Opioids derive their name from the Greek ὄπιος for poppy sap. Various preparations of the opium poppy *Papaver somniferum* have been used for pain relief for centuries. Structure and stereochemistry are essential for the analgesic actions of morphine and other opiates, leading to the hypothesis of the existence of specific receptors. Receptors were identified simultaneously by three laboratories in 1973 [1–3]. The different pharmacologic activity of agonists provided evidence for the existence of multiple receptors [4]. In the early 1980s, there was evidence for the existence of at least three types of opiate receptors: μ, κ, and δ [5,6]. A fourth “orphan” receptor (ORL1 or NOP1) displays a high degree of structural homology with conventional opioid receptors and was identified through homology with the δ receptor [7], but the endogenous ligand, orphanin FQ/nociceptin, does not interact directly...
with classical opioid receptors [8] and is not discussed further herein. Endogenous opioids have been identified [9]. These substances were originally distinguished from exogenous opiates, but the term opioids is now generally used for all ligands.

Besides their role as endogenous and exogenous pain killers, opioids and their receptors are implicated in reward, addiction, mood regulation and mood disorders, epilepsy, movement disorders, and dementia.

**Derivation, release, peptide action, and metabolism**

All opioid peptides are derived from three different gene products: proopiomelanocortin, proenkephalin, and prodynorphin. Proopiomelanocortin gives rise to β-endorphin (μ- and δ-prefering) and non-opioid peptides. Proenkephalin contains four copies of different enkephalins (most δ-prefering). Prodynorphin gives rise to several dynorphin peptides and neoendorphin (all κ-prefering) (Table 1) [10]. Endogenous opioids consist of between four (endomorphin) and 31 amino acids (β-endorphin).

Similar to other neuropeptides, opioids are stored in large dense core vesicles [11], which are distinguishable electron microscopically from small clear vesicles containing the fast-acting transmitters such as GABA and glutamate. Opioid vesicle containing neurons also contain classical fast-acting transmitters; therefore, opioids act as co-transmitters to modulate the actions of the primary transmitter. Exocytosis of the dense core vesicles is calcium dependent [12] but, unlike small clear vesicles, exocytosis is not limited to restricted zones of the plasma membrane. Exocytosis requires high-frequency stimulation of the opioid-containing neurons [13,14].

In contrast to amino acid transmitters, opioid peptides can affect the excitability of neurons at the relatively large distance of 50 to 100 μm from the site of release [15] owing to their diffusion through the extracellular space and their high affinities for their receptors, with nanomolar concentrations of the peptides being effective [16]. After release from nerve terminals, opioid peptides are rapidly degraded by a variety of peptidases [9].

The time courses of changes of transmitter peptides and receptor protein are of great interest when interpreting positron emission tomography (PET) studies of opioid function in experiments designed to show the role of neurotransmitters, usually by employing two PET scans acquired at different delays from an experimental manipulation or spontaneously occurring event.

As is true for most G-protein coupled receptors, short-term exposure of opioid receptors to agonists leads to receptor desensitization, and long-term exposure to agonists leads to receptor downregulation [17]. Desensitization is achieved through phosphorylation of agonist-activated receptors and subsequent receptor endocytosis via clathrin-coated pits. The ubiquitin/proteasome pathway seems to be important in agonist-induced downregulation as well as basal turnover of opioid receptors. Mono-ubiquinated receptors are simply internalized, whereas ubiquitin chains of more than three units target the receptor to the proteasome. Further downstream, trafficking to lysosomes may have a role.

Triggered release of endogenous opioids (eg, in seizure models) leads to major changes [18]. In laboratory animals, transmitter peptide levels generally decrease to about 40% to 50% of baseline in the hours after release. Levels of transmitter peptide mRNA tend to increase temporarily. Depending on the model, increases can be relatively minor (approximately 150% of baseline) or major (300% to 1400%). Limited data are available on opioid receptor level changes following events like epileptic seizures. Acute release leads to decreased availability of receptors as measured by [3H]diprenorphine binding [13] or [3H]U69,593 binding [14]. Over slightly longer time courses, both decreases, particularly for δ-opioid receptors [19], and increases (of the μ receptor) [20] have been found. Receptor endocytosis may be irreversible for δ receptors, requiring de novo protein synthesis [17].

Opioid receptor function changes over the hours following neuronal events have received far less attention. After 4 hours of exposure to the μ-agonist DAMGO to induce desensitization, some functional resensitization of the μ receptor occurred after 10 minutes, with 100% of control responses reached after 60 minutes [21]. In another study, μ-receptor protein reached normal levels and function after 6 hours through recycling [22]. Basal levels can be achieved through recycling in 30 to 60 minutes, with some agonists inducing receptor levels of 110% to 120% of control as measured with [3H]diprenorphine [24].

**Receptors and ligands**

**Receptors**

There are three main types of opioid receptors, μ, δ, and κ. For all, the existence of subtypes has been proposed [10], and one subtype each has been cloned in several species including man. The receptor proteins consist of about 370 amino acids [17]. They belong to the G-protein coupled receptor family and have their characteristic structure with seven hydrophobic transmembrane domains, connected by relatively short intracellular and extracellular loops. The amino acid sequences are about 60%
### Table 1: Opioid receptors and their ligands

<table>
<thead>
<tr>
<th>Receptor</th>
<th>μ (MOR)</th>
<th>δ (DOR)</th>
<th>κ (KOR)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endogenous ligand potency</strong></td>
<td>β-endorphin &gt; dynorphin A &gt; met-enkephalin, leu-enkephalin</td>
<td>β-endorphin, leu-enkephalin, met-enkephalin &gt; dynorphin A</td>
<td>Dynorphin A &gt;&gt; β-endorphin &gt; leu-enkephalin &gt; met-enkephalin</td>
</tr>
<tr>
<td><strong>Agonists</strong></td>
<td>DAMGO, DAGO, Dihydromorphine</td>
<td>DPDPE, DSLET, DADLE (δ, μ)</td>
<td>Enadoline (CI-977) U69,593</td>
</tr>
<tr>
<td><strong>Selective antagonists</strong></td>
<td>CTAP</td>
<td>Naltrindole</td>
<td>nor-BNI</td>
</tr>
<tr>
<td><strong>Nonselective antagonists</strong></td>
<td></td>
<td>Naloxone Naltrexone (orally active)</td>
<td></td>
</tr>
<tr>
<td><strong>PET ligands</strong></td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
<tr>
<td></td>
<td>[¹¹C]carfentanil (CFN) (agonist)</td>
<td>[¹¹C]methylnaltrexone (MeNTX) (antagonist)</td>
<td>[¹⁸F]cyclofectroxy (CFX) (κ, μ; antagonist)</td>
</tr>
<tr>
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<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
<tr>
<td></td>
<td>[¹¹C]diprenorphine/[¹⁸F]fluorodiprenorphine (DPN) (partial agonist at δ and κ, antagonist at μ)</td>
<td></td>
<td>[¹¹C]GR103545 (κ; agonist)</td>
</tr>
<tr>
<td></td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[¹¹C]buprenorphine (partial agonist at μ, antagonist at δ, κ)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** DAMGO, [D-Ala²,N-Me-Phe⁴,Gly-ol³]-enkephalin; DAGO, D-Ala²-MePhe⁴-Gly(ol)⁵ enkephalin; DPDPE, [D-Pen₂,⁵]-enkephalin; DSLET, [D-Ser₂, Leu⁵] enkephalin-Thr⁶; DADLE, D-Ala²-D-Leu⁵ enkephalin; CTAP, D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH₂; nor-BNI, nor-binaltorphimine.
identical between opioid receptor types and about 90% identical between the same receptor types cloned from different species. There are marked differences in the degree of sequence conservation between domains. Most transmembrane segments and the three intracellular loops are highly conserved [17], the latter probably because they mediate the G-protein coupling. Extracellular loops are more variable and are responsible for receptor selectivity and affinity. The determinants of the opioid receptor binding pocket differ from ligand to ligand for a given receptor. This difference means that there is not a single receptor binding site, as is true for ionotrophic receptors; rather, opioid receptors are capable of considerable plasticity regarding engagement of ligands and subsequent events leading to receptor activation.

**Species differences**

There are well-known species differences. In general, there is relatively less δ binding in the human compared with the rat brain and relatively more κ binding [25]. The latter finding has been corroborated by quantitative autoradiography [26,27] and by the detection of a more widespread κ-opioid receptor mRNA expression in humans compared with rodents [28]. Other examples include κ-opioid receptors found in deep cortical layers in human but not in rat brain and the lack in humans of the typical patchy distribution of μ receptors seen in rodents [29]. It is prudent to be careful in extrapolating results from rodent studies to humans.

**Regional and layer-specific subtype distributions**

The receptor subtypes are unevenly distributed between regions. Outside the medial occipital lobe, human neocortical binding consists of 40% to 50% κ, 20% to 40% δ, and 15% to 40% μ [25,29]. In the thalamus, μ binding predominates at approximately two-thirds, whereas in mesial temporal structures, approximately 60% of binding is attributed to κ receptors. Absolute levels are about 120 to 145 fmol receptors/mg protein in most of the neocortex, compared with about 72 to 74 fmol receptors/mg protein in the lateral and medial occipitotemporal gyri [26].

Importantly for PET studies, there is evidence of μ-receptor binding and, at a lower level, κ-receptor binding in the human cerebellum [30], indicating that, in contrast to many other transmitter systems, it cannot be used as a classical reference region for μ- or κ-receptor PET tracers; however, the medial occipital lobe is a potential reference region for μ-receptor PET tracers.

Mu receptors are found abundantly in the human thalamus, amygdala, striatum, and neocortex (except medial occipital), as well as some midbrain and deeper nuclei [31]. μ-opioid receptor binding is present in all of these areas but is most dense in layers I, IIa, and IV [26,28,29], suggesting a somatodendritic expression. Functional coupling of μ receptors to intracellular signal transduction mechanisms, measured as DAMGO-stimulated [35S]GTPγS-binding, was around 200% above basal levels and evenly distributed throughout human frontal gray matter [32,33]. In comparison, mRNA, receptor protein, and functional coupling are all much lower in the human hippocampus, whereas μ receptors contribute considerably to the high density of opioid receptors in the amygdala.

Delta receptors are generally less prominent in the human brain than in rodent brains. They are most numerous in the striatum and throughout the neocortex, and occur notably in medial occipital cortex [25,29]. δ-opioid receptor binding is present in all layers and shows peak densities in layers I to IIIa [26,29]. Functional coupling of δ receptors, assessed as DPDPE-stimulated [35S]GTPγS-binding, was evenly distributed throughout human frontal gray matter [32]. There is comparatively little δ-opioid receptor binding in hippocampus and amygdala, very little in the thalamus (approximately 9% of total [25]), and none in the cerebellum, making the cerebellum a possible reference region for δ-opioid receptor ligands.

Kappa receptors are most numerous in the neocortex, amygdala, and hippocampus and notably sparse in the striatum [29]. κ-opioid receptor binding is present in all layers but concentrated in layers V and VI, matching the distribution of mRNA expression [26,29]. Enadoline-stimulated [35S]GTPγS-binding, indicating functional coupling of κ receptors to intracellular signal transduction mechanisms, was present throughout human frontal gray matter but twice as strong in lamina V-VI when compared with lamina I-IV [32]. κ-opioid receptor binding in the human hippocampus is higher than μ- or δ-receptor binding [25] and restricted to the pyramidal cell layer [26]. About 60% of the high level of opioid receptor binding in the amygdala represents κ-opioid receptor binding [25,29]. In contrast to the rat, mRNA and κ-binding sites are present in the human cerebellum [30].

At least for the μ receptor, in some regions a substantial influence of age and sex on the binding potential of the μ-selective ligand [11C]carfentanil as measured by PET has been found [34] in a total of 36 men and 30 women spanning several decades and scanned in two different scanners.

**Ligands**

Selective agonists and antagonists are now available (Table 1). Selectivity is never complete, but the
Positron emission tomography imaging of opioid receptors

**Introduction PET imaging**

PET enables tomographic imaging of local concentrations of injected biologically active substances that have been radioactively labeled (radioligands or tracers) [35–37]. Subjects can be investigated in the resting state or in relationship to an event such as the injection of a substance, the induction of pain, or the occurrence of a seizure. Positron-emitting isotopes used for opioid receptor imaging have half-lives of approximately 20 minutes (11C) and 110 minutes (18F); therefore, the use of PET is limited by the need for an on-site cyclotron to produce 11C, whereas 18F-labeled substances can be transported off site. Only minimal or “tracer doses” of neuroreceptor radiotracers (in the microgram range) need to be injected to estimate receptor parameters; importantly for potent substances like opioid agonists, such small doses are usually without clinical effect. Radiotracers for receptor imaging need to have certain physical properties (ie, a suitable lipophilicity [measured as logP octanol-water] for penetrating the blood brain barrier), and biologic properties (ie, they should not be substrates for multidrug transporters). The radiation dose depends on the tracer used and the activity injected but is typically in the range of 2.5 to 5 mSv using contemporary tomographs and three-dimensional scanning, which should always be used for brain imaging. The raw data collected by the PET scanner is mathematically reconstructed to produce tomographic images of tissue radioactivity concentration. Requirements for absolute quantification are normalization for the current efficiency of detector blocks, correction for nonuniform efficiency along the longitudinal (z) axis, and scatter and attenuation correction.

**Quantification of images**

The distribution of the radioligand changes over time, and scanning is subdivided into time frames. Shortly after injection, radioactivity distribution reflects blood flow and radioligand delivery. Later, radioactivity levels and their changes will gradually reflect the ligand-receptor interaction more than blood flow and delivery.

Radiotracer availability in the brain depends on the blood concentration of the parent radioligand, its partitioning between blood cells and plasma, its binding to plasma proteins, and its metabolism. A measure of the brain availability of the parent radiotracer is desirable. This measurement can be achieved with arterial blood sampling in which total radioactivity and radiolabeled metabolites in plasma are assayed in blood samples throughout the scanning period. These data can be used to produce an “input function” for the available unmetabolized ligand in arterial plasma. Mathematical modeling techniques are then used to derive parameters of interest from the data. These models make the assumption that the time-activity curve in each region or voxel of interest represents the convolution of the input function and the tissue response function. Compartmental models are typically used, usually with two tissue compartments, of which one represents free and nonspecific binding and the other specific binding. Outcome parameters are the volume-of-distribution (VD) or sometimes individual rate constants of radioligand exchange between different compartments. When a brain region devoid or nearly devoid of specific binding is available, for example, in the medial occiptal lobe for µ receptors and the cerebellum for δ receptors, an image-based input function can be derived by measuring the tissue kinetics in this “reference region” [38]. A simpler method is to use late summed images, subtract a reference region’s integrated radioactivity concentration from that of the target region, and divide by the reference region’s integrated radioactivity concentration (ratio images). Radioligand properties, the radiation dose to patients and particularly healthy volunteers, accuracy, bias, reproducibility, reliability, and the sensitivity to clinically meaningful changes should always be considered before choosing a particular method of analysis. In addition, in more clinically orientated settings, it may not be possible to implement more complex acquisition techniques, such as those involving arterial cannulation and metabolite analysis. Simpler methods may often be less sensitive to changes and require the scanning of larger numbers of subjects, with additional cost and radiation protection implications [39–41].

Spectral analysis [42] is an alternative modeling technique to compartmental modeling that has the advantage of not requiring a predefined number of compartments to produce estimates of VD and has been widely used in the analysis of opioid receptor PET data. Spectral analysis convolves a multiexponential function with the parent tracer arterial plasma input function to generate an optimum fit for the observed tissue data and can often be used at the voxel level for the generation of parametric maps. The number and components of the exponentials represent the spectral contributions of the impulse function. The integral of the impulse response function (IRF), extrapolated to infinity,
corresponds to the total VD of the tracer; however, for tracers with slow kinetics, VD estimates may be noisy owing to poor extrapolation of the late time courses. In this situation, suitable restrictions of the expected range of slow exponential decays can be used but will lead to underestimation of high binding regions. Alternatives or extensions currently under study include derivations of spectral analysis such as rank shaping [43], basis pursuit [44], bootstrapped techniques to derive error estimates simultaneously [45,46], or the use of a specific time point on the fitted time course of the unit IRF (eg, IRF_{60} at 60 minutes), which is less dependent on the extrapolation to infinity than VD.

Information derived from a reference region can be used to correct VD for nonspecific binding, yielding binding potentials (BP). VD and BP are proportional to the available receptor density (B_{max}) over receptor affinity (K_{d}). More complex experimental protocols usually requiring multiple injections may be used to derive B_{max} and K_{d} separately. Region-and voxel-based methods (the latter when tracer characteristics allow the generation of parametric maps) are used for the analysis of changes; both are complementary [47].

Quantitative or semi-quantitative evaluation of datasets, typically in comparison with healthy controls, is often more sensitive to abnormalities than the qualitative assessment of PET images [47–50]. Automatic methods for the co-registration of MR imaging and PET data are readily available and allow the interpretation of functional data compared with higher resolution structural data [51].

Partial volume effects arise owing to the restricted spatial resolution PET provides. When structural changes are present, that is, the anatomy differs between patients and controls, these effects are particularly important because they can lead to spurious differences. In these situations, they should be corrected for [52,53]. Region-based correction methods [54–56] and voxel-based methods [57] are used.

Even in the absence of partial volume effects differing between conditions or groups, interpretation of binding estimates tends not to be unequivocal. For example, reductions in binding estimates can be due to reduced receptor concentration, reduced receptor affinity, increased occupancy by endogenous ligands, or opposite changes in the control group or control condition. In the following sections, the interpretation thought to be most likely in the circumstances of the study has been indicated.

**Available ligands and their quantification**

Available ligands are tabulated in Table 1. Diprenorphine (DPN) binds with similar, approximately 1 nmol/L, affinities at all three major opioid receptor subtypes [58] and does not have a reference region because binding in all putative reference regions can be blocked with naloxone [59]. It is a partial agonist at δ and κ receptors and an antagonist at the μ receptor [60]. It has been labeled with ^{11}C [61,62] and ^{18}F [63], with the ^{18}F derivative having similar properties [63]. Ratio images derived from the use of an occipital reference region despite the presence of δ and κ receptors [25,28,29] have repeatedly been shown to have less sensitivity to detect changes in patients than images quantified with spectral analysis [40,41].[^{11}C]buprenorphine is a partial agonist at μ receptors and an antagonist at δ and κ receptors but has not found widespread use compared with[^{11}C]DPN. In rats, the signal is smaller than that of[^{11}C]DPN, with more nonspecific binding.

The agonist[^{11}C]carfentanil (CFN) is used for imaging μ receptors. Its affinity for μ receptors (K_{i} at 37°C, 0.051 nM) is approximately 100-fold higher than for δ receptors and more than 200-fold higher than for κ receptors. Medial occipital cortex can be used as a reference region, and quantification has often been performed as the specific-nonspecific ratio on late summed images (eg, 35–70 minutes) as (region of interest – occipital)/occipital [64]. More recently, the use of graphic methods and the simplified reference tissue model [65] have been more accurate in quantifying[^{11}C]CFN binding [66]. Similar[^{18}F]-labeled tracers are under development for use by centers without access to a cyclotron [67].

Delta receptors are visualized with the antagonist[^{11}C]methyl naltrindole (MeNTI). Its affinity for δ receptors of 0.02 nM is approximately 700-fold higher than for μ receptors and more than 3000-fold higher than for κ receptors [68]. Cerebellum has been used as a reference region, and quantification has been performed on late summed images (eg, 34–90 minutes [68] or 50–90 minutes [69]) as (region of interest – cerebellum)/cerebellum.[^{11}C]MeNTI binding is essentially irreversible over the time course of a 90-minute PET scan [68,70]. It is unclear how far flow effects will enter these binding estimates, and the ratio method was abandoned by the same researchers in a later systematic modeling study [70].

[^{18}F]cyclofoxy (CFX) is an antagonist at μ and κ receptors. Affinities were reported as a Ki of 2.6 nM for μ sites, 9.3 nM for κ sites, and 89 nM for δ sites [71]. The medial occipital cortex may contain some κ receptors [28] but has been successfully used as a reference region following estimation of total VD with an arterial input function and kinetic modeling with a one tissue compartment model [72–74].[^{11}C]GR103545 is the active (−) enantiomer of the racemate GR89696 and a selective κ agonist, with subnanomolar to low nanomolar affinities
It has shown promise as the first \( \kappa \)-selective PET tracer in mice [76] and baboons [75] but will require radiochemistry improvements to increase specific activity and reduce co-injected unlabeled ligand to become useful in humans.

**Opioid receptor imaging in healthy volunteers**

The marked regional differences in overall opioid receptor concentrations and subtype distributions described previously are reproduced by in vivo imaging techniques. Regarding total opioid receptor binding, Jones and coworkers [77] commented on a marked difference, estimated from \( ^{11}C \)DPN ratio images, between the “medial” or “affective” pain system (which comprises the medial thalamus, amygdala, caudate nucleus, insula, cingulate gyrus, and orbitofrontal cortex) (Fig. 1) with high opioid receptor binding and the “lateral” or “discriminating” pain system (projecting to the somatosensory cortex) with much lower binding. This difference is also obvious on parametric maps of \( ^{11}C \)DPN VD (Fig. 2). Other areas now thought to be part of the lateral nociceptive system, such as parts of the insula and the parietal operculum, have high \( ^{18}F \)DPN BPs [78]. Genotype has an important role in regional binding estimates [79]. There is a common co-dominant valine/methionine polymorphism in the gene encoding catechol-O-methyl-transferase (COMT), leading to higher activity of this enzyme that metabolizes catecholamines like dopamine, adrenaline, and noradrenaline in the order of val/val > val/met > met/met carrying individuals. The more met polymorphism, the less breakdown of catecholamines, presumably leading to more continuous stimulation of the dopaminergic system. In animal models, such chronic activation has been shown to lead to reduced enkephalin (Table 1) levels and compensatory increases in regional \( \mu \) receptors. Although global \( ^{11}C \)CFN BP, reflecting \( \mu \) receptor concentration, was not influenced by the polymorphism in 18 subjects of whom 11 were heterozygous, marked differences in the expected direction were seen regionally in the thalamus [79]. Comparison of the various groups showed further effects of genotype on baseline \( ^{11}C \)CFN BP in ventral pallidum and nucleus accumbens, in the order of about 30% (Fig. 3).

**Fig. 1.** Localized clusters of negative correlations between \( ^{11}C \)diprenorphine (DPN) VD (n = 14) and restless legs syndrome severity, measured on the International Restless Legs Scale, analyzed with SPM99 and displayed at \( P < .01 \) uncorrected threshold, cluster extent of 50 voxels. All clusters throughout the whole brain are demonstrated (top left) in the maximum intensity projection “glass brain.” The top right and bottom two panels show significant clusters overlain on a single subject brain [133]. Color bar represents Z-values. Many structures of the medial pain system show this negative correlation. (From von Spiczak S, et al. The role of opioids in restless legs syndrome: an \( ^{11}C \)diprenorphine PET study. Brain 2005;128(Pt 4):911; with permission.)
individuals with the met/met phenotype was confirmed by a different group in postmortem autoradiographic studies in the caudate and accumbens nucleus and some thalamic nuclei; however, that study showed increased mRNA levels for pre-proenkephalin, contrary to predictions [80].

Other factors affect receptor concentrations. Age may regionally increase or decrease receptor concentrations as seen in postmortem studies and using [11C]CFN BP via a ratio method in subjects aged between 19 and 79 years [34]. Women had generally higher μ receptor BP, thought to relate at least partly to hormonal status [34]. A high estradiol state induced with patches led to higher μ-receptor availability in eight women ranging between 15% and 32% [81]. These effects are likely to be region specific, and some regions may show interactions between age and sex, adding to difficulties in interpretation. In the thalamus, μ receptor BP was higher in premenopausal women when compared with their male contemporaries, whereas the opposite relationship was seen in postmenopausal women [34]. Similarly, total VD and specific VD (regional total VD minus occipital VD) of [18F]CFX, reflecting μ- as well as κ-opioid receptor binding, were reduced in nine postmenopausal women compared with 15 men in the thalamus but not in several other regions of interest, including caudate and putamen [74].

The opioid system is involved in mood regulation. μ agonists tend to be rewarding, whereas κ agonists induce dysphoria. In 14 healthy women scanned with a bolus-infusion paradigm [82], neutral and sad mood states were induced in a counterbalanced order between 5 and 45 and 45 and 100 minutes, respectively, after the start of the radiotracer infusion. Sadness was induced by instructing subjects to concentrate on an autobiographical event that, before scanning, had been established to be associated with profound sadness. Logan plot derived VD ratios (over occipital lobe) for the periods of 10 to 45 and 50 to 100 minutes were compared using statistical parametric mapping. Increased availability of μ receptors (ie, a “deactivation” of the opioid system) was seen in the perigenual anterior cingulate gyrus,

Fig. 2. Parametric maps of [11C]diprenorphine (DPN) VD in a healthy male volunteer, acquired after injection of approximately 185 MBq of [11C]DPN on a Siemens/CTI HR++ scanner displayed alongside co-registered MR imaging slices. (Top) Coronal slices at the level of the amygdala. (Bottom) Superior transverse slices. Note the heterogeneity of binding in the coronal slices and the relative lack of binding centered on the postcentral gyrus in the transverse slices. Color bar represents the VD.
left inferior temporal cortex, and amygdala and ventral pallidum bilaterally. Most of these changes were more clearly seen after global normalization of VD ratios. The magnitude of deactivations correlated with the increase in negative affect ratings or the decrease in positive affect ratings in the perigenual cingulate gyrus, ventral pallidum bilaterally, and on the left in the amygdala, insula, and hypothalamus. The study provides direct in vivo evidence for a role of the µ-opioid receptor system in affect regulation in humans. In another study in 12 healthy men, higher baseline Logan plot VD ratios were weakly associated with smaller blood flow responses to aversive visual stimuli in the left anterior middle temporal gyrus [83].

Opioid imaging in the wider sense also includes indirect measures of opioid neurotransmission, that is, studies investigating cerebral blood flow in relation to opioid receptor agonists. Although these studies are not discussed herein, a recent detailed review is available elsewhere [84].

### Changes in receptor availability in pain and discomfort: between-group comparisons

Four patients with central neuropathic pain (mainly resulting from strokes) when compared with an undisclosed number of healthy controls...
had reduced $[^{11}C]DPN$ VD centered on medial pain system structures (cingulate gyrus, insula, and thalamus), with some reductions in the lateral pain system (inferior parietal cortex) [85]. The effect sizes were around 15% to 30%. In this particular study, it was thought to be unlikely that the reductions were due to occupancy of receptors by endogenous opioids, and the interpretation of a disease-related loss of binding sites with subsequent failure of the opioid system to suppress pain was favored, partly supported by the lack of clinical change after prolonged infusions of naloxone in two patients. A different group reported a similar finding of regionally reduced $[^{11}C]DPN$ ratios in medial (contralateral thalamus, insula, cingulate gyrus, midbrain) and lateral (parietal, somatosensory, and lateral frontal cortices) pain system structures in five patients with poststroke pain compared with 12 controls using the occipital lobe as a reference region [86], expanding on an earlier case report [87]. In contrast to the previous study [85], all images had been preprocessed to show the anatomic lesion on the same side, and decreases showed lateralization to the side contralateral to the perceived pain.

When compared with healthy controls, 15 patients with meticulously evaluated primary restless legs syndrome had no global or regional differences in $[^{11}C]DPN$ VD [40]; however, the more severe the restless legs syndrome, the less $[^{11}C]DPN$ VD in much of the medial pain system (Fig. 1), suggesting enhanced release of endogenous opioids. Similarly, ratings on the McGill Pain Questionnaire correlated inversely with $[^{11}C]DPN$ VD in the orbitofrontal cortex and anterior cingulate gyrus.

Overall, these studies are remarkable for delineating the medial pain system irrespectively of the type of pain or anatomic localization of lesions. They offer no explanation of how such systematic changes could be mediated.

Six men with left-sided cluster headache when compared with eight healthy controls had decreased signal of $[^{11}C]DPN$ (quantified as IRF$_{60}$) in the pineal gland, and binding in hypothalamus and cingulate gyrus correlated negatively with disease duration [88]. The validity of quantifying $[^{11}C]DPN$ with IRF$_{60}$ for the pineal gland, which is situated outside the blood-brain barrier, was not explicitly examined. Although the changes are in brain regions previously implicated in the pathophysiology of cluster headache [89], this makes them harder to interpret.

Ultimately, patients should be the beneficiaries and subjects in imaging research; however, experimental paradigms offer the possibility of tightly controlling several variables that would be impossible to standardize in real patients. In one such experiment [90], pain was induced in eight healthy volunteers by applying a heat stimulus (approximately 44°C) from 60 to 70 minutes after injection of $[^{18}F]DPN$. A VD from data 80 to 120 minutes post injection was calculated using an invasive Logan plot and compared with the findings in another eight healthy volunteers of similar age and sex distribution scanned with the same protocol but without pain induction. In the group with pain stimulation, there were decreases in $[^{18}F]DPN$ VD in the ipsilateral nucleus accumbens and amygdala and bilaterally in the middle frontal gyrus, the anterior insulae, the thalami, and the perigenual anterior cingulate gyrus.

**Direct intrasubject comparisons of periods with pain and pain-free states**

Such studies are more complex to perform, because paired studies need to be obtained for patients in two different states and ideally compared with paired control scans. Nevertheless, they allow the attribution of changes in a neurotransmitter system to changes in clinical or experimental stage and provide more powerful evidence than studies comparing groups of patients and controls.

Four patients with rheumatoid arthritis were examined twice with $[^{11}C]DPN$ [91]. Global increases in opioid receptor availability and additional regional increases in the frontal and temporal lobe and cingulate gyrus were seen in the reduced pain state, consistent with the idea that occupancy by endogenous ligands, triggered by pain, may lead to reduced availability. The same group obtained similar results in six patients undergoing thermocoagulation for refractory trigeminal neuralgia. Regional increases of $[^{11}C]DPN$ VD after surgical pain relief were seen in frontal, insular, perigenual, midcingulate, and inferior parietal cortices, basal ganglia, and thalamus bilaterally [92]. Although the preceding studies suffer from the lack of a control group scanned twice, they have the merit of being the only ones attempting intrasubject comparisons in patients so far.

More work has been done in healthy controls in experimental paradigms. When pain was induced through capsaicin application to the hand, eight healthy volunteers showed reductions in contralateral thalamic $[^{11}C]CFN$ 34- to 82-minute tissue ratios over the occipital lobe when compared with baseline; in addition, the magnitude of the decrease of μ-receptor binding correlated directly with pain intensity [93].

All studies discussed hereafter used healthy controls in a paradigm whereby sustained deep tissue pain was induced by infusion of 5% hypertonic saline into the masseter muscle, with nonpainful infusion of 0.9% (isotonic) saline in the control condition into the other masseter muscle. By controlling the rate of infusion, a similar level of pain...
was maintained, around 50 or slightly below on a visual analogue scale where 0 denotes no pain and 100 the most intense pain imaginable. Both scans were acquired on the same day, separated by at least six isotope half-lives. Intramusseter infusions were administered from 20 to 40 minutes after radiotracer administration \cite{79,81,94,95} or from 40 to 60 minutes \cite{96}. A bolus-infusion radiotracer administration protocol was used. Modified Logan graphical analysis with occipital cortex as a reference region on data from 20 to 70 \cite{79,81,94,95} or 40 to 90 minutes \cite{96} post radiotracer injection was used to quantify \( \mu \)-receptor binding as DVR, a VD ratio (ie, the slopes of the Logan plot for voxels of interest divided by the reference region slope).

Using this paradigm, the Ann Arbor group has initially shown in 20 healthy volunteers that sustained pain can induce a release of endogenous opioids, measurable as a decrease in \(^{[11]}\)CFN DVR, indicating interaction of endogenous opioids with \( \mu \)-opioid receptors in specific brain regions. \( \mu \)-opioid receptor mediated activation is associated with reductions in pain ratings in the sensory domain (ipsilateral nucleus accumbens, thalamus, and amygdala) and affective domain (bilaterally in the cingulate gyrus and thalamus, and ipsilaterally in the nucleus accumbens) \cite{94}. Having established this principle, the researchers went on to demonstrate sex differences in 14 men and 14 women aged between 20 and 30 years, with the women scanned during the low-estrogen, low-progesterone early follicular menstrual cycle phase \cite{95}. The intensity-controlled paradigm did not show any differences in subjective positive and negative affect scores or pain questionnaire ratings. Nevertheless, men activated the \( \mu \)-opioid receptor system contralaterally to pain infliction in the anterior thalamus, ventral pallidum/substantia innominata, and anterior insula, as well as ipsilaterally in the amygdala and ventral basal ganglia, whereas the latter was the only area with significant DVR decreases in women who, in addition, showed DVR increases following pain in the ipsilateral nucleus accumbens. Across all subjects, greater activation of \( \mu \)-opioid receptors in ipsilateral nucleus accumbens and amygdala was correlated with less pain perception, a correlation in the expected direction. Baseline \(^{[11]}\)CFN DVR indicated higher binding in women only in one amygdala, in contrast to the group’s earlier study showing more widespread and impressive increases in women compared with men \cite{34}. Characteristics of this earlier study which might explain some of these differences include a wider age range, a ratio method of quantification, no correction for multiple comparisons, and a less objective method of region definition. Sex differences in the same direction were replicated later for the nucleus accumbens and hypothalamus (but not the amygdala) \cite{81}.

To further characterize the role estradiol has in women’s \( \mu \)-opioid transmission in relation to pain, eight women were subjected to two paired studies—one pair in the low-estrogen, low-progestosterone early follicular menstrual cycle phase and one pair after transdermal delivery of estradiol for 7 to 9 days, which increased estradiol plasma levels about fivefold \cite{81}. In the high estradiol state, baseline DVRs of \(^{[11]}\)CFN increased an average of 15% to 32% in the thalamus, amygdala, nucleus accumbens, and hypothalamus. These increases were correlated with estradiol plasma levels in the latter two. The high estradiol state also led to 12% to 19% decreases of pain-induced \(^{[11]}\)CFN DVRs (ie, activation of \( \mu \) receptors through endogenous opioid receptors) in the hypothalamus/nucleus accumbens and the amygdala ipsilateral to pain. Even more strikingly than in the earlier study \cite{95}, women in the low estradiol state did not show any activation of \( \mu \) receptors but, on the contrary, “deactivations” in these same regions and the medial thalamus; these effects ranged from 11% to 16%.

Such increases are difficult to interpret, because opioid release generally requires high frequency firing \cite{97}, and the system overall does not seem tonically active, however, the authors discuss \cite{81,95} some evidence that there may be a tonic release of endogenous opioids at baseline, particularly in the nucleus accumbens, but possibly also in the brainstem, amygdala, and other regions.

The group went on to demonstrate an effect of genotype on \( \mu \)-receptor neurotransmission and used the common COMT val/met polymorphism described above in the section on opioid imaging in healthy volunteers. In addition to the substantial influence on baseline \( \mu \)-receptor binding, with individuals with the met/met phenotype showing higher levels of \( \mu \)-opioid receptors in the thalamus, ventral pallidum, and nucleus accumbens, these individuals also had substantially smaller activations of the \( \mu \)-receptor system (with a gene “dose” effect and met/val heterozygotes intermediate between homozygotes) and, in parallel, higher negative ratings on affect scores and pain questionnaires.

Interestingly, the same group provided evidence that the placebo response is mediated via \( \mu \)-opioid receptors. Fourteen healthy male volunteers underwent a three-scan paradigm consisting of a baseline scan and two scans with pain induction. During one of these, a placebo was administered with the expectation of pain relief. Placebo did relieve pain and also led to release of endogenous opioids in the middle frontal gyrus and nucleus accumbens ipsilateral to the pain and in the perigenual anterior
Opioid imaging in epilepsy

Epilepsy is the most common serious neurologic disorder, affecting 0.5% to 0.8% of the population. Epileptic seizures can arise from one part of the brain (focal epilepsies) or can appear generalized from the outset (generalized epilepsies). Among the focal epilepsies, temporal lobe epilepsy is the most common, and hippocampal sclerosis is the most frequent underlying cause. There is considerable interest in opioidergic mechanisms in epilepsy.

Two recent reviews covering opioid imaging in epilepsy are available [98,99].

Focal epilepsies

Interictal studies

Overall opioid receptor binding has been studied with $[11C]CNF$ in two studies. Using $[11C]DPN$ ratios (region – occipital/occipital) for four regions (amygdala, anterior temporal/midtemporal/posterior temporal, ie, not hippocampus) derived from standard-sized rectangular regions of interest in a single PET plane in 11 patients with a video electroencephalogram confirmed temporal lobe epilepsy, no differences between the focus and nonfocus sides were seen [100]. Eleven controls were scanned but no results reported. A later study confirmed the absence of asymmetries of $[11C]DPN$ binding in two patients [101]. In contrast, work in progress has shown abnormalities of $[11C]DPN$ binding in 80% of patients with focal epilepsies due to malformations of cortical development [102].

$[11C]CNF$ was used in the same 11 patients studied with $[11C]DPN$ [100] and quantified in the same way in the same regions. In contrast to opioid receptors overall, increases of $\mu$-receptor binding ratios were seen on the side of the epileptogenic focus in lateral midtemporal and posterior temporal neocortex, whereas binding in the amygdala was decreased. The latter finding might be due to partial volume effects that could be corrected for in contemporary studies [103]; however, the finding of lateral neocortical increases confirms an earlier study [104]. These increases of $[11C]CNF$ binding coincided with areas of hypometabolism seen on $[18F]FDG$ PET in the earlier [104] but not the later study [103]. Temporal neocortical binding increases were also seen in ten patients using the $\delta$-receptor subtype selective antagonist $[11C]MeNTI$. Region definition was similar to that in the earlier study [100] but performed on three slices, and binding was quantified via ratios on 50- to 90-minute concentration images as (region – cerebellum)/cerebellum [69]. Because of $[11C]MeNTI$'s essentially irreversible binding, this quantification method is likely suboptimal and was later abandoned [70]. Ratio increases for $\delta$ receptors were seen over all of the temporal neocortex ipsilateral to the seizure focus and included the amygdala. Significance was reached for the superior temporal pole, reaching more posteriorly in the most inferior of three PET slices available [69]. The increases in the inferior most slice coincided with increases of $[11C]CNF$ ratios derived from the same patients. In contrast to the earlier study, no increases were seen in temporal neocortex at the level of the amygdala, although the amygdala $[11C]CNF$ ratio decrease was replicated.

One speculative explanation is that an increase in $\mu$ receptors may be a manifestation of a tonic antiepileptic system in the temporal neocortex to limit the spread of epileptic activity, while the amygdalar decrease of $[11C]CNF$ ratios could be related to mesial temporal hyperexcitability. Opposite changes of $[11C]CNF$ and $[11C]MeNTI$ ratios in the amygdala, implying increases of $\mu$ receptors but decreases of $\delta$ receptors, may explain why no asymmetries were detected with the nonselective tracer $[11C]DPN$.

Using $[18F]CFX$, the $\mu$ and $\kappa$ antagonist, quantified with a kinetic model as a VR, there was no overall asymmetry in a group of 14 patients with temporal lobe epilepsy, but, in some individual patients, binding was increased in the ipsilateral temporal lobe [72]. Taken together with the temporal neocortical increases of $\mu$ receptors described previously, this finding would be consistent with a decrease in the affinity or number of $\kappa$ receptors, or decreased availability of $\kappa$ receptors through occupation by an endogenous ligand.

Ictal studies

Ictal studies have been performed in five patients with reading epilepsy compared with six controls...
with $^{[11}C$DPN and a two-scan (rest-activation) paradigm [105]. Quantification was performed using a metabolite-corrected arterial plasma input function and spectral analysis. This approach allowed the computation of parametric maps of the IRF$_{60}$ and use of statistical parametric mapping. Reading epilepsy provides a model for focal epilepsy and has the advantage that seizures can be provoked easily through reading yet do not lead to significant head movement. Comparison of the resting condition in patients with that in controls revealed no significant differences. Reading-induced seizures were associated with reduced $^{[11}C$DPN IRF$_{60}$ in the left parietotemporo-occipital cortex and to a lesser extent in the left middle temporal gyrus and posterior parieto-occipital junction (Fig. 4), implying release of endogenous opioids at the time of seizures. These areas are known to be involved in reading, visual processing, and the researchers have since provided fMR imaging measures of increased cerebral blood flow during reading overlapping with these regions (Fig. 4).

Further evidence for the release of endogenous opioids at the time of seizures comes from a fortuitous ictal $^{[18}F$CFX PET scan from the National Institutes of Health laboratory reported as a personal communication [106]. Frequent intermittent right medial temporal discharges developed about 6 minutes after injection, and the time-activity curve for the right medial temporal lobe remained constantly below that of the contralateral side for the remaining 60 minutes.

Taken together, these findings suggest an involvement of the opioid system in several forms of focal epilepsy. Currently, PET opioid studies are confined to research in a few centers and are not used clinically.

**Idiopathic generalized epilepsy**

$^{[11}C$DPN VD was used to image all opioid receptor subtypes in eight patients with childhood and juvenile absence epilepsy compared with eight controls [107]. No significant differences were found, suggesting no overall interictal abnormalities of the opioid system in idiopathic generalized epilepsy. Nevertheless, changes were seen in a dynamic $^{[11}C$DPN study investigating eight patients with primary generalized epilepsy and eight controls. In patients, absence seizures were induced by hyperventilation 30 to 40 minutes after tracer injection, and hyperventilation maintained for 10 minutes led to generalized spike-wave discharges for 10% to 51% of this period. After this provocation of serial absence seizures, a faster elimination of $^{[11}C$DPN from association cortices but not thalamus, basal ganglia, or cerebellum was seen, suggested by simulations and a two-compartment model to correspond to an estimated 15% to 41% decrease in the specific tracer uptake rate constant (k3) [108], compatible with the release of endogenous opioids in association cortices at the time of seizures.

**Summary**

Opioid receptor alterations seem to be involved in temporal lobe epilepsy and other epilepsy syndromes. Some of the earlier studies could be replicated to benefit from the rapid progress in the

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*Fig. 4.* Diprenorphine binding during reading-induced seizures compared with control conditions and cerebral blood flow during reading. (Left) Statistical parametric map on a normalized MR brain image. Areas with significantly decreased $^{[11}C$dprenorphine (DPN) impulse response function at 60 minutes (IRF$_{60}$) during seizures are shown in yellow ($Z = 5.22; P < .05$ corrected) in the left parietotemporo-occipital cortex and red (uncorrected $P < .001$; not significant when corrected for multiple comparisons) in the right inferior parietal lobe ($Z = 4.12$), left superior temporal gyrus ($Z = 4.02$), left fusiform gyrus ($Z = 4.00$), left middle temporal gyrus ($Z = 3.51$), and left temporoparietal junction ($Z = 3.37$). (From Koepp MJ, Richardson MP, Brooks DJ, et al. Focal cortical release of endogenous opioids during reading-induced seizures. Lancet 1998;352:954; with permission.) (Right) fMR imaging results in healthy volunteers reading sentences versus pseudofont [134]. The activated areas overlap with those in which endogenous opioid release is seen.
fields of MR imaging and PET in the last decade. No detailed studies of extratemporal epilepsies and malformations of cortical development have been reported. In the absence of suitable κ-selective radioligands, the question of the interictal and ictal role of the κ-opioid system in the epilepsies remains open.

There is good evidence for ictal release of endogenous opioids in several syndromes. Because many of the opioid receptor ligands seem to be displaceable by endogenous transmitters, further systematic comparisons of the ictal state with the interictal state will be highly relevant. Future studies aiming to elucidate the pathophysiology of the epilepsies may benefit from the use of subtype-specific opioid ligands.

### Opioid imaging in other specialties

#### Movement disorders

The high concentration of opioid peptides and receptors in the basal ganglia has prompted investigations in movement disorders. Parkinson’s disease is the most common akinetic rigid movement disorder and is due to degeneration of nigral dopaminergic neurons projecting to the striatum; consequently, major differences have been shown in the dopaminergic system [109]. Several atypical parkinsonian syndromes, including multiple system atrophy and Steele-Richardson-Olszewski syndrome (SRO) (supranuclear palsy), typically do not respond to levodopa replacement therapy.

There were no differences in standard-sized putaminal and caudate region of interest 

[^11C]DPN ratios over occipital cortex in eight normal controls and eight patients with a clinical diagnosis of Parkinson’s disease [110]. In contrast, the same study showed that mean putaminal ratios were reduced in seven patients with multiple system atrophy (striatonigral degeneration subtype), with three of seven patients showing significant abnormalities defined as binding greater than 2.5 standard deviations below the control mean. Six patients with SRO syndrome (supranuclear palsy) showed decreased ratios in caudate and putamen as a group, with abnormal caudate binding in four of six patients and abnormal putaminal binding in all six. In the SRO group, mean binding was also significantly decreased when compared with that in Parkinson’s disease patients.

Another study by the same group investigated six Parkinson’s disease patients with dyskinesias (involuntary movements that are a complication of long-term treatment with levodopa therapy) compared with seven Parkinson’s disease patients without dyskinesias and ten controls [111]. They used a region of interest approach on ratio images of 

[^11C]DPN radioactivity concentration between 30 and 90 minutes postinjection, dividing caudate, putamen, and thalamus standard-sized region of interest values by occipital values, and in addition used spectral analysis to generate parametric images of IRF_{50} values. Again, no differences between non-dyskinetic Parkinson’s disease patients and controls were seen. Dyskinetic patients showed reduced uptake ratios when compared with controls and their non-dyskinetic counterparts in the putamen and thalamus. A smaller difference was found between dyskinetic patients and controls in the caudate nucleus; this finding did not differentiate the dyskinetic from the non-dyskinetic group. There was a nonsignificant (P < .1) but large (1 1 years on average) age difference between the patient groups; however, the same group had seen no age effect on striatal[^11C]DPN ratios in the earlier study [110]. The findings have some support in a decreasing putaminal uptake ratio with increased dyskinesia severity, defined as the summed scores (1–4) of the Unified Parkinson’s Disease Rating Scale (UPDRS) items 32 and 33 (duration and disability), which just reached significance using a Pearson correlation, and the (nonsignificant) greater reduction of contralateral uptake ratios in two patients with unilateral dyskinesias. This study was one of the first to harness the power of statistical parametric mapping, developed in the same institution originally for blood flow studies, for the investigation of receptor binding studies. The statistical parametric mapping investigation of IRF_{50} not only confirmed the differences between dyskinetic Parkinson’s disease patients and controls but also showed additional decreases in the pallidum and anterior cingulate gyrus, as well as increases in the middle and superior frontal gyri. Based on experimental evidence, the authors favored the interpretation that the striatal and thalamic reductions in opioid receptor binding were due to increased occupancy due to increased endogenous opioid transmission.

In view of no differences in dopamine D1 or D2 receptors between dyskinetic and non-dyskinetic Parkinson’s disease patients and increased blood flow in basal ganglia and frontal cortex during levodopa-induced dyskinesias, this study overall suggests that dyskinesias are instead associated with alterations of opioid transmission in the basal ganglia, with resulting overactivity of basal ganglia–frontal connections [112].

Another condition in which opioid neurotransmission has been investigated is Huntington’s disease, an autosomal dominantly transmitted neurodegeneration predominantly affecting the caudate nucleus which leads to psychiatric abnormalities, involuntary movements, and, ultimately, death in early middle age. In five patients with
Huntington’s disease compared with nine controls, decreases of $[11^C]DPN$ binding measures were seen with a variety of approaches. Importantly, $IRF_{40}$ images outperformed ratio images (striatal regions over occipital lobe for 30- to 90-minute radioactivity images), and statistical parametric mapping outperformed regional approaches in the sense that peak changes were more marked, and additional nonhypothesized decreases were found in the mid-to-anterior cingulate gyrus as well as nonhypothesized increases in thalami and pregenual cingulate gyrus/superior frontal gyrus [41], arguing strongly for the use of kinetic modeling techniques. Huntington’s disease is accompanied by major atrophy, particularly of the caudate nucleus. The study did not correct for partial volume effects, and it is likely that this factor accounts at least for some of the observed decreases but not for the thalamic and cortical increases.

The findings in dyskinetic patients with Parkinson’s disease and patients with Huntington’s disease prompted investigation of carriers of a mutation in the DYT1 gene manifesting with primary torsion dystonia. There were no regional differences in $[11^C]DPN$ VD in seven patients compared with 15 healthy controls and no correlation of opioid receptor binding with disease severity, arguing against a major role of opioid neurotransmission across all dyskinesias [113].

In the movement disorder restless legs syndrome, no differences between 15 patients and 12 age-matched healthy controls were seen using $[11^C]DPN$ and either ratio methods or $[11^C]DPN$ VD ($n = 14$ patients) [40]. $[11^C]DPN$ VD was negatively correlated with the severity of restless legs syndrome in structures belonging to the medial pain system, and negatively correlated with the affect score of the McGill Pain Questionnaire in orbitofrontal cortex and anterior cingulate gyrus; both findings were interpreted as occupancy by endogenous opioids. Similar changes were seen using ratio images, but effect sizes and spatial extents were much smaller, underlining the value of quantitative kinetic analysis.

Tourette’s syndrome is a tic disorder with motor and vocal tics starting before the age of 18 years. In five patients with this syndrome, basal ganglia total opioid receptor binding was not different from that in an undisclosed number of controls. Spectral analysis and statistical parametric mapping localized decreased binding in the cingulate gyrus, as well as increased binding in the insula bilaterally, the left premotor cortex and perigenual cingulate cortex [114]. These findings, although etiologically unclear, are interesting in the context of case reports of therapeutic utility of opioid neurotransmission modifying drugs (both antagonists and agonists).

**Dementia**

Opioid receptor alterations have been described in the most frequent dementia, Alzheimer’s disease. A quantitative autoradiographic study investigated 11 brains of patients with Alzheimer’s disease compared with 10 brains of age-matched controls who had had normal neurological status in life. There was decreased $\mu$-receptor binding in the hippocampus (−48%) and subiculum (−46%), decreased $\delta$-receptor binding in the amygdala (−51%) and ventral putamen (−30%), decreased $\kappa$-receptor binding in the hippocampus (−39%), and increased $\kappa$-receptor binding in the dorsal (+57%) and ventral (+93%) putamen and cerebellar cortex (+155%, but on the background of relatively low absolute binding) [27]. Quantifying receptor binding decreases in neurodegenerative diseases in vivo requires partial volume effect correction, which can be difficult. Nevertheless, the effect sizes shown in this postmortem study are remarkable, and the large increases in $\kappa$-receptor binding could potentially be useful for early diagnostic and therapeutic studies.

Early reports of $\mu$-opioid receptor decrease in the amygdala in Alzheimer’s disease measured by $[11^C]CFN$ have not been pursued [115]. Work with $[18^C]CFX$ in 12 patients with Alzheimer’s disease and 12 age-matched controls using specific VD calculated as total VD minus medial occipital VD and standard-sized circular regions of interest in both groups revealed decreased specific VD in the Alzheimer patients in a global fashion but accentuated in parietal, frontal, and limbic areas [73]. It is unclear how partial volume effects affect kinetic modeling of time-activity curves derived from standard-sized regions of interest, and the global nature of changes as well as their smaller magnitude compared with cerebral blood flow changes make clinical usefulness of $[18^C]CFX$ in Alzheimer’s disease unlikely, particularly in view of the recent availability of in vivo markers of Alzheimer’s pathology like $[11^C]PIB$ or $[18^F]FDDNP$ [116].

**Cardiology**

There are other areas in which opioid imaging could potentially be useful. For example, a recent preliminary study in five subjects showed BPs in myocardium of about 4 with $[11^C]CFN$ and $[11^C]MeNTI$. High doses of naloxone, however, when given as a single pre-PET bolus without subsequent infusion, only reduced BPs by about 20% [117]. Nonetheless, this study shows the potential of opioid imaging techniques used outside the brain.

**Opioid imaging in psychiatry: addiction**

In contrast to the widespread use of PET/single-photon emission computed tomography (SPECT)
imaging of dopaminergic systems in psychiatric disorders, opioid imaging has not been widely applied. The exception is in the addiction field. The opioid system is the target for opioid drugs of abuse and has a key role in modulating the dopaminergic system, mediating, for example, the pleasurable and reinforcing effects of alcohol [118].

The first study to explore the opioid system in addiction used $[1^{11}C]$CFN to investigate the $\mu$-opioid receptor in cocaine addiction. Increased levels of the $\mu$-opioid receptor were found in ten cocaine addicts who were 1 to 4 days abstinent when compared with six controls [119]. Increased $[1^{11}C]$CFN binding was seen throughout the brain in the anterior cingulate, frontal and temporal cortices, caudate, and thalamus. The increased levels in the amygdala, anterior cingulate, and frontal cortex positively correlated with the severity of cocaine craving reported. When rescanned at approximately 4 weeks of abstinence, in some but not all of the individuals, the increased levels of $[1^{11}C]$CFN binding were reduced to levels comparable with those in healthy controls. A later study in 17 cocaine addicts compared with 16 controls also reported increased $\mu$-opioid receptor levels as measured with $[1^{11}C]$CFN in the anterior cingulate and frontal and lateral temporal cortices in cocaine addicts after 1 day of abstinence from cocaine [120]. Similar to the earlier study, the increased $\mu$-opioid receptor levels correlated with self-reported craving in several brain regions (eg, the anterior cingulate, dorsolateral frontal cortex) but not ratings of depression or anxiety. Interestingly, the increased levels of $[1^{11}C]$CFN binding in some frontal cortical regions (eg, dorsolateral frontal cortex) positively correlated with the amount of cocaine recently used. This finding suggests that the increased levels occur in response to cocaine use. Cocaine addicts were then scanned at 1 week and 12 weeks after monitored abstinence. The levels of $[1^{11}C]$CFN binding and changes over time varied, depending on the brain region. For instance, $\mu$-opioid receptors remained elevated in the anterior cingulate cortex after 1 week but in the dorsolateral cortex had reduced to control levels. Over the 12-week period, binding of $[1^{11}C]$CFN decreased in all brain regions with the exception of the anterior cingulate cortex. Craving was still positively correlated with increased $\mu$-opioid receptors at 1 week; however, after 12 weeks, craving was negligible, so correlational analysis was not performed.

The results of these two studies support a role for the opioid system in cocaine addiction. The level of $\mu$-opioid receptor availability seems to be related to cocaine use and craving. $[1^{11}C]$CFN is sensitive to endogenous opioid levels, and the reported increase in early abstinence from cocaine in dependent individuals could be due to an increase in receptor number or reduced endogenous opioid levels. The preclinical literature has reported that both may occur.

The opioid system has a key role in mediating the pleasurable effects of alcohol, and several lines of evidence suggest that altered function in the opioid system may be involved in vulnerability to alcohol dependence in humans [121]. In addition, the opioid antagonist naltrexone is an effective medication in treating alcohol misuse [122]. To test the hypothesis that increased opiate receptor levels may also mediate craving for alcohol, Heinz and coworkers [123] used $[1^{11}C]$CFN PET to assess $\mu$-opioid receptor levels in alcohol dependent individuals who had been abstinent for 1 to 3 weeks. Using regional analysis and statistical parametric mapping to compare $[1^{11}C]$CFN levels in patients with that in controls, increased binding was seen in the ventral striatum, including the nucleus accumbens (Fig. 5). This area is involved in mediating reward-based learning, and its dopaminergic function is modulated by the opioid system. No differences were seen in other areas, including the cortex. Using statistical parametric mapping, a positive correlation between $[1^{11}C]$CFN binding levels in the ventral striatum and self-reported craving was seen (Fig. 5). This finding is similar to observations in cocaine dependence. In addition, a positive correlation was seen between $\mu$-opiate receptor levels in the frontal cortex and craving, as measured by the obsessive-compulsive drinking scale. This area of the cortex is involved in executive functioning; therefore, a dysfunctional $\mu$-opioid system may contribute to the poor decision making often seen in dependent patients. Several other clinical variables were studied, including a family history of alcoholism, age, early versus late alcoholism (established dependency before and after the age of 25 years), and smoking, but none of these were significantly associated with $\mu$-opioid receptor availability.

Some individuals maintained their sobriety and were rescanned 5 weeks later. Unlike in cocaine dependence, the higher $[1^{11}C]$CFN binding levels persisted. It was not possible to determine whether the increased binding was due to reduced endogenous opioid levels or increased receptor levels; however, the researchers speculated that if receptor levels are increased, naltrexone may work through normalizing this increase and reducing the rewarding effects of alcohol.

In opiate addiction, a similar increase in opioid receptor availability in early abstinence has been reported. A preliminary study of three heroin dependent patients used $[1^{11}C]$CFN PET to measure $\mu$-opioid receptor availability during and after...
a brief 6-week ascending and descending regimen of the substitute drug, buprenorphine [124]. Buprenorphine is a partial agonist at the μ-opiate receptors and a κ antagonist. After withdrawal from buprenorphine, a significant increase in $[^{11}C]$CFN binding was seen compared with that in controls in the inferofrontal and anterior cingulate cortices. The small number of patients studied likely contributed to the restricted areas showing a significant increase. Using $[^{11}C]DPN$ PET, higher binding
levels throughout the brain have been reported in recently abstinence methadone-maintained heroin dependent patients compared with control subjects [125]. Unlike for cocaine or alcohol dependence, no significant correlation with craving was found.

Together these studies suggest that early abstinence from dependence is associated with increased availability of μ-opioid receptors. Because this increase has been shown for alcohol, cocaine, and opioid dependence, it suggests that the opioid system may have a fundamental role in addiction and that changes occur regardless of the substance of abuse. Although an increase in receptor availability is associated with craving in alcohol and cocaine dependence, this is not true in opioid dependence. Perhaps the direct action of the opioid abuse on the opioid receptors obscures any more subtle effects on the opiate system involved in reward and addiction. An important issue that needs to be fully explored is the contribution of the effects of substances of abuse on the opioid system, the effects of abstinence, or underlying vulnerability to substances of abuse. Although an increase in receptor availability is associated with craving in alcohol and cocaine dependence, this is not true in opioid dependence.

PET imaging has been widely used to explore the dose-occupancy relationship for a number of drugs in psychiatry, in particular D2 occupancy by the antipsychotic drugs [126]. In clinical practice, it became clear that high doses of these drugs were unnecessary because most D2 receptors were already blocked, and the risk of adverse side effects outweighed any small potential increase in antipsychotic efficacy. In the management of opioid addiction, patients are generally put on a substitute drug such as methadone or buprenorphine. In clinical practice, the dose of the substitute drug given is generally based on converting how much "street" opioid drugs such as heroin the patient is using, or intoxification once on the substitute drug. There can be a wide variation in the dose of substitute drug, particularly methadone, that the patient eventually is maintained on (eg, tens of milligrams to over 100 mg). In addition, patients can request more substitute drug when it is not clear to the clinician that they are in withdrawal or need more. PET imaging can assess the dose-occupancy relationship for these two substitute drugs at the opioid receptor in an attempt to determine how much drug to give to a patient.

Methadone, a μ agonist, is the most widely prescribed substitute drug. A group of opioid dependent patients who had been maintained on methadone for many years (2–27 years) and were stable underwent a [18C]CFX PET scan [127]. In regions of interest such as the anterior cingulate cortex, thalamus, caudate nucleus, and amygdala, reduced [18C]CFX binding (19% to 32%) was seen in these methadone-maintained opiate addicts when compared with controls. The scans were performed 22 hours after the last dose of methadone, almost at a time when their next daily dose was due. Plasma levels of methadone only weakly correlated with [18C]CFX binding in the caudate and putamen and not significantly in any other region. It was speculated that only a small number of opioid receptors were occupied by clinically efficacious doses of methadone (30–90 mg/day), leaving a significant number of opioid receptors available for other functions such as pain relief.

Another study using [11C]DPN PET has also explored the dose-occupancy relationship for methadone [128]. In this study, no significant relationship between the dose of methadone and [11C]DPN binding was evident, and complementary preclinical work revealed that [11C]DPN binding was not altered by an acute dose of methadone despite having profound behavioral effects. The conclusion was that methadone needs to occupy a small percentage of receptors to be clinical efficacious and at a level that is undetectable using [11C]DPN PET. These findings were slightly at odds with a previous study from the same group that had shown blockade of opioid agonist effects in a similar group of methadone-maintained patients [129], suggesting that few opioid receptors were available.

Although both studies suggest that methadone does not need to occupy a large percentage of opioid receptors for efficacy, neither can help with rational prescribing, that is, how much methadone to give to reach a maximal clinical effect. The studies suggest that the tolerance seen to opiates in such patients is due to altered opioid receptor function other than removal of the opioid receptor binding site, because this site is clearly still available for the radiotracers to bind to. It is likely that mechanisms involved in transmembrane signaling underlie this phenomenon. Evidence from preclinical or human postmortem studies is also not consistent as to what happens to the opioid receptor in opioid dependence, with increases, reductions, or no change reported [130,131]. It is notable that opioid withdrawal can be ameliorated with non-opioid drugs, suggesting that this system is not critical in mediating all of the effects of abused opioid drugs [132].

By contrast, a clear dose-occupancy relationship is apparent with buprenorphine, a partial agonist at the μ receptor and κ antagonist. In a preliminary study of three heroin dependent patients using [11C]CFN PET, 2 mg of buprenorphine, a relatively low clinical dose, occupied 36% to 50% of opioid receptors [124]. A relatively large clinical dose, 16 mg, resulted in 79% to 95% occupancy; however,
there was a large variation in the percentage occupancy seen. In a follow-up study using more patients, buprenorphine resulted in significant reduction of $[^{11}C]\text{CFN}$ binding by 41% with 2 mg, 80% with 16 mg, and 84% with 32 mg [132]. This degree of occupancy was seen throughout the brain, including areas of interest such as the anterior cingulate gyrus, amygdala, and frontal cortical regions, and was correlated with plasma buprenorphine levels. In addition, blockade of opioid agonist effects correlated with buprenorphine occupancy. PET opioid receptor imaging has been informative, showing the amount of buprenorphine required to result in almost complete occupancy of opioid receptors.

**Summary**

The excitement of the 1970s, when opioid receptors and the various subtypes were described, has been matched in the 1990s and early years of this century by the application of ever higher resolution whole-brain PET scanners, the availability of several radioligands, the combination of PET with advanced structural imaging, advances in modeling of macro-parameters of PET ligand binding, and efforts in large-scale statistical analysis of imaging datasets. Suitable SPECT tracers are lacking, but with the current increase in the number of available PET (or PET/CT) cameras and cyclotrons owing to the clinical success of PET in oncology, PET may become widespread enough to overcome this.

Many breakthrough scientific discoveries have been made using opioid imaging. It is to be hoped that, in the coming decade, there will be a more widespread application of the available techniques to patients and an impact in clinical medicine.

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