Opioidergic regulation of human affiliative behavior

Evidence from positron emission tomography studies
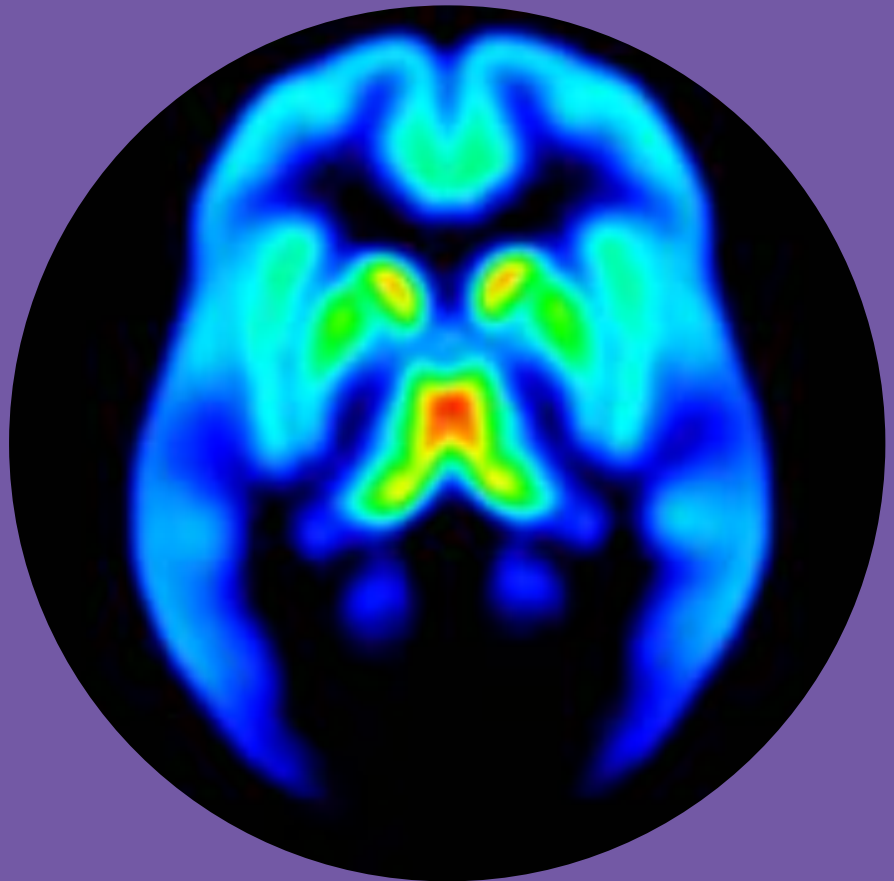
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Tomi Karjalainen

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Abstract

Humans display a remarkable pattern of affiliative behavior. We talk, laugh, play games, and celebrate various events, such as birthdays. Our social networks are also much larger than those of our evolutionary closest cousins, nonhuman primates. Social life is so important for humans that social problems, such as isolation and loneliness, are detrimental to our mental and somatic health. Sociality is indeed one of the basic human needs, similarly as food, water, and safety. Despite the fundamental role of sociality to humans, the neurobiological mechanisms influencing human affiliative behavior are still poorly understood.

Animal models of social behavior suggest that endogenous opioid system–a neurotransmitter system modulating pain and pleasure in all mammals–regulates also affiliative behavior. Proposedly, motivation for social interaction partly arises from decreased opioidergic activity in the brain, and various forms of social behavior increase opioidergic processing. This increase results in pleasant affective states and facilitates inter-personal bonding between the interacting individuals. While the results from animal studies are mostly consistent with this model, it is still unknown whether and how the opioid system regulates affiliative behavior of humans.

The aim of this Thesis was to characterize the role of the opioid system in human affiliative behavior using neuroimaging with positron emission tomography (PET) and functional magnetic resonance imaging (fMRI). The Thesis focuses on individual differences in self-reports of approach–avoidance behavior and affective responses measured with fMRI. The Thesis also tested if laughing induces release of endogenous opioids. [11C]carfentanil, a selective µ-opioid receptor (MOR) agonist tracer, was used to quantify cerebral MOR availability in all four studies.

The first study showed that cerebral MOR availability is positively associated with approach motivation. This finding is in line with data from animal studies, suggesting that baseline opioidergic activity influences how actively humans seek reward. The second study showed that laughing with friends induces endogenous opioid release, consistent with the hypothesis that laughing facilitates interpersonal bonding in humans via opioidergic mechanisms. The third and fourth studies showed that individuals with high MOR availability, particularly in rostral anterior cingulate cortex and insular cortex, have blunted hemodynamic responses to painful and otherwise arousing movie scenes. These findings are consistent with the opioid system's role in regulation of pain and anxiety, suggesting that inter-individual differences in MOR availability may explain why some humans often find themselves highly aroused, while others may be perfectly calm in the same situations. In sum, results of the Thesis support the involvement of opioids in transmitting not only signals related to pain and pleasure but also to sociality in humans.

Tekijä
Tomi Karjalainen

Väitöskirjan nimi
Ihmisen sosiaalisen käytöksen opioidivälitteinen ohjaus

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Tiivistelmä

Ihmisten sosiaalinen käytös on hyvin monimuotoista. Me puhumme, nauramme, pelaamme pelejä ja juhlimme erilaisia tapahtumia, kuten syntymäpäiviä. Meillä on myös merkittävästi suuremmat sosiaaliset verkostot kuin muilla kädellisillä eläimillä, jotka ovat evolutiivisesti lähimpiä serkkujamme. Sosiaalinen elämä on meille niin tärkeää, että erilaiset sosiaaliset ongelmat, kuten eristyneisyys ja yksinäisyys, aiheuttavat paitsi psyykkisiä myös somaattisia sairauksia. Sosiaalisuus katsotaankin yhdeksi ihmisen perustarpeista siinä missä ruoka, vesi ja turvallisuuskin. Vaikka sosiaalisuus on meille näin tärkeää, ihmisen sosiaaliseen käytökseen vaikuttavia neurobiologisia tekijöitä tunnetaan yhä huonosti.

Eläintutkimukset ovat osoittaneet, että endogeeninen opioidijärjestelmä, jolla on keskeinen rooli kivun ja mielihyvän säätelyssä nisäkkäillä, säätelee myös sosiaalista käytöstä. On esitetty, että alhainen opioidijärjestelmän aktiivisuus motivoisi yksilöitä sosiaaliseen toimintaan. Sosiaalinen toiminta puolestaan aktivoi opioidijärjestelmää, mikä tuntuu miellyttävältä ja edistää sosiaalisten suhteiden muodostumista. Toistaiseksi ei kuitenkaan tiedetä onko myös ihmisillä samanlaisia opioidivälitteisiä sosiaalisen käytöksen ohjausmekanismeja.

Väitöskirjan tavoitteena oli tutkia opioidijärjestelmän roolia ihmisen sosiaalisen käytöksen ohjaamisessa. Tutkimuksissa käytettiin toiminnallista magneettikuvantamista sekä positroniemissiotomografiaa (PET). Väitöskirja keskittyy erityisesti yksilöiden välisiin eroihin lähestymis–välttelykäyttäytymisessä sekä aivojen tunnevasteissa. Väitöskirjassa testattiin myös hypoteesia, jonka mukaan nauraminen vapauttaa opioideja aivoissa. Kaikissa neljässä tutkimuksessa keskiössä oli μ-opioidireseptori (MOR). Sen saatavuutta aivoissa mitattiin käyttäen PET-merkkiainetta [11C]karfentaniili, joka on selektiivinen MOR-agonisti.

Ensimmäinen tutkimus osoitti, että MOR-saatavuus aivoissa on positiivisesti yhteydessä lähestymismotivaatioon. Opioidijärjestelmän perusaktiivisuus aivoissa voi siis vaikuttaa siihen, miten herkästi yksilö etsii luonnollisia palkkioita. Toinen tutkimus osoitti, että ystävien kanssa nauraminen vapauttaa opioideja aivoissa. Tämä tulos tukee hypoteesia, jonka mukaan nauraminen edistää sosiaalisten suhteiden muodostumista opioidivälitteisten mekanismien avulla. Kolmas ja neljäs tutkimus osoittivat, että niillä henkilöillä, joilla on korkea MOR-saatavuus, aivojen aktiivisuus on keskimäärin muita heikompää kivuliaiden tai muutoin tunteita herättävien elokuvakohtausten aikana. Nämä tulokset ovat sopusoinnussa opioidijärjestelmän osallisuudelle kivun ja ahdistuksen säätämisessä. Yksilöiden väliset erot MOR-järjestelmän toiminnassa voivat selittää miksi jotkut henkilöt ovat helposti kiihtyviä, kun taas toiset voivat samoissa tilanteissa pysyä täysin rauhallisena. Kokonaisuudessaan väitöskirjan tulokset viittaavat siihen, että opioidit välittävät kipuun ja mielihyvään liittyvien signaalien lisäksi myös sosiaalisia signaaleja ihmisillä.

Contents

List of publications

This Thesis consists of a compiling part and four journal publications that are referred to by their roman numerals.

I.   Karjalainen, T., Tuominen, L., Manninen, S., Kalliokoski, K., Nuutila, P., Jääskeläinen, I.P., Hari, R., Sams, M., Nummenmaa, L. (2016) Behavioural activation system sensitivity is associated with cerebral µ-opioid receptor availability. *Social Cognitive and Affective Neuroscience*, 11, 1310–1316. doi:10.1093/scan/nsw044.

II.   Manninen, S., Tuominen, L., Dunbar, R.I.M., Karjalainen, T., Hirvonen, J., Arponen, E., Hari, R., Jääskeläinen, I.P., Sams, M., Nummenmaa, L. (2017). Social laughter triggers endogenous opioid release in humans. *The Journal of Neuroscience*, 37, 6125–6131. doi:10.1523/JNEUROSCI.0688-16.2017.

III. Karjalainen, T., Karlsson, H.K., Lahnakoski, J.M., Glerean, E., Nuutila, P., Jääskeläinen, I.P., Hari, R., Sams, M., Nummenmaa, L. Dissociable roles of cerebral µ-opioid and type 2 dopamine receptors in vicarious pain: A combined PET–fMRI study. *Cerebral Cortex*, 27, 4257–4266. doi:10.1093/cercor/bhx129.

IV.   Karjalainen, T., Seppälä, K., Glerean, E., Karlsson, H.K., Lahnakoski, J.M., Nuutila, P., I.P. Jääskeläinen, Hari, R., Sams, M., Nummenmaa, L. Opioidergic regulation of emotional arousal: A combined PET–fMRI study. *Cerebral Cortex*, 11 p. doi.org/10.1093/cercor/bhy281.

Author's contribution

Study I: The candidate analyzed the data and wrote the manuscript. All co-authors contributed to writing of the manuscript.

Study II: The candidate analyzed the data and contributed to writing of the manuscript with the other co-authors.

Study III: The candidate analyzed the data and wrote the manuscript. All co-authors contributed to writing of the manuscript.

Study IV: The candidate analyzed the data and wrote the manuscript. All co-authors contributed to writing of the manuscript.

List of Abbreviations and Symbols

ACC	Anterior cingulate cortex
B_0	Magnitude of static magnetic field
BAS	Behavioral activation (approach) system
BIS	Behavioral inhibition system
BOLD	Blood-oxygen-level dependent
B_{avail}	Receptor density
BP_{ND}	Nondisplaceable binding potential
C_r	Radioactivity concentration in the reference tissue
C_t	Radioactivity concentration in the target tissue
D_2R	Dopamine type 2 receptor
FDR	False discovery rate
fMRI	Functional magnetic resonance imaging
f_{ND}	Free fraction of the radioligand in the nondisplaceable compartment
FWE	Family-wise error
GLM	General linear model
HRF	Hemodynamic response function
k_2	Effective efflux rate constant
K_D	Dissociation constant
M1	Primary motor cortex
MNI	Montreal neurological institute
MOR	μ-opioid receptor
MR	Magnetic resonance
MRI	Magnetic resonance imaging
NAcc	Nucleus accumbens
OFC	Orbitofrontal cortex
PET	Positron emission tomography
PFC	Prefrontal cortex
PRECG	Precentral gyrus
R_1	Influx ratio
rACC	Rostral anterior cingulate cortex
RF	Radiofrequency
ROI	Region of interest
S1	Primary somatosensory cortex
S2	Secondary somatosensory cortex
SMA	Supplementary motor area
SNP	Single-nucleotide polymorphism
SRTM	Simplified reference-tissue model
STS	Superior temporal sulcus
γ	Gyromagnetic ratio
ω	Larmor-frequency

1 Introduction

1.1 Affiliative behavior

Humans, like many primates, are social animals. Relative to their body size, primates typically have large brains compared to other mammals [1], and humans have unusually large brains even among primates [2]. The human brain weighs approximately 2 % of the body mass, yet it consumes up to 20 % of the body's oxygen, indicating that the human brain is metabolically extremely active [3]. In fact, the human brain is metabolically the costliest among hominoids [4]. As the energy cost of maintaining nervous tissue is so massive, there must have been extraordinary evolutionary pressure for the human brain to develop that large. While there are several hypotheses explaining the exceptional size of the human brain [5], of particular interest here is the social brain hypothesis proposing that the computational requirements arising from the complexities of social life has been one of the driving forces. This hypothesis is supported by the observation that the size of the neocortex correlates positively with the social network size in mammals. Consistent with this finding, it has been proposed that the neocortex size sets an upper limit for the size of the social network an individual can live in. [6] The enlarged human neocortex has presumably enabled co-operation to facilitate gathering of resources, such as food, as well as division of labor that optimizes resource allocation. The evolutionary development of human sociality has gone so far that feeling socially connected is regarded as one of the basic human needs [7], providing happiness and meaning to life [8]. On the contrary, social deprivation can cause severe stress to humans and have detrimental effects on mental and physical health [9].

Humans have a wide repertoire of behaviors aimed at forming and maintaining social bonds. Playing, verbal communication, social touching, and laughing all have important roles. Such behavior is referred to as *affiliative behavior*. It is also one of the main sources of pleasure for humans [10], and people spend a significant proportion of their lives socializing with others. The social dimension of life is so important for humans that termination of a social relationship can feel devastating, and even less salient social rejections often cause misery. The negative feelings associated with social rejection resemble pain and are associated with enhanced brain activity in anterior cingulate cortex, secondary somatosensory cortex and posterior insula that are also involved in processing of nociceptive signals [11, 12]. Furthermore, genetic variation influencing the function of the endogenous opioid system–a neurotransmitter system fundamentally involved in pain modulation–is associated with sensitivity to social rejection [13]. These observations indicate similarities in the neurobiological processes underlying sensory pain and social rejection, suggesting that the evolutionary ancient brain circuits processing noxious stimuli may have been exapted for processing information unrelated to tissue damage. In other words, social pain has presumably developed during human evolution from the more rudimentary sensation of sensory pain.

Animal studies have highlighted how specific neurotransmitter systems regulate social behavior. Most important of these systems are the oxytocin, vasopressin, dopamine, and opioid systems. While inter-dependent, each neuromolecular system is associated with their own roles in the process [14, 15]. Extensive studies focusing on oxytocin and vasopressin have shown their fundamental in maternal bonding and formation of reproductive relationships [16-18]. Dopamine is crucial in generating motivation to seek reward, such as social interaction [19]. The opioid system governs the hedonic value associated with affiliative behavior [20] and has been argued to be important in maintenance rather than formation of social bonds [21]. Given the substantial amount of evidence linking opioid peptides to affiliation in animals [22-24], and the notable scarcity of evidence on humans, this Thesis focuses on the endogenous opioid system and how it contributes to human social behavior.

1.2 The endogenous opioid system

Endogenous opioid system is well known for its role in modulation of nociceptive signals [25]. Its name is derived from opium, the dried latex of *Papaver somniferum,* also known as the opium poppy. Opium was the most powerful analgesic substance available for thousands of years before modern understanding of pharmacology. In addition to its essential role in the history of pain treatment, opium has also been one of the most common compounds causing addiction. However, opioids have not been relevant only historically. Even today, the most powerful analgesic substances, such as oxycodone and fentanyl, target the opioid system. Furthermore, echoing the infamous problems with opium addiction in China, there is currently a massive problem with opioid abuse in the United States, with opioid overdose causing 20 % of deaths among young adults [26]. These facts make the opioid system as topical as ever.

Despite this long history with opioids, it was not until the 1970s that humans discovered that exogenous opiates act on multiple opioid receptors. Soon after, also the endogenous opioid peptides binding to these receptors were identified. [27] Today, it is known that the constituents of the endogenous opioid system are three families of opioid peptides–endorphins, enkephalins, and dynorphins–as well as three families of receptors, namely, μ-, δ-, and κ-opioid receptors. Endomorphins have also been identified as opioid peptides, but their function is not yet properly understood [28]. The opioid peptides have different affinity profiles for the opioid receptors: β-endorphin and endomorphins have highest affinity to the μ-opioid receptor (MOR), the main target of dynorphins is the κ-opioid receptor, while enkephalins primarily bind to the δ-opioid receptor [29].

The main focus of the Thesis is on the MOR system, i.e. the receptor and the opioid peptides binding to it. MOR is a G-protein coupled receptor whose activation starts a cascade of events that inhibit presynaptic neurotransmission and decrease postsynaptic excitability. [28] In humans, MOR is encoded by the OPRM1 gene, and a similar gene sequence is presumably found in all vertebrates but not in invertebrates, suggesting that the receptor developed early in vertebrate evolution [30]. The neuroanatomical distribution of MOR in rats closely resembles that of humans [31], indicating high functional similarity of the receptor at least in mammals. OPRM1 has a widely investigated single-nucleotide polymorphism (SNP) A118G [32]. The A118G SNP of OPRM1 changes one of the amino acids of MOR, and thus carriers of the G-allele have a different version of the receptor compared to A-homozygotes. Carriers of the A118G SNP have lowered concentration of MOR messenger RNA [33] and elevated affinity for β-endorphin [34], indicating that the SNP is functionally relevant.

While MORs are also expressed outside the central nervous system [35], the Thesis focuses on the brain. MORs are expressed abundantly throughout the brain except in occipital cortex. They are particularly densely populated in thalamus, amygdala, striatum, and cingulate cortex, i.e. brain regions involved in processing of noxious and rewarding information, as well as in hypothalamus that controls the autonomic nervous system (Figure 1). Activating MOR using exogenous opiates, such as morphine, has analgesic, euphoric and anxiolytic effects. Similarly, also endogenous activation of MOR by β-endorphin produces profound analgesia [36], and the descending pain control system is largely driven by opioidergic mechanisms [37].
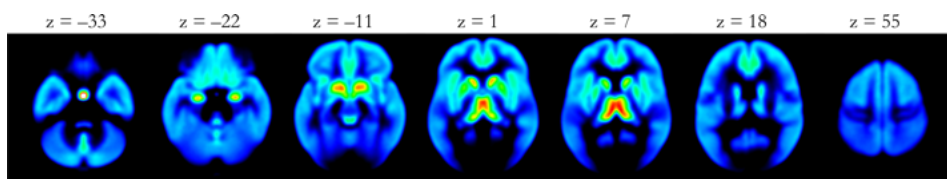
Figure 1. Spatial distribution of μ-opioid receptors in seven axial slices of the human brain. Population average derived from a sample of 380 [^{11}C]carfentanil PET scans. Z-values denote the slice in the MNI152 space.

1.3 Opioidergic regulation of affiliation

In addition to regulating pain and pleasure, it has been proposed that opioids could also regulate motivation for social affiliation. This hypothesis was inspired by the striking similarities observed between opioid addiction and romantic relationships, such as strong emotional attachment and distress following withdrawal of narcotics or social companion [22]. The dual role of endogenous opioids in transmitting

signals related to both pain and pleasure makes this hypothesis particularly attractive, as the most thrilling moments as well as the most painful tragedies humans experience are typically related to social life. Testing this hypothesis begun already in the 1970s using animal models, and today there is an extensive scientific literature describing how opioids regulate affiliation.

1.3.1 Animal data suggest a negative feedback loop between social and opioidergic activity

Social behavior of animals has been mostly studied by monitoring separation distress, social interaction, such as rough-and-tumble play, as well as mother–infant relationships [21]. In many mammals including humans, infants start vocalizing distressfully when separated from their mothers [22]. These reflex-like vocalizations have powerful effects on their caregivers who respond to them by accompanying the infant [38]. The separation elevates stress hormone levels, and reuniting the infant with the mother ends the vocalizations and decreases the stress hormone levels back to baseline level [39, 40]. Administration of μ-opioid agonist morphine markedly reduces separation-induced distress, while an opioid antagonist naloxone increases it. Furthermore, the morphine-induced alleviation of separation distress can be blocked with naloxone. These findings–observed in many species from rodents [41] to primates [39]–suggest that separation distress is modulated by the opioid system, and that separation distress possibly reflects a state of endorphin withdrawal. These data thus suggest that relief from the aversive sensations associated with social separation results from activation of the same ancient opioidergic circuits that were developed during evolution for pain alleviation.

Further support for the opioidergic regulation of social behavior comes from rat studies investigating how social isolation influences subsequent affiliative behavior. On behavioral level, socially isolated rats are socially active when returned to their familiar environment, while socially housed individuals have reduced motivation to play [42]. Autoradiographic studies using [³H]diprenorphine suggest that social interaction enhances opioidergic activity in the rat brain [43, 44]. Opioid antagonism has also been shown to increase grooming–an important form of behavior facilitating social bonding [45, 46]–and grooming invitations in adult monkeys [23, 47]. Pharmacologically reducing opioid-mediated signal transmission also makes young monkeys more actively seek maternal contact, with infants also showing increased attempts to suckle [23]. These observations suggest that the opioidergic mechanism regulating social behavior is not limited to rodents but may also be conserved in primates. Indeed, it has been shown that grooming elevates monkeys' concentration of β-endorphin in cerebrospinal fluid [48], further supporting the hypothesis that affiliative behavior is regulated by opioidergic mechanisms also in primates.

These data suggest a negative feedback loop between affiliative behavior and opioidergic activity (Figure 2): Similarly as decreased metabolic rate makes humans hungry [49], reduced opioid tone may make individuals more 'hungry' for social contact [42]. These sensations motivate a behavioral response, namely, eating to restore the metabolic rate and affiliation to satisfy the social needs. The behavioral response, in turn, triggers a biological response: Eating elevates metabolic rate, while affiliative behavior triggers opioid release in the brain [43, 44], terminating the negative feedback loops. These biological responses are associated with perceptions of satiety and calmness, signaling to the individual that the metabolic rate and affiliative behavior are again within biologically safe limits. Thus, endogenous opioid tonus can be seen as a biological tracker of an individual's social activity.

	Affiliative behavior	Energy demand
Driven by	Opioidergic activity	Metabolic rate
Sensation	Social 'hunger'	Hunger
Behavioral response	Affiliation	Eating
Biological response	Opioid release	Elevated metabolic rate
Perception	Calmness	Satiety

Figure 2. Negative feedback loop between affiliative behavior and opioidergic activity.

1.3.2 Human data link the opioid system with negative and positive social feelings

As summarized above, extensive literature suggests that the endogenous opioid system regulates social behavior in many animals, including rats, guinea pigs, chicks, dogs, and monkeys. In contrast, direct evidence for opioidergic regulation of affiliation in humans is parsimonious. Even if animal models can provide approximations that are useful also for understanding human behavior, humans have significantly larger social networks compared to other primates and our social behavior is markedly different from that of rats or chimpanzees. Furthermore, the social relationships of many other species are mostly related to reproduction or nurturing of the offspring, but humans also have friendships with varying levels. Friendships typically do not involve sexual interaction. Yet, they may last decades despite long physical distances, suggesting that humans have evolved brain mechanisms regulating social behavior that are unique for our species. More detailed understanding of the biological mechanism regulating human affiliative behavior thus requires direct investigation of humans.

Social and vicarious pain are under opioidergic control

Most compelling evidence for opioidergic contribution to human social behavior have been collected using 1) a social-pain-model focusing on the aversive feelings arising from social rejection or loss and 2) vicarious pain, i.e. the negative sensations associated with knowing or seeing that somebody else is suffering from pain. Functional neuroimaging studies have repeatedly shown that anterior cingulate cortex and insular cortex, brain regions that are activated in response to noxious stimuli, also exhibit increased brain activity during social and vicarious pain [11, 50]. The neuroanatomical overlap between brain regions displaying enhanced activity during pain and social pain has prompted the hypothesis that social pain shares some of the brain mechanisms with sensory pain, providing a neurobiological explanation for the similarities between the affective experiences of physical, social, and vicarious pain. Given the fundamental role of endogenous opioids in modulation of sensory pain, this idea of shared brain mechanisms [51] would predict that opioids also regulate social and vicarious pain.

There is indeed evidence linking the opioid system to social pain: Carriers of the A118G SNP of OPRM1 need larger doses of opiates to achieve similar levels of analgesia compared to A-homozygotes [52], suggesting that the SNP influences how well humans cope with physical pain. Interestingly, the G-carriers are also more sensitive to social rejection: Compared to A-homozygotes, they report elevated disposition to social rejection, and consistent with their reports, social rejection in a virtual ball-tossing task elicits greater brain activity in dorsal anterior cingulate cortex and anterior insula that are also commonly activated during vicarious pain [13, 50]. This study shows that not only does the chemical makeup of the MOR influence sensitivity to physical pain, it also explains inter-individual differences in how people cope with social rejections. Using positron emission tomography, a more recent study showed that opioidergic circuits in thalamus, amygdala, ventral striatum and periaqueductal grey are activated in response to social rejection, and that the magnitude of the opioid response in amygdala, periaqueductal grey and anterior cingulate cortex positively correlates with questionnaire-derived index of trait resiliency [53]. These findings suggest that opioid peptides are released not only in response to noxious stimuli [54] but also to "socially noxious" stimuli such as interpersonal rejection, and that the opioidergic activity attenuates distress arising from negative social events.

The opioid system has also been implicated in vicarious pain. Placebo analgesia, known to be partially mediated by the opioid system [55], dampens brain activity elicited by noxious stimuli as well as knowing that another person is in pain. Furthermore, blocking the placebo analgesia using an opioid antagonist also reduces the negative affect associated with vicarious pain. [56] These results suggest that activity in opioidergic circuits influences how humans respond to information about other's harm. Altogether, these evidence consistently suggest that the opioid system helps people cope with stressing social situations.

Human opioid system responds to social activities

In addition to the negative social events such as social rejection and the associated social pain, the opioid system has also been implicated in a wide range of social behavior unrelated to pain. To begin with, various social activities have been shown to elevate pain threshold in humans, suggesting that the social activities have triggered release of opioids [36]. For example, social laughter [57], music performance [58], viewing of emotionally engaging movies [59] and rowing with others [60] elevate pain threshold, suggesting that various forms of social affiliation induce enhanced opioidergic processing in humans. While the evidence from these studies is indirect, it has also been shown that the dance-induced elevation of pain threshold can be blocked

with naltrexone-pretreatment [61]. This finding suggests that opioidergic activity indeed contributes to the analgesic effect of affiliation.

Data from studies pharmacologically manipulating the opioid system are also consistent with the hypothesis that social interaction triggers release of opioids [62]. An early study compared the effects of viewing an affiliative and a neutral film clip on pain tolerance and feelings of warmth and affection under placebo and naltrexone treatments. Under the placebo-treatment, the affiliative film reliably evoked stronger feelings of affiliation than the neutral film. However, the naltrexone-treatment abolished the effect. Similarly, while the affiliative film clip made the participants more tolerant to heat-pain under the placebo-condition, heat-pain tolerance was not affected after the naltrexone-treatment. These results were observed in females with high but not low trait affiliation. [15] Subsequently, it has been shown that opioid antagonist naltrexone downgrades feelings of social connection triggered by reading affective letters written by friends and family members, as well as daily feelings of social connection [63]. Overall, these data indicate that the pleasant sensations evoked by affective social stimuli may arise from increased opioidergic activity.

Three related positron emission tomography (PET) studies have been conducted in humans. The first study showed that trait impulsivity correlates positively with availability of MORs in the brain's motivational circuits [64], indicating that between-individual variation in the function of the MOR system may explain why some people are more prone than others to risky behaviors, such as substance abuse. Indeed, MOR availability in ventral striatum has also been shown to correlate with alcohol-craving following detoxification [65], suggesting that high MOR availability in this region may reflect increased sensitivity to rewards. As social interaction is one form of natural reward, it is possible that individuals with high MOR availability in ventral striatum are also more sensitive to social rewards. More recently, social touching has been shown to elevate availability of MORs in thalamus, striatum, frontal, cingulate, and insular cortices [66], suggesting that cerebral MORs are upregulated widely in the brain after social touching, possibly to magnify the opioid response to forthcoming stimuli. Elevated MOR availability has also been linked to secure romantic attachment-style [67], indicating that interpersonal bonding may depend on opioidergic mechanisms. These data thus suggest that the opioid system responds to social activities also in humans, that the opioid-release evokes affiliative sensations, and that individual differences in the function of the opioid system may alter long-lasting behavioral habits.

1.4   Objectives

The aim of this Thesis was to reveal if and how the endogenous opioid system regulates human social behavior. Most of the Thesis focused on how individual differences in the function of the opioid system influence human behavior in social situations. The Thesis also investigated opioidergic involvement in social laughing. The first study investigated how baseline availability of cerebral MORs influences approach–avoidance behavior, as assessed by self-reports. The second study tested the hypothesis that social affiliation via laughing triggers release of endogenous opioids. The third and fourth studies investigated if MOR availability explains inter-individual differences in the brain's hemodynamic responses elicited by affective movie scenes.

2 Methods

2.1 Positron emission tomography

2.1.1 Data acquisition and image formation

PET is a medical imaging technique that uses unstable positron-emitting isotopes, such as carbon-11. In PET imaging, biologically interesting molecules are radiolabeled with such isotopes. These radioactive molecules are mixed with saline to produce a tracer that is injected to the blood stream of patients. The injected tracer is transported via blood flow all over the body, binding to the tissue either reversibly or irreversibly, continuously decaying. The emitted positrons resulting from the decay collide with the electrons that are abundant in the surrounding tissue (Figure 3a). When they collide, the particles are annihilated, releasing energy in form of gamma rays that the scintillators of PET scanners detect. The annihilation of a positron and an electron produces a pair of 511 keV photons that travel in opposite directions. Because the photons travel at the speed of light, they are detected almost simultaneously. Thus, PET scanners are designed to efficiently detect pairs of photons with 511 keV energy. Such detections can be used to infer the most likely origins of photons. Thus, PET images reflect spatial distribution of tracer accumulation (Figure 3b).
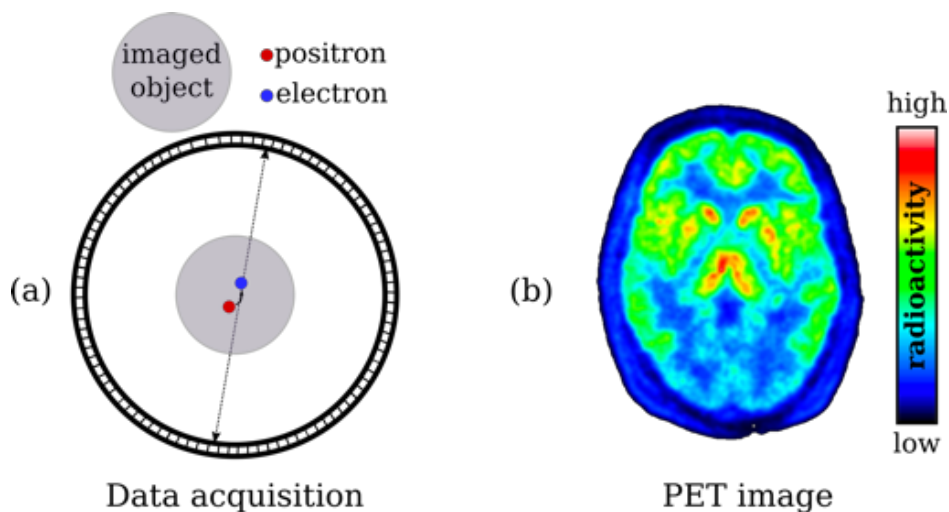
Figure 3. (a) The principle of PET measurements. The radioactive isotopes decay, producing positrons that collide with surrounding electrons. This collision results in annihilation of both particles. In the annihilation, the energy of the colliding positron and electron are converted into two 511 keV photons, traveling in opposite directions. (b) A representative axial slice from a single native space [^{11}C]carfentanil PET image. [^{11}C]carfentanil binds to μ-opioid receptors, and the PET image roughly reflects their spatial distribution.

PET scans can be either static or dynamic. In static PET images, all the counts during data acquisition are used to reconstruct one image. This has the advantage of having a large number of counts, providing high signal-to-noise ratio and enabling accurate estimation of the spatial distribution of tracer accumulation. The drawback of static images is that they only produce one image and do not reveal the time-course of tracer accumulation, which prohibits pharmacokinetic analyses that can yield accurate quantitative information about biologically relevant variables, such as receptor density. In dynamic PET images, the data acquisition period is divided into non-overlapping time frames, and image is reconstructed for each of these frames

separately. With sufficient number of counts per frame, the reconstruction process can be done reliably. Dynamic images also provide information about the kinetics of the tracer. Pharmacokinetic models or their derivatives can then be used to estimate how the tracer travels between plasma and tissue.

While the raw PET images are useful because they reflect where the tracer accumulates and to what extent, the tracer may not be bound to the molecule it was designed to bind to. For example, the tracer can be free or bound to plasma proteins. Measuring radioactivity of such molecules does not tell anything about *specific binding*. There are numerous methods for estimating specific binding, and how it is estimated in practice depends on the tracer. The following section will explain how specific binding is often estimated in neuroreceptor studies.

2.1.2   Imaging neuroreceptors

Binding of tracer to a receptor is often quantified using *binding potential (BP)* that is defined as ratio of specifically-bound-tracer concentration to some reference concentration in equilibrium [68]. The reference concentration can always be calculated based on arterial blood samples obtained during the PET scan. However, often the receptor of interest is not expressed everywhere in the brain. In these cases, the regions devoid of the receptor can be used as reference regions, allowing calculation of nondisplaceable binding potential that is defined as

$$(1) \quad BP_{ND} = \frac{f_{ND}B_{avail}}{K_D},$$

where f_{ND} is free fraction of the radioligand in the nondisplaceable compartment, B_{avail} is density of receptors available to bind radioligand, and K_D is the dissociation constant between the receptor and the tracer. Equation (1) states that binding potential is directly related to receptor availability. Receptor availability, on the other hand, depends on density of the receptors and their occupancy by endogenous ligands. The concentration of free radioligand and its affinity ($1/K_D$) to the receptor also influence binding potential. Nondisplaceable binding potential thus quantifies the radioligand concentrations in receptor-rich and receptor-free regions. From here onwards, the term binding potential refers specifically to nondisplaceable binding potential. Binding potential is often estimated using the simplified reference-tissue model [69] that describes the kinetics of the tracer in terms of compartments:

$$(2) \quad C_t(t) = R_1 C_r(t) + \left(k_2 - \frac{R_1 k_2}{1+BP_{ND}}\right)\int_0^t C_r(\tau)e^{\frac{-k_2\tau}{1+BP_{ND}}} \, d\tau,$$

where $C_t(t)$ is radioactivity concentration of the target tissue at time t, $C_r(t)$ is radioactivity concentration of the reference tissue at time t, and R_1, k_2, and BP_{ND} are the estimated parameters. R_1 reflects how quickly the tracer is delivered to the tissue, and k_2 reflects how quickly the tracer washes out from the tissue. The parameters can be estimated using nonlinear optimization. For calculation of parametric images, a linearization is typically used as the signal-to-noise ratio of single-voxel time-activity curves is very low [70].

2.1.3   Imaging neurotransmitter release

PET can also be used to image neurotransmitter release *in vivo*. This is typically done by imaging the same person twice using the same neuroreceptor tracer: One of the scans serves as a baseline scan, which provides an estimate of the individual's nominal receptor availability. In contrast, the second scan provides information about receptor availability during or after an activation. Equation (1) states that the difference in binding potential estimates derived from these two scans may be due to changes in receptor density, affinity, or concentration of endogenous ligands. Based on the two binding potential estimates alone it is impossible to tell how much each of these factors contributes to the observed difference. A commonly used occupancy model [71], however, assumes that the affinity and the receptor density are constant over time. This assumption implies that any differences between the two estimates originate from varying synaptic concentration of endogenous ligands competing for receptor binding (Figure 4).
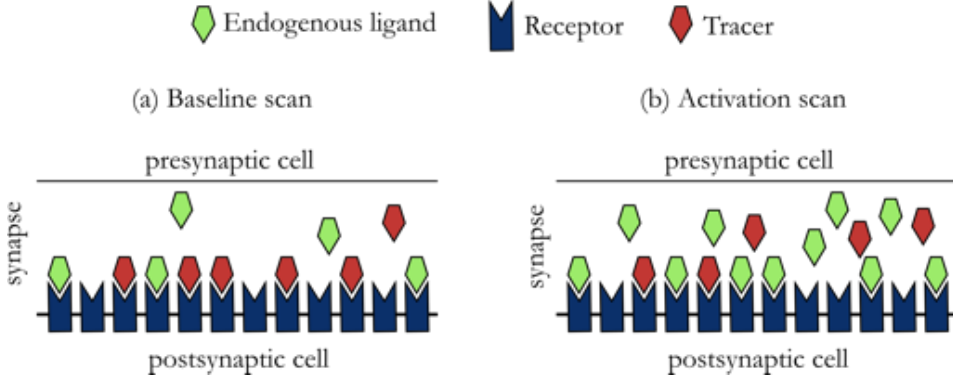
Figure 4. Occupancy model. (a) During baseline scan, there are many receptors available for the tracer to bind to because there is little competition from endogenous ligands. (b) During activation scan, there are more endogenous ligands bound to the receptors, leaving less receptors available for the tracer to bind to.

Challenge PET studies investigate within-subject differences in binding potential estimates to detect neurotransmitter release. In these studies, there is an activation task before or during one of the scans, and the aim is to test if the activation triggers release of neurotransmitters binding to the receptor of interest. It has been estimated that for a dopamine type 2 receptor ligand [123I]IBZM, a decrease of 1 % in binding potential reflects approximately 40 % increase in synaptic dopamine concentration [71]. While the temporal resolution of challenge PET studies is poor compared to many other functional neuroimaging methods, such as functional magnetic resonance imaging (discussed below), PET is the state-of-the-art method for imaging targeted biological systems *in vivo*.

2.2 Magnetic resonance imaging

2.2.1 Data acquisition and image formation

Magnetic resonance imaging (MRI) devices are used to create tomographic images of living human tissue, such as the brain. As the following sections reveal, the MRI devices use strong static magnetic fields to reveal the otherwise hidden magnetic properties of the tissue. The devices apply radiofrequency (RF) pulses and gradient fields to manipulate the magnetic elements and, they have coils for measuring the MR-signal. This much is common for all MRI. However, exactly what kind of information the MR-signal transmits depends on the used contrast. In brain imaging, most of the contrasts aim to differentiate between different brain tissue types. For example, T1-weighted imaging is good at differentiating between grey and white matter. In addition to such anatomical contrasts, a number of contrasts have been developed to facilitate investigation of brain function.

2.2.2 MR-signal

Understanding the origin of MR-signal requires introduction of *spin*, that–like charge–is a fundamental property of an elementary particle. Spins and their relation to the MR-signal are discussed in detail in [72]. For the purposes of this Thesis, it is enough to know that some of the particles with spin, such as proton, possess a magnetic moment. Magnetic fields distort the behavior of such particles. Static magnetic field has two effects on particles with spin: First, the magnetic moments become more likely to be aligned with the direction of the static magnetic field. The stronger the magnetic field is, the bigger this effect is. Second, the magnetic moments start precessing about the magnetic field. The precession frequency is called Larmor frequency ω, and its magnitude is defined by

$$(3) \quad \omega = \gamma B_0,$$

where γ is a constant called gyromagnetic ratio, and B_0 is the magnitude of the static magnetic field. In essence, Equation (3) shows that Larmor frequency depends linearly on the magnitude of the static magnetic field.

Exposing a precessing spin to a dynamic magnetic field oscillating with the Larmor frequency–these are the RF pulses–causes the precession axis to rotate. When the dynamic magnetic field disappears, the precession axis starts realigning itself with the static magnetic field, emitting electromagnetic waves on its way to the energetically more stable state. This is the MR signal that can be measured. In addition to its intensity, MR signal carries with it the frequency and phase of precession. Thus, MRI devices combine static and dynamic magnetic fields to produce images of proton-containing matter, such as living human brain.

2.2.3   Spatial encoding of the MR-signal

MR images may have very precise anatomical resolution: The brain is divided into tiny cubes called voxels, each of which can then emit its own MR-signal whose properties are dependent on the voxel's anatomical location. The division is done using magnetic gradients in three orthogonal directions. The gradient fields are magnetic fields that are location-dependent. MR images are typically acquired one slice at a time. The first gradient is applied along the main static magnetic fields which is typically denoted as z-direction. According to Equation (3), this makes the Larmor frequencies of the spins dependent on the z-axis. Thus, each slice is defined by a Larmor frequency band. Applying a dynamic magnetic field with frequency components within the Larmor frequency band will excite the spins within a particular slice. Once a slice has been selected, all the spins within it are precessing at the same frequency. Thus, without further spatial encoding, it would be impossible to calculate how much each voxel of the slice contributed to the observed MR signal. The within-slice spatial encoding is done by applying two more gradients in a controlled sequence. The first of the gradients encodes the frequency of the MR-signal, while the second gradient encodes its phase This procedure generates a unique combination of frequency and phase for each cube within a slice, making image reconstruction possible. [72]

2.3   Functional magnetic resonance imaging

2.3.1   Blood-oxygen-level dependent contrast

By far the most commonly used form of functional magnetic resonance imaging (fMRI) uses the blood-oxygen-level dependent (BOLD) contrast [73]. BOLD–fMRI takes advantage of the fact that magnetic properties of hemoglobin depend on how much oxygen is bound to it [74]. The cardiovascular system supplies neuronally active brain regions more blood. This increases the amount of regionally oxygenated hemoglobin, changing the magnetic properties of the surrounding tissue. In brief, this is how neuronal activity propagates to the hemodynamic BOLD-signal. The BOLD-signal thus only indirectly reflects neuronal activity. It also carries components without neural origin, such as cardiac pulsation and respiration, further decreasing its signal-to-noise ratio [75].

2.3.2   Hemodynamic response function

Brief stimuli, such as sudden flashes of light, trigger a hemodynamic response measurable with BOLD–fMRI that typically has a particular shape across humans [76]. The canonical *hemodynamic response function* (HRF) is visualized in Figure 5a. In 2–3 seconds after the stimuli, the hemodynamic signal measured with BOLD–fMRI starts sharply increasing. The signal peaks at around 5 seconds, after which it declines quickly back to the baseline level or even below it. The cascade is over in approximately 25 seconds.
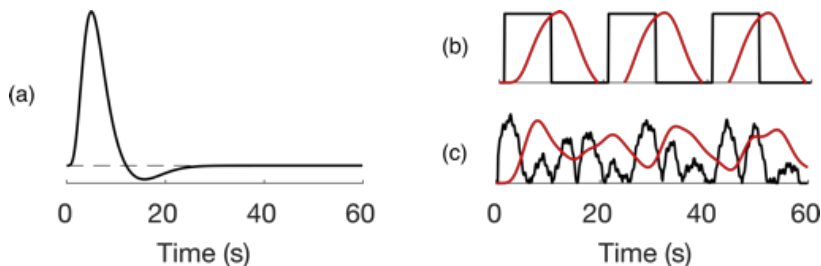

Figure 5. (a) Canonical hemodynamic response function. (b) Boxcar design. (c) Continuous stimulus magnitude. The original stimuli are shown in black, while the red curves depict the regressors obtained after convolving the stimuli models with the HRF.

2.3.3 Estimating hemodynamic responses to stimuli

The BOLD–fMRI signal is often modeled as output of a linear system whose impulse response function the HRF is [77]. In simple block and event-related designs, the input to the system is considered to be a constant stimulus that is either on or off. Given a controlled input and the impulse response function, linear systems theory predicts the measured output. The predicted output is used as a regressor in general linear model (GLM), making it possible to statistically infer how much each stimulus contributed to the signal. This approach is well-suited for studying sensory processes, such as vision, audition and pain, where it is natural to consider a stimulus to be either on or off (Figure 5b). Yet, more naturalistic stimuli, such as movies, are poorly modeled using the on-off structure, because they are associated with time-varying intensity, often in multiple dimensions [78]. For example, some movie scenes may evoke much stronger feelings than other scenes in most individuals. Modeling such variation makes it possible to investigate the neuronal regions whose hemodynamic activity increases or decreases as a function of stimulus *intensity*. Compared to block-designs, this approach not only localizes brain regions involved in processing of a stimulus, but also informs how the stimulus intensity is mapped to hemodynamic activity. Given the parametric representation of a stimulus, linear systems theory can again be used to estimate the predicted outcome that can be fed into GLM analysis (Figure 5c). The fMRI data presented in the Thesis were analyzed using such approach.

2.4 Fusion imaging with PET and fMRI

fMRI is good at localizing the brain regions involved in sensory processing. However, knowing the brain regions that are activated in response to a task or a stimulus is only a superficial description of the underlying neural computations. Deeper understanding of the neural computations requires knowledge about the neurotransmitters involved in them. fMRI alone does not provide such information, because the hemodynamic signal reflects the sum of all contributing neurons, irrespective of the neurotransmitters involved. Neuroreceptor estimates derived from PET studies can be used for modeling the hemodynamic responses obtained from fMRI experiments, making it possible to investigate how inter-individual differences in functioning of a single neurotransmitter system propagate into differences in sensory processing. Such information elaborates the neuromolecular mechanisms of the sensory processing and can identify targets for pharmacological interventions. In this Thesis, such combination of PET neuroreceptor imaging and fMRI is referred to as *fusion imaging*. Fusion imaging was used in the Studies III and IV of the Thesis.

Figure 6 illustrates the approach to fusion imaging implemented in this Thesis. During fMRI, participants were shown movie clips with varying emotional content. The hemodynamic signal obtained from fMRI was modeled with a multidimensional stimulus model using GLM. The dimensions included arousal and valence associated with the scenes, as well as the brightness of the frames and the loudness of the audio track. The same participants also underwent a PET scan. The PET data were used to derive regional binding potential estimates. The fMRI and PET data were then combined in the fusion analysis, where the binding potential estimates (PET) were correlated with the regression coefficients (fMRI). Results from the fusion analysis inform how individual differences in binding potential translate into individual differences in hemodynamic activity.
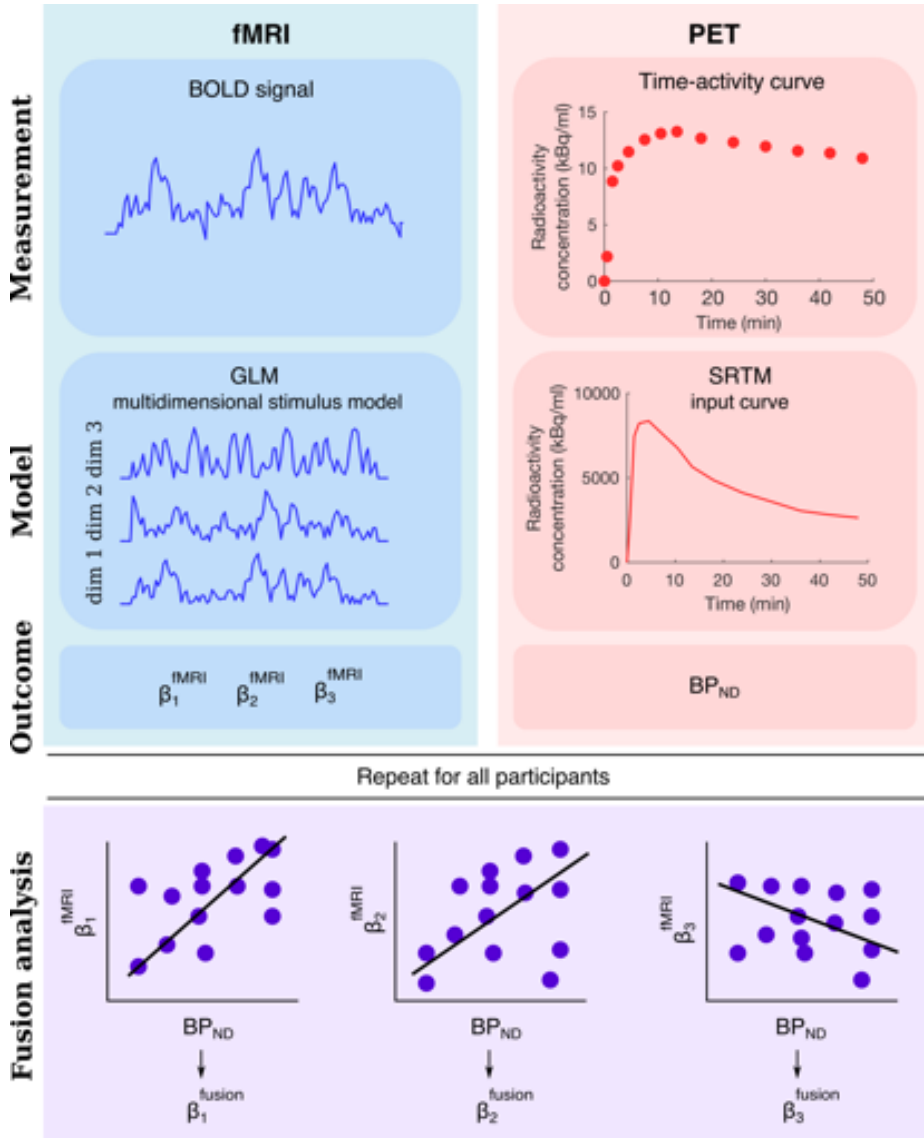
Figure 6. Fusion imaging with PET and fMRI. BOLD-signal was modeled with general linear model (GLM) to produce estimates of regression coefficients. Time-activity curves measured with PET were modeled using simplified reference-tissue model (SRTM) to produce binding potential estimates. These two outcome measures, the regression coefficients and the binding potentials, were finally correlated against each other in the fusion analysis step.

2.5 Materials and Methods used in Studies I–IV

The Materials and Methods used in Studies I–IV are summarized in Table 1. All studies included a PET scan with [11C]carfentanil, a selective μ-opioid receptor (MOR) agonist [79, 80]. Studies III and IV also included a PET scan with [11C]raclopride that binds preferably to dopamine type-2 receptors. All study protocols were approved by the ethics board of the hospital district of Southwest Finland, and were conducted in accordance with the Declaration of Helsinki.

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Table 1. Participants of the studies included in the Thesis. PET–MRI = Philips Ingenuity PET-MR; D690 = GE Healthcare Discovery TM 690 PET–CT.

	Study I	Study II	Studies III & IV
N (females)	49 (20)	12 (0)	35 (35)
age in years (mean ± sd, range)	32 ± 6.4, 19–58	23 ± 3.3, 20–32	44 ± 10, 19–58
scanner(s)	PET-MRI & D690	PET–MRI	D690
PET 1: Tracer	[11C]carfentanil	[11C]carfentanil	[11C]carfentanil
PET 1: Description	rest	rest	rest
PET 2: Tracer	-	[11C]carfentanil	[11C]raclopride
PET 2: Description	-	social laughter	rest
fMRI task	-	-	movie watching

In Study I, 49 healthy adults were scanned at rest using [11C]carfentanil PET to quantify baseline MOR availability. They also filled the BIS–BAS questionnaire [81]. The questionnaire provides an index for an individual's avoidance (BIS) and approach (BAS) behavior motivations. The BAS scale is split into three subscales: Drive, Fun Seeking, and Reward Responsiveness. The BIS and BAS scores, along with a scanner covariate, were used to model binding potential using linear regression.

In Study II, twelve healthy adult males were scanned in two conditions using [11C]carfentanil PET. During the *social laughter condition,* the subjects watched a compilation of short comedy clips together with their friends. These sessions were recorded, and these recordings were used to obtain rates of social laughter for each participant. During the *baseline* condition, the participants spent half an hour alone. MOR activation was assessed for each participant by contrasting the laughter and baseline binding potential estimates. In addition, associations between baseline MOR availability and the laughter-rates were explored.

Studies III and IV used fusion imaging. In both studies, 35 female participants watched a series of Hollywood movie scenes during fMRI [82]. In a separate behavioural experiment, 17 participants watched the scenes and simultaneously rated them in three dimensions: painfulness, arousal, and valence. These ratings were averaged across the participants to provide a multidimensional model of the scenes for GLM analysis. The BOLD-signals were modeled with the painfulness regressor in Study III, while the ratings for arousal and valence were used in Study IV. In both studies, also the brightness of the scenes as well as the intensity of the audio track were used as nuisance covariates. After standard fMRI preprocessing, individual hemodynamic responses related to pain, arousal, and valence were estimated using GLM. The subjects who participated in the fMRI experiment were also scanned with [11C]carfentanil and [11C]raclopride PET in rest. Regional receptor availabilities were estimated using SRTM. In linear regression analysis, the receptor availabilities were used to model the hemodynamic responses related to pain, arousal, and valence.

2.5.1 Processing of PET data

The PET data were preprocessed using SPM12 and then modeled using SRTM to obtain binding potential estimates. During preprocessing, the movements between frames were mitigated by realigning the images using a rigid transformation. The PET images were then coregistered to the participant's MRI. The PET data were modeled using SRTM, providing binding potential maps. The binding potential maps were normalized to a standard anatomical space (MNI 152). Finally, the maps were smoothed using an isotropic 8-mm Gaussian kernel.

2.5.2 Processing of fMRI data

The fMRI data were preprocessed with FSL. During preprocessing, the data were slice-time-corrected, rigidly motion-corrected and coregistered to a standard anatomical 2-mm template (MNI 152), after which they were spatially smoothed using an isotropic 8-mm Gaussian kernel. In addition, low-frequency components in the BOLD-signals were filtered using a 240-s-long Savitzky–Golay filter [83]. Finally, the motion parameters obtained from the motion-correction step were regressed out [84] to control for head-motion related confounds in the BOLD-signals. GLM analyses were performed in SPM12.

3 Summaries of the original studies

3.1 Study I: Behavioural activation system sensitivity is associated with cerebral μ-opioid receptor availability

3.1.1 Aim of the study

According to the reinforcement-sensitivity theory, two opposing motivational systems govern human behavior in survival-salient situations. The behavioral activation (or approach) system (BAS) is an appetitive-motivational system whose activity increases upon reward consumption or its expectation. BAS thus facilitates approach behavior. The behavioural inhibition system (BIS), in contrast, is an aversive-motivational system that is activated by information signaling possible punishment. BIS inhibits behavior that could have harmful consequences. The reinforcement-sensitivity theory proposes that relative activities in these approach and avoidance systems determines an individual's behavior in approach–avoidance situations [85]. Animal studies have shown that endogenous opioids regulate approach behavior and reward functions [86]. The opioid system also processes noxious signals [87]. The opioid system is thus in a good position to regulate behavior with potentially rewarding and painful consequences. The study aimed to investigate if individual differences in the MOR system associate with approach or avoidance motivation. This was done by collecting BIS–BAS questionnaire scores from 49 individuals and correlating regional MOR availabilities with them in linear regression analyses.

3.1.2 Results

In the full-volume analysis, the BAS scores were positively correlated with availability of MORs in insula, amygdala, thalamus, brainstem, and temporal cortex (Figure 7). Consistent with these findings, the ROI-level analyses revealed that the strongest effects were observed in anterior cingulate cortex, amygdala, insula, and orbitofrontal cortex (Figure 8). The BIS scores were not associated with availability of MORs in any brain region.
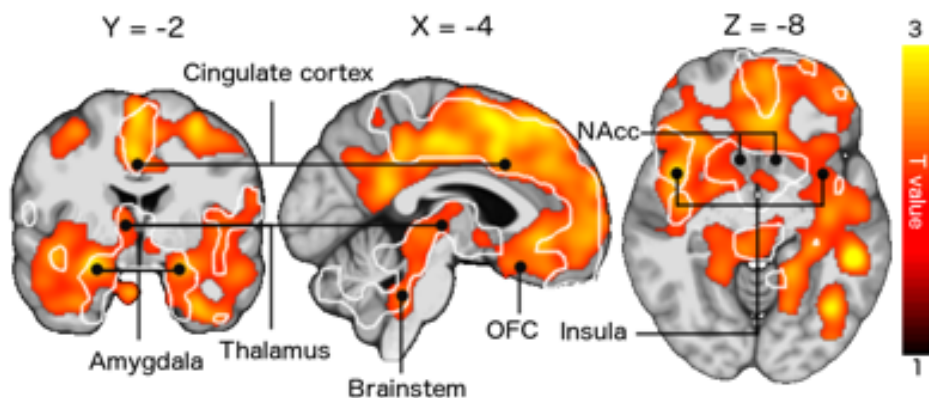

Figure 7. Brain regions where [11C]carfentanil BP_{ND} correlated with BAS total scores. Clusters were defined with p < 0.05, and only the clusters surviving FDR-correction (p < 0.05) are shown. The white outlines display clusters where the BAS subscale Fun Seeking was correlated with MOR availability. OFC = orbitofrontal cortex; NAcc = nucleus accumbens.
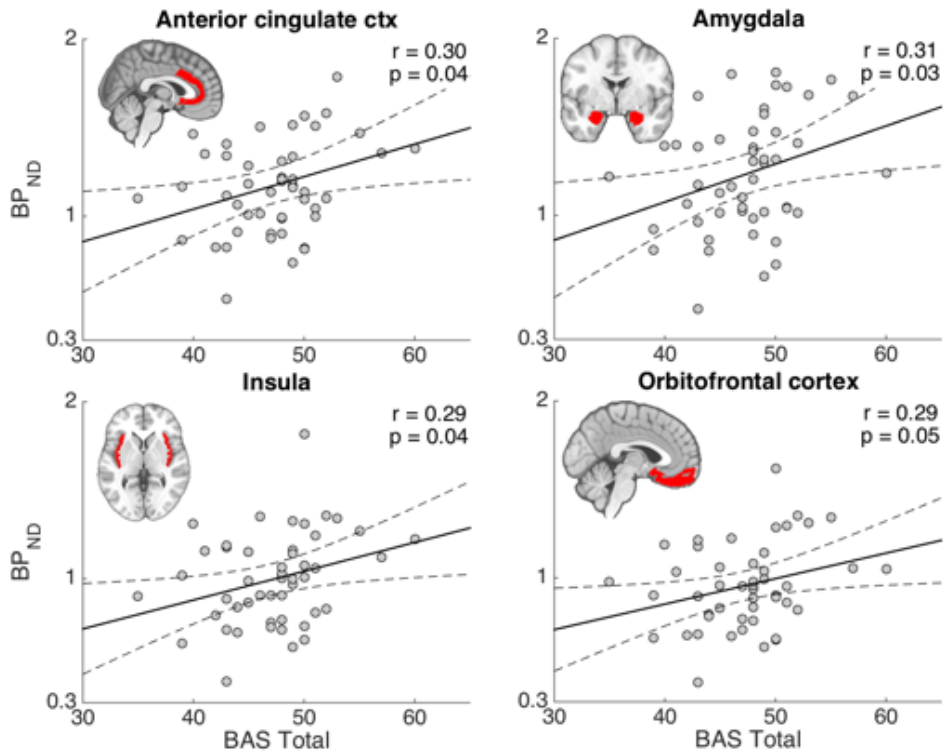
Figure 8. ROI-level data displaying the correlation between BAS and availability of MORs in anterior cingulate cortex, amygdala, insula and orbitofrontal cortex. Least-square regression lines, along with their 95 % confidence intervals, are shown on top of the raw data points.

3.1.3 Conclusions

The results suggest that endogenous opioids mediate approach behavior in humans. The effect was observed in brain regions processing rewarding and noxious signals, including amygdala, insula, anterior cingulate cortex and orbitofrontal cortex. BIS, in contrast, was not associated with availability of MORs anywhere in the brain. Different neurotransmitter systems thus appear to govern BIS and BAS. The results are consistent with animal data showing that reduced opioidergic activity in the brain is associated with increased motivation for social interaction. Thus, inter-individual variation in baseline activity of the MOR system may explain why there are stable inter-individual differences in how humans behave in approach–avoidance situations.

3.2 Study II: Social laughter triggers endogenous opioid release in humans

3.2.1 Aim of the study

Primates spend a significant proportion of their life grooming in-group members, presumably to facilitate interpersonal bonding via opioidergic mechanisms. However, the social network of humans may consist of up to 150 individuals [88], making the maintenance of inter-personal human relationships via grooming impossible. Accordingly, it has been proposed that humans have evolved other types of behavioral mechanisms that serve the same function as grooming does in other primates. Laughing, in particular, has been suggested to serve that function. Laughing is uplifting and relaxing. It also elevates pain-thresholds [57] and facilitates inter-personal bonding. Compared to grooming that is limited to pairs of individuals, laughing is much more efficient in facilitating bonding because it can simultaneously bind together even a large group of individuals. Because the effects of laughing have similarities to effects resulting from use of exogenous opioids [89], namely pleasantness and calmness, it has been proposed that laughing triggers release of endogenous opioids. This study aimed to test this hypothesis. 12 males were PET scanned using

[11C]carfentanil before and after a social laughing session to quantify changes in MOR availability, indicative of opioid release.

3.2.2 Results

The social laughter condition reliably evoked bursts of laughter and was associated with elevated feelings of amusement and calmness in the participants. Compared to the baseline condition, social laughter decreased MOR availability in several brain regions, including thalamus and caudate (Figure 9). This decrease presumably reflects laughter-induced release of endogenous opioids in these regions. In contrast, the social laughter condition was associated with increased MOR availability in cortical midline regions, possibly as a consequence of receptor externalization or conformational changes. Social laughter rate in the video viewing condition also correlated positively with baseline MOR availability (Figure 10).
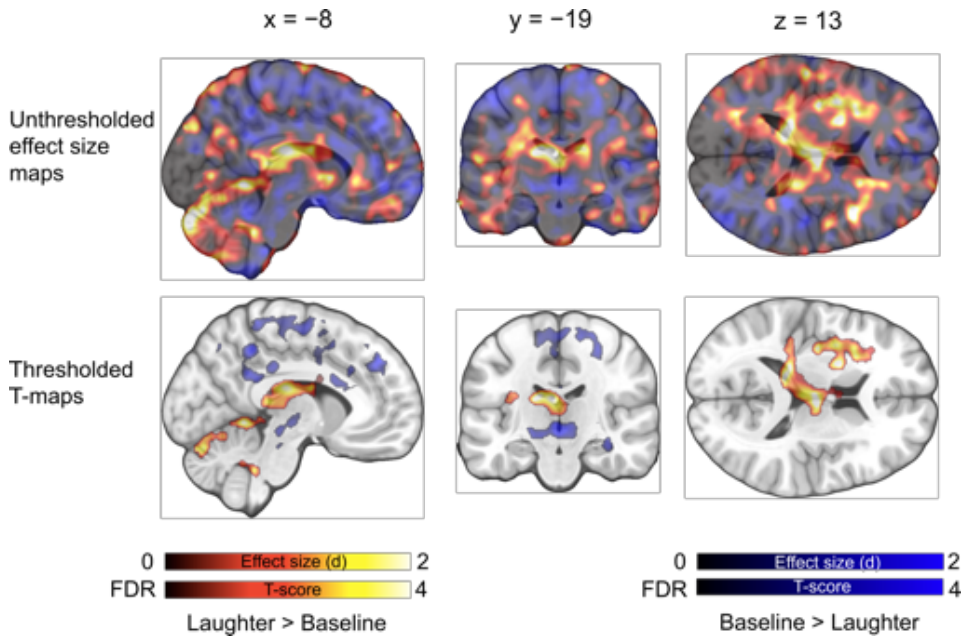
Figure 9. Brain regions showing enhanced (red-yellow) and decreased (blue) release of endogenous opioids during the social laughter versus baseline conditions. The top row shows unthresholded maps of effect size; the bottom row shows maps of t-statistic thresholded at p < 0.05, FDR-corrected at the cluster level.
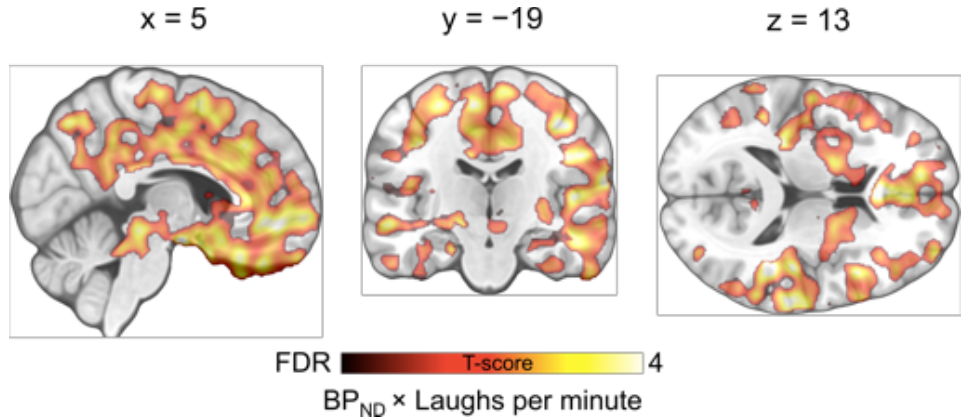
Figure 10. Brain regions in which baseline MOR availability correlated with rate of social laughter (laughs per minute). Only statistically significant (p < 0.05, FDR-corrected) clusters are shown.

3.2.3   Conclusions

In agreement with the opioidergic theory of social attachment, social laughter with friends triggered release of endogenous opioids. As a complement to social touching, laughing may have evolved as a behavioral bonding mechanism for large groups of individuals. Because baseline MOR availability was correlated with laughing rate, it is possible that cerebral MOR availability reflects how receptive individuals are to the group-bonding benefits of laughing.

3.3   Study III: Dissociable roles of cerebral μ-opioid and type 2 dopamine receptors in vicarious pain: A combined PET–fMRI study

3.3.1   Aim of the study

Empathy allows understanding the perspective of others. In line with the perception–action model of empathy [51], dorsal anterior cingulate cortex and insular cortex are activated during pain and vicarious pain [50]. These findings have been interpreted to support existence of shared neuronal circuits processing first-hand and vicarious pain. Consistent with this hypothesis, pharmacologically activating the opioid system not only alleviates pain but also the distress associated with vicarious pain [56], suggesting the shared circuits rely on opioidergic processing. However, direct support for opioidergic contribution to vicarious pain and empathy is still missing. The study aimed to investigate if the endogenous opioid and dopamine systems that are implicated in first-hand pain also contribute to vicarious pain. 35 females first watched violent movie scenes during fMRI. They were also scanned twice with PET using [11C]carfentanil and [11C]raclopride to quantify baseline availability of MOR and dopamine type 2 receptors (D$_2$Rs), respectively. The regional receptor availabilities obtained from the PET studies were then used to predict individual hemodynamic responses to the violent scenes.

3.3.2   Results

In the fMRI experiment, viewing scenes with painful content activated brain regions that are responsive to noxious stimuli. These regions included anterior insulae, thalamus and secondary somatosensory cortices. Activity in posterior superior temporal sulci also correlated with the painfulness ratings. In the fusion PET–fMRI analysis, MOR availability correlated negatively with these responses across subjects in insulae, thalamus, the somatosensory cortices, primary motor cortex, and superior temporal sulci (Figure 11). In contrast, availability of MORs in several brain regions was positively correlated with the hemodynamic responses in orbitofrontal cortex (Figure 12). D$_2$R availability in striatum was not correlated with the brain responses.
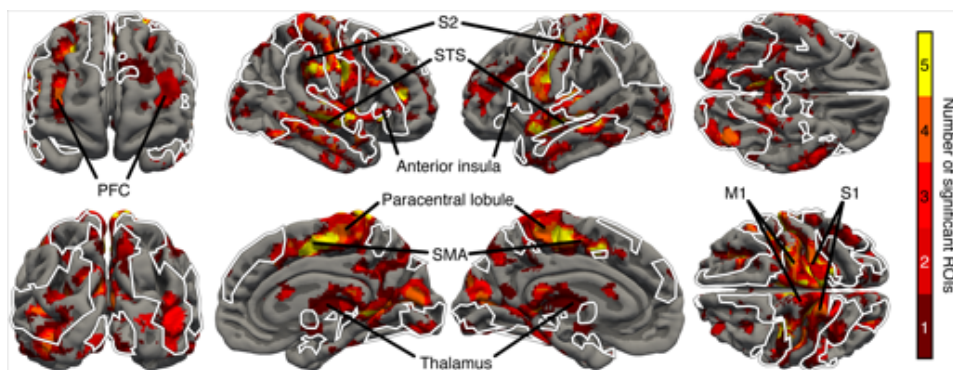
Figure 11. Maps displaying the brain regions whose hemodynamic activity was dependent on regional MOR availability. The red-to-yellow colors reflect the number of ROIs (out of 13) where MOR availability was correlated with BOLD responses to painful movie scenes. White outlines show regions where the hemodynamic signal also correlated with the painfulness ratings. PFC, prefrontal cortex; S1, primary somatosensory cortex; S2, secondary somatosensory cortex; M1, primary motor cortex; SMA, supplementary motor area; STS, superior temporal sulcus.
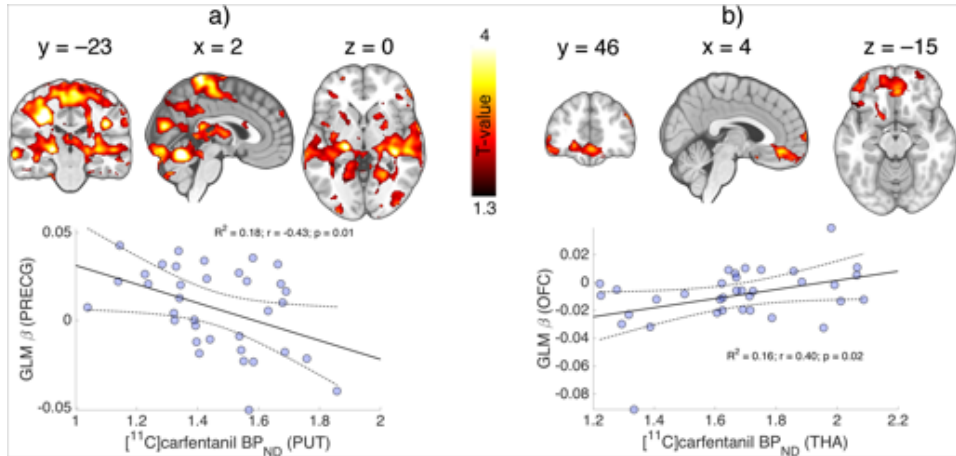
Figure 12. a) Brain regions where MOR availability in putamen correlated negatively with hemodynamic activity during painful movie scenes (p < 0.05, FWE-corrected at cluster level). b) Thalamic MOR availability correlated positively with hemodynamic activity in orbitofrontal cortex during painful movie scenes (p < 0.05, uncorrected, cluster size > 8 cm^3). The scatterplots show ROI-level data with regression lines and their 95 % confidence intervals. PRECG = precentral gyrus; OFC = orbitofrontal cortex.

3.3.3 Conclusions

The results support opioidergic involvement in vicarious pain, extending the similarities between the neurobiological mechanisms underlying sensory and vicarious pain to neurotransmitter level. Specifically, these results suggest that the endogenous opioids modulating nociceptive signals [25] also transmit signals related to vicarious pain. Elevated availability of MORs may protect individuals against negative emotions resulting from distressing social signals, possibly making individuals with high cerebral MOR availability more capable for helping individuals suffering from pain.

3.4 Study IV: Opioidergic regulation of emotional arousal: A combined PET–fMRI study

3.4.1 Aim of the study

Emotions can be expressed in terms of arousal and valence (pleasantness). High-arousal states are characterized by elevated skin conductance and dilated pupils [90, 91], whereas increased activity in the zygomatic muscle is a physiological indicator of high valence [91]. Functional neuroimaging studies have shown that states of high-arousal are typically associated with enhanced brain activity in the sensory cortices, amygdala, and thalamus. High-valence states, on the other hand, are associated with enhanced hemodynamic activity in orbitofrontal cortex. [92-96] Even if the neuroanatomical correlates of emotional arousal and valence have been extensively mapped, the neurotransmitter systems governing them still remain largely unexplored. The endogenous opioid and dopamine systems have been linked to reward and motivational processes. This study aimed to investigate if they would also contribute to emotional arousal or valence.

3.4.2 Results

The fMRI experiment revealed that the arousal and valence dimensions of emotions were associated with separable patterns of hemodynamic activity in brain regions previously linked to affective processing. Hemodynamic signals in amygdala, thalamus, superior temporal sulci, anterior insula, inferior frontal gyri, and precuneus best tracked the arousal ratings of the scenes. The valence ratings of the stimuli, on the other hand, were positively correlated with brain activity in posterior superior temporal sulci and sensorimotor regions. In the fusion PET–fMRI analysis, MOR availability in the brain correlated negatively with the arousal responses in amygdala, hippocampus, thalamus and hypothalamus (Figure 13). The associations were mostly linear (Figure 14). Positive correlations were not observed anywhere in the brain. Neither emotional arousal or valence were associated with striatal D$_2$R availability.
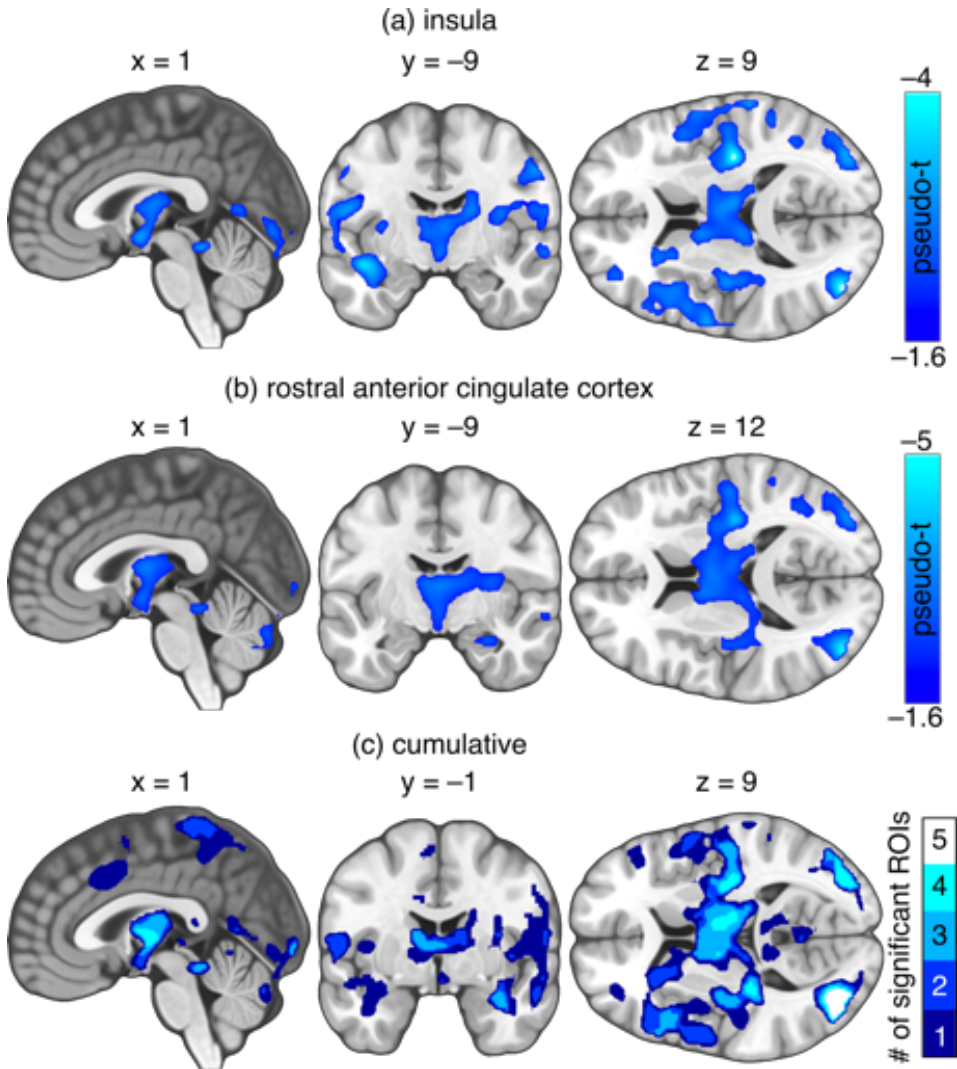
Figure 13. a) Brain regions where hemodynamic responses to arousing scenes were negatively correlated with availability of MORs in insula (FWE-corrected at cluster level, p < 0.05). b) Brain regions where hemodynamic responses to arousing scenes were negatively correlated with availability of MORs in rostral anterior cingulate cortex (FWE-corrected at cluster level, p < 0.05). c) Maps displaying the brain regions where hemodynamic responses to the arousing scenes were mostly influenced by cerebral MOR availability. The blue-to-white colors reflect the cumulative number of ROIs (out of 9) in which MOR availability was negatively correlated (p < 0.05, FDR-corrected at cluster level) with hemodynamic responses to the arousing scenes.
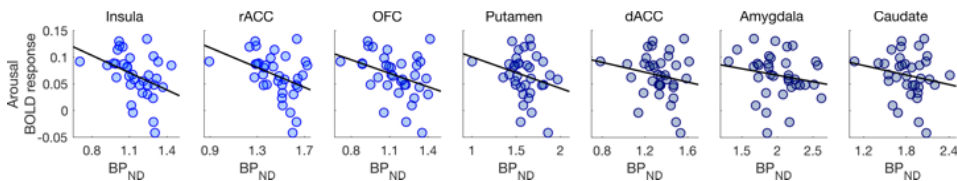
Figure 14. Visualization of the relationship between the brain responses to arousing scenes and regional MOR availability in seven representative ROIs.

3.4.3 Conclusions

Individuals with elevated MOR availability had reduced hemodynamic activity during arousing movies scenes, indicating that differences in the function of the opioid system may partly explain why humans react so differently to emotionally salient stimuli. These data support the involvement of endogenous opioids in regulation of emotional arousal in naturalistic conditions. In addition to modulating pain, pleasure, stress and anxiety, the MOR system may thus also regulate the arousal dimension of emotions irrespective of their valence.

4 Discussion

The primary findings of the Thesis are threefold. First, MOR availability correlates with human tendency to engage in approach behavior. In other words, humans who tend to choose approach over avoidance in potentially rewarding and punishing situations have, on average, elevated MOR availability in their brain. Second, laughing with friends triggers endogenous opioid release in the brain, possibly to facilitate bonding between the laughing individuals. Third, cerebral MOR availability correlates negatively with brain responses to viewing violent and more generally arousing scenes. These findings accord with the inhibitory role of opioidergic neurons, as well as with the opioid system's role in regulation of pain, anxiety, and stress. The results are also consistent with the hypothesis that opioidergic mechanisms influence motivation for affiliative behavior in humans.

4.1 Approach behavior and μ-opioid receptors

The main finding of the Study I is that cerebral MOR availability in healthy humans correlates positively with the BAS total score of the BIS–BAS questionnaire [81]. BAS measures an individual's general approach motivation that, in previous studies, has been associated with prosocial personality traits including extraversion and gregariousness, as well as impulsiveness and excitement seeking in general [97, 98]. This finding suggests that individuals with high MOR availability are more eager to seek rewards, also in the social domain. This finding fits well within the negative feedback loop between opioidergic activity and social behavior: Individual differences in MOR availability likely result mostly from differences in receptor density (see section 4.3.1, page 22); yet *in vitro* studies suggest that opioid receptor density itself is influenced by long-term opioid tonus, with low opioidergic activity leading to upregulation of opioid receptors [99-101]. Consequently, individuals with elevated MOR availability may have decreased baseline opioid tonus, thus motivating them towards approach behavior to increase the opioidergic activity.

The results of the Study II are also consistent with the negative feedback loop. The social laughter session was associated with decreased MOR availability in thalamus, caudate, and anterior insula, suggesting that laughing with friends triggers release of endogenous opioids in these regions. The social laughter session was also associated with elevated sensations of pleasantness and calmness–effects that are associated with pharmacological administration of MOR agonists–further supporting the interpretation of laughing-induced opioid release. Finally, the rate of social laughter correlated positively with baseline availability of MORs widely in the brain, suggesting that individuals with elevated MOR availability have increased disposition towards behavior facilitating social bonding, such as laughing. This correlation is also consistent with the results of the Study I. The effects observed in Studies I and II overlap most strongly in cortical midline regions, more specifically in orbitofrontal cortex and cingulate cortex that are key parts of the emotional circuits of the brain. Among many functions [102], the orbitofrontal cortex is well known for its involvement in reward [103]. The cingulate cortex is cytoarchitectonically heterogenous and has been implicated in a wide range of different functions, such as regulation of affect [104, 105]. Together, these findings suggest that individuals with high MOR availability in cortical midline regions, such as orbitofrontal cortex and cingulate cortex, are more attracted by rewarding stimuli, such as social interaction. The increase in MOR availability presumably reflects low baseline opioidergic activity that subsequently leads to upregulation of the receptors. Thus, individuals whose baseline opioid activity is low may have increased need for sociability to keep the opioid activity within limits that signal safety.

The results from Studies I and II are consistent with previously published data showing that availability of MORs in medial frontal cortex, amygdala, and striatum correlates positively with impulsiveness [64], a trait that reflects an individual's tendency to act on cravings instead of delaying gratification. The same study found that high impulsiveness was also associated with magnified opioidergic response to noxious stimulus in orbitofrontal cortex, suggesting that individuals with high opioid receptor availability in orbitofrontal cortex can better cope with pain, possibly making them better equipped to cope with the consequences of

their impulsive decisions. Another related study found that intensity of alcohol-craving following detoxification of alcohol addicts correlates positively with MOR availability in Broadmann area 10 of frontal cortex as well as in ventral striatum [65]. Both of these studies are consistent with the proposed hypothesis that cerebral MOR availability, particularly in medial frontal cortex, influences approach behavior tendency of an individual. However, results from these studies cannot be regarded as conclusive, as they rely on modest sample sizes (N ≤ 25). Furthermore, one study has found that MOR availability in frontal cortex correlates positively with trait harm avoidance [106]. As harm avoidance correlates negatively with impulsiveness, sociability and extraversion [107], this finding seems to be contradicting the previously mentioned evidence linking elevated opioid receptor availability in frontal cortex to such traits. Thus, the available data are not completely consistent about the effects of high MOR availability in frontal cortex on social behavior, and more research is needed to evaluate the reliability of the hypothesis that frontal MORs promote social behavior.

4.2   Emotional responsivity and μ-opioid receptors

Studies III and IV found that MOR availability correlates negatively with brain responses elicited by naturalistic audiovisual stimuli depicting humans in both painful and otherwise arousing circumstances. Previous human PET studies have shown that the opioid system is responsive to both positively and negatively valenced emotional stimuli [54, 108-110]. This Thesis extends these observations by demonstrating that mere baseline MOR availability correlates with affective brain responses that can be measured with fMRI. The brain responses to arousing audiovisual stimuli were mostly affected by MORs in insula and rostral anterior cingulate cortex (rACC). Insula has an important role in interoceptive processing [111], it reliably responds to noxious stimuli [112] and is also activated during vicarious pain [50, 113]. Insular activity during pain and vicarious pain presumably reflects the negative affect related to both states. The rACC has also been associated with the negative affect of pain [114], as well as its regulation via opioidergic mechanisms [115, 116]. Opioid release in subgenual ACC relieves negative affect, regardless of how the affect was induced [53, 110]. These findings suggest that MORs in insula and anterior cingulate cortex also process socially distressing signals and that high-MOR individuals may be less sensitive to them.

The findings also dovetail with neuroimaging studies showing that placebo analgesia–known to be opioid-dependent [117]–significantly attenuates affective brain responses during vicarious pain [56, 118]. Furthermore, availability of MORs in striatum correlates positively with pain threshold [119], suggesting that high density of striatal MORs protects individuals from excessive pain-induced stress. Altogether, the evidence suggest that individuals with high MOR availability respond more calmly to distressing stimuli, possibly because they have higher capacity for MOR-mediated attenuation of negative affect. Consequently, individuals with high MOR availability may be more resilient to environmental stress signals.

MOR-dependent attenuation of brain responses may also extend to positively valenced stimuli, although the evidence is not as strong as it is for distressing stimuli. First, the stimulus set used in the Study IV contained both negatively and positively valenced stimuli, suggesting that the arousal-attenuating effect of MORs is indeed independent of valence. Second, MOR availability in thalamus has been recently shown to correlate negatively with brain responses to pictures of appetizing foods [120], suggesting that thalamic MORs attenuate brain responses to positively valenced stimuli. The same study did not find association between MOR availability and brain responses to seeing pictures of cars, suggesting that MOR availability does not attenuate brain responses irrespective of the contents of the stimuli. Instead, these pieces of evidence suggest that only emotionally salient stimuli trigger MOR-dependent brain activity. However, the evidence for opioidergic regulation of positive emotions is not as strong as opioidergic regulation of negative emotions.

4.2.1   Emotional responsivity has implications for approach behavior

There is evidence from multiple studies showing that cerebral MORs have a protective role in humans. The MOR system is activated by noxious and "socially noxious" stimuli, and the magnitude of activation correlates with the perceived relief [53, 54]. MOR availability in the brain also correlates with pain threshold [119]. The Studies III and IV of this Thesis suggest that individuals with high MOR availability in rACC and insula have attenuated brain responses to vicariously painful and arousing stimuli. As MOR availability correlates widely within the brain, humans with high receptor availability in one region typically also have high receptor availability also elsewhere in the brain [121]. Overall, these results thus paint a picture of

individuals with high MOR availability as humans with high resiliency, possibly explaining why they also tend to have secure romantic attachment-styles [67].

The results from Study I suggest that individuals with high MOR availability are predisposed towards approach rather than avoidance. Presumably, how humans behave in a typical approach–avoidance situation is influenced by what could happen, given a choice of approach or avoidance. Approach can either succeed or fail; both of these have some probabilities. All else being equal, individuals who can better cope with the consequences of failed approach should be more likely to select approach over avoidance. Given the massive bias of humans towards loss aversion [122]–humans generally evaluate losses approximately twice as important as wins–how well an individual can cope with losses can have significant effects on their behavior. As individuals with high MOR availability seem to be very resilient to various stressors, it seems likely that they can better cope with the consequences of failed approach, thus predisposing them towards approach behavior, thus explaining the association observed in the Study I.

4.3   Limitations

4.3.1   Interpretation of between-individual variation in [11C]carfentanil binding potential

By definition, nondisplaceable binding potential is influenced by receptor density, occupancy of the receptors by endogenous ligands, and affinity of the radioligand. It is not possible to quantify the contributions of each of these variables to binding potential using a single PET scan. Thus, single-scan PET studies do not provide sufficient information for assessing how much each of these variables contributes to inter-individual differences between binding potential estimates derived for a particular brain region. It is, however, possible to separately estimate receptor density and radioligand affinity using PET if data are acquired from multiple PET scans using varying specific activity of the injected radioligand. In these studies, presence of endogenous ligands influences the estimated radioligand affinity.

Unfortunately, there is no published data on the question for [11C]carfentanil. However, published data exists for [11C]raclopride that preferably binds to dopamine type 2 receptors [123]. In that data set, the variation coefficients for receptor density and affinity in striatum were 0.24 and 0.18, respectively, implying that there is approximately 1.3 times as much between-individual variation in striatal receptor density relative to affinity. Thus, these data suggest that receptor density and affinity would, respectively, explain approximately 60 % and 40 % the variation observed in [11C]raclopride binding potential in striatum. Unlike carfentanil, raclopride is an antagonist, and it may more efficiently bind also to receptors in low-affinity states [124]. However, compared to carfentanil, its affinity toward the target receptor is substantially lower ($K_D = 0.08$ nM vs. 1.2 nM) [125, 126]. In fact, carfentanil has extremely high affinity towards MOR [127], not just among exogenous opiates but even compared to endogenous opioid peptides [128]. Consequently, carfentanil may be able to effectively displace endogenous peptides better than raclopride can displace dopamine. For this reason, the receptor-density explanation seems particularly feasible for [11C]carfentanil despite its agonist nature. The contributions of receptor density and affinity are, however, likely to vary between brain regions. In any case, the definition of binding potential implies that all of its constituent variables contribute to individual differences. Explaining all variation with a single variable would be unrealistically simplistic. How much each of these variables contributes to between-individual variation in binding potential of any brain region is an empirical research question that should be resolved in future studies.

4.3.2   Imaging neurotransmission

Even under optimal conditions using an ideal tracer, binding potential estimates for two consecutive scans acquired without head motion would still not be exactly the same because of imperfect measurements and reconstruction algorithms. In reality, the situation is even worse because the kinetics of any tracer are never perfectly modeled with any model and the subject is never perfectly still. This discrepancy does not cause serious problems if the activation between or during one of the scans is powerful enough, as with many pharmacological challenges [129]. However, the design may not work well when the activation is less powerful, as the noise provided by the two-scan design may mask an effect or amplify it, especially with the low sample sizes typical in PET studies. Furthermore, the two scans may be acquired on different days, providing a possibility of neurobiological changes unrelated to the activation in question. One possible way to solve this problem would be to start the activation during a scan, estimate baseline binding potential from the first part and then estimate time-varying binding potential after the activation has begun [130].

Another problem in detection of neurotransmission *in vivo* is that changes in binding potential may not only represent changes in synaptic concentrations of endogenous ligands, but also changes in receptor density or affinity. There are, in fact, some PET studies showing that a challenge does not decrease binding potential but instead increases it [66, 131]. While such findings can be explained by reduced synaptic neurotransmitter concentrations, another explanation is that the increased binding potential results from increased receptor density or affinity. These factors also need to be taken into account when interpreting within-individual binding potential differences.

4.3.3 Fusion imaging with PET and fMRI

Fusion PET–fMRI imaging allows correlating binding potential and hemodynamic responses with each other, providing information about neurotransmitter-level contributions to the fMRI signal. Estimates obtained from the PET and fMRI scans contain uncertainty. While measuring the same individual twice with PET often produces very similar binding potential estimates [132], they are not perfectly accurate. The situation is much worse for the hemodynamic responses [133]. For example, even an extraordinary long data-acquisition period of 24 minutes for resting-state fMRI is not sufficient to match the test–retest reliability of [11C]carfentanil PET [134]. Because uncertainty in the PET and the fMRI estimates propagate into the fusion analysis estimates, they are even *more* noisy than the *worse* of the inputs. This means that fusion imaging has very low statistical power, and it is probably the method's primary limitation. As always, and even more so for fusion imaging, high-quality measurements along with sufficient sample size are needed to get precise estimates.

A further problem in fusion imaging as implemented in this Thesis is the delay between the PET and the fMRI scans. The delay may be hours or even days. Thus, it is possible that the binding potential estimates obtained from the PET scan are no longer completely valid during fMRI. This is especially true when the scans are acquired on separate days. For example, if the PET data are acquired two days after the fMRI scan, it is possible that the subject consumes large doses of alcohol between the scans, possibly changing the measured neuroreceptor levels [135], adding noise to the fusion image analyses. Fortunately, recent technological developments have made it possible to acquire fMRI and PET data simultaneously [136], providing a complete solution to the problem.

The associations obtained from fusion imaging studies do not imply that baseline receptor availability would deterministically influence the individual hemodynamic activity. That would be an unrealistically simplistic model, even if binding potential were regarded as a direct measure of receptor density. This is because receptor density reflects structure rather than function. However, because structure can also inform about function, the fusion approach can yield statistical associations indicating that a particular neurotransmitter is likely to be involved in processing a given stimulus. An obvious improvement to the current fusion imaging approach would be to investigate how neurotransmission influences hemodynamic activity recorded with fMRI [137]. In other words, the hemodynamic responses could be correlated with magnitude of neurotransmitter release that reflects function more than receptor availability does. Even this has its limitations, as simultaneous fMRI and electrophysiological recordings suggest that the BOLD-signal reflects local field potentials rather than neuronal firing or neurotransmission [138].

4.4 Future directions

4.4.1 Open questions for opioidergic imaging

If high MOR availability in cortical midline regions promotes social behavior, as proposed in this Thesis, then individuals with high MOR availability should have larger social networks and spend more time with their friends. This hypothesis would be easy to test in a future PET study. Another prediction stemming from this Thesis is that how much humans are affected by social exclusion depends on their baseline MOR availability, particularly in insula and rACC. Outside the social domain, high approach-sensitivity combined with low emotional responsivity should associate with more frequent risk-taking. Presumably, baseline MOR availability should be positively correlated with risk-taking tendencies in economic contexts.

Compared to μ-opioid receptors, the other opioid receptors have been less extensively studied in humans. However, also the δ [139] and κ [140] opioid receptors can be investigated *in vivo in* the human brain using PET. Thus, it would be possible to estimate the contributions of each of these receptors to a phenomenon of interest. Due to the intimate involvement of the opioid system in modulation of pain, both acute and chronic pain would be interesting areas of research. The κ-opioid system has also been implicated

in regulation of social behavior: κ-opioid agonism dose-dependently suppresses playful behavior of juvenile rats. While rats are normally less playful in an unfamiliar environment, rats pre-treated with a κ-opioid receptor antagonist nor-binaltorphimine display normal play behavior also in novel environments. [141] The κ-opioid system also regulates responses to social stress [142], suggesting that in addition to the MOR system also the κ-opioid system's role in regulation of social behavior should be more thoroughly investigated.

The time-dynamics of MOR activation, as measured with [11C]carfentanil PET, is also an underinvestigated area. Only one study has directly investigated the topic. That study focused on pain-induced decrease in [11C]carfentanil binding potential, and found that the decrease is still detectable 20–50 minutes after the challenge. [143] Lacking knowledge about the time-course of MOR activation hinders efficient experimental design. Knowing the time-dynamics of typical MOR activation related to the challenge would make it possible to start the challenge at a time where the challenge is known to elicit large effects. Currently, researchers do not have such information available.

4.4.2 How to improve fusion imaging?

One of the main obstacles for fusion imaging is its cost: For example, in Turku PET Centre, the list price for PET and fMRI studies are 5000 e and 500 e, respectively. Thus, the data acquisition costs alone for a sample of 50 participants–typical in fMRI–would be 275 000 e. Such sums are financially prohibitive for many research groups. Collecting smaller samples cannot be recommended, as inter-individual correlation fMRI studies are already now underpowered with typically used sample sizes and scan durations [144]. The low statistical power results from the noisy BOLD-signal as well as from the subjectivity of the questionnaire scores that are often used in studies of individual differences. Compared to such metrics that suffer from subjectivity, binding potential estimates derived from PET imaging are objective and reliable [132, 145], slightly mitigating the statistical concerns for fusion imaging. It is also possible to increase the reliability of fMRI-based estimates by adding more repetitions for each participant. This is recommended for all fMRI studies, and is especially helpful for fusion imaging, because increasing the number of participants is so costly. A complementary option to increase reliability of fMRI-based estimates would be to use an MRI device with ultra-high static magnetic field strength that increases the signal-to-noise ratio of the BOLD-signal [146].

Statistical power of PET–fMRI fusion imaging could also be increased by using within-subject experimental designs. While they are commonly used in PET studies, they are rarely used in fMRI. In PET–fMRI fusion imaging, it would be interesting to see how within-subject manipulations of binding potential influence brain responses to a stimulus. Pharmacological agents could be used to manipulate the binding potential levels. Such a within-subject design could consist, for example, of three PET scans and three fMRI scans per participant. The first PET and fMRI scans could be performed without pharmacological intervention, and the second and third scans could use increasing doses of substances that can displace the tracer. For [11C]carfentanil, these substances could be opioid antagonists, such as naltrexone [147]. If the effect lasts sufficiently long, the fMRI and PET scans could be acquired after each other. However, the design would significantly benefit from simultaneous data acquisition for PET and fMRI to speed up the data acquisition process as well as to validate that the pharmacological manipulation works as expected also during fMRI.

In addition to the costs and the statistical issues, fusion imaging with PET and fMRI also has other issues. Compared to hemodynamic responses obtained from fMRI, baseline binding potential is the product of receptor density and radioligand affinity thus reflecting more structure than function. While it is interesting to investigate how brain structure influences hemodynamic activity, even a better way to assess how much a specific neurotransmitter system contributes to the hemodynamic signal measured by fMRI would be to correlate the fMRI responses with magnitude of neurotransmission [137], another form of activity that could be quantified using a stimulus during the PET scan. While traditionally neurotransmission estimates are obtained using a two-scan design, as was done in Study II of the Thesis, it is also possible to estimate how the binding potential fluctuates during a single PET scan [130]. Furthermore, if the measurements are done using simultaneous PET–MRI, it is possible to measure changes in binding potential during an fMRI experiment. Thus, recent developments in instrumentation of PET and MRI as well as modeling of PET data provide unprecedented possibilities for fusion imaging. Yet, even these developments are of little use unless the studies are carefully designed and sufficient numbers of high-quality measurements are collected.

4.4.3 Towards generative Bayesian modeling in PET studies

The Study I of the Thesis used simple bivariate linear regression for investigating how MOR availability associates with the BIS–BAS scores. Bivariate linear regression quantifies how a single variable influences another variable, forgetting about everything else. From the perspective of generative modeling, the model used in Study I states that the binding potential is generated by a single questionnaire score plus noise. The model is highly unrealistic. A better model would assume, for example, that binding potential is generated by sex, age, version of the OPRM1 gene and smoking status in addition to the questionnaire scores and noise. Such modeling is natural in Bayesian framework.

Bayesian models are always generative by nature, thus forcing the analyst to think about the variables influencing the outcome. The Bayesian approach makes it possible to use prior information about the possible range of effects a single variable could potentially have on the outcome. For example, even before collecting any data, it is possible to state with confidence that [11C]carfentanil binding potential does not increase or decrease by 50 % every year as an individual gets older. This is obvious, and not using such information often leads to significantly inflated effect sizes, especially when sample size is small.

The Bayesian framework also makes building of multilevel models natural [148]. Multilevel models can trade information across groups. For example, in neuroimaging ROI-level analyses are often done separately for each ROI, implicitly assuming that knowing an estimate from one region tells nothing about estimates in other regions. This is disadvantageous, because the ROI-wise explanatory variables are often highly correlated, causing dependencies also in the ROI-wise estimates. Multilevel modeling takes advantage of such dependencies and thus uses the data more effectively. Another advantage of Bayesian multilevel modeling is that adding ROIs in an analysis is beneficial because the model then better learns how much information should be transferred across the ROIs. Furthermore, because Bayesian multilevel model provides estimates that are already shrinked towards zero, correction for multiple comparisons becomes practically unnecessary [149]. The only downside of these models is that they often have to be estimated using Markov chain Monte Carlo sampling methods, and consequently accurately estimating a model may take hours. Thus, for full-volume analyses the multilevel approach may be computationally too intensive. However, even full-volume analyses can be performed with regularized regression with closed-form solutions, such as Ridge regression [150].

5 Conclusions

In conclusion, this Thesis provides evidence that opioids transmit information related to sociality also in humans. Humans differ in their dispositions in approach–avoidance situations, and the results of the Study I suggest that cerebral MOR availability explains some of this variation. Following the interpretation of findings in animal research on social behavior, the MOR-dependency on approach behavior is proposed to result from differences in baseline opioid tonus. More specifically, low baseline opioidergic activity presumably motivates individuals to engage in approach behavior more frequently, subsequently enhancing the opioidergic activity. Consistent with this explanation, laughing was shown in Study II to induce release of endogenous opioids in the human brain, providing a neurobiological correlate for the pleasant and calming effects associated with laughing. This finding also supports the hypothesis that laughing facilitates interpersonal bonding via opioidergic mechanisms. PET–fMRI fusion data from the Studies III and IV suggest that cerebral MORs regulate affective brain responses. More specifically, the results suggest that individuals with high availability of MORs in rostral anterior cingulate cortex and insula have blunted responses to highly arousing stimuli, such as witnessing a heated conversation or seeing others in severe pain. Such insensitivity to arousing stimuli can also explain the association between cerebral MOR availability and approach sensitivity: High MOR availability can be seen as a biomarker of resiliency, i.e. individuals with high MOR availability may have more capacity for opioid-mediated relief of the negative consequences that sometimes results from failed approach, thus making the failures less demotivating for them than for others.

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