. In a PET study assessing the effects of intravenous alfentanil, a potent μ-opioid agonist, on [^{11}C]raclopride and [^{11}C]FLB 457 binding, a significant increase in BP_{ND} was found in the ACC, dorsolateral prefrontal cortex, medial frontal cortex, superior temporal cortex and medial thalamus as well as in the striatum (Hagelberg et al. 2002a & 2004a). These studies suggest that opiates administered in pharmacologically relevant concentrations reduce D2/D3 receptor activation in the striatal and extrastriatal areas in humans. The only so far known functional polymorphism of COMT results in a valine (*val*) to methionine (*met*) substitution at position 158 of dominant transcript MB-COMT, and this substitution has a drastic effect on the enzymatic activity of the enzyme (Männistö & Kaakkola 1999). The finding that this abundant functional polymorphism affects response to pain and μ-opioid receptor activation during sustained pain also indicates the important involvement of brain catecholamine metabolism, and possibly dopamine, in μ-opioid analgesia: individuals with the *met* allele, and consequently a low COMT enzyme activity and high dopamine levels, demonstrated diminished μ-opioid system activation in the basal ganglia during a sustained painful stimulus. Moreover, these changes were accompanied by higher sensory and affective ratings as measured using McGill Pain Questionnaire (MPQ), and a more negative internal affective state as measured using the Positive and Negative Affectivity Scale (PANAS) (Zubieta et al. 2003).

2.2.3. The role of dopamine in clinical pain and treatment of pain

Parkinson's disease (PD) results from degeneration of the dopaminergic cells of the SNc that causes a lack of dopaminergic nerve terminals in the striatum, where the putamen is most severely affected (Hornykiewicz 2001). The resulting abnormality in basal ganglia function leads to the well-known motor signs of bradykinesia, rigidity, tremor and postural instability. The standard treatment of PD is levodopa, which effectively counteracts the loss of dopaminergic input to striatum. Unfortunately, most PD patients develop motor fluctuations as a complication of long-term levodopa therapy. In addition, those patients experiencing motor fluctuations also tend to have non-motor fluctuations, such as pain that are occasionally more disabling than the motor fluctuations (Witjas et al. 2002). Sensory symptoms are common in PD and affect approximately 40-75% of patients, pain being the most common complaint (Goetz et al. 1986, Koller 1984, Wasner & Deuschl 2006). In a recent study with home-living PD patients, 83% of PD patients reported pain (Beiske et al. 2009). The pain often tends to fluctuate with motor manifestations and responds well to levodopa therapy (Ford et al. 1996, Goetz et al. 1986). However, several lines of evidence support a view of spontaneous pain in PD as an independent symptom resulting from the aberrant basal ganglia function. In addition to spontaneous pain, which is often a poorly localized, cramplike or aching sensation, PD patients frequently exhibit burning pain symptoms typical of a central pain syndrome (Ford 1998, Ford et al. 1996, Koller 1984, Schott 1985, Snider et al. 1976, Witjas et al. 2002). In addition, experimental studies on PD patients support the hypothesis of a primary sensory disturbance of pain perception and modulation in PD. Djaldetti et al. (2004) reported a lower heat pain threshold in PD patients with pain than healthy controls, while PD patients with spontaneous pain had lower heat pain thresholds than PD patients without pain. A lower pain threshold and an increased activation of pain-processing cortical areas during pain in PD patients during the OFF condition has been reported, and the pain threshold as well as pain-induced cortical activation was normalized with levodopa administration (Brefel-Courbon et al. 2005). On the basis of these findings it is tempting to explain the abnormalities in pain perception in PD with dysfunction of the dopaminergic pain regulation in the basal ganglia (Chudler & Dong 1995) and diminished dopamine-mediated analgesia. However, PD also leads to changes in striatal neurotransmitters other than dopamine (e.g. Albin et al. 1989, Hornykiewicz 2001) and dysfunction in several brain areas critically involved in pain regulation (e.g. Scherder et al. 2005), raising the possibility that the pain in PD may arise from the interaction of multiple factors. Still, other disorders relating to a disturbance in dopamine regulation also exhibit abnormalities in pain. Restless legs syndrome (RLS) is a disorder characterized by unpleasant leg sensations and an inner urge to move the legs (Allen et al. 2003). Dopamine agonists effectively relieve the symptoms of RLS, while dopamine antagonists worsen the symptoms, suggesting a role for the dopaminergic system in RLS. The dopaminergic system may also be involved in the aetiology and clinical presentation of other pain syndromes, such as migraine (Akerman & Goadsby 2007, Peroutka 1997) and chronic orofacial pain (Hagelberg et al. 2003a & 2003b, Jääskeläinen et al. 2001).

Considering the large body of evidence suggesting a role for dopamine in pain, surprisingly few studies have addressed the role of brain dopamine in chronic pain in humans. There is evidence from PET studies indicating that in two chronic orofacial pain syndromes, burning mouth syndrome (BMS) and atypical facial pain (AFP), patients have abnormal striatal dopamine

function. In patients with BMS, [^{18}F]FDOPA uptake in the striatum is decreased, suggesting a decrese in striatal dopaminergic tone (Jääskeläinen et al. 2001). Moreover, BMS patients have a higher binding of [^{11}C]raclopride (D2/D3 receptor selective radioligand) in the putamen than healthy controls while uptake of [^{11}C]NNC 756 (D1 receptor selective radioligand) is in normal range (Hagelberg et al. 2003b). Correspondingly, in AFP the D2/D3 BP_{ND} as measured with [^{11}C]raclopride is increased in the left putamen, with no difference in [^{11}C]NNC 756 binding or [^{18}F]FDOPA uptake in comparison to healthy controls (Hagelberg et al. 2003a). In both studies, the D1/(D2/D3) BP_{ND} ratio in the putamen was bilaterally decreased. The high striatal D2/D3 BP_{ND} and the decreased D1/(D2/D3) BP_{ND} ratio among chronic orofacial pain patients may have two alternative explanations: a decline in the level of endogenous striatal dopamine resulting in diminished striatal pain-inhibition and an increased sensitivity to pain, or upregulation of D2/D3 receptor expression reflecting activation of endogenous pain-controlling mechanisms. Considering that the high D2/D3 BP_{ND} in chronic pain is associated with low [^{18}F]FDOPA uptake, the former explanation seems more plausible. In RLS, PET studies have provided considerable support to the theory of an abnormal dopamine system (Paulus et al. 2007). PET studies have shown reduced [^{18}F]FDOPA utilization in the striatum (Ruottinen et al. 2000, Turjanski et al. 1999), and reduced BP_{ND} in the striatum with [^{11}C]raclopride and in extrastriatal areas with [^{11}C]FLB 457 (Červenka et al. 2006, Turjanski et al. 1999), possibly pointing to a reduced dopaminergic tone in RLS. While RLS has been proposed to be a disorder of abnormal central somatosensory processing (Schattschneider et al. 2004), abnormalities in the D2 receptor function in RLS may be related to the proposed role of D2 receptors in pain regulation (Hagelberg et al. 2004b). Recently, healthy subjects have been demonstrated to release dopamine in the basal ganglia during painful stimulation (as indicated by a decrease in D2/D3 BP_{ND}) (Scott et al. 2006, Wood et al. 2007), but in fibromyalgia patients this dopamine release is absent (Wood et al. 2007), suggesting that the abnormal dopamine response to pain might be involved in the clinical picture of fibromyalgia.

The mounting evidence suggesting a pivotal role for dopamine in the regulation of pain raises the question of whether drugs acting on dopamine receptors could be used in the treatment of pain in humans. Indeed, drugs acting on dopamine receptors have been shown to have analgesic properties in clinical studies. The dopamine agonist apomorphine has analgesic effects in thalamic pain (Miley et al. 1978), and bupropion, which acts as a dopamine reuptake inhibitor, has been found effective in neuropathic pain (Semenchuk et al. 2001). In a study with patients with herpes zoster, the patients receiving levodopa had lower pain intensity ratings during acute pain and less post-herpetic neuralgia than those patients receiving the placebo (Kernbaum & Hauchecorne 1981). Furthermore, pain arising from diabetic polyneuropathy has been successfully treated with levodopa (Ertas et al. 1998). In general, drugs increasing dopaminergic neurotransmission have been found to have analgesic effects. However, dopamine receptor antagonists, such as neuroleptics, also exhibit analgesic effects in various pain syndromes such as migraine (Honkaniemi et al. 2006, Silberstein 2003), postherpetic neuralgia (Taub 1973) and trigeminal neuralgia (Lechin et al. 1989), but the effects are inconsistent (Graff-Radford et al. 2000, Zitman et al. 1991), and adverse effects are common (for a review, see Seidel et al. 2008). The varying effects of dopaminergic drugs in the treatment of pain may be related to the complexity of the dopaminergic system and the

existence of several receptor subtypes, which have potentially conflicting effects on pain perception.

2.3. Dopaminergic mechanisms of placebo analgesic response

Medical treatments can have a dual effect on the patient: the effect of the actual treatment itself, and the effect resulting from the perception of receiving the treatment. The latter effect, arising from the expectations of the patient, is called the placebo effect, research on which offers an interesting model to the study of some aspects of psychological top-down modulation of pain (Benedetti 2009, Pollo & Benedetti 2004). Placebo analgesic agents and opioids activate a shared brain network, which suggests that the opioid system and the placebo may have similar mechanisms (Petrovic et al. 2002). Indeed, the analgesic placebo effect has been shown to be dependent on opioid receptor activation (Levine et al. 1978, Zubieta et al. 2005). Recent studies suggest that the placebo response may also be under the control of the brain dopaminergic reward circuitry (de la Fuente-Fernández et al. 2002, Lidstone & Stoessl 2007). Striatal dopamine release as assessed by a decrease in the BP_{ND} of [^{11}C]raclopride has been demonstrated during a placebo-induced relief of motor symptoms and placebo-rTMS (repetitive transcranial magnetic stimulation) in PD (de la Fuente-Fernández et al. 2001, Strafella et al. 2006), and in placebo-induced arousal in healthy subjects expecting to receive caffeine (Kaasinen et al. 2004). These findings suggest that the dopaminergic system and striatal dopamine D2/D3 receptors may contribute to the biochemical basis of the placebo response. In fact, it has been proposed that opioid-dopamine interactions in the reward circuitry of the brain may play an important role in placebo analgesia (de la Fuente-Fernández et al. 2002). As striatal dopamine D2/D3 receptors may play an important role both in the regulation of pain (Hagelberg et al. 2004b) and placebo response, it may be speculated that striatal D2/D3 receptors might play a role in placebo analgesia. Indeed, a recent PET study found that D2/D3 receptors in the ventral basal ganglia are involved in the analgesic effects of placebo (Scott et al. 2008).

2.4. Serotonergic system

2.4.1. Serotonin

The biogenic amine neurotransmitter serotonin (5-hydroxytryptamine or 5-HT) is derived from the essential amino acid tryptophan and belongs to a group of aromatic compounds called indoles. Serotonin is found in many cell types, such as platelets, mast cells and enterochromaffin cells; only 1-2 % of the whole body serotonin is found in the brain (Cooper et al. 2003). However, serotonin is one of the most ancient signaling molecules, and serotonin is an important regulator of a large variety of behaviors (Nichols & Nichols 2008). The synthesis of serotonin requires two enzymes: tryptophan hydroxylase (TPH) and AADC, TPH being the rate-limiting enzyme in the synthesis. First, the amino acid tryptophan, which is primarily derived from the diet, is actively transported across the blood-brain barrier and hydroxylated by TPH. The resulting 5-hydroxytryptophan is decarboxylated into serotonin by AADC.

In the catabolism of serotonin, the first step is the oxidation to 5-hydroxyindoleacetaldehyde by MAO_A. Aldehydedehydrogenase catalyzes the oxidation of 5-hydroxyindoleacetaldehyde to the main metabolite of serotonin, 5-hydroxyindoleacetic acid (5-HIAA) (Nestler et al. 2001). Depending on the ratio of the oxidized to the reduced form of nicotinamide adenine

dinucleotide (NAD^+/NADH) in the tissue, 5-hydroxyindoleacetaldehyde may also be reduced to 5-hydroxytryptophol (Cooper et al. 2003). As with dopamine and other catecholamines, reuptake is the main mechanism for the termination of synaptic actions of serotonin and regulation of serotonin level in the synapse. This is accomplished by the serotonin transporter (5-HTT or SERT), which is ubiquitous in the CNS, consistent with the extensive projections of the serotonergic neurons (Hornung 2003).

2.4.2. Serotonin receptors

Mammalian serotonin receptors form seven families, 5-HT_{1-7}, and a total of 14 different receptor subtypes with different structural and pharmacological properties (Barnes & Sharp 1999, Hoyer et al. 1994 & 2002). Multiple splice variants, RNA-edited isoforms and receptor homo- and heterodimerization have been described, adding to the diversity of serotonin receptors (Hoyer et al. 2002). With the exception of the 5-HT_3 receptor, which is a ligand-gated ion channel, the serotonin receptors belong to the group of seven transmembrane spanning G-protein coupled receptors. The serotonin receptors are divided into receptor families based on the coupling to second messengers and amino acid homology (Barnes & Sharp 1999, Hoyer et al. 1994, Nichols & Nichols 2000). The 5-HT_1 family consists of five receptor subtypes that are negatively coupled to adenylate cyclase, and generally couple to $G_{i/O}$ proteins. In mammalian CNS, 5-HT_1 receptors are highly expressed in the basal ganglia, neocortex, hippocampus and raphe nuclei (Barnes & Sharp 1999, Hoyer et al. 1994 & 2002, Palacios et al. 1990). The 5-HT_2 receptors are a homologous group of receptors that activate phospholipase C, couple to G_q proteins, and are highly expressed in the neocortex, basal ganglia, choroid plexus, cortex, facial motor nucleus, hippocampus and medulla (Barnes & Sharp 1999, Hoyer et al. 1994, Palacios et al. 1990). 5-HT_4, 5-HT_6, and 5-HT_7 receptors are a heterogenous group of receptors which are positively associated with adenylate cyclase and couple to G_s proteins. 5-HT_4 receptors are mainly expressed in the basal ganglia, colliculi and hippocampus, 5-HT_6 receptors in the amygdala, hippocampus, olfactory tubercle, cortex, NAcc and striatum, and 5-HT_7 receptors in the amygdala, thalamus and hypothalamus (Barnes & Sharp 1999, Hoyer et al. 1994 & 2002). The 5-HT_5 receptors form a recently-described receptor family that does not resemble 5-HT_1 or $5HT_2$ receptors in their structure or transduction system, and its expression in an endogenous setting has not been confirmed (Hoyer et al. 2002). 5-HT_5 receptor expression has been reported to occur in the cortex, hypothalamus, hippocampus, habenula, olfactory bulb and cerebellum, as well as in the corpus callosum, cerebral ventricles and glia (Hoyer et al. 1994 & 2002). The 5-HT_3 receptor belongs to the same molecular receptor family of ligand-gated ion channels as the $GABA_A$, glycine and nicotinic cholinergic receptors (Hoyer et al. 1994, Kandel & Siegbaum 2000), and activation of the 5-HT_3 receptor leads to the opening of a cation channel and rapid depolarization. 5-HT_3 receptor expression is high in peripheral neurons, where 5-HT_3 receptors are found both peripherally and centrally in the substantia gelatinosa of the spinal cord (Palacios et al. 1990), and in discrete nuclei in the brain stem (e.g. area postrema) as well as in the cortex and limbic areas, such as the amygdala, hippocampus and entorhinal cortex (Barnes & Sharp 1999, Hoyer et al. 1994 & 2002). Due to the differences in distribution of the receptor subtypes, cellular localization and effects on the target neuron, the end effects of serotonin vary significantly depending on the receptor subtype and the anatomical location of the activated receptor (Cooper et al. 2003, Hoyer et al. 1994).

Of all serotonin receptors discovered, the 5-HT_{1A} receptor is one of the best characterized (Hamon et al. 1990). The human 5-HT_{1A} receptor is composed of 421 amino acid residues, and the receptor gene is localized on chromosome 5 (5q11.2-q13) (Barnes & Sharp 1999, Hoyer et al. 1994, Lanfumey & Hamon 2000). 5-HT_{1A} receptors are distributed throughout the CNS, and their expression is essentially similar in rodents and humans (Barnes & Sharp 1999). The receptor protein and mRNA expression is high in the frontal cortex, limbic system (amygdala, cingulum, entorhinal cortex, hippocampus, lateral septum) and dorsal and medial raphe nuclei (Barnes & Sharp 1999, Farde et al. 1998, Hall et al. 1997, Hamon et al. 1990, Hoyer et al. 1994, Lanfumey & Hamon 2000). 5-HT_{1A} receptors are also widely found in the neocortex, the thalamus, hypothalamus and the substantia gelatinosa of the spinal cord (Hall et al. 1997, Hoyer et al. 1994, Lanfumey & Hamon 2000). In raphe nuclei, the 5-HT_{1A} receptors are located presynaptically mainly in serotonergic neurons, whereas in the forebrain the 5-HT_{1A} receptors are located postsynaptically (Lanfumey & Hamon 2000, Wright et al. 1995). The activation of a 5-HT_{1A} receptor inhibits adenylate cyclase, decreases production of cyclic adenosine monophosphate (cAMP), and causes neuronal hyperpolarization by opening G_i-protein coupled inward-rectifying K^+ channels and closing voltage-gated Ca^{2+} channels (Barnes & Sharp 1999). In accordance with this, 5-HT_{1A} receptor agonists activate the raphe 5-HT_{1A} autoreceptor, slow down the pacemaker activity of raphe neurons, and induce a drop in release of serotonin in the forebrain (Sharp & Hjorth 1990). In experiments with animals, 5-HT_{1A} receptor activation with the 5-HT_{1A} receptor agonist 8-OH-DPAT increases the release of acetylcholine in the cortex and hippocampus and also increases the release of noradrenaline in several brain areas, such as the hypothalamus, hippocampus, frontal cortex and VTA (Barnes & Sharp 1999). In rats, activation of brain 5-HT_{1A} receptors by application of 8-OH-DPAT induces a wide range of effects on behavior, including antidepressive effects and anxiolysis, hyperphagia, hyperthermia, and an increase in locomotion and sexual behavior as well as a decrease in blood pressure and heart rate (Barnes & Sharp 1999, Hoyer et al. 1994).

2.4.3. Serotonergic neurons and pathways

Cell bodies of mammalian serotonergic neurons are mainly localized in several clusters in the midline, or raphe, of the brain stem, with extensive projections to both the forebrain and spinal cord (Azmitia & Segal 1978, Dahlström & Fuxe 1964, Hornung 2003, Jacobs & Azmitia 1992, Törk 1990). Although first described in rats, the main nuclei are also present in the monkey and human brain (Jacobs & Azmitia 1992). These clusters, the raphe nuclei, are functionally divided in two groups. The rostral group comprises the nucleus linearis, dorsal raphe nucleus (DRN), medial raphe and raphe pontis (Hornung 2003, Jacobs & Azmitia 1992). The caudal group includes the nucleus raphe magnus (NRM), raphe pallidus and raphe obscurus (Hornung 2003, Jacobs & Azmitia 1992). The main source of afferent input to the raphe nuclei is from the raphe nuclei themselves (Jacobs & Azmitia 1992). The rest of the afferents to the raphe nuclei derive from the brain stem, hypothalamus, prefrontal cortex, and limbic forebrain (Jacobs & Azmitia 1992). The caudal nuclei, mainly the NRM, project predominantly to the spinal cord, whereas the more rostral nuclei, such as the DRN, project to the brain and cerebellum (Hornung 2003, Jacobs & Azmitia 1992, Törk 1990). The NRM has serotonergic projections to the spinal dorsal horn neurons giving rise to the spinothalamic tract, and these projections are important in the modulation of nociception (Jacobs & Azmitia 1992). Although the terminal fields of the dorsal and median raphe are somewhat overlapping, the DRN sends projections mainly to the cortex,

thalamus and striatal regions, and the median raphe projects mainly to the limbic system, including the hippocampus (Jacobs & Azmitia 1992).

The raphe nuclei have a critical role in the general regulation of the serotonergic activity in the CNS: the projections of raphe nuclei are so extensive that virtually all neurons in the brain may be in contact with a serotonergic fiber (Jacobs & Azmitia 1992, Nestler et al. 2001). Thus, despite the fact that serotonergic neurons number only in the thousands (Jacobs & Azmitia 1992), the serotonergic system exerts an important modulatory effect on behavior. The serotonergic neurons exhibit slow and highly regular neuronal activity, leading to a tonic modulatory influence on the projection areas (Jacobs & Azmitia 1992). In general, an increase in the tonic activity of serotonergic neurons is observed during waking arousal and a decrease of activity is observed during sleep, possibly reflecting the effects of the serotonergic system in enhancement of motor neuron excitability and suppression of distracting sensory cues (Cooper et al. 2003, Lucki 1998). Together, the highly regulated pacemaker pattern of activity and widespread projections give the serotonergic system a strategic position to modulate a great variety of behaviors, such as sleep, food intake and sexual behavior (Buhot 1997, Lucki 1998). In addition, a variety of cognitive functions such as anxiety, attention, emotion, mood states, learning and memory are regulated by serotonin (Buhot 1997, Dayan & Huys 2009, Lucki 1998). Serotonin also plays a key role in the perception of pain (Fields et al. 2006, Millan 2002, Sommer 2006, Wang & Nakai 1994). Dysfunction of the serotonergic system has been demonstrated in a variety of psychiatric disorders, such as anxiety, affective disorders including major depression and suicidal behavior, eating disorders, hyperaggressive states, obsessive-compulsive disorder, phobic disorders, sleep disorders and schizophrenia (Hoyer et al. 2002, Lucki 1998).

2.5. Serotonin and pain

2.5.1. Experimental studies in animals and humans

A large body of evidence suggests a role for serotonin in pain perception and modulation (Basbaum & Fields 1978, Fields et al. 2006, Kayser et al. 2007, Lopez-Garcia 2006, Messing & Lytle 1977, Millan 2002, Richardson 1990, Sommer 2006, Yaksh & Wilson 1979). Several studies have demonstrated analgesic effects for serotonin (e.g. Akil & Liebeskind 1975, Bardin et al. 2000, Kilkens et al. 2004, Vogel et al. 2003, Yaksh & Wilson 1979), and the role of descending serotonergic pathway in pain inhibition is well established (Fields et al. 2006, Yaksh & Tyce 1979, Yoshimura & Furue 2006). In the periphery, serotonin released from platelets is able to activate nociceptors (Lang et al. 1990), and many studies have suggested pronociceptive effects for serotonin (e.g. Pickering et al. 2003, Richardson & Engel 1986, Zeitz et al. 2002, Zhang et al. 2001). Additionally, bulbospinal serotonergic pathways have also been shown to mediate facilitation and potentiation of pain (Millan 2002, Suzuki et al. 2004). In summary, both antinociceptive and pronociceptive pain-modulatory actions for serotonin have been described, and the final effects of serotonin on pain depend on a variety of factors, such as the level of the neuraxis (primary sensory neuron, spinal cord or brain), receptor subtype, and pain condition (Millan 2002, Sommer 2006). Consequently, the effects of serotonin on pain are complex; for instance, knock-out mice lacking serotonergic neurons show normal thermal and visceral pain responses, decreased mechanical sensitivity and enhanced inflammatory pain sensitivity (Zhao et al. 2007).

Experimental animal studies indicate that the bulbospinal pathways originating from the raphe nuclei have an important role in serotoninergic pain modulation (e.g. Lopez-Garcia 2006, Millan 2002, Rivot et al. 1984). Neurons in the rostroventromedial medulla (RVM), particularly in the NRM, give rise to important inhibitory and facilitatory serotonergic projections, which travel in the dorsolateral funiculus and terminate mainly in the spinal dorsal horn neurons in laminas I, II and V (Basbaum & Fields 1978, Fields et al. 2006). This endogenous pain control system is regulated by neurons in the periaqueductal grey, which is a critical part in many pain-regulating mechanisms (Basbaum & Fields 1978, Fields et al. 2006). Both acute and chronic pain activates the serotonergic neurons in the RVM and increases serotonin transmission in the spinal cord (Millan 2002). In addition to the important role of the NRM in pain, the serotonergic neurons in the dorsal (Wang & Nakai 1994) and medial (Millan 2002) raphe nuclei have also been shown to be important in pain regulation. On the other hand, the serotoninergic raphe nuclei also innervate extensively various parts of the forebrain (Azmitia & Segal 1978, Hornung 2003). The effect of these ascending serotonergic projections on pain is less well known, but there is some evidence suggesting that the ascending serotoninergic fibers might also contribute to pain regulation by a mechanism different from that of the NRM (Inase et al. 1987, Messing & Lytle 1977, Wang & Nakai 1994). Furthermore, there is some initial evidence from experiments with animals indicating that cortical serotonin receptors are involved in descending pain modulation (Pini et al. 1996, Qu et al. 2008). Although serotonin clearly has a critical role in pain regulation, there is a surprising lack of experimental studies relating the role of serotonin receptor function in humans to pain perception.

2.5.2. Serotonin 1A receptors and pain

Among the many types of serotonin receptors associated with pain, the 5-HT_{1A} receptor appears to be one that plays a significant role in mediating pain regulatory effects (e.g. Colpaert et al. 2002 & 2006, Mico et al. 2006). As with serotonin in general, a dual action of 5-HT_{1A} receptors on pain has been described: 5-HT_{1A} receptor activation may lead to both pro-nociceptive and anti-nociceptive effects (Colpaert et al. 2002, Sommer 2006). Moreover, 5-HT_{1A} receptors both in the spinal cord and supraspinal areas seem to differentially regulate pain, adding to the complexity of the 5-HT_{1A} receptor-mediated pain regulation (e.g. Fasmer et al. 1986, Millan 2002).

5-HT_{1A} knock-out mice and mice treated with systemic 5-HT_{1A} antagonist exhibit high heat pain sensitivity, suggesting that 5-HT_{1A} receptors mediate analgesia to heat pain (Kayser et al. 2007). Correspondingly, systemic administration of 5-HT_{1A} agonists produces antinociception in a variety of pain models in animals (Bardin et al. 2001, Cervo et al. 1994, Colpaert et al. 2002, Fasmer et al. 1986, You et al. 2005). In addition, spinal administration of 5-HT_{1A} agonists produces a significant reduction in pain-related responses (Danzebrink & Gebhart 1991, Eide & Hole 1991, el-Yassir et al. 1988, Garraway & Hochman 2001, Hains et al. 2003, Jeong et al. 2004). Indeed, a substantial amount of evidence suggests that 5-HT_{1A} receptors at the spinal cord dorsal horn have a role in nociceptive processing in mediating anti-nociceptive effects. This is also supported by the high expression of 5-HT_{1A} receptors in the dorsal horn, especially in the superficial layers but also in deeper laminae, but negligible densities in intermediolateral cell column or ventral horn (Millan 2002). However, 5-HT_{1A} receptors also mediate pro-

nociceptive effects (Alhaider & Wilcox 1993, Ali et al. 1994, Millan et al. 1996, Zemlan et al. 1983, Zhang et al. 2001, Zhang et al. 2002). Several experimental animal studies indicate that the spinal 5-HT_{1A} receptors are central in descending raphe-spinal pain regulation (el-Yassir & Fleetwood-Walker 1990, Lin et al. 1996, Liu et al. 2002, Wei & Pertovaara 2006). In addition to the well-established effects in the spinal cord, the supraspinal 5-HT_{1A} receptors have also been suggested to modulate pain. Intracerebroventricular administration of 8-OH-DPAT in mice produces hypoalgesia in the hot-plate test and the formalin test (Fasmer et al. 1986). Specifically, 5-HT_{1A} receptors in the brainstem seem to be important in pain regulation. 5-HT_{1A} receptors in the RVM are involved in the regulation of neuropathic hypersensitivity (Wei & Pertovaara 2006). Furthermore, the DRN is important in pain control, and the activity of the DRN is mainly under the control of 5-HT_{1A} somatodendritic autoreceptors (Hopwood & Stamford 2001, Sotelo et al. 1990, Wang & Nakai 1994). Although experimental studies generally find 5-HT_{1A} receptor agonists to be antinociceptive, it is often unclear whether the main antinociceptive actions are spinal or supraspinal.

Several brain areas involved in pain perception, such as the raphe nuclei, amygdala, cingulate cortex, insula and prefrontal cortex (Bushnell & Apkarian 2006, Casey & Tran 2006), have a high density of 5-HT_{1A} receptors (e.g. Azmitia et al. 1996, Hirvonen et al. 2007, Palacios et al. 1990, Parsey et al. 2002, Pazos & Palacios 1985, Rabiner et al. 2002, Tiihonen et al. 2004), as indicated by studies in non-human primates and humans. Apart from the well-established role of raphe 5-HT_{1A} receptors in pain, little is known about the effects of brain 5-HT_{1A} receptors on pain. The 5-HT_{1A} receptors in ventrolateral orbital cortex are involved in descending modulation of pain in the rat (Qu et al. 2008), suggesting that cortical 5-HT_{1A} receptors, too, might be involved in pain regulation. So far, no studies have investigated the role of brain 5-HT_{1A} receptors in the regulation of pain in humans.

2.5.3. The role of serotonin in clinical pain and treatment of pain

Considering the complexity of serotonergic pain modulatory systems, it is not surprising that studies assessing the association of blood or tissue serotonin with pain have resulted in partly conflicting results (e.g. Ernberg et al. 1999, Kopp & Alstergren 2002, Wolfe et al. 1997). Healthy subjects with low blood serotonin have high pain thresholds (Kopp & Alstergren 2002, Pickering et al. 2003); correspondingly, patients with seropositive rheumatoid arthritis have high blood serotonin (Kopp & Alstergren 2002). Nonetheless, low serotonin has been associated with chronic pain, such as chronic tension headache (Anthony & Lance 1989) and fibromyalgia (Wolfe et al. 1997). In migraine, the platelet serotonin drops during the migraine attack, and a serotonin infusion relieves attacks (Goadsby 2000). Apart from studies on the role of serotonin in migraine, there is a clear lack of studies concerning the effect of serotonin and the effect of various serotonin receptors on clinical pain, although animal studies suggest that 5-HT_{1A} receptors (Bonnefont et al. 2005, Mico et al. 2006) and brain serotonin (e.g. Pini et al. 1996) may be involved in the effects of clinical analgesics. In a study with patients with irritable bowel syndrome, an increased synthesis of serotonin in the right medial temporal cortex was demonstrated (Nakai et al. 2003), which may be associated with abnormal visceral pain processing at the supraspinal level. Recently, a functional polymorphism of the 5-HTT gene promoter region (5-HTTLPR) has been shown to be involved in clinical pain, as

migraineurs with aura were shown to carry the 5-HTTLPR S-allele significantly more often than the control population (Borroni et al. 2005, Marziniak et al. 2005).

Drugs that modulate serotonergic neurotransmission have analgesic properties in patients with pain, supporting the hypothesis that serotonin has a role in the modulation of pain. Triptans, which exert their effects mainly by 5-$HT_{1B/1D}$ receptor agonism, have an important role in the treatment of migraine (Goadsby 2000). Drugs acting on serotonin receptors also have a role in the treatment of pain in other pain syndromes: this is suggested by the analgesic effect induced by selective serotonin reuptake inhibitors (SSRIs) in various clinical conditions such as irritable bowel syndrome (Tack et al. 2006, Vahedi et al. 2005), chronic rheumatic (Rani et al. 1996) and neuropathic pain (Rowbotham et al. 2005, Sindrup et al. 1990), fibromyalgia (Arnold et al. 2002, Goldenberg et al. 1996), somatoform pain disorder (Aragona et al. 2005) and oesophageal hypersensitivity (Broekaert et al. 2006). However, there is only limited evidence for the effectiveness of SSRIs in chronic pain conditions and the observed analgesic effects tend to be modest (Otto et al. 2008, Saarto & Wiffen 2007, Watson et al. 2006). Moreover, in an acute setting, the effects of SSRIs may be even pronociceptive (Dirksen et al. 1998), again emphasizing the complexity of serotonergic influences on pain.

2.6. Positron Emission Tomography

Positron emission tomography (PET) is a non-invasive tomographic imaging method based on the use of positron-emitting isotopes (tracers) and cameras with detectors for gamma rays. Computer analysis is used for the reconstruction of 3-dimensional images of tracer concentration in the target tissue. This method allows *in vivo* imaging of many physiological processes, such as blood flow, oxygen consumption, glucose metabolism, and neurotransmitter and receptor functions. As receptors are key players in brain function, brain receptor imaging has become an important tool in neuroscience research (Heiss & Herholz 2006).

Radioligands are biologically active molecules labelled with a radioisotope, which is produced in a cyclotron. After quality control, the radioligand is introduced into the body by inhalation or intravenous injection. The radioisotopes have relatively short half lives: the most commonly used radioisotopes ^{11}C, ^{15}O and ^{18}F have respective half lives of 20 min., 2 min. and 110 min. (Lammertsma 1992). An isotope with an excess of protons reaches stability by emitting a positron (an antimatter counterpart to the electron with the opposite charge), in a process called positron emission decay (Turkington 2001):

$$ {}^{A}_{Z}X_{N} \rightarrow {}^{A}_{Z-1}Y_{N+1} + e^{+} + \nu $$

Positron decay results in a new nuclide with 1 fewer proton and 1 more neutron. Simultaneously, a positron and a neutrino are emitted. Depending on its energy and tissue density, the positron may travel up to a few millimetres before encountering an electron. The collision annihilates both particles, and the masses of both particles are converted into the energy of a pair of 511 keV annihilation (gamma) photons moving in opposite directions. The photons are detected by scintillator crystals in the detector ring of the scanning device. Coincidental detection of photons at opposite sides of the detector ring makes a true count, and the annihilation is interpreted to have taken place somewhere along the line between the

coincidental detections (line of response). PET images are reconstructed according to the spatial and temporal distribution of the coincidence events. The theoretically optimal spatial resolution of PET (2-3 mm) is limited by physical factors such as positron path and the non-collinearity of the annihilation photons, as well as the structure of the detector system (Turkington 2001).

Tracer modeling is needed in order to obtain quantitative information on the specifically bound tracer, as the regional radioactivity in PET is composed of radioactivity from not only the specifically bound tracer, but also from unbound and nonspecifically bound tracers; additionally, intravascular activity contributes to the total regional radioactivity (Lammertsma 2002). All the modelling approaches assume a compartmental system (Lammertsma 1992). A compartment is a physiological or biochemical (theoretical) space in which the tracer concentration is homogenous at all times (Slifstein & Laruelle 2001). There are several different methods available for tracer modelling for reversibly binding radioligands (Ichise et al. 2001, Slifstein & Laruelle 2001). The methods generally used can be divided into three categories: kinetic (iterative), equilibrium and graphical methods. In kinetic compartmental models, the pharmacokinetics of the tracer is described by rate constants between different compartments, and the radioactivity of the unbound tracer in arterial plasma is used as an input function. In reference region methods, a reference region (a region with no specific binding) is used as an input function and arterial plasma sampling is not needed. True equilibrium methods, which necessitate a constant infusion of the tracer, enable measurements at equilibrium (unlike other methods that infer equilibrium concentrations with mathematical models from nonequilibrium data). Graphical methods allow estimation of distribution volume (V_T) without *a priori* compartmental model specification. The different methods have characteristic advantages and biases which have to be taken into account when planning the study and interpreting the data (Slifstein & Laruelle 2001). The principal outcome measure for radioligand uptake in receptor studies is binding potential (*BP*), which quantifies the equilibrium concentration of specific binding as a ratio to a reference concentration. Essentially, specific binding is compared to free plasma concentration (BP_F), total plasma concentration (BP_P), or nondisplaceable uptake (BP_{ND}) (Innis et al. 2007).

PET data can be analyzed by region of interest (ROI) or voxel-based methods. In the ROI method, ROIs are manually drawn on coregistered individual magnetic resonance (MR) images. For each ROI, a time activity curve is calculated and fitted into the tracer model. Voxel-based analysis utilizes parametric images calculated from PET data with models such as the simplified reference tissue model (Gunn et al. 1997). In parametric images, each pixel represents a measure of interest (e.g. *BP*). The parametric images are smoothed and spatially normalized onto a common stereotactic space, allowing comparisons between subjects or experimental groups. Receptor parametric maps visualizing differences in tracer uptake between subjects/experimental conditions are produced by application of a general linear model on a voxel-by-voxel basis (Friston et al. 1995).

2.7. Psychophysical methods in pain research

Psychophysics is the scientific study of the relation between stimulus and sensation. Because pain is a subjective experience, it can only be measured by studying the subject´s reports of his sensations. The use of calibrated painful stimuli and psychophysical analysis of the responses

enables the study of individual pain sensitivity and comparison of pain sensitivity between individuals in a laboratory setting, where many external confounding factors influencing sensation may be eliminated (Gracely 2006, Gracely & Eliav 2009). Thus, in order to increase our understanding of pain, the use of classical and modern psychophysical methods is of fundamental importance.

A peripheral nociceptor may be activated by mechanical, thermal or chemical stimuli (Meyer et al. 2006), and all these different stimulus modalities are used in experimental pain research (Gracely 2006). Mechanical stimuli are easy to apply but activate both nociceptors and mechanoreceptors. Thermal stimuli selectively activate a well-characterized set of nociceptors with specific signal detection and transduction molecules (Julius & Basbaum 2001, Julius & McCleskey 2006), without contamination from other sensory modalities. Multiple graded stimuli are easily applied to the skin, and pain is relieved quickly after stimulation. Heat stimuli are commonly used and delivered mainly with contact heat (usually contact thermode) or a radiant source (e.g. laser); cold stimuli are usually delivered with a contact thermode or by immersing a body part in ice water in the cold pressor test (CPT) (Chen et al. 1989, Walsh et al. 1989, Wolf & Hardy 1941). The low relative unpleasantness makes contact heat stimulation suitable for studying sensory-discriminative aspects of pain (Rainville et al. 1992), whereas CPT induces a deep aching pain probably originating from deep structures (Fruhstorfer & Lindblom 1983) which mimicks clinical pain by evoking high relative unpleasantness (Rainville et al. 1992), making it an interesting model in experimental pain research. However, CPT also induces also autonomic vasomotor responses in the immersed body part, which are correlated with the experienced pain (Wolf & Hardy 1941). The autonomic vasomotor responses in the immersed body part may influence pain and provide a hypothetical source of bias (Handwerker & Kobal 1993). Additionally, pain may be elicited by applying electrical or chemical stimuli to skin, or by causing temporary ischaemia in a limb (Gracely 2006). Electrical stimuli elicit pain by directly activating afferent nerve fibers. This causes serious methodological problems, as not only nociceptive, but also different non-nociceptive fibers are activated. Moreover, the activation is not physiological, as the transduction process in sensory nerve ending is bypassed and the fibers activated are unnaturally synchronized (Handwerker & Kobal 1993). Ischaemic pain (such as the tourniquet pain test), in contrast, leads undoubtedly to a physiological activation of nociceptors and, additionally, evokes high unpleasantness (Rainville et al. 1992), mimicking pathological pain (Sternbach 1983). However, a paradigm with many graded stimuli is obviously impossible. Painful stimuli may also be classified according to the duration as either phasic and tonic stimuli. Phasic stimuli are usually easy to apply in a graded manner, may be reproduced many times during a session and have a clear onset that enables stimulus-locking in e.g. electroencephalography (EEG) experiments. Tonic stimuli lack a clear time course but may be closer to clinical pain (Handwerker & Kobal 1993, Rainville et al. 1992).

The measurement of pain provides a significant challenge for pain research. Perhaps the most fundamental problem is the semantic question: when can a burning or pricking sensation be labeled as "painful"? This problem cannot fully be solved, and many pain measurement paradigms simply allow the subject to establish his own arbitrary criterion to report pain and measure changes in the criterion in different settings. Many different procedures have been

employed to measure experimental pain, with characteristic advantages and limitations (Chapman et al. 1985). A common measure of pain in experimental settings is the pain threshold. The psychophysical concept of threshold (absolute *or* stimulus threshold) is based on the philosopher Herbart´s assumption that in order to be consciously experienced, the mental events had to be stronger than some critical amount (Gescheider 1997). The pain threshold is defined as the least intensity of pain that a subject can recognize. However, in common usage the term pain threshold refers to the least stimulus energy that produces pain, and the psychophysical pain threshold is defined as the stimulus intensity that produces pain in 50% of stimulus deliveries (Gescheider 1997, IASP Task Force on Taxonomy 1994, Wolff 1983). The basic psychophysical measurements are often easy to perform, and the concept of "pain threshold" is well known to all healthcare professionals (although often misinterpreted as being synonymous with pain sensitivity). Unfortunately, pain threshold procedures have serious shortcomings and these methods may often be unsuitable for pain assessment, in particular if psychological variables play a major role (Chapman et al. 1985). Other measures include pain tolerance, which is defined as the greatest level of pain that a subject is prepared to tolerate (Wolff 1983). As with the pain threshold, pain tolerance is commonly defined by the energy of the noxious stimulus (IASP Task Force on Taxonomy 1994). The sensation elicited by any stimulus intensity can be assessed by mainly two scales, category and ratio scales: the category scales consist of fixed categories, whereas a ratio scale is a response continuum, as in the visual analogue scale (VAS) (Handwerker & Kobal 1993, Huskinsson 1983). Pain can be divided into sensory-discriminative, affective-motivational and cognitive-evaluative dimensions, and these different aspects of pain can be separately measured and analyzed (Handwerker & Kobal 1993).

Pain sensitivity determined with traditional methods, such as threshold theory, is dependent both on the subject's discriminative capacity and the subject's attitude towards reporting the sensory experience (Clark & Yang 1983, Gescheider 1997). Discriminative capacity is dependent on sensory function, whereas the subject's attitude (response criterion) reflects non-sensory functions such as personality traits (Gescheider 1997, Swets 1973). Although differences in pain threshold are often attributed to differences in the nociceptive system mediating the sensory signal, differences in pain threshold may also be due to complex psychological factors, such as the motivational state of the subject. Assessment of sensory responses by advanced psychophysical methods that are based on the signal detection theory (SDT) allows dissociation of the subject's discriminative capacity from the subject's response criterion (Clark 1974 & 1994, Clark & Yang 1983, Swets 1973). One component, discriminative capacity (e.g. as measured by the area under the receiver operating characteristic curve, ROC [AUC]), provides a relatively pure measure of sensory discriminability, which is independent of attitude or expectation, whereas the second measure of the subject's performance, the response criterion, gives an estimate of the subject's response bias or attitude toward reporting a sensation (Clark & Yang 1983, Gescheider 1997).

3. RATIONALE FOR THE STUDY

Animal studies and studies with healthy human subjects suggest that the striatum and particularly striatal dopamine D2/D3 receptors are involved in the perception of pain (Chudler & Dong 1995, Hagelberg et al. 2004b). Animal studies have indicated that administration of dopamine D2/D3 receptor agonists in the striatum suppresses pain-related responses, whereas dopamine D2/D3 receptor antagonists in the striatum enhance pain (Ben-Sreti et al. 1983, Lin et al. 1981, Magnusson & Fisher 2000). The activation of the striatum during painful stimulation in human brain imaging studies suggests that the striatum is involved in the perception of pain in humans (Casey et al. 1996, Jones et al. 1991). The dopamine D2/D3 receptor may also be mediating the pain regulatory effects of striatal dopamine in humans, as shown by the finding that patients with a chronic orofacial pain syndrome have increased dopamine D2/D3 receptor availability (Hagelberg et al. 2003a & 2003b) and diminished uptake of [^{18}F]FDOPA (Jääskeläinen et al. 2001) in the putamen. In an experimental study with healthy humans, D2/D3 BP_{ND} in the putamen was inversely correlated with the cold pressor pain threshold (Hagelberg et al. 2002b). On the other hand, the dopaminergic system has been implicated in emotional, motivational and cognitive functions including placebo response (de la Fuente-Fernández et al. 2001, Nieoullon & Coquerel 2003). All this raises the hypothesis that striatal dopamine D2/D3 receptors mediate analgesic effects in humans, and potentially the analgesic effect of placebo.

Correspondingly, evidence from animal studies indicates that 5-HT_{1A} receptors are involved in the regulation of pain (Colpaert 2002 & 2006, Mico et al. 2006). Systemic application of 5-HT_{1A} receptor agonists suppresses pain-related responses, whereas application of 5-HT_{1A} receptor antagonists enhances pain (Colpaert 2002, Fasmer et al. 1986). In humans, serotonergic drugs have been found effective in the treatment of pain (Saarto & Wiffen 2007). In the human brain, there is a high density of 5-HT_{1A} receptors in the dorsal raphe nucleus, which is critical in the regulation of overall brain serotonergic activity, as well as in many other pain-related areas in the forebrain, raising the question of whether brain 5-HT_{1A} receptors could be involved in the perception of pain in humans. In addition, brain areas related to memory, such as the hippocampus, have a high density of 5-HT_{1A} receptors, and experimental studies in animals and in humans suggest that 5-HT_{1A} receptors may be involved in the regulation of memory (Meneses & Perez-Garcia 2007, Yasuno et al. 2003). This, in turn, raises the hypothesis that brain 5-HT_{1A} receptors are involved in the regulation of memory for pain.

The purpose of this thesis was to assess whether baseline (resting) D2/D3 BP_{ND} and 5-HT_{1A} $BP_{ND/P}$ in the brain in healthy humans is associated with response to pain versus touch, and whether the potential associations could be explained by effects on discriminative versus non-sensory (evaluative) aspects of pain and touch perception. Additionally, we assessed whether brain D2/D3 BP_{ND} is associated with an analgesic response to placebo, and whether brain 5-HT_{1A} receptor $BP_{ND/P}$ is associated with autonomic control and short-term memory for pain. The results of this thesis increase the current understanding of the role of D2/D3 receptors and 5-HT_{1A} receptors in the perception of pain in humans and provide a rational basis for further human imaging studies.

4. AIMS OF THE STUDY

The aim of the study was to assess the role of striatal dopamine 2/3 (D2/D3) receptors and brain serotonin 1A (5-HT_{1A}) receptors in regulating responses to experimental pain in healthy human subjects. The specific objectives were:

1. To assess whether striatal D2/D3 BP_{ND} is associated with the response to pain and touch, and with the analgesic effect of placebo on heat pain (**II**).

2. To assess whether brain D2/D3 BP_{ND} is associated with discriminative capacity and response criterion for heat pain (**I**).

3. To assess whether brain 5-HT_{1A} BP_P is associated with response to cold pressor pain and autonomic control (**III**).

4. To assess whether brain 5-HT_{1A} BP_{ND} is associated with discriminative capacity and response criterion for heat pain and for touch, and with short-term memory for pain (**IV**).

5. SUBJECTS AND METHODS

5.1. Subjects

A total of 39 healthy, right-handed volunteers were recruited for the studies. The subjects had previously participated as healthy controls in PET studies in which their resting (baseline) BP_{ND}/BP_P had been assessed, and were later recruited for the psychophysical testing. The time interval between the PET scanning and psychophysical testing was: 817 ± 180 (**I**), 2197 ± 110 (**II**), 1938 ± 330 (**III**), and 2662 ± 670 (**IV**) days (mean ± SD) (Table 1). Of the 8 subjects of study **II,** 6 subjects had participated in study **I**. Of the 11 male subjects in the study **III**, 9 males also participated in study **IV** (Table 1). All subjects were non-smoking, had no history of alcohol or drug abuse, chronic pain, or psychiatric (Axis I disorders according to DSM-IIIR or DSM-IV) or somatic illness. The subjects had been screened for history of first-degree relatives with psychosis and abuse and were life-time naïve for psychotropic medicines, as based on self-reports given in an interview before the PET scanning. The subjects were interviewed by an experienced clinician about their psychiatric and somatic health status and potential use of medication before both psychophysical testing and PET scanning, to ensure that the health status of the subjects involved in the study had not changed, and that they had not developed drug or alcohol abuse. Subjects abstained from alcohol and any medication for 48 h before the psychophysical testing. To exclude structural brain abnormalities and for anatomical reference, a 1.5 T magnetic resonance (MR) image of the brain was obtained from each participant (Magnetom, Siemens, Erlangen, Germany). No laboratory tests were taken. The studies were performed in accordance with the Declaration of Helsinki, and the Joint Ethical Committee of Turku University Hospital and University of Turku approved the study protocols. Written, informed consent was received from the subjects before both PET scanning and psychophysical testing. In one subject, the pain threshold was not reached even at 48°C and this subject was excluded from all further analyses (**IV**).

Table 1. Demographic data of the subjects and general characteristics of the studies I-IV

Study	**I**		**II**	**III**	**IV**
Subjects	19		8[a]	11	16[b,c]
Sex (M/F)	19/0		8/0	11/0	9/7
Age	33 ± 6	26 ± 3	33 ± 4	33 ± 6	34 ± 6
- range	26-41	22-31	29-42	25-48	25-49
Tracers					
- [^{11}C]raclopride	N = 8		N = 8		
- [^{11}C]FLB 457		N = 11			
- [^{11}C]WAY-100635				N = 11	N = 16

The data are presented as mean ± SD.

[a] Of the 8 subjects of study **II**, 6 subjects had also participated in study **I**.

[b] Of the 11 male subjects of study **III**, 9 males also participated in study **IV**.

[c] In one subject, the pain threshold was not reached, and this subject was excluded from all further analyses.

5.2. PET imaging

5.2.1. Imaging of brain dopamine D2/D3 receptor binding

The availability of dopamine D2/D3 receptors in the living human brain can be studied by determining dopamine D2/D3 receptor binding potential (D2/D3 BP_{ND}) with PET. Since in study **I** we determined D2/D3 BP_{ND} in both the striatal and extrastriatal regions of interest (ROIs) which have D2/D3 receptor densities far from each other (Hall et al. 1994), PET imaging was performed with two D2/D3 receptor specific radioligands: the low-affinity ligand [^{11}C]raclopride for striatal ROIs and the high-affinity ligand [^{11}C]FLB 457 for extrastriatal ROIs (Table 2). Study **II** focused on striatal D2/D3 receptors, and included only the subjects that had been studied with [^{11}C]raclopride.

Scanning protocols (Hietala et al. 1999, Vilkman et al. 2000) and details of the PET method (Hagelberg et al. 2002b) have been previously published. The methods for the preparation of the radioligands [^{11}C]raclopride (Hietala et al. 1994) and [^{11}C]FLB 457 (Lundkvist et al. 1998) have also been described in detail earlier. The specific radioactivity of [^{11}C]raclopride was 31 ± 5.3 MBq/nmol, injected dose 220 ± 19 MBq and mass 2.2 ± 0.5 μg (mean ± SD). For [^{11}C]FLB 457, the specific radioactivity was 47 ± 7.6 MBq/nmol, injected dose 210 ± 19 MBq and mass 1.4 ± 0.3 μg (mean ± SD). PET scans were conducted using a GE Advance PET scanner (General Electric Medical Systems, Milwaukee, WI, USA) in 3D mode with 35 slices of 4.25 mm thickness covering the whole brain. A transmission scan for the measurement of

attenuation of soft tissues preceded the dynamic PET scan. The radioligands were injected intravenously as a rapid bolus, and the uptake was measured for 51 min. using 13 time frames with [^{11}C]raclopride and 69 min. using 16 time frames with [^{11}C]FLB 457 (Vilkman et al. 2000). ROIs were defined on individually realigned 1.5 T MRI images, and ROI analysis was performed to determine striatal dopamine D2/D3 BP_{ND} with [^{11}C]raclopride in eight subjects (**I** and **II**), and cortical and thalamic D2/D3 BP_{ND} with the high affinity tracer [^{11}C]FLB 457 in eleven subjects (**I**). Striatal ROIs were defined on the putamen and caudate nucleus (**I** and **II**), and the extrastriatal ROIs on the medial and lateral thalamus, the medial and lateral frontal cortex, the medial and lateral temporal cortex, and the anterior cingulum (**I**) (Table 2). D2/D3 receptor BP_{ND} was determined for each ROI in both the right and left hemisphere individually. For the calculation of D2/D3 BP_{ND}, a simplified reference tissue model was used for [^{11}C]raclopride (Lammertsma & Hume 1996), and a reference tissue model for [^{11}C]FLB 457 (Olsson et al. 1999) with the cerebellum as reference region, as the cerebellum is devoid of D2/D3 receptors (Hall et al. 1994).

Table 2. Regions of Interest (ROIs)

I[a]	II[a]	III[b]	IV[b]
Anterior cingulate cortex Caudate nucleus Cerebellum Medial frontal cortex Lateral frontal cortex Putamen Medial temporal cortex Lateral temporal cortex Medial thalamus Lateral thalamus	Caudate nucleus Cerebellum Putamen	Amygdala Angular gyrus Cerebellum Dorsal part of the anterior cingulate cortex Ventral part of the anterior cingulate cortex Posterior cingulate cortex Superior part of the posterior cingulate cortex Hippocampus Anterior insula Posterior insula Dorsolateral prefrontal cortex Medial prefrontal cortex Dorsal raphe nucleus Inferior temporal gyrus Middle temporal gyrus Superior temporal gyrus	Amygdala Angular gyrus Cerebellum Dorsal part of the anterior cingulate cortex Ventral part of the anterior cingulate cortex Posterior cingulate cortex Superior part of the posterior cingulate cortex Hippocampus Anterior insula Posterior insula Orbitofrontal cortex Dorsolateral prefrontal cortex Ventrolateral prefrontal cortex Medial prefrontal cortex Dorsal raphe nucleus Inferior temporal gyrus Middle temporal gyrus Superior temporal gyrus Supramarginal gyrus

[a] ROIs were delineated in the right and left hemisphere, and analyzed separately in statistical analysis.
[b] ROIs were delineated in both hemispheres, but as there was no difference in binding potential (BP_{ND}/BP_P) between right and left hemisphere, BP_{ND}/BP_P values from right and left were pooled for the statistical analysis.

5.2.2. Imaging of brain serotonin 5-HT_{1A} receptor binding

The imaging of brain 5-HT_{1A} receptors was performed using [*carbonyl*-^{11}C]WAY-100635. PET experiments were performed using a whole-body 3D PET scanner (GE Advance; GE, Milwaukee, Wis., USA) with 35 slices of 4.25 mm thickness covering the whole brain. The scanning procedure and preparation of [*carbonyl*-^{11}C]WAY-100635 have been described in detail previously (Hirvonen et al. 2007). The radioligand was administered intravenously as a rapid bolus. The injected dose of [*carbonyl*-^{11}C]WAY-100635 was 290 ± 74 MBq, specific radioactivity 62 ± 34 MBq/nmol and mass of radiotracer 2.3 ± 0.8 μg (means ± SD). The uptake of [*carbonyl*-^{11}C]WAY-100635 was measured for 57 minutes using 14 time frames of increasing duration. To obtain arterial input function for modelling, the left radial artery was cannulated for taking arterial samples. For arterial input function, an automated continuous blood sampling system was used for the first 3.5 minutes, and manual samples (2 ml) were obtained thereafter at 4.5, 7.5, 11, 14, 18, 24, 30, 36, 42, 48 and 54 minutes after radioligand injection. To measure the fraction of unchanged [*carbonyl*-^{11}C]WAY-100635 in arterial plasma, arterial blood samples (2 ml) were collected at 2, 6, 12, 20 and 30 minutes. The unchanged [*carbonyl*-^{11}C]WAY-100635 in arterial plasma was measured as previously described (Hirvonen et al. 2007).

For the calculation of regional time-activity curves, regions of interest (ROIs) (Table 2) were delineated (**III**). ROIs were drawn on the MR images that had been coregistered according to the mean image of PET-PET coregistered summed PET images using the normalized mutual information method as implemented in SPM2. ROIs were drawn manually on the MR images using Imadeus software (version 1.2, Forima Inc., Turku, Finland). All ROIs were drawn on 3-4 planes. In study **IV**, an automated region of interest (ROI) analysis was carried out to calculate regional time-activity curves as previously described in detail (Hirvonen et al. 2008). Briefly, spatial normalization parameters into standard space were estimated from integral (summed) PET images and a ligand-specific template for [*carbonyl*-^{11}C]WAY-100635. These spatial normalization parameters were then applied to individual dynamic PET images to transform them into standard space. These preprocessing steps were done with SPM2 (Friston et al. 1995). A predefined set of ROIs (Table 2) in the standard space was then applied to spatially normalized dynamic PET images, and regional time-activity curves were calculated using Imadeus software. Thus, individual MR images were not used for normalization or ROI definition in study **IV**. The ROI for the DRN was drawn directly on the PET images, since this structure is not readily visible in MR images. Cerebellar white matter was delineated as a reference region (Hirvonen et al. 2007, Parsey et al. 2005). No correction for partial volume effects or segmentation of white and grey matter was applied. Prior to modeling, contribution of total blood radioactivity to regional tissue time-activity curves was eliminated by assuming 5% blood volume in ROI and subtracting it directly from regional radioactivity. Distribution volumes for the standard two-tissue compartmental model were estimated directly without division using a linearized method based on non-negative least squares optimization, using the metabolite-corrected arterial plasma time-activity curve as the input function (Hirvonen et al. 2007, Zhou et al. 2004). Binding potential (*BP*) values were then indirectly estimated from the V_T values of ROIs and the reference region. Two commonly used estimates of *BP* were considered: $BP_P = V_T[\text{ROI}]-V_T[\text{REF}]$ and $BP_{ND} = V_T[\text{ROI}]/V_T[\text{REF}]-1$ ($BP_P/V_T[\text{REF}]$). BP_P is

proportional to $f_P B_{Avail}/K_D$ (where f_P is the fraction of free or non-protein bound radiotracer in the plasma, B_{Avail} is the total concentration for receptors, and K_D is the apparent equilibrium dissociation constant), whereas BP_{ND} is proportional to $f_{ND} B_{Avail}/K_D$, where f_{ND} is the fraction of radioactivity originating from free radiotracer in the non-displaceable tissue compartment (Innis et al. 2007). The free fraction f_P was not measured in the current study. In study **III**, V_T[REF] (V_T in the reference region), used to approximate free and non-specific binding in the brain, had a significant inverse association with the intensity of CPP (β = -0.62, t = -2.33, p = 0.048) but not with other psychophysical measures (data not shown). Consequently, BP_P was chosen as the outcome measure of choice given the relative independence of this measure concerning reference region V_T as compared with BP_{ND}. Since V_T[REF] was not associated with any of the psychophysical variables (data not shown) in study **IV**, BP_{ND} was chosen as the primary outcome measure since it is independent of the plasma protein binding of the parent compound (f_P; Innis et al. 2007). In publication **III**, BP_P is referred to as BP_1, and BP_{ND} is referred to as BP_2.

In order to confirm the results from the ROI-based analysis in study **III**, an independent voxel-based analysis was performed (Rabiner et al. 2002). First, V_T values for the two-tissue compartmental model were estimated voxel-wise using a linearized method based on non-negative least squares optimization, as was done at the ROI-level. These parametric two-tissue V_T maps were then converted to parametric BP_P maps by applying the equation $BP_P = V_T$[ROI] - V_T[REF] to each voxel in the V_T map, that is, by subtracting the ROI-based cerebellar white matter V_T value from each voxel. Preprocessing and statistical analysis was performed using SPM2 (Friston et al. 1995) running on Matlab 6.5 for Windows (Math Works, Natick, MA). Parametric BP_P maps were spatially normalized into standard space using normalization parameters estimated from summated PET images and a ligand-specific template, created as previously described (Meyer et al. 1999). Spatially normalized parametric BP_P maps were then smoothed using a 12-mm Gaussian kernel. The association between voxel-wise BP_P and pain intensity was examined using the simple regression (correlation) option in SPM2. As BP_P values are quantitative in nature, no scaling of voxel-wise BP_P values was performed. Images were masked using the image global value as threshold, and zeros were ignored. A cluster-level corrected p-value of 0.05 was considered a criterion for statistical significance.

5.3. Psychophysical testing

5.3.1. Psychophysical testing sessions

Before the psychophysical testing, all subjects abstained from any medication or alcohol for 48 h. The psychophysical testing sessions started at 9 a.m. – 3 p.m. During the testing session, the subjects were sitting comfortably in a quiet room. Before all experiments, the subjects were familiarized with the experimental conditions and all stimulus intensities were introduced to the subject once.

5.3.2. Assessment of tactile sensitivity

Sensitivity to tactile stimuli was tested with von Frey monofilaments (18011 Semmes-Weinstein Aesthesiometer Kit; Stoelting Co., Wood Dale, IL, USA). While the subject was sitting blindfolded in a comfortable chair, monofilaments of five strengths (1 = 0.226 mN, 2 = 0.270 mN, 3 = 0.667 mN, 4 = 1.63 mN and 5 = 3.99 mN) and a sham monofilament (no

stimulus) were presented to the tip of the subject's right index finger (monofilament 5 was not used in study **IV**). All conditions were introduced 10 (**IV**) or 20 (**II**) times in a random order. The time of stimulus presentation was told to the subject. While sitting blindfolded, the subject answered with a category rating scale with three possibilities: no stimulus (score 0), a possible stimulus (score 1), or a definite stimulus (score 2). For the analysis of the detection thresholds the scores 1 and 2 were pooled together. The traditional detection threshold was defined as a 50% detection rate and was depicted from psychometric function curves.

5.3.3. Assessment of heat pain sensitivity

In studies **I** and **II**, the cutaneous heat pain threshold was assessed with a Medoc TSA-2001 Thermal Sensory Analyzer using a 16 mm x 16 mm probe, and in study **IV** with a Medoc TSA-2 NeuroSensory Analyzer using a 30 mm x 30 mm probe (Medoc Inc., Rehovot, Israel). All stimuli were delivered to the right volar forearm, and the subject was instructed to change the stimulus site after each stimulus presentation (in the heat pain short-term memory task, after each pair of stimuli; see below) to avoid sensitization. In the assessment of heat pain sensitivity, two methods were employed: the method of constant stimuli (**I**, **IV**) and the method of limits (**II**). The method of limits was chosen in study **II** due to the shorter duration of the pain sensitivity testing with this method, as this study included other time-consuming psychophysical assessments. The constant stimuli method procedure was modified from the study by Clark (1974). In the method of constant stimuli, the thermode adaptation temperature was 34.5°C (**I**) or 35°C (**IV**), stimulus rise rate was 3°C/s and the duration of the peak stimulus temperature was 4 s, after which the temperature returned to the baseline level. Six predetermined stimulus temperatures were used: 45.8, 46.3, 46.8, 47.3, 47.8 and 48.3°C in study **I** and 43, 44, 45, 46, 47 and 48°C in study **IV**. In study **I**, the nominal temperatures given by the computer were calibrated by measuring the stimulus temperature on the surface of the thermostimulator with a thermometer (TES-1300; E & E Process Instrumentation, Concord, Ontario, Canada). The interval between successive stimulations was 15 s (Clark 1974), and the suitability of this interval for this study was tested in a pilot study before the actual study. Each stimulus temperature was applied eight times, and the order of presentation of the stimuli was randomized. After presentation of each stimulus, the subject was asked to rate the sensation evoked by the stimulus using the following verbal rating scale: faintly warm, warm, hot, very hot, very faint pain, faint pain, painful and very painful. In the method of limits, the adaptation temperature of the thermode was 32°C, and the linear rate of heating of the thermode during the stimulation was 1.5°C/s for all stimuli. The subject reported the pain threshold by pressing a button that reversed the stimulation back to adaptation temperature. The cutaneous heat pain threshold was measured three times in both the right and left volar forearm in order to rule out possible side difference between the two hands. The interstimulus interval between two stimuli was 30 s.

5.3.4. Assessment of cold pressor pain sensitivity

In study **III**, pain sensitivity was assessed with cold pressor pain (CPP). At the beginning of the session, CPP threshold was determined by measuring the latency to the first pain sensation after immersion of the right hand up to the wrist level in ice water (Martikainen et al. 2004). Using only the right hand for application of the test stimuli should not be a problem, since we have shown previously that the CPP thresholds are not significantly different between the right and left hand in healthy subjects (Hagelberg et al. 2002b). Measurements of the response latencies

were made with a stopwatch. The temperature of ice water was 3.8 ± 1.1°C and it was continuously measured with a TES-1300 Thermometer. CPP threshold was determined four times: at the beginning of the session, at the end of the session, and two times at various time points during the session. With repeated exposures to cold water, the CPP threshold was very stable during the experiment. This is indicated by the finding that the mean slope of the regression line calculated from CPP threshold values obtained at four different time points was 1.1 ± 5.8 (± S.E.M., n = 11) and not significantly different from a horizontal line or slope value 0 (t-test); indicating that the CPP threshold was not significantly changed during the experiment. To test CPP tolerance, the subjects were told to withdraw the hand from the ice water when the pain became intolerable; the latency from the immersion to this point of intolerable pain was defined as CPP tolerance. CPP tolerance was measured only once in order to avoid unnecessary distress.

5.3.5. Assessment of autonomic control and central modulation of CPP

The cutaneous vasoconstriction response, which is a sympathetic reflex (Wallin 1990), was induced by CPP and the Valsalva maneuver (**III**). Assessments of CPP-induced pain sensation and CPP-induced sympathetic vasoconstriction response provide separate measures for regulation of supraspinal versus spinal pain-related responses. Furthermore, responses to an innocuous Valsalva maneuver allow comparison of painful versus non-painful stimulation, and dissociation of potential influences on afferent versus efferent limb of the vasoconstrictor reflex. For this purpose, peripheral blood flow in the tip of the left index finger was measured with a laser Doppler flowmeter (Periflux PF2, Perimed, Stockholm, Sweden). The analogue output of this device gives no absolute values but detects relative changes of cutaneous blood flow. The maximum output of the gain level used was taken as 100 (arbitrary) blood flow units. A detailed discussion of the method is presented elsewhere (Öberg 1990). During the Valsalva maneuver, the subject took a deep breath and then held his breath for 10 s. To study habituation of the sympathetic reflex, the Valsalva maneuver was performed twice with a 20 s interval. These stimulation parameters were chosen in a pilot study as they produced optimal vasoconstriction and its habituation. Five minutes later, cutaneous vasoconstriction induced by CPP was determined by assessing the blood flow in the tip of the left index finger, while the right hand was immersed in ice water. The right hand was kept in ice water for a predetermined time that was 1.1 x CPP threshold measured at the beginning of the session. In order to study habituation of the CPP-induced vasoconstriction, ice water exposure was repeated after a 60 s interval. The maximum blood flow dip from the baseline induced by Valsalva or CPP was determined in each condition and used in further calculations. While studying the effect of CPP on cutaneous blood flow, the subject also reported the intensity and unpleasantness of CPP. These reports were made immediately after removing the hand from ice water using separate 0-10 cm visual analogue scales for pain intensity and unpleasantness (0 representing no pain or no unpleasantness, and 10 representing the maximum pain intensity or unpleasantness imaginable). To assess the magnitude of central modulation of CPP by conditioning noxious stimulation, CPP threshold was measured in the right hand during contralateral suprathreshold conditioning CPP. The test hand was immersed in ice water immediately after the conditioning ice water stimulation in the contralateral hand had produced the first sensation of pain. The increase of CPP threshold by conditioning CPP was calculated by subtracting the CPP threshold measured prior to conditioning stimulation from that measured during conditioning stimulation; i.e., Δ

CPP threshold > 0 s represents an increase of CPP threshold by conditioning stimulation. Because the CPP threshold with repeated exposures to ice water was very stable from the start to the end of the session (see above), habituation or sensitization of the sensory response to ice water is not likely to contribute to the pain modulatory effect induced by conditioning CPP.

5.3.6. Assessment of the effect of placebo on pain sensitivity

The placebo trial started with the heat pain threshold measurement (method of limits) with the contact thermode in the right volar forearm as described above (see 5.3.3. Assessment of heat pain sensitivity). After the control heat pain threshold was determined, the subjects received the placebo drug in a solutab form (Calcium-Sandoz, Sandoz, Switzerland). The subjects were told that they had received a novel and effective analgesic drug. The heat pain threshold was measured again 15 minutes after the placebo administration. The analgesic response to placebo was defined as the increase in heat pain threshold (ΔT) after the administration of placebo. Testing the effect of placebo was the last session for each subject in study **II**.

5.3.7. Assessment of short-term memory for heat pain

Objective short-term memory capacity for heat pain and subjective certainty of performance were assessed with a procedure modified from Rainville et al. (2004). The explicit, episodic memory of heat pain sensation was tested with a delayed-discrimination paradigm by delivering pairs of heat stimuli of same or different temperatures. The subject had to compare the intensities of the two consecutive stimuli and decide whether the two stimuli were of same or different temperature. The first stimulus was always 47°C and the second stimulus 47°C, 47.5°C or 48°C, thus the temperature differences (ΔT) in the discrimination were 0°C, 0.5°C or 1.0°C. All stimulus temperatures were found painful by the subjects, possibly reflecting a slight sensitization after the first heat pain session. After each pair of stimuli, the subject had to report his certainty of giving a correct answer with a 0-10 VRS, in which 0 represented being completely unsure (the subject was merely guessing) while 10 represented the highest imaginable level of certainty of correct discrimination. All stimulus temperature pairs were presented 10 times in a randomized order with an 8 s inter-stimulus interval. Three different variables were chosen to represent objective performance in the pain memory task: hit rate (the percentage of correct detections when comparing 47°C to 48°C), false alarm rate (the percentage of false alarms, i.e. reporting "different intensity" to a pair of two 47°C stimuli), and the SDT discrimination index (ROC [AUC]; see below). Average certainty ratings were used as indices of subjective performance in the pain memory task.

5.4. Psychophysical analyses

5.4.1. Conventional psychophysical analysis

In all studies, the psychophysical data was primarily analyzed by conventional psychophysical methods. For determination of the heat pain detection threshold in the method of constant stimuli (**I**, **IV**), psychometric function curves were constructed by plotting reports of pain (very faint pain, faint pain, painful, very painful pooled together) as percentages on the ordinate and stimulus intensity on the abscissa and depicting individual heat pain thresholds from the psychometric functions. The pain threshold was defined as the stimulus temperature at which the subject reported pain of any strength to 50% of stimulus deliveries, as described in detail elsewhere (Pertovaara et al. 1988). Correspondingly, the tactile detection threshold was

depicted from psychometric function curves, the threshold representing a 50% detection rate (score 1 and 2 pooled together). In studies **II** and **III**, the pain threshold was assessed with the method of limits, and the threshold was defined as the average temperature at which the subject reported the first sensation of pain by pressing a response button (**II**) or as the time to the withdrawal of the hand from ice water at the first sensation of pain (**III**).

5.4.2. Analysis based on the Signal Detection Theory

For the assessment of the subject's discriminative capacity, a receiver operating characteristic (ROC) curve analysis was performed using MedCalc software (MedCalc, Mariakerke, Belgium; www.medcalc.be). The area under the ROC curve (ROC [AUC]) was used as an index of the subject's discriminative capacity. When entering data, the category rating scale was transformed into numerical form ('one' representing faint warmth and 'eight' representing very painful; with touch, 'zero' representing no stimulus, 'one' representing a possible stimulus and 'two' a definite stimulus). The analysis focused on results obtained using stimulus temperatures/forces at and near the average detection threshold. Since the assessment of pain thresholds using psychometric function curves indicated that the mean pain threshold was 47.3°C in study **I** and 46.5 °C in study **IV**, the ROC curve analysis focused on responses elicited by stimulus temperatures of 46.8°C versus 47.3°C (**I**) and 46°C versus 47°C (**IV**). Additionally, to determine the dependence of discriminative capacity on the strength of the stimulus, the ROC curve analysis was performed with responses elicited by the stimulus temperatures of 46.8°C versus 47.8°C and 48.3°C (**I**). The mean detection threshold for touch was 1.09 mN in study **II** and 0.983 mN in study **IV**, and the ROC curve analysis focused on responses elicited by stimulus forces of 0.667 mN versus 1.63 mN. The subject's response criterion along the sensory magnitude, or decision, axis was determined from responses to stimuli near the detection threshold as described in detail elsewhere (Gescheider 1997). In calculations, the probability of rating a stimulus of 47.3°C in **I** and 47°C in **IV** as painful (rating categories 5-8 pooled together) was converted to a Z score, Z_{SN}, as described by Gescheider (1997, pp.122-123 and Table A). The probability of rating a stimulus of 46.8°C in **I** and 46°C in **IV** as non-painful (rating categories 1-4 pooled together) was converted to a Z score, Z_N. With touch, the analysis focused on results obtained using a stimulus strength of 0.667 mN, which was the closest to the average psychophysical tactile detection threshold (1.09 mN in **II**, 0.983 in **IV**). Similarly, the location of the response criterion on the noise distribution was found by converting the probability of correctly rejecting a sham monofilament (giving a rating 0) to a Z score (Z_N), and the location of the response criterion on the signal plus noise distribution was found by converting the probability of correct detection of a stimulus of 0.667 mN (rating categories 1-2 pooled together) to a Z score, Z_{SN}. The numeric values of the response criterion represent the number of standard deviation units (Z score units) that the response criterion is above or below the zero bias point, where the distributions of the response probabilities (Z_{SN} = signal plus noise and Z_N = noise) to two stimulus intensities cross. The response criterion (C) was defined as: $C = 0.5 [Z_{SN} + Z_N]$. The response criterion, as defined above, is statistically independent of the discriminative capacity (Gescheider 1997).

5.5. Statistical analyses

In studies **I** and **II**, the statistical analyses were performed with GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA, USA). Associations between

psychophysical results (pain and touch threshold, ROC [AUC], response criterion) and dopamine D2/D3 receptor BP_{ND} were determined using Pearson's coefficient of correlation, and the association between response criterion for tactile stimuli and D2/D3 receptor BP_{ND} in study **II** was determined using Spearman's coefficient of correlation. The dependence of the ROC [AUC] on stimulus strength (**I**) and heat pain threshold on repetition (**II**) was tested using one-way analysis of variance (ANOVA) followed by Tukey's test. Student's t-test was used to compare differences between the two conditions. In studies **III** and **IV**, statistical analyses were performed using SPSS 13.0 for Windows (Release 13.0.1, copyright SPSS Inc., 1989-2004). The data were primarily analyzed by means of repeated measures analysis of variance (rmANOVA) with region (ROI) and hemisphere as within-subject factors and age (**III**) or sex (**IV**) and each psychophysical measure at a time as between-subject predictor of BP_{ND}/BP_P. In study **III**, an interaction between region and psychophysical measure in the prediction of BP_P would allow for the assessment of the magnitude of association between psychophysical data and 5-HT_{1A} BP_P in each brain region separately using Pearson's coefficient of correlation. In study **IV**, a partial correlation analysis, covarying for the confounding effects of sex, was carried out as a secondary analysis for those psychophysical variables that showed associations with global 5-HT_{1A} density in the overall model. While sex has a significant effect on 5-HT_{1A} BP_{ND} as measured with [*carbonyl*-^{11}C]WAY-100635 (Jovanovic et al. 2008), the effects of aging within the narrow age range of the studies **III** and **IV** are negligible (Rabiner et al. 2002): thus, age was not included in the primary covariate analysis in study **IV**. The DRN was analyzed by partial correlation only and not by rmANOVA because it is a midline structure. As a methodological validation, a regression model was built predicting cerebellar white matter V_T (as representing free and non-specific binding in the brain) with psychophysical measures, with age as a covariate (**III**). Statistical analysis of ROI-based correlations was primarily performed with ANOVA followed by a *post hoc* test that did not take multiple comparisons into account (see 7.1. Methodological considerations). Before determining the partial correlations between psychophysical data and 5-HT_{1A} BP_{ND}/BP_P in each ROI in studies **III** and **IV**, Grubb's outlier test (www.graphpad.com/quickcalcs/) was used to confirm that the study population did not include significant outliers. In all studies, $p < 0.05$ was considered a criterion for statistical significance.

6. RESULTS

6.1. Psychophysical characteristics of the subjects

The average tactile detection threshold depicted from psychometric function curves was 1.09 ± 0.34 mN (mean ± SD) (**II**) and 0.98 ± 0.64 mN (**IV**). The heat pain threshold determined with method of constant stimuli from the psychometric function curves was 47.3 ± 0.87°C (**I**; 16 mm x 16 mm probe) and 46.5 ± 0.89°C (**IV**; 30 mm x 30 mm probe). When determined with method of limits, the heat pain threshold of the left arm was 46.9 ± 2.6°C and right arm 45.7 ± 2.9°C (**II**) (16 mm x 16 mm probe). There was no statistical difference between the heat pain threshold of the right and left arm ($p = 0.096$) (**II**). Administration of placebo induced a significant increase in the heat pain threshold from the baseline level of 45.7 ± 1.0°C to 47.5 ± 0.84°C after the placebo ($p = 0.0066$). Repetition of the heat pain threshold measurement *per se* prior to the administration of placebo did not change the threshold (not shown). In the heat pain sensitivity task of study **IV**, the subjects could easily discriminate the two temperatures (47 and 48°C) later used in the short-term memory task for heat pain of the same study ($p < 0.001$; t-test with a Bonferroni correction). In the short-term memory task, the mean hit rates and certainty ratings of responses were lowest when the difference in the temperatures of the stimulus pair was 0.5°C, instead of 0°C or 1.0°C. For example, the mean certainty ratings of the responses in the heat pain memory task were 6.1 ± 1.7 at ΔT 0°C, 5.2 ± 1.3 at ΔT 0.5°C, and 6.8 ± 1.2 at ΔT 1.0°C (± SD). In the CPP challenge (**III**), the mean pain threshold (time from the immersion of the hand to the first sensation of pain) was 41.8 ± 12.7 s and mean tolerance (time from the immersion of the hand to the point when the pain turned intolerable and the subject had to withdraw the hand from ice water) was 168.6 ± 35.1 s (mean ± SD). The mean CPP intensity, as measured with a 0-10 cm VAS at time point 1.1 x the pain threshold, was 4.7 ± 0.6 units (cm), and unpleasantness was 5.1 ± 0.6 units (cm) (**III**). Contralateral suprathreshold conditioning CPP lead to an increase of 36.3 ± 13.6% (9.30 ± 20.5 s) in CPP threshold in the test hand (**III**).

Autonomic nervous system responses to CPP and the Valsalva maneuver varied considerably between subjects. Interestingly, although the mean vasoconstriction responses of the skin evoked by CPP and the Valsalva maneuver were of equal magnitude, there was no correlation between the CPP- and Valsalva-induced vasoconstriction ($r = 0.117$). In other words, an individual with a strong Valsalva-induced vasoconstriction could have a weak, moderate or strong CPP-induced vasoconstriction (**III**).

The relationship between different psychophysical measures and heat pain sensitivity was further analyzed in study **I**. The index of the subject's discriminative capacity, ROC [AUC], varied over a wide range between the subjects. Discriminability between the painful test stimuli was increased with an increase of the stimulus temperature difference ($F_{2,56} = 16.0$, $p < 0.0001$). Over all subjects ($n = 19$), the subject's discriminative capacity was not significantly correlated with the pain threshold ($p > 0.13$). In contrast, the index of response bias, the criterion, was significantly associated with the subject's heat pain threshold ($p < 0.0001$) (**I**).

6.2. Brain D2/D3 receptor binding and psychophysical characteristics

A summary of the results is presented in Table 3. Pain threshold as assessed with the method of constant stimuli (**I**) was inversely correlated with D2/D3 BP_{ND} in the right putamen ($p = 0.006$, $r = -0.86$), and this finding was replicated in the second study with the method of limits ($p = 0.042$, $r = -0.73$) (**II**). The inverse correlation between the pain threshold and D2/D3 BP_{ND} in the right putamen also remained significant after a correction for multiple comparisons in study **I**, but not in study **II** (four striatal ROIs; Bonferroni correction). The inverse correlation between the pain threshold and D2/D3 BP_{ND} in the right caudate nucleus was close to significance ($r = -0.705$, $p = 0.05$) (**I**). The correlations between the pain threshold and D2/D3 BP_{ND} in the left putamen, the left caudate nucleus, or D2/D3 BP_{ND} in other brain regions were not significant (**I** and **II**, not shown). An index of the subject's discriminative capacity, ROC [AUC], was not correlated with D2/D3 BP_{ND} in the right putamen ($p > 0.4$) or with D2/D3 BP_{ND} in any other brain region (**I**). In contrast, the inverse correlation of the subject's response criterion with D2/D3 BP_{ND} in the right putamen was significant ($p = 0.041$) (**I**). Correlation of the response criterion with D2/D3 BP_{ND} in other brain regions was not significant (**I**). Although the placebo administration induced a significant increase in the heat pain threshold, the placebo-induced increase in the heat pain threshold was not correlated with dopamine D2/D3 receptor BP_{ND} in any of the striatal regions of interests (**II**). The tactile detection threshold, the response criterion or the index of tactile discriminability (ROC [AUC]) were not associated with dopamine D2/D3 receptor BP_{ND} in any of the striatal regions (**II**).

6.3. Brain 5-HT_{1A} receptor binding and psychophysical characteristics

A summary of the results is presented in Table 3. Specific radioactivities or masses of injected radiotracer were not associated with psychophysical variables, but in study **III** the injected dose (MBq) was negatively correlated with pain intensity ($r = -0.62$, $p = 0.04$); however, this association was due to an outlier (injected dose 126.9 MBq) removal of which diminished the correlation ($r = -0.44$, $p = 0.17$). In study **III**, the distribution volume of cerebellar white matter, used to approximate free and non-specific binding in the brain, had a significant inverse association with the intensity of CPP ($\beta = -0.62$, $t = -2.33$, $p = 0.048$) but not with other psychophysical measures (data not shown). Consequently, in study **III,** BP_P was chosen as the outcome measure of choice given the relative independence of this measure on reference region V_T as compared with BP_{ND}. The rmANOVA predicting BP_P with the intensity of CPP revealed a significant effect of CPP intensity ($F = 10.7$, $p = 0.011$) and no effects of age ($F = 0.02$, $p = 0.898$). A significant interaction between region and CPP intensity was also observed ($F = 4.9$, $p < 0.001$) but not between region, CPP intensity, and hemisphere ($F = 1.3$, $p = 0.238$). Intensity of CPP was significantly correlated with 5-HT_{1A} BP_P in all ROIs (Table 2). All associations of 5-HT_{1A} BP_P with CPP intensity were inverse ones; i.e., the higher the intensity of CPP, the lower the availability of 5-HT_{1A} receptors. The most significant correlations between the intensity of CPP and 5-HT_{1A} BP_P were observed in the posterior cingulate cortex, posterior insula and the dorsal raphe. An independent, voxel-based analysis on parametric BP_P maps confirmed the ROI-based results. An exploratory analysis with voxel-level uncorrected $p < 0.05$ and extent threshold at 17 000 voxels (corresponding to a cluster-level corrected $p < 0.045$) revealed a large cluster spanning throughout the brain. An analysis using stricter criteria, voxel-level uncorrected $p < 0.0003$ and extent threshold at 260 voxels (corresponding to a cluster-level corrected $p < 0.043$), suggested that among the brain regions with the most pronounced

associations with the intensity of CPP are the posterior cingulate cortex and the left posterior insula, consistent with the ROI-based results. CPP intensity measures were also correlated with regional total tissue distribution volumes (V_T), which are free of any assumptions about a receptor-free reference region. Similar results were seen: the correlation coefficients and levels of statistical significance were comparable with those yielded using BP_P in nearly all brain regions (data not shown). While the intensity of CPP assessed at time point 1.1 x the subject's CPP threshold was significantly associated with 5-HT_{1A} BP_P in all ROIs (see above), CPP threshold or CPP tolerance were not significantly correlated with 5-HT_{1A} BP_P in any of the ROIs (not shown). When assessing unpleasantness scores, one subject proved to be an outlier (Grubb's test) and therefore, his results were not taken into account in the final analysis. Following removal of the outlier, unpleasantness induced by CPP was not significantly correlated with 5-HT_{1A} BP_P in any of the ROIs (not shown) (**III**). Increase of CPP threshold by conditioning CPP in the contralateral hand was a significant predictor of 5-HT_{1A} BP_P ($F = 8.26$, $p = 0.021$), with a significant interaction with region ($F = 9.32$, $p < 0.001$). No interactions with hemisphere or effect of age were observed (data not shown). Increase of CPP threshold by conditioning CPP was directly correlated with 5-HT_{1A} BP_P in the amygdala and medial prefrontal cortex; i.e., the higher the availability of 5-HT_{1A} receptors in these ROIs, the stronger the magnitude of the pain threshold increase by contralateral conditioning stimulation (**III**).

The magnitude of the cutaneous vasoconstriction in the fingertip evoked by CPP in the contralateral hand was not significantly correlated with 5-HT_{1A} receptor BP_P in any of the ROIs (not shown). The magnitude of the cutaneous vasoconstriction induced by Valsalva, however, had an association with BP_P approaching statistical significance ($F = 4.50$, $p = 0.067$), with a significant interaction with region ($F = 2.01$, $p = 0.023$), but with no hemispheric differences or contributions of age (data not shown). The blood flow decrease induced by Valsalva had a significant direct correlation with 5-HT_{1A} BP_P in the anterior insula and ventral part of the anterior cingulate cortex. Repetition of the Valsalva maneuver or CPP within one minute produced a marked attenuation (habituation) of the vasoconstrictor response. The magnitude of habituation of the Valsalva- or CPP-induced vasoconstriction was not correlated with 5-HT_{1A} BP_P (not shown) (**III**).

The possible association of brain 5-HT_{1A} BP_{ND} with response to heat pain, tactile stimuli and heat pain short-term memory was studied in study **IV**. The rmANOVA suggested associations between 5-HT_{1A} BP_{ND} and the tactile discrimination index (ROC [AUC]) ($F = 4.58$, $p = 0.054$) and response criterion of heat pain ($F = 3.55$, $p = 0.084$), but the interaction with brain region was not significant in either case. These psychophysical variables were further analyzed using linear regression models. The tactile discrimination index ROC [AUC] was inversely correlated with 5-HT_{1A} BP_{ND} in the ventral anterior cingulate cortex (partial $R = -0.63$, $p = 0.016$), inferior temporal gyrus (partial $R = -0.53$, $p = 0.050$), medial prefrontal cortex (partial $R = -0.65$, $p = 0.023$), and posterior cingulate cortex (partial $R = -0.60$, $p = 0.012$). When age was included in the statistical model, similar significant associations were observed in the ventral anterior cingulate cortex (partial $R = -0.61$, $p = 0.027$), medial prefrontal cortex (partial $R = -0.58$, $p = 0.037$) and posterior cingulate cortex (partial $R = -0.61$, $p = 0.027$), while the correlation in inferior temporal gyrus did not reach statistical significance (partial $R = -0.48$, $p = 0.099$). The tactile detection threshold and response criterion for touch were not correlated with 5-HT_{1A}

BP_{ND} in any of the ROIs (not shown). The response criterion for heat pain correlated negatively with 5-HT_{1A} BP_{ND} in the middle temporal gyrus (partial R = -0.55, p = 0.044), orbitofrontal cortex (partial R = -0.57, p = 0.035), posterior cingulate cortex (partial R = -0.62, p = 0.018), and DRN (partial R = -0.77, p = 0.001). Again, when age was covaried for in the analysis, similar significant associations were observed in the orbitofrontal cortex (partial R = -0.56, p = 0.046), posterior cingulate cortex (partial R = -0.59, p = 0.033), and DRN (partial R = -0.80, p = 0.001), while the correlation in the middle temporal gyrus did not quite reach statistical significance (partial R = -0.53, p = 0.066). Neither the heat pain threshold nor the heat pain discrimination index (ROC [AUC]) was correlated with 5-HT_{1A} BP_{ND} (not shown). In the heat pain memory task, 5-HT_{1A} BP_{ND} in the DRN was positively correlated (partial R = 0.57, p = 0.035) with the certainty rating of discrimination of two 47 ^{o}C stimuli. The objective indices of memory function, i.e. false alarm rate, hit rate and the index of discriminability (ROC [AUC]), were not correlated with 5-HT_{1A} BP_{ND} in any of the ROIs (not shown). The correlations between 5-HT_{1A} BP_{ND} and psychophysical results were not dependent on sex (not shown) (**IV**).

Table 3. Summary of the Results

Psychophysical variable	Association	Receptor BP_{ND}/BP_P (Study)	Region of Interest (ROI)
Heat pain			
Threshold[a]	-	D2/D3 (**I**, **II**)	Putamen (right)
Response criterion	-	D2/D3 (**I**)	Putamen (right)
	-	5-HT_{1A} (**IV**)	DRN, MTG, OFC, PCC
ROC [AUC]	N.S.	D2/D3 (**I**), 5-HT_{1A} (**IV**)	Any
Placebo response (ΔT)	N.S.	D2/D3 (**II**)	Any
Heat pain memory			
Hit rate	N.S.	5-HT_{1A} (**IV**)	Any
False alarm rate	N.S.	5-HT_{1A} (**IV**)	Any
ROC [AUC]	N.S.	5-HT_{1A} (**IV**)	Any
Certainty rating	+	5-HT_{1A} (**IV**)	DRN
Cold pressor pain			
Threshold	N.S.	5-HT_{1A} (**III**)	Any
Tolerance	N.S.	5-HT_{1A} (**III**)	Any
VAS intensity	-	5-HT_{1A} (**III**)	All ROIs (see Table 2)
VAS unpleasantness	N.S.	5-HT_{1A} (**III**)	Any
ΔCPP threshold during conditioning CPP	+	5-HT_{1A} (**III**)	Amygdala, MPFC
Touch			
Threshold	N.S.	D2/D3, 5-HT_{1A} (**II**, **IV**)	Any
Response criterion	N.S.	D2/D3, 5-HT_{1A} (**II**, **IV**)	Any
ROC [AUC]	-	5-HT_{1A} (**IV**)	vACC, ITG, MPFC, PCC
Autonomic control			
Valsalva-induced VR	+	5-HT_{1A} (**III**)	Anterior insula, vACC
CPP-induced VR	N.S.	5-HT_{1A} (**III**)	Any
Habituation of VR (CPP/Valsalva)	N.S.	5-HT_{1A} (**III**)	Any

[a] Heat pain threshold was determined with method of constant stimuli (**I**) and limits (**II**).

Abbreviations: BP_{ND}/BP_P, binding potential; CPP, cold pressor pain; DRN, dorsal raphe nucleus; ITG, inferior temporal gyrus; MPFC, medial prefrontal cortex; MTG, middle temporal gyrus; OFC, orbitofrontal cortex; PCC, posterior cingulate cortex; ROC [AUC], area under the receiver operating characteristic curve; ROI, region of interest; vACC, ventral anterior cingulate cortex; VAS, visual analogue scale; VR, vasoconstriction response. **Associations**: +/- = positive/negative correlation, N.S. = non significant.

7. DISCUSSION

7.1. Methodological considerations

The question concerning the stability and repeatablity of responses to experimental pain is of critical importance to most psychophysical studies, as numerous studies rely on an assumption of the stability of experimental pain sensitivity measures rather than actual experimental data concerning this issue. The existing evidence suggests that test protocols that employ the application of contact heat stimuli provide relatively stable estimates of individual heat pain sensitivity (Quiton & Greenspan 2008, Rosier et al. 2002), although one study with a somewhat different approach found significant variability (Yarnitsky et al. 1996). The structure of the test protocol seems to be a critical factor determining the repeatability. Rosier et al. (2002) suggest a list of methods for maximizing the reproducibility of heat pain sensitivity measurements, all of which were used in studies **I** and **IV**: 1) multiple assessments 2) minimal use of prolonged stimuli 3) a training period before the experiment 4) repeated presentations of scale instructions (in studies **I** and **IV**, the subject had the scale and instructions in front of him throughout the experiment). Additionally, the individual pain estimates obtained with repetition of the CPP test seem to be highly reproducible (Hagelberg et al. 2002b, Peckerman et al. 1991). Unfortunately, there is no data available concerning the test-retest repeatability of experimental pain measurements over years and this provides a potential source of bias in this thesis. However, as reviewed above, the existing data suggests that heat and cold pressor pain sensitivity measurements are reproducible. Interestingly, a very recent study indicated that the subject's response to experimental cold pain predicts the pain response of his own family members (Birklein et al. 2008), giving further support to the proposal that the response to experimental pain stimulus is a stable characteristic of the subject. There is absence of consistent aging effect on pain, and generally age-related changes in sensitivity to experimental pain are small (Gibson & Farrell 2004). It should also be pointed out that our subjects were young adults (Table 1). In this age group, aging by 1-9 years *per se* is not expected to produce changes in heat pain sensitivity (Lautenbacher et al. 2005).

The subjects reported that they were healthy, they were not taking any medication, and that their general health status had been similar during the interval from the PET scanning to the psychophysical experiments. Nevertheless, it is possible that this assessment of health status, based on self-reports, was not sensitive enough to rule out all potential changes in health status which might have an effect on sensitivity to pain, such as mood disorders, changes in drinking habits and smoking, thereby providing an additional potential source of bias in this study. In study **IV**, the study population included 7 females, which might theoretically provide an additional source of variability to reproducibility of pain sensitivity measurements. None of the female subjects received hormonal replacement therapy, but hormonal contraception or state of menstrual cycle was not controlled. However, it should be pointed out that the effects of female hormones are found very inconsistently, and if found, the effects tend to be small. Specifically, as with heat pain, there is little evidence indicating that female hormone levels affect pain sensitivity (Sherman & LeResche 2006).

There are some limitations in the psychophysical study protocols employed in this thesis. First, although testing protocols used in pain measurement in the laboratory setting are designed to control for biases that may influence pain assessments (Gracely & Eliav 2009), the different testing procedures have inherent weaknesses in terms of vulnerability to biases. In the method of limits procedure, the subject may become accustomed to reporting a sensation in a similar manner over different stimulus intensities (habituation), or may anticipate a sensation and report detection prematurely (expectation). The method of constant stimuli is regarded to be relatively insensitive to these biases, but the application of a large amount of painful stimuli may predispose the subject to sensitization. However, sensitization may be limited by adjusting the interstimulus interval and by varying the location of the stimuli (Clark 1974), and this was performed with heat stimuli in studies **I**, **II** and **IV**. On the other hand, the application of multiple painful stimuli may also trigger activation of endogenous pain modulatory systems (Ren & Dubner 2009), which may change the individual pain experience. In study **II**, the placebo response was studied without the use of a control group to rule out any order effect as in the study by Scott et al. (2008). However, in study **II,** the repetition of heat pain measurement *per se* before the placebo administration did not have an effect on pain threshold, arguing against a significant order effect. The pain memory task measured the subject's objective and subjective accuracy in remembering pain sensations (**IV**). Still, it is possible that the task was not pain-specific, as memory for pain may be stored in a modality non-specific coding system (Rainville et al. 2004). Additionally, ability to discriminate painful stimuli can be assumed to contribute significantly to the memory for experimental pain measured in the pain memory task in study **IV**. However, the subjects could easily discriminate between the two temperatures (47°C and 48°C) in the heat pain sensitivity task, suggesting that sensitivity to pain did not significantly contribute to the performance in the pain memory task. Memory for pain may also be biased by other factors, such as attention and mood (Erskine et al. 1990).

The conventional detection threshold is a composite of purely sensory and various psychological (non-sensory) components that affect the subject's attitude toward reporting the sensory experience. The sensory and non-sensory components can be separately analyzed by methods based on the Signal Detection Theory (SDT) (Clark 1974 & 2007, Gescheider 1997, McNicol 1972, Swets 1973). The SDT provides separate measures for the discriminative and attitudinal components of perception, and these two measures are statistically independent of each other (Gescheider 1997). The discriminative capacity measures the subject's ability to detect a target stimulus from backgroud events and is dependent on sensory function, whereas the response criterion reflects the subject's tendency to favour one response over another and is dependent on non-sensory functions, such as personality traits (Swets 1973). Manipulation of psychological variables, such as attitude and motivation, has an effect on the response criterion, while discriminative capacity remains unaltered. Essentially, a low discriminative capacity means that the subject tends to confuse lower and higher intensity stimuli (or, stimulus and noise). A low response criterion, in turn, means that the subject may be sensitive in discriminating stimuli, but has a low cutoff point to report a sensation. While it is easily conceivable that different pain-modulatory systems may have an effect on pain report by either modulating the sensory or non-sensory aspect of pain (or both), SDT provides an important tool in the assessment of pain-modulatory mechanisms in the human brain.

There is a general agreement that the traditional psychophysical methods in pain research are vulnerable to extraneous factors that may augment or reduce reported pain (Gescheider 1997, Gracely & Eliav 2009). Moreover, traditional detection theory is often interpreted as a measure of sensory sensitivity, although this assumption clearly lacks empirical evidence (Clark 2007). Taking into account these pitfalls in traditional psychophysical methods, it is surprising that there have been so few attempts to overcome these problems by developing new methods in pain psychophysics. SDT has provided psychophysicists with a tool to dissociate the sensory report into separate components representing discrimination of two stimulus intensities and the setting of the response criterion (Gescheider 1997, Swets 1973). However, there has been an ongoing debate on the interpretation of the measures of SDT and their applicability to pain research (e.g. Chapman 1977, Chapman et al. 1985, Gracely 2006, Gracely & Eliav 2009, Rollman 1977). The criticism has been directed toward both the interpretation of discriminability component (d' or its equivalent, ROC [AUC]) as a purely sensory factor and interpretation of the response criterion component as a purely non-sensory factor (Coppola & Gracely 1983, Gracely & Eliav 2009). Theoretically, the response criterion in pain measurement could be influenced by a sensory factor, if the sensory modulatory mechanism had an effect on the location of the stimulus-response function (i.e., a left- or rightward shift) and did not influence the function itself (e.g. Carstens et al. 1980, Cervero & Laird 1996). For instance, an intervention that reduces the affective component of pain could conceivably result in such a change in response criterion (Gracely & Eliav 2009), and as pain sensation by definition emcompassess both sensory and affective dimensions, it might be considered misleading to label the effect as "non-sensory". Thus, the potential limitations of SDT have to be taken into account when interpreting results from studies employing SDT methods. However, it should be borne in mind that the criticism has been usually directed toward the interpretations of the SDT parameters, not toward the applicability of the SDT model itself (Coppola & Gracely 1983): moreover, the significance of the theoretical limitatitions of SDT in practice is not yet known (Gracely & Eliav 2009). Further studies on the role of striatal D2/D3 receptors in pain and on the role of brain 5-HT_{1A} receptors in pain and touch are needed to clarify the effect of these neurotransmission systems on discriminative capacity and sensory function, sensory decision-making and eventual reporting of sensations.

Dopamine D2/D3 receptor availability in the brain as assessed with [^{11}C]raclopride is dependent on the number of free receptors available for binding, and the affinity of the tracer to the receptor. The number of free receptors, in turn, is affected by the concentration of endogenous dopamine in the synaptic cleft (Koepp et al. 1998, Laruelle 2000). Therefore, the individual differences in D2/D3 BP_{ND} as assessed with [^{11}C]raclopride in **I** and **II** may represent differences in i) receptor density ii) the level of endogenous dopamine in the synaptic cleft or iii) the affinity of the tracer to the receptors. It has been shown that D2/D3 BP_{ND}, but not K_D, varies considerably between individuals as assessed with [^{11}C]raclopride (Farde et al. 1995). This lends support to the hypothesis that the differences in D2/D3 BP_{ND} in this study are mainly due to differences in receptor density and the level of endogenous ligand (dopamine) available to compete in binding with [^{11}C]raclopride. The dopamine receptor ligand [^{11}C]raclopride binds to D2-like receptors and thus does not represent solely binding to D2 receptors, but also to D3 receptors. D3 receptors are found mainly in the ventral striatum and islands of Calleja (Hall et al. 1996, Murray et al. 1994). Therefore, [^{11}C]raclopride BP_{ND} in the dorsal striatum may be

considered to represent mainly D2 receptor BP_{ND}. The subjects in studies **I** and **II** were right-handed, non-smoking males within a relatively narrow age range (22-42 years) and PET imaging was performed in a resting state, excluding the possibility that the brain D2 receptor availability was biased due to differences in these variables (Antonini & Leenders 1993, Pohjalainen 1998, Rinne 1993).

WAY-100635 is the first 5-HT_{1A} receptor silent antagonist to demonstrate high affinity and selectivity (Forster et al. 1995, Khawaja et al. 1995). Its isotope, [*carbonyl*-^{11}C]WAY-100635, is a well-documented tracer that shows feasible properties for PET imaging of 5-HT_{1A} receptor binding in the living human brain (Farde et al. 1998, Hirvonen et al. 2007, Pike et al. 1996, Rabiner et al. 2002). The 5-HT_{1A} BP_{ND} in the brain, as assessed by PET, represents the product of total number of receptors (B_{Avail}), the apparent affinity for the radioligand (K_D), and the fraction of radioactivity originating from free (non-protein bound) fraction of the radioligand in the nondisplaceable tissue compartment (f_{ND}). Correspondingly, 5-HT_{1A} BP_P in the brain represents the product of the total number of receptors (B_{Avail}), the apparent affinity for the radioligand (K_D), and the fraction of radioactivity originating from free (non-protein bound) radioligand in arterial plasma (f_P) (Innis et al. 2007). 5-HT_{1A} BP_{ND} is not sensitive to changes in endogenous serotonin levels (Bhagwagar et al. 2004, Rabiner et al. 2002, Sargent et al. 2000). Therefore, 5-HT_{1A} BP_{ND}/BP_P as measured with [*carbonyl*-^{11}C]WAY-100635 is a reflection of receptor number (B_{avail}), or, hypothetically, receptor function (via conformational changes in the receptor protein structure affecting the affinity of each receptor to [*carbonyl*-^{11}C]WAY-100635). When interpreting the BP_{ND}/BP_P values from different ROIs, it should be taken into account that 5-HT_{1A} receptors in the raphe are likely to be somatodendritic autoreceptors, while in other areas 5-HT_{1A} receptors are predominantly postsynaptic heteroreceptors (Wright et al. 1995). The subjects in studies **III** and **IV** were mainly young adults and their age range was relatively narrow (25-49 years), and it is not expected that age is a significant factor within our subjects (Rabiner et al. 2002). In contrast, sex seems to have a significant effect on 5-HT_{1A} BP_{ND} as measured with [*carbonyl*-^{11}C]WAY-100635 (Jovanovic et al. 2008). In this study, sex did not have a significant effect on the results, since in study **III** only males were studied and in study **IV**, correlations between psychophysical results and 5-HT_{1A} BP_{ND} proved not to vary with sex.

In study **III**, BP_P was preferred over the more commonly-used estimate of specific binding, BP_{ND} because a significant association was seen between CPP intensity and cerebellar white matter V_T, the latter being used to approximate free and non-specific binding in the brain. Since BP_P is less dependent on reference region V_T than BP_{ND}, it is more suitable in situations where changes in reference region V_T occur (Meltzer et al. 2004, Parsey et al. 2006). We were also able to replicate our findings concerning association between CPP intensity and [*carbonyl*-^{11}C]WAY-100635 binding using regional V_T, a measure that is not dependent on any assumptions regarding the validity of the reference region (**III**). In addition, both BP_P and V_T should be independent of changes in cerebral blood flow that is canceled out in the definitions of both outcome variables. Derivation of *BP* estimates by kinetic analysis using the arterial plasma input function is the method of choice because of its higher test-retest reproducibility, lower vulnerability to experimental noise, and absence of bias (Parsey et al. 2000). Numerous [*carbonyl*-^{11}C]WAY-100635 PET studies use no arterial input but rather use the cerebellum

input which may result in biased binding estimates. While changes in the free fraction f_p could theoretically contribute to 5-HT_{1A} BP_P results, an association between psychophysical measures and plasma protein binding of [*carbonyl*-^{11}C]WAY-100635 would seem counterintuitive. Measurement of f_p of [*carbonyl*-^{11}C]WAY-100635 is not reliable (Parsey et al. 2000) and therefore, it was not performed (**III**).

Dopamine D2/D3 receptor availability as measured with [^{11}C]raclopride and [^{11}C]FLB 457 varies between subjects, but it is highly reproducible within individuals (Farde et al. 1995, Hietala et al. 1999, Nordström et al. 1992, Schlosser et al. 1998, Sudo et al. 2001, Vilkman et al. 2000, Volkow et al. 1993). Moreover, the estimated age-related decline in [^{11}C]raclopride BP_{ND} in the putamen is only 7.9-8.2% per decade (Volkow et al. 1996, Wang et al. 1996). Thus, in spite of the long time interval between the PET scan and the psychophysical tests in studies **I** and **II**, the confounding effect of a small systemic annual decline in D2/D3 receptor BP_{ND} on the observed correlations is probably minimal. We were able to reproduce the original finding (Hagelberg et al. 2002b) of an inverse correlation between D2/D3 BP_{ND} and sensitivity to experimental pain (**I, II**): moreover, this finding has been recently successfully reproduced (Scott et al. 2006, Wood et al. 2007), lending corroborative evidence to this methodological point of view. Correspondingly, also 5-HT_{1A} BP_{ND} in the brain as measured with [*carbonyl*-^{11}C]WAY-100635 is also stable even over long time intervals (Rabiner et al. 2002), and the effects of aging on 5-HT_{1A} BP_{ND} are not a relevant source of bias in adult populations within such a narrow age range as in **III** and **IV** (Rabiner et al. 2002). The results from the earlier studies and the results presented in this thesis suggest that sensitivity to painful stimulation and D2/D3 BP_{ND} and 5-HT_{1A} BP_{ND}/BP_P are relatively stable characteristics of an individual, and it is reasonable to assess correlations between these parameters even if they are determined over intervals of a few years. Nonetheless, there are two important caveats to be taken into account. First, it has been reported that the age-related decline in D2/D3 receptor availability is more pronounced in extrastriatal brain areas (Inoue et al. 2001), providing a potential source of bias in the lack of association between D2/D3 BP_{ND} in extrastriatal brain areas and response to pain. In addition, the age range of the subjects (22-49 years) may make the subject group heterogenous in terms of D2/D3 and 5-HT_{1A} receptor availability.

In studies **I** and **II**, only 4 striatal ROIs (right and left caudate nucleus and putamen; Table 2) were analyzed based on the results of the previous PET study (Hagelberg et al. 2002b). If a conventional correction for multiple comparisons ($p < 0.0125$; Bonferroni correction) is applied, only the inverse correlation of heat pain threshold with D2/D3 BP_{ND} in the right putamen remains significant (**I**). However, although the inverse correlation of heat pain criterion with D2/D3 BP_{ND} in the right putamen does not reach significance if corrected for multiple comparisons ($p = 0.04$), it may be a noteworthy finding since it could explain the inverse correlation with heat pain threshold found in the same study (**I**). Additionally, since we were able to reproduce the significant inverse correlation of heat pain threshold with D2/D3 BP_{ND} in the right putamen in a second study (**II**), this result can be regarded as a significant finding supporting the first study although it does not pass a conventional correction for multiple comparisons. Similarly, in studies **III** and **IV**, statistical assessment of ROI-based correlations was performed with ANOVA followed by a *post hoc* test that did not take into account multiple comparisons. If a conventional correction for multiple comparisons (15 ROIs

in **III**, 18 ROIs in **IV**) is taken into account, then only four of the associations remain significant ($p < 0.0034$ in **III**, $p < 0.0028$ in **IV**; Bonferroni correction): the inverse correlations of CPP intensity with 5-HT_{1A} BP_P in the dorsal raphe, the posterior insula and the posterior cingulate cortex (**III**), and the inverse correlation of 5-HT_{1A} BP_{ND} in the DRN with the criterion for heat pain (**IV**). Still, since regional 5-HT_{1A} binding values are highly inter-correlated and cannot be regarded as independent observations, the conventional correction for multiple comparisons may lead to type II error, i.e. underestimation of the amount of regions associated with a variable. Moreover, the large number of brain regions in which an inverse correlation between 5-HT_{1A} BP_P and CPP intensity was found (**III**) suggests that an underlying mechanism may be a non-specific effect of overall brain serotonin on pain, as our results resemble previous findings of associations between brain 5-HT_{1A} BP_{ND} and various other behavioral aspects (e.g. Borg et al. 2003, Hirvonen et al. 2008).

7.2. Striatal D2/D3 receptors in pain sensitivity

The involvement of the striatum in pain in humans is supported by its frequent activation (as assessed by increased regional cerebral blood flow) during painful stimulation in human brain imaging studies (Casey et al. 1996, Coghill et al. 1999 & 2001, Derbyshire et al. 1997, Iadarola et al. 1998, Jones et al. 1991, Svensson et al. 1997). However, this activation is not always detected (Casey et al. 1999, Peyron et al. 2000). The striatal activation has often been attributed to inhibition or preparation of motor activity during pain, although the relationship between changes in regional cerebral blood flow and dopaminergic activity is not fully established (Black et al. 1997, Cumming et al. 2003, Hassoun et al. 2003). Recently, a decrease in regional cerebral blood flow and a negative functional magnetic resonance imaging (fMRI) signal in the CPu was demonstrated during nociceptive stimulation in rats, providing a potential explanation for difficulties in finding pain-induced hemodynamic responses in humans (Shih et al. 2009).

In an experimental study with healthy humans, D2/D3 BP_{ND} in the putamen was inversely correlated with the cold pressor pain threshold, while the association between D2/D3 BP_{ND} in the right putamen and heat pain threshold just failed to reach significance ($p = 0.05$) (Hagelberg et al. 2002b). The present findings showing a significant inverse correlation of the heat pain threshold with dopamine D2/D3 receptor BP_{ND} in the right putamen (**I**, **II**) provide corroborative evidence that striatal dopamine D2/D3 receptors have an effect on pain (Hagelberg et al. 2004b). Furthermore, our findings suggest that the association of the heat pain threshold with striatal dopamine D2/D3 receptor BP_{ND} is explained by dopaminergic modulation of the subject's response criterion rather than discriminative capacity (**I**). This interpretation is supported by the finding that the inverse correlation of D2/D3 BP_{ND} in the right putamen was significant with the pain threshold (**I**, **II**) and with the subject's response criterion (**I**). In line with this, the correlation of D2/D3 BP_{ND} was not significant with an index of the subject's sensory-discriminative function, the area under the ROC curve (ROC [AUC]), indicating that the subject's discriminative capacity is not a critical factor underlying the association between striatal D2/D3 BP_{ND} and pain responses. The tactile detection threshold, the subject's response criterion to tactile stimulation, or the index of tactile discriminative capacity were not correlated with dopamine D2/D3 receptor BP_{ND} in any of the striatal regions studied (**II**). Thus, the influence of striatal dopamine D2/D3 receptors on sensory responses

appears to be selective for the modality of pain, despite the fact that many nociceptive striatal neurons have been shown to be multisensory (Chudler & Dong 1995).

In study **I,** the heat pain threshold was not significantly correlated with D2/D3 BP_{ND} in the thalamus or in any of the cortical regions studied. When a traditional pain threshold is used in calculations, the correlations of D2/D3 BP_{ND} with the subject's discriminative capacity (sensory function) or response criterion of pain (non-sensory function) might be missed, since the traditional threshold is influenced by a variety of sensory and non-sensory factors. However, although there is a dense innervation of the thalamus by dopaminergic nerve fibers (García-Cabezas et al. 2007, Sánchez-González et al. 2005), the D2/D3 BP_{ND} was not correlated with the subject's response criterion or capacity to discriminate heat pain in the thalamic or cortical ROIs (**I**). In our earlier study, D2/D3 BP_{ND} in the right medial temporal cortex was correlated with the tolerance to cold pressor pain (Hagelberg et al. 2002b). Since the cold pressor test induces a stronger affective component of pain than heat applied to the skin (Rainville et al. 1992), cortical dopamine D2/D3 receptors may have a more important role in the regulation of affective-motivational component of pain or suprathreshold pain. D2/D3 BP_{ND} in the brain as assessed by PET represents the ratio between the total number of receptors (B_{Avail}) and the affinity for the radioligand (K_D), reduced by competition from endogenous dopamine (see 7.1. Methodological considerations). The interindividual variability in striatal D2/D3 BP_{ND} may be caused by a constitutional difference in D2/D3 receptor density, a difference in D2/D3 receptor affinity, or a difference in the endogenous levels of synaptic dopamine (Koepp et al. 1998, Laruelle 2000). Since it has been shown that D2/D3 receptor affinity does not vary considerably between the subjects (Farde et al. 1995), a difference in D2/D3 receptor affinity is not a likely explanation. Further studies are needed to confirm whether a difference in D2/D3 receptor density, in endogenous release of dopamine, or both are underlying the association of pain with D2/D3 BP_{ND}. Since activation of striatal dopamine D2/D3 receptors suppresses pain in animals (e.g. Ben-Sreti et al. 1983, Magnusson & Fisher 2000), it may be proposed that a high basal level of endogenous dopamine in the striatum might explain the high pain threshold in subjects with low striatal D2/D3 BP_{ND}. The correlations of D2/D3 BP_{ND} with the subject's attitude towards rating pain in this study and with detachment, anxiety and novelty-seeking in earlier studies (Breier et al. 1998, Farde et al. 1997, Suhara et al. 2001) suggest that dopamine receptor availability might be a covariant with response to pain and personality traits. The present (**I**, **II**) and previous findings (Hagelberg et al. 2002b) suggesting that the pain threshold is inversely correlated with D2/D3 BP_{ND} in the putamen, and that the capacity to suppress pain by concurrent conditioning pain is directly correlated with D2/D3 BP_{ND} in the putamen may be explained by considering the dynamics of tonic and phasic dopamine signalling. Tonic dopamine release regulates the responsiveness of the phasic dopamine system to stimuli: high tonic release attenuates phasic release, and correspondingly, low tonic release facilitates phasic release (Grace 1991, West et al. 2003). From this point of view, a subject with a low D2/D3 BP_{ND} in the putamen may have a high tonic release of dopamine, raising the pain threshold, but making the dopamine system insensitive to phasic release and leading to poor capacity to recruit additional pain inhibition.

The placebo-induced increase in the heat pain threshold was not correlated with striatal dopamine D2/D3 receptor BP_{ND}, suggesting that striatal dopamine D2/D3 receptors may not be

involved in placebo analgesia. We have previously demonstrated that individuals with a low dopamine D2/D3 receptor BP_{ND} in the putamen have a low pain modulation capacity induced by noxious conditioning stimulation (Hagelberg et al. 2002b). Thus, placebo analgesia and noxious conditioning stimulation-induced analgesia may not share a common role for striatal dopamine D2/D3 receptors. However, we only assessed the baseline availability of striatal dopamine D2/D3 receptors: therefore, we cannot exclude the possibility that the expectation of placebo induces a striatal release of dopamine. This could have contributed to placebo analgesia in the same way as placebo contributes to relief of motor symptoms and arousal effect in subjects expecting to receive caffeine (de la Fuente-Fernández et al. 2001, Kaasinen et al. 2004). On the other hand, opioidergic mechanisms outside of the striatum may be critical for placebo analgesia, since it has been shown that placebo and opioids activate the same opioidergic pain modulatory nuclei in the brainstem and forebrain (Petrovic et al. 2002).

During the preparation of this thesis, two additional experimental PET imaging studies with [^{11}C]raclopride examining the role of striatal D2/D3 receptors in pain have been published (Scott et al. 2006, Wood et al. 2007). Both studies successfully reproduced the earlier finding that baseline dopamine D2/D3 receptor binding potential (BP_{ND}) in the putamen in healthy humans is inversely correlated with sensitivity to experimental pain (**I**, **II**) (Hagelberg et al. 2002b, Scott et al. 2006, Wood et al. 2007). Baseline dopamine D2/D3 receptor binding potential (BP_{ND}) in the putamen was inversely correlated to tolerance to sustained pain (Scott et al. 2006), which conforms with the earlier findings of inverse correlation with the capacity to modulate pain by noxious conditioning stimulation (Hagelberg et al. 2002b). In addition, Scott et al. (2006) were able to demonstrate striatal dopamine release during sustained pain, which strongly suggests that striatal dopamine release is associated with dynamic modulation of pain: dopamine release (as assessed by a decrease in [^{11}C]raclopride BP_{ND}) was observed during pain challenge in healthy volunteers in both the ventral and dorsal basal ganglia (caudate nucleus, putamen, NAcc) (Scott et al. 2006).

In the study by Scott et al. (2006), tonic pain was delivered via a hypertonic saline injection in the masseter muscle. Imaging with [^{11}C]raclopride was done at the baseline, during the pain and during an isotonic, non-painful saline control. In the first study, the decrease in BP_{ND} during the pain was compared with baseline (resting) BP_{ND}. Significant effects were obtained in the caudate nucleus and putamen bilaterally, and in the right (contralateral) NAcc. Pain tolerance, an objective measure of pain sensitivity defined as the total volume of injected hypertonic saline to maintain pain for each subject, was inversely correlated with D2-receptor activation in the right caudate. Right caudate activation was also positively correlated with the subjective aspects of pain, such as the total MPQ score, the MPQ sensory subscale score, an increase in the PANAS negative affect score and VAS unpleasantness (Scott et al. 2006). In a second study, the changes in [^{11}C]raclopride BP_{ND} during the pain were compared with BP_{ND} during saline control without pain expectation, and a similar effect of pain on D2/D3 BP_{ND} in the basal ganglia was observed: a decrease in D2/D3 BP_{ND} in the caudate nucleus and putamen bilaterally and in the contralateral (right) NAcc. The gain in D2/D3 receptor activation was restricted in the dorsal caudate and putamen, with no gain in activation in the NAcc. D2/D3 receptor activation in the right caudate and right putamen was positively correlated with MPQ total scores and MPQ sensory and pain affect subscale scores (Scott et al. 2006). Finally, the role of

D2/D3 receptors in pain stress was examined by comparing the baseline D2/D3 receptor BP_{ND} with a saline control with pain expectation. This analysis found an association between the right NAcc and pain stress, and D2/D3 activation in the NAcc was positively correlated with an increase in PANAS ratings of pain affect and fear (Scott et al. 2006). This study suggests that the nigrostriatal D2/D3 receptor-mediated neurotransmission is involved in both sensory and affective components of pain, whereas mesolimbic D2/D3 receptors are involved in the emotional responses to pain. In another study, the essential findings of Scott et al. (2006) concerning dopamine release were confirmed, and striatal dopamine D2/D3 activation during tonic pain was found in the globus pallidus, caudate nucleus and putamen (Wood et al. 2007). Moreover, the D2/D3 receptor activation in all subregions was correlated with perceived pain intensity (measured as difference in pain ratings during hypertonic vs. isotonic saline). In a second study by Scott et al. (2008), the potential role of μ-opioid receptors and D2/D3 receptors in placebo and nocebo effect in pain was studied with PET. This study found that, in addition to significant increase in μ-opioid neurotransmission in many brain areas, placebo induced an activation of D2/D3 neurotransmission in the ventral basal ganglia, and there was a positive correlation between placebo analgesia and D2/D3 activation in the ventral basal ganglia. These results suggest that striatal D2/D3 receptors, particularly in the right NAcc, participate in the regulation of placebo analgesia by interactions with the endogenous μ-opioid receptor system.

The study by Wood et al. (2007) showed that patients with fibromyalgia have a dysfunction in striatal D2/D3 receptor-mediated neurotransmission. As healthy subjects release dopamine in striatum during noxious stimulation and this release correlates with the perceived pain (Scott et al. 2006, Wood et al. 2007), PET imaging with [^{11}C]raclopride in fibromyalgia patients revealed neither striatal dopamine release nor correlation of the dopamine release with perceived experimental pain (Wood et al. 2007). Despite the abnormal striatal dopamine function during sustained pain, fibromyalgia patients display a correlation between D2/D3 BP_{ND} in the right putamen and perceived pain intensity. Interestingly, the D2/D3 BP_{ND} among fibromyalgia patients was also correlated with their clinical pain, the tender point index (Wood et al. 2007). The possible dysfunction in the dopamine system might be involved in the clinical features of fibromyalgia pain, which is characterized by tenderness and spontaneous chronic widespread pain (Wolfe et al. 1990). However, fibromyalgia patients have also other abnormalities in central neurotransmission. Recently, fibromyalgia patients have been shown to have decreased μ-opioid receptor availability in the brain (Harris et al. 2007). Alternations in opioid receptor availability have been demonstrated in several other chronic pain syndromes as well (Maarrawi et al. 2007, Willoch et al. 2004), which possibly indicates that the alternations in opioidergic neurotransmission are secondary phenomena, not the aetiological factors underlying the chronic pain itself. Together, these recent PET imaging studies support the role of the striatum and D2/D3 receptors in pain (**I**, **II**) and fit the hypothesis that striatal D2/D3 receptor activation is associated with pain suppression (Hagelberg et al. 2004b).

7.3. Striatal D2/D3 receptors in non-sensory influences on pain

The correlation of the pain threshold with the subject's response criterion was strong, whereas the correlation of the pain threshold with the index of the subject's sensory function was weak (**I**), which is in accordance with earlier findings (Clark & Mehl 1971). Consequently, the subjects with widely different sensory-discriminative capacities may have pain thresholds in the

same range. This finding might be explained by a major contribution by non-sensory factors (e.g. personality) to the individual pain threshold as well as to the subject's response criterion. This explanation is in line with the proposals that the subject's response criterion is a measure of non-sensory factors and that non-sensory functions have an important role in the variability of pain ratings between subjects (Clark 1994). Furthermore, previous studies have shown that D2/D3 BP_{ND} is correlated with personality traits such as detachment (Farde et al. 1997), anxiety (Breier et al. 1998) and novelty seeking (Suhara et al. 2001). This suggests that the correlation of striatal D2/D3 BP_{ND} with the pain threshold and the subject's response criterion may be explained by non-sensory factors. On the other hand, it has been proposed that a change in pain sensation may occur without an accompanying change in discriminability between two painful stimuli (Rollman 1977). Accordingly, it may be argued that a change in the subject's response criterion has an underlying neurobiological mechanism influencing the sensation, although in a different way to a mechanism influencing the subject's discriminative capacity. Experimental animal studies have demonstrated separate pain inhibitory pathways that differentially influence the slope or the intercept of stimulus-response functions of ascending pain signals (Carstens et al. 1980). An inhibitory pathway producing a change in the slope of the stimulus-response functions (a shift in gain) will also cause a change in the discriminability of sensory signals. However, an inhibitory pathway producing a selective change in the intercept of the stimulus-response function for pain (a parallel shift) may produce a selective change in the index of the subject's response criterion. This type of mechanism, causing a change in the index of the subject's response criterion but not in discriminative capacity, would also influence sensation; i.e., it should be classified as a sensory factor. On the basis of the present results, it is not possible to determine whether the association of striatal D2/D3 BP_{ND} is due to sensory factors producing a parallel shift in stimulus-response functions for pain, non-sensory factors (e.g. personality), or both. Thus, the proposal that striatal D2/D3 receptors do not influence the subject's discriminative capacity need not be in discrepancy with the possibility that striatal D2/D3 receptors influence sensory mechanisms that have an effect on the index of the subject's response criterion.

The potential mechanism for the non-sensory effects of striatal D2/D3 receptors on pain remains elusive. However, the striatum receives important projections from several brain areas involved in emotion and affective modulation of pain, such as the ACC, amygdala, and prefrontal cortex, which raises the possibility that these projections are associated with the proposed role of the striatum in the affective dimension of pain (Chudler & Dong 1995). On the other hand, a large body of evidence has linked brain dopaminergic pathways with incentive motivational processes, reward and reinforcement of behavior (Kupfermann 2000, Schultz 1997, Wise 2004). Many lines of evidence indicate a major role for the ventral (e.g. Kupfermann et al. 2000, Mobbs et al. 2003, Schweinhardt et al. 2009), and also for the dorsal striatum (e.g. Barrett et al. 2004, Zald et al. 2004) in reward processing (for reviews, see Schultz 1997, Wise 2004). Moreover, the ventral striatum, which has often been suggested to play a key role in reward and motivation, innervates most of the dopaminergic neurons projecting to the dorsal striatum (Haber 2003, Joel & Weiner 2000). Importantly, dopaminergic neurons not only respond to reward-related stimuli, but also to aversive events, including punishment (Cools 2008) and pain (Horvitz 2000). Further, there seems to be considerable overlap in brain regions associated with the regulation of reward and motivation, and pain

processing (Leknes & Tracey 2008). All this raises the hypothesis that the striatal dopamine regulates pain perception by affecting pathways that modulate motivational processes, or more precisely, by affecting the interaction between motivation and motor functions. From this point of view, our results suggesting that striatal D2/D3 receptors modulate the evaluative but not the discriminative component of pain perception could be explained by interactions between the decision making-action processes and motivation-related circuits (Haber 2003, Kupfermann 2000) or dopamine-opioid system interactions in motivation-based decision-making in pain (Fields 2004). Such mechanisms could also explain why striatal D2/D3 receptors do not seem to regulate response to touch (**II**): sensation of a stimulus of neutral valence, such as a tactile stimulus, probably does not initiate the unconscious decision process primarily concerned with estimations of salient and arousing environmental stimuli (Horvitz 2000). Indeed, there is evidence suggesting that strong, but not mildly aversive or neutral events have the capability to activate the dopaminergic system (Horvitz 2000). On the other hand, dopamine has an important role in the general regulation of cognition and attention (Cropley et al. 2006, Nieoullon 2002, Nieoullon & Coquerel 2003). In a recent PET imaging study, placebo and nocebo responses to pain challenge were associated with corresponding activation and deactivation in striatal D2/D3 neurotransmission in healthy humans (Scott et al. 2008). As placebo and nocebo responses to pain are, by definition, based on purely cognitive manipulation of the pain response, this study supports the view that striatal dopamine D2/D3 receptors may be associated with cognitive-evaluative (non-sensory) rather than sensory-discriminative aspects of pain.

7.4. Brain 5-HT$_{1A}$ receptors in pain sensitivity and pain memory

Intensity of CPP had an inverse correlation with 5-HT$_{1A}$ BP_P in multiple brain regions, including the prefrontal, cingulate and insular cortices, suggesting that brain 5-HT$_{1A}$ receptors influence perception of pain in humans (**III**). Those brain areas with a significant association with pain belong to the group of brain areas that are frequently activated in brain imaging studies and have well-documented effects on pain ('pain matrix') (Apkarian et al. 2005, Tracey & Mantyh 2007). Activations of the cingulate cortex and the insula have been associated with the affective-motivational component of pain, whereas activation of the prefrontal cortex may be related to cognitive aspects of pain perception (Bushnell & Apkarian 2006, Casey & Tran 2006, Petrovic & Ingvar 2002, Peyron et al. 2000). In the cingulate cortex, the strongest correlation with pain was in the posterior part, which is in line with earlier results indicating that noxious skin stimulation activates the posterior cingulate cortex (Vogt 2005). Subcortically, 5-HT$_{1A}$ BP_P in the amygdala and the dorsal raphe was inversely correlated with CPP intensity. The amygdala is known to contribute to emotional-affective pain modulation and response to pain (Neugebauer 2006), while serotonergic neurons of the dorsal raphe contribute to descending and ascending modulation of pain (Wang & Nakai 1994). However, not all brain regions, in which 5-HT$_{1A}$ BP_P was inversely correlated with the CPP intensity, have an established role in the perception or modulation of pain. This type of phenomenon, correlation of 5-HT$_{1A}$ *BP* in multiple brain regions with some behavioral characteristics of subjects, has been described earlier (e.g. Borg et al. 2003, Lanzenberger et al. 2007, Tiihonen et al. 2004), and may be related to the widespread projections of the highly bifurcated serotonergic neurons in raphe nuclei (Hornung 2003, Lucki 1998). This raises the possibility that 5-HT$_{1A}$ receptor availability might influence some general behavioral parameter that indirectly influences perceived CPP

intensity: such a hypothesis is in line with earlier results indicating that 5-HT_{1A} receptors regulate a number of factors with a general influence on behavioral responses, such as stress, depression and attention (Carli & Samanin 2000, Graeff et al. 1996). Curiously, although 5-HT_{1A} BP_P in multiple brain regions was significantly associated with the intensity of CPP assessed at time point 1.1 x CPP threshold, the CPP threshold itself did not have significant correlations with 5-HT_{1A} BP_P in any of the ROIs. This finding suggests that supraliminal CPP versus CPP threshold are dissociatively influenced by 5-HT_{1A} receptors. On a par with these findings, a recent PET imaging study examined the role of brain 5-HT_{2A} receptors in pain in healthy volunteers, and similarly found a significant correlation between 5-HT_{2A} receptor binding and tonic pain, but not pain threshold, tolerance or phasic pain measures (Kupers et al. 2009). The dissociative effect found in study **III** might be explained by a 5-HT_{1A} receptor-driven mechanism influencing predominantly the gain or slope of the stimulus-response curves leading to modulation of supraliminal pain, with little influence on the pain threshold. The cingulate cortex, amygdala and posterior insula are known to be involved in the affective component of pain (Kulkarni et al. 2005, Treede et al. 1999, Vogt 2005). Nonetheless, 5-HT_{1A} BP_P in these regions was associated with CPP intensity but not with CPP unpleasantness (an index of the affective component of pain), although the relatively high unpleasantness of CPP (Rainville et al. 1992) makes it an appropriate model for studying affective responses to pain. The magnitude of the CPP threshold increase by contralateral conditioning stimulation was directly correlated with 5-HT_{1A} BP_P in the amygdala and medial prefrontal cortex (**III**), suggesting that these brain areas are involved in 5-HT_{1A} receptor-mediated dynamic modulation of pain. Together, these results indicate that subjects with low brain 5-HT_{1A} BP in the amygdala and medial prefrontal cortex, possibly due to low 5-HT_{1A} receptor density, have a low capacity to recruit supraspinal pain-inhibition by conditioning painful stimulation, and give a high intensity rating to suprathreshold CPP.

The results from the correlation analysis between brain 5-HT_{1A} BP_{ND} and response to heat pain provide further support to the role of brain 5-HT_{1A} receptors in the perception of pain in humans (**IV**). In this study, 5-HT_{1A} BP_{ND} in the middle temporal gyrus, orbitofrontal cortex, posterior cingulum and DRN was inversely correlated with the criterion to report pain (C), but not with an index of the discriminative capacity, ROC [AUC], or the heat pain threshold. This finding fits our earlier results and supports our hypothesis that 5-HT_{1A} receptors of the brain influence non-sensory mechanisms underlying the subject's response to pain, such as response criterion, rather than actual pain sensitivity (**III**). Direct comparison of the results from studies **III** and **IV** is difficult, most importantly from the pain modality point of view (heat vs. CPP), but also due to other differences between the two experimental protocols. For instance, brief heat pain stimuli were applied cutaneously in study **IV**, whereas in study **III** cold pain was tonic and arises probably from deep structures, such as veins (Fruhstorfer & Lindblom 1983), which obviously results in a different kind of pain as widely known from experimental studies (e.g. Casey et al. 1996, Rainville et al. 1992). Nevertheless, it may be hypothesized that a subject with a low brain 5-HT_{1A} BP_{ND}/BP_P, exhibits a conservative bias, but gives a high intensity rating to a suprathreshold painful stimulus (i.e., a steep stimulus-response curve). Furthermore, the direct comparison of results from our studies with human subjects and previous studies with animals is complicated, as no animal studies have studied the association between pain-related responses and 5-HT_{1A} binding/receptor density in brain. In general, extremely few animal

studies have attempted to dissociate the animal's capacity to discriminate painful or tactile stimuli from its response criterion, and none of these few studies has addressed the role that the serotoninergic system might play in this respect. Nevertheless, the present findings lend support to the earlier suggestion that 5-HT_{1A} receptor-mediated analgesic effects in experimental animals may, at least partly, be explained by an effect on the emotional-motivational component of behavior (Korneyev & Seredenin 1993). The dense ascending projections of the DRN to the limbic system (Hornung 2003) might offer a mechanism for the possible non-sensory modulation of pain by 5-HT_{1A} receptors.

There is considerable comorbidity between anxiety disorders, depression and chronic pain (Bair et al. 2003, Bond 2006), implying that these syndromes may share abnormalities in neural pathways. This is supported by the effectiveness of antidepressants in the treatment of both depression and chronic pain (Saarto & Wiffen 2007). In a fMRI study with patients with unmedicated major depressive disorder (Strigo et al. 2008), the patients with depression showed an abnormal activation/deactivation pattern during pain and pain anticipation in the many brain areas involved in the modulation of pain, including the anterior insula, ACC, amygdala and prefrontal cortex. This study raises the hypothesis that concurrent abnormalities in pain and emotion-modulating circuits in major depression result in affective biasing of the pain experience. Interestingly, dysfunction of the 5-HT_{1A} receptor is assumed to play a role in anxiety-related behavior and the genesis of major depression (Savitz et al. 2009). In PET studies, patients with major depression show decreased 5-HT_{1A} BP_{ND}/BP_P in many brain areas (Hirvonen et al. 2008, Sargent et al. 2000). The results of the present study, showing that low 5-HT_{1A} BP_P is associated with high pain rating, may explain increased pain sensitivity among depressed patients and moreover, offer a potential mechanism for the analgesic effects of antidepressants.

In the short-term memory task for pain, the subject's certainty ratings of his performance were correlated with 5-HT_{1A} BP_{ND} in DRN (**IV**), adding to the established role of brain 5-HT_{1A} receptors in memory regulation (Buhot et al. 2000). As with heat pain sensitivity, the objective index of memory performance, as revealed by the area under the ROC curve (ROC [AUC]), was not associated with 5-HT_{1A} BP_{ND} in any of the ROIs. Previous microinjection studies in experimental animals suggest that 5-HT_{1A} receptors in the DRN do not have significant effects on learning and memory performance *per se* (Egashira et al. 2006, Warburton et al. 1997), while they may modulate non-mnenomic components of working memory (Ruotsalainen et al. 1998) or hippocampal functions related to learning and memory (Carli et al. 2000, Squire 1986, Squire et al. 2004). Importantly, the subjects could easily discriminate between the two temperatures used in the pain memory task (47°C and 48°C) in the first experiment assessing heat pain sensitivity, suggesting that the differences in performance in the short-term memory task can be attributed to subjective differences in the short-term memory of pain rather than pain perception *per se*.

7.5. Brain 5-HT_{1A} receptors in touch and autonomic control

Recent brain imaging studies have challenged the traditional view that the sensory-discriminative aspects of somatosensation are confined within the somatosensory cortex (Coghill et al. 1999, Oshiro et al. 2007, Pleger et al. 2006, Romo & Salinas 2001). The

discriminative aspect of touch (ROC [AUC]), but not response criterion or detection threshold, was inversely correlated with 5-HT_{1A} BP_{ND} in the anterior cingulate cortex, inferior temporal gyrus, medial prefrontal cortex and posterior cingulate cortex (**IV**). Earlier studies suggest a role for the prefrontal and cingulate cortices in decision-making in vibrotactile discrimination tasks (Pleger et al. 2006, Preuschhof et al. 2006, Romo & Salinas 2001). The cingulate and prefrontal cortices are also involved in cognitive functions, such as selective attention, working memory and guidance of goal-directed behavior, which might influence performance in a tactile discrimination task (Devinsky et al. 1995, Miller & Cohen 2001). It might be speculated that such cognitive factors have an impact on response criterion rather than on the index of discriminative capacity that reflects the sensitivity of the sensory system (Swets 1973). The present results suggest, however, that the response criterion for touch is not influenced by cortical 5-HT_{1A} receptors. On the other hand, somatosensory-evoked potentials induced by electrical stimulation of tactile nerve fibers were enhanced in patients that had prefrontal damage (Yamaguchi & Knight 1990), and navigated transcranial magnetic stimulation of cortico-cortical fiber tracts from prefrontal to S1 cortex attenuated somatosensory-evoked responses in healthy subjects (Hannula et al. 2009). These findings indicate that the prefrontal cortex gates tactile inputs to the somatosensory cortex and thus influence the subject's sensory capacity to discriminate between tactile stimuli, providing a potential underlying mechanism for the significant association of the discriminative capacity for touch with 5-HT_{1A} BP_{ND} in the prefrontal cortex. Although the present results indicate that 5-HT_{1A} receptors in the cingulate cortex, inferior temporal gyrus and medial prefrontal cortex influence discriminative aspects of tactile sensitivity, not response criterion, it should be noted that the results do not exclude the possibility that 5-HT_{1A} receptor-independent mechanisms in these brain regions play a role in modulating the subject's response criterion in a tactile perception task.

Activation of the sympathetic nervous system produces a vasoconstriction response in the skin, and this response is particularly prominent in the fingertips. This sympathetic vasoconstrictor reflex can typically be activated by mental stress, arousal or deep breaths as in the Valsalva maneuver (Wallin 1990) and by pain (e.g. Kemppainen et al. 1994). In this study, the magnitude of the vasoconstriction response induced by Valsalva, but not by CPP, was directly correlated with 5-HT_{1A} BP_P in the anterior insula and the ventral part of the anterior cingulate cortex (**III**). These findings are in line with the role of central 5-HT_{1A} receptors in the regulation of sympathetic and parasympathetic nerve activity (Ramage 1990) and, on the other hand, with earlier fMRI results in humans indicating that the anterior cingulate cortex and the insula are involved in the sympathetic regulation of cardiovascular responses during non-painful mental or motor tasks (Critchley et al. 2003) or the Valsalva maneuver (Henderson et al. 2002, Macefield et al. 2006). It should be noted, however, that a number of brain structures that have a major contribution to regulation of vasoconstriction, such as the ventrolateral medulla, nucleus tractus solitarius, locus coeruleus and midbrain periaqueductal gray (Green et al. 2006, Richerson 2003), were not included in the analysis of the present study. In accordance with the established role of the brainstem in autonomic control, results from an experimental human study suggest an important role for the brainstem in the regulation of pain-induced galvanic skin response, which is a measure of sympathetic response (Petrovic et al. 2004). In contrast to the correlation of the CPP intensity rating with many ROIs, the Valsalva-induced vasoconstriction was

associated with 5-HT_{1A} BP_P only in restricted brain regions suggesting that these associations might reflect specific modulatory mechanisms influenced by 5-HT_{1A} receptors.

7.6. Implications and future prospects

The results of this thesis suggest novel roles for brain dopamine D2/D3 and serotonin 5-HT_{1A} receptors in regulating somatosensory responses. The results suggest that striatal D2/D3 receptors regulate pain by modulating the subject's response criterion, without affecting response to innocuous stimuli. The association of suprathreshold CPP and the response criterion to heat pain with 5-HT_{1A} BP_{ND}/BP_P in multiple brain areas implies that 5-HT_{1A} receptors, too, may be related to factors affecting subjective (attitudinal) aspects of pain rather than the discriminative capacity of the sensory system mediating pain. Furthermore, the study found that brain 5-HT_{1A} BP_{ND}/BP_P was also associated with autonomic control, subjective memory for pain, and touch discrimination. These new findings may have important implications in explaining differences in pain sensitivity between individuals and offering potential molecular targets for new pain therapies. The results also provide a potential mechanism for the analgesic effects of dopaminergic and serotonergic drugs, and the results may explain the significant comorbidity between some syndromes related to abnormalities in brain dopamine and serotonin, such as Parkinson's disease and major depressive disorder, and chronic pain.

It should be borne in mind that this study presents associations between dopamine D2/D3 and serotonin 5-HT_{1A} receptor binding in the brain and several psychophysical variables, but cannot determine, whether the relationships are causal, although the results from earlier studies, and for dopamine D2/D3 receptors results from the study by Scott et al. (2006) and Wood et al. (2007) seem to corroborate this point of view. Thus, more imaging studies are needed to clarify the relationship between brain D2/D3 and 5-HT_{1A} receptors and response to pain. The results of this thesis also raise several other questions for further studies. Additional studies are needed to address the role of brain D2/D3 and 5-HT_{1A} receptors in susceptibility to chronic pain and whether there are chronic pain-related changes in brain D2/D3 and 5-HT_{1A} receptor binding, as well as the significance of brain D2/D3 and 5-HT_{1A} receptor binding in determining individual sensitivity to pain therapies. Since there seem to be significant interactions between different neurotransmitter systems in the regulation of pain in the human brain (e.g. Scott et al. 2008), such interactions need to be taken into account in future studies by performing PET imaging with different radioligands in the same subjects. On the other hand, there is rapidly mounting evidence suggesting that genetic factors associated with both dopaminergic and serotonergic neurotransmission (Kosek et al. 2009, Potvin et al. 2009) are related to sensitivity to experimental pain. Hence, future studies are also needed to clarify the potential role of genetic factors in determining both brain D2/D3 and 5-HT_{1A} receptor binding and response to pain. In addition, the results of this thesis warrant further consideration in terms of the psychophysical methods used in pain research, and the role of different brain areas in pain perception and modulation.

8. SUMMARY AND CONCLUSIONS

The conclusions of this thesis can be summarized as follows:

1. The heat pain threshold (**I**, **II**) and response criterion (**I**) are inversely correlated with D2/D3 BP_{ND} in the right putamen, but striatal D2/D3 BP_{ND} is not correlated with response to touch or placebo (**II**). These findings support the hypothesis that the striatal D2/D3 receptors are involved in the perception of pain in humans. The lack of association with response to touch suggests that the effect of striatal D2/D3 receptors on somatosensory responses may be modality-specific, or dependent on stimulus saliency.

2. Brain 5-HT_{1A} BP_P is inversely correlated with cold pressor pain intensity (**III**), and 5-HT_{1A} BP_P in the amygdala and medial prefrontal cortex is directly correlated with cold pressor pain threshold increase by conditioning cold pressor pain (**III**), suggesting that brain 5-HT_{1A} receptors are also involved in the perception of pain in humans.

3. The skin vasoconstriction response induced by the Valsalva maneuver is correlated with 5-HT_{1A} BP_P in the ventral anterior cingulate cortex and anterior insula (**III**). This indicates that brain 5-HT_{1A} receptors may be involved in autonomic control.

4. The heat pain response criterion is inversely correlated with 5-HT_{1A} BP_{ND} in the dorsal raphe, middle temporal gyrus, orbitofrontal cortex and posterior cingulate cortex (**IV**). This finding implies that the effects of brain 5-HT_{1A} receptors on pain are, at least in part, dependent on modulation of non-sensory aspects of pain.

5. The subjective, but not objective, aspect of short-term memory for heat pain is correlated with 5-HT_{1A} BP_{ND} in the dorsal raphe (**IV**), suggesting a role for 5-HT_{1A} receptors in the dorsal raphe in the subjective aspect of memory for pain.

6. The discriminative capacity for touch is inversely correlated with 5-HT_{1A} BP_{ND} in the anterior and posterior cingulate cortex, inferor temporal gyrus, and medial prefrontal cortex (**IV**). This finding suggests that brain 5-HT_{1A} receptors differentially regulate response to pain and touch by having an effect on non-sensory and discriminative aspects of perception, respectively.

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