Time-Course of Change in [11C]Carfentanil and [11C]Raclopride Binding Potential After a Nonpharmacological Challenge

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ABSTRACT   Positron Emission Tomography (PET) with appropriate radiotracers and quantification methods allows the detection of changes in endogenous neurotransmission by determine the reduction in the binding potential (BP) of receptors before and after experimental challenges. These have typically employed psychostimulants and PET with dopamine (DA) receptor radiotracers. However, reductions in BP persist far beyond the increases in the release of the endogenous neurotransmitter, an effect ascribed to receptor internalization and recycling, a possible confound in repeated studies. Here we examined the time-course of changes in BP during a nonpharmacological challenge, moderate levels of sustained pain, shown to induce robust reductions in μ-opioid and DA D2 BP, as measured with [11C]carfentanil and [11C]raclopride. It was hypothesized that, contrary to pharmacological probes, the use of a more "physiological" stimulus would not be associated with persistent changes in the BP measures. The pain challenge was associated with reductions in μ-opioid receptor BP in several cortical and subcortical regions. These did not persist in a subsequent scan. Similar results were obtained for DA D2 receptor BP, where the pain challenge induced significant reductions in the caudate nucleus. These data demonstrate that changes in receptor BP induced by a nonpharmacological challenge did not persist into subsequent scans. They further suggest differences in the effect of pharmacological and nonpharmacological probes on PET BP measures. These may reflect varying levels of change in receptor affinity, receptor internalization, and recycling depending on the type of challenge employed. **Synapse 61:707–714, 2007.**   © 2007 Wiley-Liss, Inc.

INTRODUCTION

A significant development in recent functional neuroimaging studies has been the ability to characterize the response of endogenous neurotransmitter systems to a variety of experimental challenges. Specifically, external imaging [single-photon emission tomography [single photon emission computed tomography (SPECT)] and positron emission tomography (PET)] with receptor-specific radiotracers and appropriate quantification methods have allowed the detection of changes in the concentration or affinity of receptors [typically described as the ratio between the two, B_{max}/K_d, also termed binding potential (BP)], providing an indirect measure of neurotransmitter release. Such work was pioneered over a decade ago in primates, where reductions in DA D2 receptor BP were observed following an amphetamine challenge, and further related to the concentration of dopamine (DA) in extracellular fluid as measured with microdialysis (Innis et al., 1992). Similar observations were obtained with other DA-releasing agents (e.g., methylphenidate) and with a variety of radiopharmaceuticals targeting DA D2 receptors (Dewey et al., 1991; Endres et al., 1997; Laruelle et al., 1995; Narendran et al., 2004; Riccardi et al., 2006a, 2006b; Volkow et al., 1994).

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However, it has been further observed that the reduction in DA D2 receptor BP persisted over a prolonged period of time (Carson et al., 1997; Laruelle et al., 1995), which exceeded the increases in DA release as measured with microdialysis. This has led to the hypothesis that competition between radiotracer and receptor protein alone could not explain the observed results, and that other mechanisms such as receptor internalization might be responsible (Laruelle, 2000). In addition, recent work has examined the changes in DA BP during amphetamine administration using agonist radiotracers that may preferentially bind to G-protein coupled, high affinity conformational states of the receptors (Narendran et al., 2004). In these studies it was observed that the change in the BP measure was indeed more prominent after the pharmacological challenge for an agonist tracer, compared with the more traditionally employed antagonists. This information then suggests that multiple processes are reflected by the change in BP after pharmacological challenges known to release endogenous neurotransmitters: competition of radiotracer and endogenous ligand, receptor internalization and recycling, and in the case of agonist radiotracers, possibly changes in the conformational state of the receptors after interaction with the endogenous ligand.

These mechanisms still reflect the "activation" of endogenous neurotransmission, and provide a measure of this process. However, the persistence of receptor BP changes over time may represent a confounding factor in repeated studies within subjects and limits the experimental designs that could be practically utilized in humans.

The present investigation explores whether persistent changes in the BP measure are also present in studies employing nonpharmacological challenges. Data from various laboratories have reported reductions in the BP measure in response to pain using the μ-opioid receptor agonist radiotracer [11C]carfentanil (Bencherif et al., 2002; Zubieta et al., 2001), and with the DA D2/D3 receptor antagonist [11C]raclopride (Scott et al., 2006). Similar effects have been reported with [11C]raclopride using a social stress challenge (Pruessner et al., 2004), a reward task (Zald et al., 2004), and placebo administration (de la Fuente-Fernandez et al., 2001). Of interest, and in a number of these studies, the magnitude of the BP changes observed during these challenges was substantial. However, and in animal models, the level of DA release, as measured with microdialysis is typically substantially greater for pharmacological (e.g., amphetamine administration, from 50 to 400%, depending on the dose administered) (Heidbreder and Feldon, 1998; Hernandez et al., 1987; Hooks et al., 1992), than nonpharmacological challenges (e.g., sustained pain, 10–50%, depending on the models) (Marinelli et al., 2005; Rouge-Pont et al., 1998; Schmidt et al., 2002). These differences may account for variations in receptor internalization or
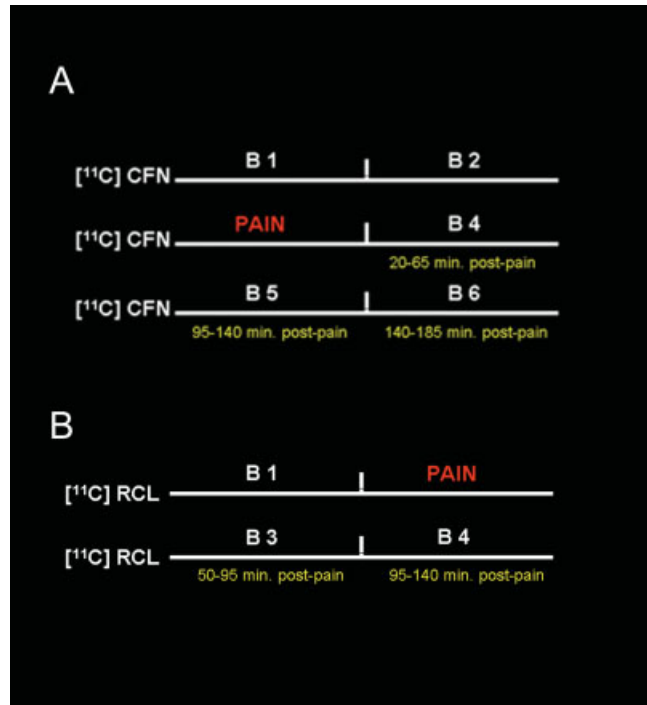

Fig. 1. Carfentanil and Raclopride scan design. Volunteers participated in either three 90-min PET scans with [11C]carfentanil (**A**) or two 90-min scans with [11C]raclopride (**B**); each scan consisted of two 45-min experimental periods. The [11C]carfentanil scans consisted of an initial scan with two baseline conditions (B1 and B2), a second scan with the pain challenge in the first period followed by a baseline condition (B4), and a third scan with two additional baseline conditions (B5 and B6). [11C]raclopride scans consisted of one scan with a baseline condition (B1) followed by the pain challenge in the second 45-min period, and a second scan with two additional baseline conditions (B3 and B4). The timing (in minutes) of post-challenge baseline conditions is displayed in yellow.

affinity induced by the released neurotransmitter, impacting on the capacity of the receptor sites to recycle after the challenges.

Here we utilize a challenge, moderate levels of sustained pain, known to activate both dopaminergic and endogenous opioid neurotransmission and to reduce the BP measure in [11C]raclopride and [11C]carfentanil PET studies (Zubieta et al., 2001, 2003). Baseline studies preceded and followed the challenge scan to examine the persistence of the BP changes. It was hypothesized that, contrary to studies using psychostimulant challenges, more "physiological," nonpharmacological challenges would not be associated with a prolonged reduction in the BP measure. This information would be of importance in PET study design and interpretation, particularly when randomized studies (e.g., challenge, control condition) are desirable and performed within subjects.

MATERIALS AND METHODS
Subjects

Volunteers were 14 healthy, medication free, right-handed men 27 ± 5 years of age, with an educational
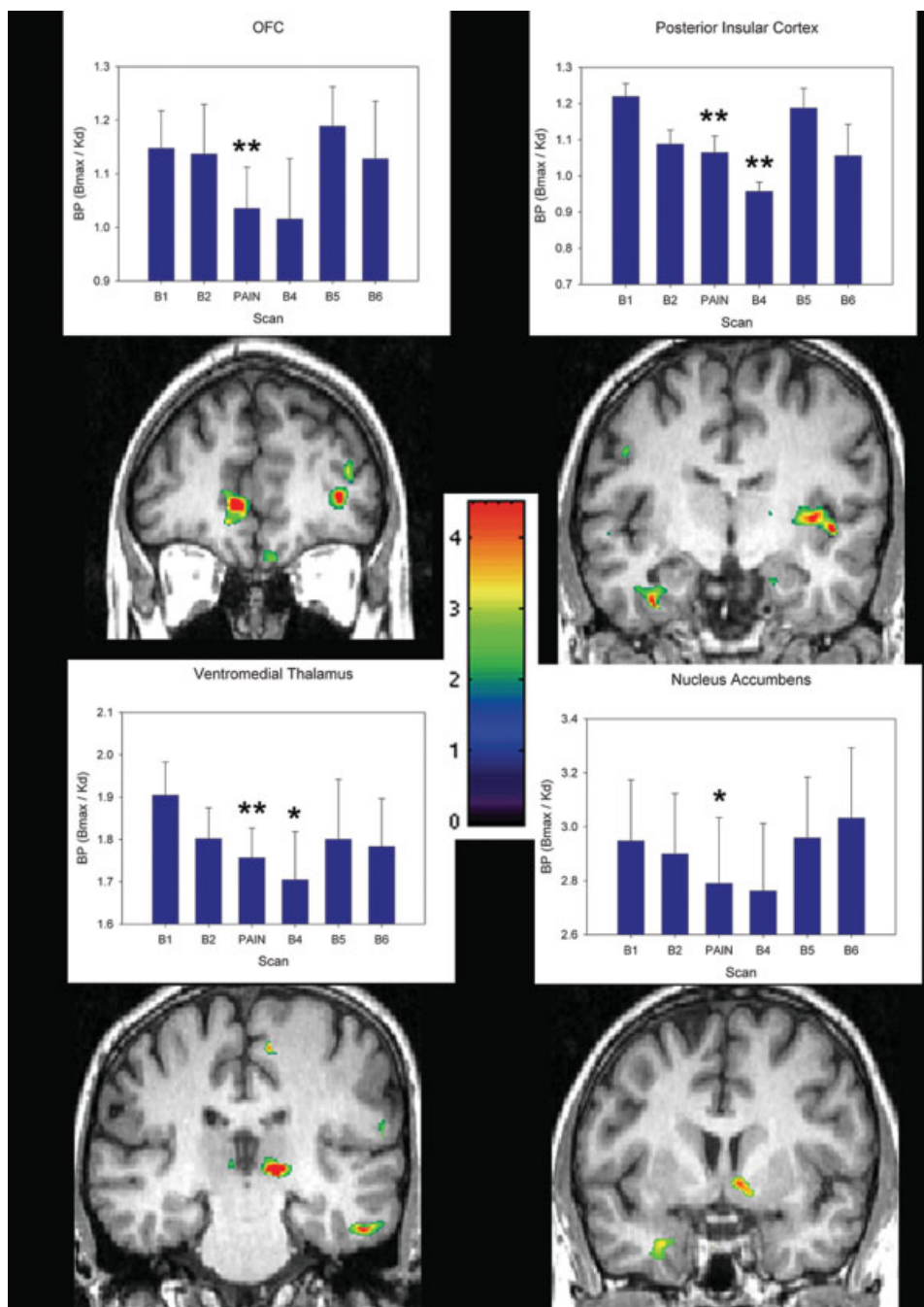
Fig. 2. Localization and time course of changes in [^{11}C]carfentanil BP following pain administration. After correction for multiple comparisons, significant pain-induced reductions in μ-opioid receptor BP were observed when compared with the first baseline condition (B1) in the pregenual anterior cingulate cortex and orbitofrontal cortex, posterior insula, thalamus, and nucleus accumbens. Z-scores of statistical significance are represented by the pseudocolor scale in the center of the image and are superimposed over an anatomically standardized MRI image. The left side of the images corresponds to the left side of the body (ipsilateral to pain; neurological convention). Plots of μ-opioid receptor BP at each time point are presented above the corresponding brain slice using the mean and SEM of the data (** $P < 0.005$; * $P < 0.05$).

level 17 ± 2 years. Subjects had no personal history of medical, psychiatric illness, substance abuse or dependence, and no family history of inheritable illnesses. Volunteers were not taking psychotropic medications or hormone treatments, were nonsmokers, and did not exercise in excess of 1 h three times a week. Written informed consent was obtained in all cases. All the procedures employed were approved by the University of Michigan Investigational Review Board for Human Subject Use and the Radiation Safety Committee.

Study design

Figure 1 shows the overall study design. Radiotracer administration for all scans was separated by 30 min to allow for radiotracer decay. Residual radioactivity between scans represented less than 1.5% of the peak activity and did not appear to influence consecutive baseline measures (Figs. 2 and 3). For [11C]carfentanil scans, 10 subjects participated in three 90-min scan periods, each of which contained two conditions. The first scan consisted of two baseline periods, referred to throughout as B1 and B2. The 20-min pain stress challenge (see later) was introduced in the first period of the second scan, 5 min post-tracer administration, and was followed by another baseline (B4). The third scan, identical to the first, again consisted of two baseline periods (B5 and B6). Given the 30-min delay between scans, the onset times for conditions B4, B5, and B6 thus correspond to 20, 95, and 140-min postchallenge, respectively. For [11C]raclopride scans, 4 subjects participated in two 90-min scan periods. One scan consisted of a baseline condition (B1) followed by the 20-min pain stress challenge. The pain challenge was introduced 45 min post-tracer administration. The second scan included two additional baseline periods (B3 and B4). Given a 30-min delay between scans, onset times for conditions B3 and B4 in this experiment were thus 50 and 95 min after completion of the 20-min challenge.

Pain stress model

A steady state of muscle pain was maintained for 20 min by a computer-controlled system through the infusion of small amounts of medication-grade hypertonic saline (5%) into the left masseter muscle. In this model of sustained deep somatic pain, the intensity of the painful stimulus is controlled using feedback from subjects every 15 s to adjust the rate of infusion of hypertonic saline, as described in detail elsewhere (Zubieta et al., 2001, 2003). Briefly, a standard 150 µl bolus of hypertonic saline is administered over 15 s to establish the initial step response of the system. An electronic version of a 10-cm visual analog scale (VAS) placed in front of the scanner gantry is then used by the subject to rate pain intensity once every 15 s. This signal is fed back to the computer via an analog-digital board, which then adjusts the infusion rate so that pain is maintained to achieve a target VAS intensity rating of 40, within a range of 30–50, for the duration of the challenge. Subjects are informed that the lower end on the scale denotes "no pain," whereas the upper bound represents the "most intense pain imaginable." This method allows for the maintenance of pain over the experimental challenge and for comparability of the stimulus across subjects. In the baseline condition, the subject was asked simply to remain still in the PET scanner
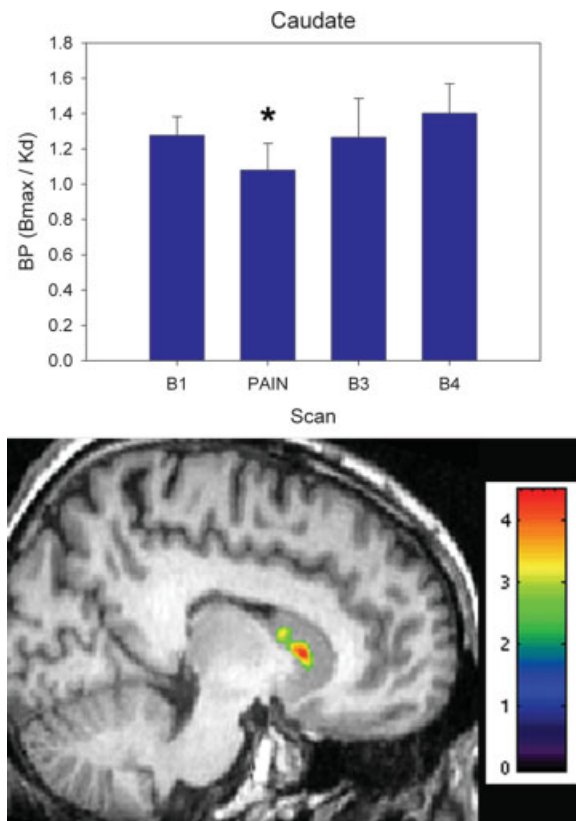

Fig. 3. Localization and time course of changes in [11C]raclopride BP following pain administration. After correction for multiple comparisons, significant reductions in Da D2 receptor BP were observed when compared with the first baseline condition (B1) in the dorsal caudate. Z-scores of statistical significance are represented by the pseudocolor scale in the center of the image and are superimposed over an anatomically standardized MRI image. The left side of the image corresponds to the left side of the body (ipsilateral to pain; neurological convention). Plots of DA D2 receptor BP at each time point are presented above the corresponding brain slice using the mean and SEM of the data (* $P < 0.05$).

for the duration of the scan, and the electronic VAS scale and infusion system were not present.

The sensory and pain-specific affective qualities of the painful stimulus were assessed after completion of the pain challenges with the corresponding subscales of the McGill Pain Questionnaire (MPQ; Melzack and Torgerson, 1971) as well as with 0–100 VAS scales of pain intensity and unpleasantness.

PET and MRI acquisition

PET scans were acquired with a Siemens (Knoxville, TN) HR+ scanner in 3D mode (reconstructed FWHM resolution ~5.5 mm in-plane and 5.0 mm axially), with septa retracted and scatter correction. Participants were positioned in the PET scanner gantry, and two i.v. (antecubital) lines were placed. A light forehead restraint was used to eliminate intrascan head movement. [11C]carfentanil was synthesized at high specific activity (>2000 Ci/mmol) by the reaction

of [^{11}C]methyliodide and a nonmethyl precursor as previously described (Jewett, 2001). [^{11}C]raclopride was synthesized at high specific activity ($>$2000 Ci/mmol) by the reaction of O-desmethyl raclopride with ^{11}C-methyl triflate. Ten to fifteen milliCurie were administered in each of the scans, with a mass of carfentanil injected of 0.048 ± 0.037 μg/kg per scan and a mass of raclopride injected of 0.089 ± 0.047 μg/kg per scan. These ensured that the compounds were administered in tracer quantities, i.e., subpharmacological doses occupying less than 1% of the available receptors. Fifty percent of the radiotracer doses were administered as a bolus, and the remaining 50% by continuous infusion for the remainder of the study.

Images were reconstructed using iterative algorithms (brain mode; Fourier rebinning with ordered subsets-expectation maximization, four iterations, 16 subsets; no smoothing) into a 128 × 128 pixel matrix in a 28.8 cm diameter field of view. Attenuation correction was performed through a 6-min transmission scan (68 Ge source) obtained prior to the PET study, also with iterative reconstruction of the blank/transmission data followed by segmentation of the attenuation image. Small head motions during emission scans were corrected by an automated computer algorithm for each subject before analysis, and the images coregistered to each other with the same software (Minoshima et al., 1993). These procedures have coregistration accuracy within 0.5° and 0.5 mm. Time points were then decay-corrected during reconstruction of the PET data.

Image data were then transformed on a voxel-by-voxel basis into two sets of parametric maps: (a) a tracer transport measure (K$_1$ ratio), and (b) a receptor-related measure (distribution volume ratio, DVR). To avoid the need for arterial blood sampling, these measures were calculated using a modified Logan graphical analysis (Logan et al., 1996) using the occipital cortex (an area devoid of μ-opioid receptors) as the reference region for [^{11}C]carfentanil and the cerebellum (an area devoid of DA D2 receptors) as the reference region for [^{11}C]raclopride. With the partial bolus—continuous infusion radiotracer administration protocol used, the Logan plot becomes linear by 4–5 min after the start of radiotracer administration, allowing the calculation of receptor measures early after tracer administration. The slope of the Logan plot is equal to the ($f_2 \ B_{max}/K_d$) + 1 for this receptor site (receptor concentration divided by its affinity for the radiotracer) and it has been referred to as the DVR; $f_2 \ B_{max}/K_d$ (or DVR − 1) is the "receptor related" measure (also termed BP, BP (Mintun et al., 1984)]. The term f_2 refers to the concentration of free radiotracer in the extracellular fluid and is considered to represent a constant and very small value. As changes in B_{max}/K_d will cause a change in the slope of the Logan plot, we measured DVR during both the early and late phases of each scan. The slope during the early

phase was estimated from 5 to 40 min postinjection, while the slope for the second phase was estimated from 45 to 90 min postinjection.

Anatomical MRI scans were acquired prior to PET scanning on a 1.5 Tesla scanner (Signa, General Electric, Milwaukee, WI). Acquisition sequences were axial SPGR Inverse Recovery-Prepared MR [echo time (TE) = 5.5 ms, repetition time (TR) = 14 ms, inversion time (TI) = 300 ms, flip angle = 20°, number of excitations (NEX) = 1, 124 contiguous images, 1.5 mm-thickness], followed by axial T2 and proton density images (TR = 4000, TE = 20 and 100, respectively, NEX = 1, 62 contiguous images, 3 mm thick). K1 images were then first coregistered to the MR images and the transformation matrix applied to the DVR data, followed by MR, K1, and DVR anatomical standardization the International Consortium for Brain Mapping (ICBM) stereotactic atlas orientation. Statistical parametric maps of differences between conditions (pain vs. baseline) were generated by anatomically standardizing the T1-SPGR MRI of each subject to the ICBM stereotactic atlas coordinates, with subsequent application of this transformation to the DA D2 and μ-opioid receptor binding maps. The accuracy of coregistration and nonlinear warping algorithms was confirmed for each subject individually by comparing the transformed MRI and PET images to each other and the ICBM atlas template.

Image data analysis

Differences between conditions were mapped into stereotactic space using z maps of statistical significance with SPM99 (Welcome Department of Cognitive Neurology, University College, London, UK) and Matlab (MathWorks, Natick, MA) software, with a general linear model and correction for multiple comparisons (Friston et al., 1995). No global normalization was applied to the data, and therefore the calculations presented are based on absolute BP ($f_2 \ B_{max}/K_d$) estimates. Only regions with specific μ-opioid or DA D2 receptor binding were included in the analyses (voxels with DVR values > 1.2 times the mean global image value as calculated with SPM99). To compensate for small residual anatomic variations across subjects and to improve signal to noise ratios, a three-dimensional Gaussian filter (FWHM 6 mm) was applied to each scan. Contrasts were performed on μ-opioid and DA D2 BP images separately to assess main effects of pain. For μ-opioid images, the early baseline 1 from scan 1 was compared with the pain condition of scan 2. For DA D2 images, the early baseline 1 from scan 1 was compared against the pain condition of scan 1. For each contrast, one sample, paired t-statistic values were calculated for each pixel, using the pooled variance across pixels (Worsley et al., 1992). Significant differences and correlations were detected using a

statistical threshold that controls a type-I error rate at $P = 0.05$ for multiple comparisons, estimated using the Euler characteristic and the number of pixels in the gray matter and image smoothness. Z-scores were also deemed significant if they reached statistical thresholds after correction for the size of the cluster under consideration with SPM (Friston et al., 1991). BP values for time-course graphs (in Figs. 2 and 3) were extracted from image data by averaging the values of voxels contained in an area where significant differences were obtained in the voxel-by-voxel analysis, down to a threshold of $P < 0.01$.

RESULTS
Psychophysical pain ratings

Across both studies, subjects received an average of 2.1 ± 1.4 ml of hypertonic saline. This infusion was associated with an average online pain report, provided every 15 s, of 34 ± 15 on a 0–100 point VAS rating scale, where 0 represented "no pain" and 100 represented "the most intense pain imaginable." Subjects also provided overall VAS ratings of pain intensity and unpleasantness at the conclusion of the challenge, reporting an average of 35 ± 15 for pain intensity and 33 ± 18 for pain unpleasantness. Overall pain ratings were also recorded using the weighted word descriptors of the MPQ, where subjects reported average MPQ total scores of 21 ± 9, MPQ sensory subscale scores of 13 ± 7, and MPQ pain affect subscale scores of 1 ± 1.

Reductions in μ-opioid receptor BP following a pain stressor

Compared with the early baseline period prior to pain (B1), significant pain-induced decreases in μ-opioid receptor BP were observed. After correction for multiple comparisons, significant effects were observed in the right ventromedial thalamus (ICBM $x \, y \, z$ coordinates in mm, 13, −24, 2; $z = 8.8$; $P < 0.0001$), the pregenual anterior cingulate cortex ($x \, y \, z$, −10, 43, 0; $z = 8.6$; $P < 0.0001$), the right orbitofrontal cortex ($x \, y \, z$, 29, 27, −14; $z = 7.0$; $P < 0.0001$), the right posterior insula ($x \, y \, z$, 38, 12, 6; $z = 6.7$; $P < 0.005$), and the right nucleus accumbens ($x \, y \, z$, 12, 12, −4; $z = 4.3$; $P < 0.005$). The average percent reductions in the in vivo μ-opioid receptor BP in these regions ranged from 6% in the nucleus accumbens to 16% in the insula. As anticipated, the regional localization and magnitude of these changes reflects previously published reports using this pain model (Zubieta et al., 2001).

We then examined the persistence of these changes in μ-opioid receptor BP into the baseline periods following the pain challenge (B4, B5, and B6). All the regions where effects were obtained during the pain challenge showed similarly reduced mean BP values during B4 (20 min after completion of the pain challenge) (Fig. 2), though these were not significant for all regions (B1 vs.

B4 comparison). Significant reductions were still observed in the insula ($t = 7.2$; $P < 0.005$) and thalamus ($t = 2.9$, $P < 0.05$), with trends in the same direction for the cingulate cortex ($t = 2.3$, $P = 0.07$) and orbitofrontal cortex ($t = 2.3$, $P = 0.07$), not reaching statistical significance. The reductions initially observed in the nucleus accumbens were no longer significant during this period ($t = 1.1$, $P = 0.18$).

Contrary to these data, subsequent baselines, with acquisitions starting at 95 min (B5) and 140 min (B6) after completion of the pain challenge, did not demonstrate reductions in BP when compared with B1. The localization of reductions in [^{11}C]carfentanil binding, as well as plots of the change in BP over time are presented in Figure 2.

Reductions in DA D2 receptor BP following pain

Compared with the first baseline condition (B1), significant pain-induced decreases in DA D2 receptor BP were observed in the left (contralateral to pain) dorsal caudate ($x \, y \, z$ coordinates in mm, 13, 6, 13; $z = 3.2$; $P < 0.005$), where BP was reduced an average of 12%, consistent with other data sets using this challenge (Scott et al., 2006). In the design used for this experiment, no significant reductions in the BP measure were observed for baselines 3 and 4 (50 and 95 min following the completion of the challenge) (caudate BP, B1 vs. B3, $t = 0.4$; $P = 0.8$; B1 vs. B4, $t = 0.4$; $P = 0.7$). These data reflect a return to prechallenge BP values in separate scans immediately following the challenge scan (50 min following the induction of caudate DA activation). The localization of reductions in [^{11}C]raclopride binding, as well as the change in BP over time are presented in Figure 3.

DISCUSSION

As previously described using pharmacological probes, a nonpharmacological challenge (sustained pain) known to induce the activation of endogenous opioid (Lapeyre et al., 2001) and DA neurotransmission (Gear et al., 1999) in animal models, also reduced μ-opioid and DA D2 BP in [^{11}C]carfentanil and [^{11}C]raclopride PET scans, as shown in larger samples (Scott et al., 2006; Zubieta et al., 2001). However, and contrary with data acquired after the administration of pharmacological agents known to release DA (typically the psychostimulants amphetamine and methylphenidate), the changes in BP did not persist in subsequent scans following the pain stressor. A full return to baseline BP levels is described 50 and 95 min postchallenge. Some residual effects, albeit with regional variability, were still observed for periods that immediately followed (20 min after its completion) the noxious challenge, dur-

ing the same PET scan. These effects were no longer observed during subsequent PET studies.

This information is of relevance for the design PET studies when multiple conditions are examined, and point to important differences in the behavior of external imaging measures when "pharmacological" and "physiological" probes are used to stimulate endogenous neurotransmission.

Molecular (neurochemical) PET techniques have been in use for approximately two decades for the quantification of receptor concentrations and enzyme activity. More recent studies also employ them to explore variations in stimulated endogenous neurotransmission across individuals and pathological states [e.g., schizophrenia, (Abi-Dargham et al., 1998; Laruelle et al., 1996); substance abuse, (Martinez et al., 2005)]. The earliest validations employed primate models to examine the effect of amphetamine on endogenous DA neurotransmission with (Innis et al., 1992), or without simultaneous microdialysis (Dewey et al., 1991). Differences in the sensitivity of radiotracers for the detection of changes in endogenous neurotransmission have also been recognized, with initial ex vivo rodent studies reporting [^{3}H]raclopride to be more sensitive than [^{3}H]N-methylspiperone to detect changes in extracellular DA (Young et al., 1991). For radioligands sensitive to endogenous neurotransmitter release (e.g., [^{11}C]raclopride), estimates of DA activation in response to pharmacological challenges have not only proven to be generalizable across drugs with similar properties (Dewey et al., 1993), but also highly reproducible within individuals in repeated studies (Mawlawi et al., 2001; Volkow et al., 1993).

However, several studies produced radiotracer time-activity curves that did not closely model the rapid, pulsatile response expected from amphetamine-induced DA release. In fact, a study using [^{11}C]raclopride observed that striatal tissue concentrations of the radiotracer did not return to baseline levels for at least an hour following amphetamine administration, exceeding the expected time course of DA activation (Carson et al., 1997). An even greater persistence in altered DA D2 receptor BP following amphetamine was observed using SPECT and [^{123}I]IBZM, in this case extending to as long as two hours after drug administration (Laruelle et al., 1995). While such findings were significant in the development of new kinetic modeling techniques that incorporated microdialysis data into the models (Endres et al., 1997), they were also theoretically important in broadening the hypothesis about what caused the changes in radiotracer BP. Competition with the endogenous ligand represented one source of the observed reduction in receptor BP, given the dose-response relationships between the drug and the endogenous ligand (Breier et al., 1997). However, several other mechanisms have been proposed to account for reductions in radiotracer BP following a drug challenge, including receptor internalization or recycling (Chugani et al., 1988), though this process might be lim-

ited in the case of PET with raclopride (Laruelle, 2000). In addition, agonist radiotracers, such as [^{11}C]carfentanil (μ-opioid receptor) or [^{11}C]N-propyl-norapomorphine (DA D2 receptor), appear to be more sensitive to challenges releasing endogenous ligands than do antagonist tracers, such as [^{11}C]raclopride (Narendran et al., 2004, 2005). This mechanism appears to arise from the increased likelihood of an agonist to selectively label high affinity sites, and the conversion of these sites from high to low affinity following the release of excess endogenous neurotransmitter (Kenakin, 1997; Narendran et al., 2004; Sastre and Garcia-Sevilla, 1994).

Regardless of whether challenge-induced changes in the externally quantified BP values for a receptor site are due to one or multiple processes, interpretation difficulties do arise in the context of multiple studies within a particular individual. While within-subject designs are desirable to reduce the statistical noise due interindividual variability in the measures, residual effects may introduce an important confound. Furthermore, residual effects may conceivably vary between individuals depending on factors such as genetic variants or effects of disease processes. For some experimental designs, it would also be desirable to introduce control and challenge conditions in a randomized fashion and in close proximity to each other.

In the present report we examined the time-course of changes in μ-opioid and DA D2 receptor BP measures following a nonpharmacological challenge. In contrast to the data acquired after the administration of amphetamine with DA D2 radiotracers, we observe that the BP changes were no longer present in sequential scans.

The information presented therefore supports the use of appropriate nonpharmacological challenges as an alternative to the more frequently performed studies with pharmacological probes. These data, together with the existing literature, further suggest that differences may exist between the biological effects of pharmacological and nonpharmacological challenges used to stimulate neurotransmitter release in human functional neuroimaging experiments.

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