Behavioral/Systems/Cognitive

Variations in the Human Pain Stress Experience Mediated by Ventral and Dorsal Basal Ganglia Dopamine Activity

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In addition to its involvement in motor control and in encoding reward value, increasing evidence also implicates basal ganglia dopaminergic mechanisms in responses to stress and aversive stimuli. Basal ganglia dopamine (DA) neurotransmission may then respond to environmental events depending on their saliency, orienting the subsequent responses of the organism to both positive and negative stimuli. Here we examined the involvement of DA neurotransmission in the human response to pain, a robust physical and emotional stressor across species. Positron emission tomography with the DA D_2 receptor antagonist radiotracer [^{11}C]raclopride detected significant activation of DA release in dorsal and ventral regions of the basal ganglia of healthy volunteers. Activation of nigrostriatal (dorsal nucleus caudate and putamen) DA D_2 receptor-mediated neurotransmission was positively associated with individual variations in subjective ratings of sensory and affective qualities of the pain. In contrast, mesolimbic (nucleus accumbens) DA activation, which may impact on both D_2 and D_3 receptors, was exclusively associated with variations in the emotional responses of the individual during the pain challenge (increases in negative affect and fear ratings). These data demonstrate that basal ganglia dopamine D_2 receptor-mediated neurotransmission is involved in responses to pain and that it contributes to individual variations in the pain experience at the levels of physical and emotional elements, albeit with different neuroanatomical substrates.

Key words: **dopamine; saliency; reward; D_2 receptors; nigrostriatal; mesolimbic**

Introduction

Persistent pain represents a frequent condition and a leading cause of health and social care service. It is also a complex form of stress, with both physical and emotional elements contributing to individual variations in pain report and pain-associated disability (Stone et al., 2004; Harris et al., 2005; Sullivan et al., 2005). The understanding of neurobiological mechanisms contributing to that variability is therefore of considerable interest.

The present study examines the involvement of dopamine (DA) neurotransmission in responses to a pain stressor. At the level of mesolimbic, ventral tegmental projections to the nucleus accumbens, dopaminergic activity has been traditionally involved in the anticipation and response to natural rewards and drug reinforcers (Robinson and Berridge, 2000; Volkow et al., 2002; Nicola et al., 2005). In this context, mesolimbic DA cells are engaged in the encoding of both reward value and its uncertainty, to appropriately orient the responses of the organism (Fiorillo et al., 2003; Hsu et al., 2005; Tobler et al., 2005). However, this system also seems to respond to stressors and negative emotional states (Thierry et al., 1976; Horvitz, 2000; Pruessner et al., 2004). In the specific case of pain stimuli, it has been noted that prolonged, but not brief, pain induces the activation of nucleus accumbens DA release in rodents (Louilot et al., 1986; Schmidt et al., 2002). This may reflect the more stressful or emotionally salient characteristics of pain as it becomes temporally sustained (Stohler and Kowalski, 1999), but these processes have not been studied directly in humans.

Experiments in animal models have also suggested that DA activity in the nigrostriatal pathway is associated with pain suppression, an effect that appears mediated though DA D_2 receptors (Lin et al., 1981; Ben-Sreti et al., 1983; Morgan and Franklin, 1991; Altier and Stewart, 1999; Magnusson and Fisher, 2000). In this regard, the administration of levodopa, an indirect DA agonist, has been reported to reduce pain ratings in painful diabetic neuropathy in humans (Ertas et al., 1998). Contrary to these results, the systemic administration of DA D_2 receptor antagonists in humans has also been shown to reduce pain ratings in clinical trials (Dundee et al., 1963; Taub, 1973; Fields, 1989; Zitman et al., 1991). The existing information as to the involvement of DA mechanisms in pain is therefore conflicting from the perspective of both directionality (DA release being associated with pronociceptive or antinociceptive responses) and their localization (nigrostriatal or mesolimbic pathways).

To clarify these issues, we used positron emission tomography (PET) with [^{11}C]raclopride, a DA D_2 receptor radiotracer, for the quantification of the *in vivo* availability of these receptors during control states and during moderate levels of sustained pain in healthy subjects. Under these conditions, reductions in regional

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receptor availability for the radiotracer reflect DA release and DA activation of D_2 receptors (Laruelle et al., 1995; Endres et al., 1997) and will be referred to here as evidencing "activation" of DA D_2 neurotransmission. This measure of DA system activation was then related to the psychophysical pain descriptors provided by the subjects.

Materials and Methods

Subjects

Volunteers were 25 healthy, medication-free, right-handed men ($n = 18$) and women ($n = 7$) 27 ± 5 years of age, with an educational level of 17 ± 2 years. Subjects had no personal history of medical, psychiatric illness, or substance abuse or dependence and no family history of inheritable illnesses, ascertained by physical examination, personal and family history, and review of systems. The Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders DSM-IV nonpatient version (First et al., 1995) was used to rule out undiagnosed psychiatric illness and substance abuse. Volunteers did not take psychotropic medications or hormone treatments, including birth control in women, for at least 6 months, were nonsmokers, and did not exercise in excess of 1 h three times a week or were involved in competitive exercise. Women were studied in the midfollicular phase of the menstrual cycle (days 4–12 after initiation of menses). Written informed consent was obtained in all cases. All of the procedures used were approved by the institutional Investigational Review Boards and Radioactive Drug Research Committees.

Pain stress model

Receptor quantification with PET requires a relatively prolonged period of time for the acquisition of kinetic measures and the determination of specific binding. Here pain was maintained from 45 to 65 min after the administration of radiotracer by the infusion of medication-grade 5% hypertonic saline into the relaxed masseter (jaw) muscle via a computer-controlled closed-loop system, as described previously (Zhang et al., 1993; Stohler and Kowalski, 1999). Initially, the subject-specific parameters of the system for maintaining muscle pain are established. This consists of measuring each subject response to a standard bolus injection of 0.15 ml of hypertonic saline, infused over 15 s. A suitable infusion rate is then estimated by comparing the individual response with the mean response of 65 individuals exposed to the same bolus. Subsequently, the adaptive controller depends on feedback from the subjects. Subjects were required to report the present pain intensity every 15 s on an electronic version of 100 mm visual analog scale (VAS), with the lower and upper bound of the scale marked with numbers 0 and 100, representing the range from "no pain" to "the most pain intensity imaginable." Based on the VAS pain intensity scores provided by the subject every 15 s for the reminder of the experiment, individual infusion requirements were continuously modeled and updated to keep the present pain intensity scores in the target range (40 VAS intensity units; range, 35–45) for the full duration of the experiment (Stohler and Lund, 1995; Zubieta et al., 2001). Infusion volumes, required to maintain the pre-set pain intensity, were recorded every 15 s, and the cumulative infusion volume required over time was used as an indicator of subjects' pain sensitivity. Using this model, pain disappears 5–10 min after completion of the algesic infusion. To avoid swelling and possible tissue damage, the maximum infusion rate was limited to 250 μl/min.

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 VASs of pain intensity and unpleasantness. The internal emotional state of the volunteers was rated at the same time with the Positive and Negative Affectivity Scale (PANAS) (Watson et al., 1988). These overall measures of the subjective experience of pain stress were then used for correlational analyses with the individual neurotransmitter responses and baseline binding values.

Experiment 1. An initial pilot study was undertaken to examine whether evidence of activation of DA neurotransmission on D_2 receptors could be ascertained. Two PET studies with [^{11}C]raclopride were completed in eight male volunteers. One was performed without any intervention (baseline), and the other included the sustained pain stress challenge. Scan order was randomized and counterbalanced between subjects. Radiotracer administrations were separated by at least 2 h to allow for radiotracer decay and eliminate any possible residual effects of the preceding challenge. This study provided DA D_2 measurements at baseline and during pain stress using equilibrium analyses. The activation of DA D_2 neurotransmission was calculated as the reduction in regional DA D_2 receptor availability *in vivo* from baseline to pain stress.

Experiment 2. In view of the possible influences of anticipatory and attentional elements of pain stress that may not be specific to nociceptive signaling, a second series of studies was conducted in a larger sample of 10 men and 7 women. In these studies, a single PET study with [^{11}C]raclopride was acquired over 90 min using two different challenge conditions, as described previously (Zubieta et al., 2003b). Pain stress challenges were introduced for 20 min starting again at 45 min after tracer administration, as noted above, but were preceded by a saline control challenge using isotonic (0.9%) saline infused at the average rate required for the pain stress challenges. This condition is invariably not associated with pain (Stohler and Kowalski, 1999; Zubieta et al., 2001, 2002, 2003b). The saline control state was introduced 5 min after tracer administration during the same scanning session, and subjects rated pain intensity every 15 s in a manner identical to that of the actual pain challenges. Subjects were blind as to when the pain challenge was to take place and were instructed to expect pain during the rating periods. The saline control challenge was followed by a 15 min resting period. The last 10 min of this resting period were used to obtain estimates of baseline binding, as described previously (Carson et al., 1997; Watanabe et al., 2000), in the absence of pain expectation. Masseter muscle needles remained in location during the entire scanning period, including saline control, resting, and pain stress periods. Baseline to pain stress binding potential (BP) changes were assessed in a manner identical to experiment 1, for replication purposes. In addition, Logan plot graphical analyses were used to estimate BP values during early (saline control) and late (pain stress) scan phases for subsequent subtraction of total and nonspecific components of pain stress.

Neuroimaging methods

Anatomical magnetic resonance imaging (MRI) scans were acquired on a 1.5 tesla scanner (Signa; General Electric, Milwaukee, WI). Acquisition sequences were axial spoiled gradient-recalled acquisition in a steady state inverse recovery prepared MR [echo time (TE), 5.5 ms; repetition time (TR), 14; inversion time, 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 ms; TE, 20 and 100 ms, respectively; NEX, 1; 62 contiguous images, 3 mm thick) to rule out intracranial pathology.

PET scans were acquired with a Siemens (Knoxville, TN) HR$^+$ scanner in three-dimensional mode [reconstructed full-width at half-maximum (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 using the orbitomeatal line aided by the scanner gantry laser lights, and two intravenous (antecubital) lines were placed. A light forehead restraint was used to eliminate intrascan head movement. [^{11}C]raclopride was synthesized at high specific activity (>2000 Ci/mmol) by the reaction of *O*-desmethyl raclopride with ^{11}C-methyl triflate; 10–15 mCi were administered in each of the two scans. Fifty percent of the [^{11}C]raclopride dose was administered as a bolus, and the remaining 50% was administered by continuous infusion for the remainder of the study. Under these conditions, equilibrium conditions are achieved after 35 min after tracer administration (Carson et al., 1997). Twenty-eight frames of images were acquired over 90 with an increasing duration (30 s up to 10 min). The total mass of raclopride injected was 0.089 ± 0.047 μg/kg per scan, ensuring that the compound was administered in tracer quantities, i.e., subpharmacological doses.

Images were reconstructed using iterative algorithms (brain mode; Fourier rebinning with ordered subsets-expectation maximization, 4 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 before the

PET study, also with iterative reconstruction of the blank/transmission data, followed by segmentation of the attenuation image. Small head motions between emission scan frames were corrected by an automated computer algorithm for each subject before analysis, and the images were coregistered to each other with the same software (Minoshima et al., 1993). 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 separate sets of parametric maps. In all cases (experiments 1 and 2), these included (1) a tracer transport measure (K_1 ratio), and (2) a receptor-related measure at equilibrium (DVeq), the latter using data obtained from the 45–90 min after tracer administration. The DVeq measure was obtained using the ratio of brain activity to activity in the cerebellum (Carson et al., 1997; Watanabe et al., 2000); f_2B_{max}/K_d (or DVeq − 1) was the "receptor related" measure (DA D_2 receptor availability, BP; 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). In the case of experiment 2, baseline BP values were obtained from 35–45 min after tracer administration using identical methods. In addition, and in experiment 2, the tracer transport and binding measures were also calculated using a Logan plot analysis (Logan et al., 1996) using the cerebellar cortex as the reference region. With the tracer administration protocol used, the Logan plot becomes linear by 4–5 min after the start of radiotracer administration, with its slope being the distribution volume ratio (DVR), a measure equal to (f_2B_{max}/K_d) + 1. The Logan analyses permitted the calculation of BP during the saline control condition (pain is expected and not received, pain is rated every 15 s as during the actual pain challenges) and used data from 5 to 35 min after tracer administration. To ensure that no systematic biases would be present when comparing early and late scan periods with Logan plots, we examined the baseline studies in the eight subjects from experiment 1. No significant differences in DA D_2 binding measures from 5–35 and 45–90 min were observed with Statistical Parametric Mapping SPM2 software (Wellcome Department of Cognitive Neurology, University College London, London, UK) up to a threshold of $p = 0.01$ uncorrected for multiple comparisons.

Raclopride is an antagonist at the level of both D_2 and D_3 receptors. In the dorsal basal ganglia, this radiotracer only binds to D_2 receptors, whereas in the ventral basal ganglia, one-third of the signal may be contributed by D_3 receptors (Seeman et al., 2006). Findings in dorsal regions will then be referred to as reflecting DA D_2 neurotransmission, and DA D_2/D_3 in ventral areas.

K_1 and BP images for each experimental period and magnetic resonance images were coregistered to each other and to the International Consortium for Brain Mapping (ICBM) stereotactic atlas orientation (Meyer et al., 1997). The accuracy of coregistration and nonlinear warping algorithms was confirmed for each subject individually by comparing the transformed MRI and PET images with each other and the ICBM atlas template.

Image data analysis

Differences between conditions were mapped into stereotactic space using *t* maps of statistical significance and random effects with SPM2 and Matlab software (MathWorks, Natick, MA), 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 f_2B_{max}/K_d estimates. Only regions with specific DA D_2/D_3 receptor BP were included in the analyses (voxels with DVR values >1.2 times the mean global image as calculated with SPM2). To compensate for small residual anatomic variations across subjects and to improve signal-to-noise ratios, a three-dimensional Gaussian filter (FWHM of 6 mm) was applied to each scan.

Significant effects were detected using a statistical threshold that controls a type I error rate at $p = 0.05$ after correction for multiple comparisons. These were estimated using the Euler characteristic (Worsley et al., 1992) based on the number of voxels in the gray matter and image smoothness and the extent of local changes (correction for cluster volume) (Friston et al., 1991). Numerical values for the changes in DA D_2/D_3 binding, as well as for the calculation of correlations and *r* values, were extracted from the image data by averaging the values of voxels contained in an area in which significant effects were obtained in the voxel-by-voxel analyses, down to a threshold of $p = 0.01$.

Results

Psychophysics

The pain maintenance system resulted in average 0–100 VAS pain intensity ratings, acquired every 15 s for the 20 min challenge, of 34 ± 13 units across the 25 subjects studied. The infusion volume of algesic substance (intramuscular 5% hypertonic saline) required for the maintenance of pain at the target level, an objective measure of pain tolerance of which the subjects were not aware, averaged 1.5 ± 0.8 ml. Ratings acquired at the completion of the pain challenge averaged 41 ± 18 VAS units for pain intensity and 42 ± 22 for pain unpleasantness. MPQ total ratings of pain were 21.9 ± 10.1, MPQ sensory subscale scores were 13.8 ± 6.0, and MPQ pain affect subscale scores were 1.8 ± 2.6. The PANAS, evaluating the internal negative affective state of the volunteers, obtained before and after pain stress scans, averaged 3.2 ± 4.4 and 5.4 ± 5.7, respectively (paired *t* test; df = 24; $t = 2.40$; $p < 0.03$).

DA D_2 system activation during pain stress compared with baseline

In an initial pilot study, eight male volunteers were studied under baseline (no intervention) and sustained pain stress conditions in two separate PET studies to ascertain the feasibility of detecting enhancements in DA D_2 neurotransmission with pain stress (ascertained as reductions in DA D_2 BP). Pain challenges were introduced in the left masseter muscle.

Voxel-by-voxel *t* tests using SPM2 and the ICBM stereotactic coordinate system demonstrated significant pain stress-induced activation of DA neurotransmission in the basal ganglia. Significant effects, after correction for multiple comparisons, were obtained in the putamen, bilaterally [left, (all coordinates are *x*, *y*, *z*, shown in mm throughout), −26, 4, 9; $z = 7.73$, $p < 0.0001$; right, 30, 4, 1; $z = 4.52$, $p < 0.05$], the caudate nucleus, bilaterally (left, −14, 4, 18; $z = 6.57$, $p < 0.0001$; right, 16, 13, 13; $z = 5.28$, $p < 0.01$), as well as in the right (contralateral to pain) nucleus accumbens (coordinates, 11, 16, 5; $z = 5.34$, $p < 0.01$). The average percentage reductions in the *in vivo* DA D_2 receptor availability in these regions were 16 and 13% in left and right putamen, respectively, 19 and 12% in left and right caudate, respectively, and 12% in the right nucleus accumbens. which in the latter region may reflect both D_2 and D_3 receptor signal. These are substantial changes comparable with or superior to those observed in response to pharmacological challenges known to induce massive release of DA into synaptic and extracellular spaces (Martinez et al., 2003; Narendran et al., 2004).

Receptor availability data were then extracted for the regions above and then compared with pain ratings and a measure of pain tolerance. The objective measure of sustained pain tolerance used (the total volume of algesic stimulus required to maintain the pain for each subject) was inversely correlated with the activation of DA D_2 neurotransmission in the right caudate ($r = -0.70$; $p < 0.05$).

Positive correlations were obtained for MPQ total ($r = 0.74$; $p = 0.04$) and MPQ sensory subscale ($r = 0.74$; $p = 0.04$) scores, VAS intensity ($r = 0.84$; $p < 0.01$) and VAS unpleasantness ($r = 0.84$; $p < 0.01$), and DA activation in the right caudate. Right caudate activation was also positively correlated with the increase in negative PANAS affect scores from baseline to pain stress conditions ($r = 0.88$; $p < 0.005$), with a trend in the same direction for the right nucleus accumbens ($r = 0.68$; $p = 0.06$).

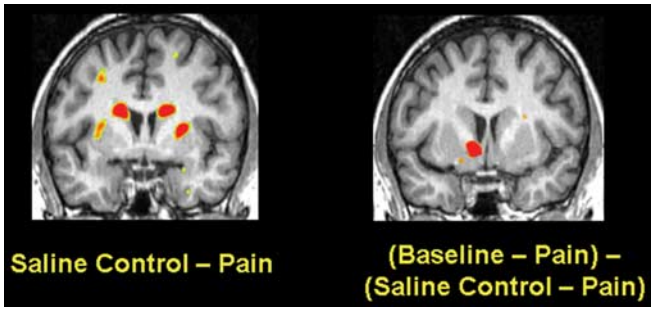

Figure 1. Localization of DA D_2 receptor activation during pain in humans. Areas of significant activation of DA D_2 neurotransmission during pain, superimposed over an anatomical MRI in coronal views. The left image shows the activation attributable to pain signal (pain − saline control subtraction), localized in the dorsal nucleus caudate and putamen. The magnitude of activation is correlated with sensory and pain affect ratings of the pain. The right image depicts the signal remaining from the activation of DA D_2 neurotransmission during pain after pain-specific elements are subtracted: (baseline − pain) − (saline control − pain). The latter is localized in the nucleus accumbens region and associated with the internal negative affective state experienced during pain.

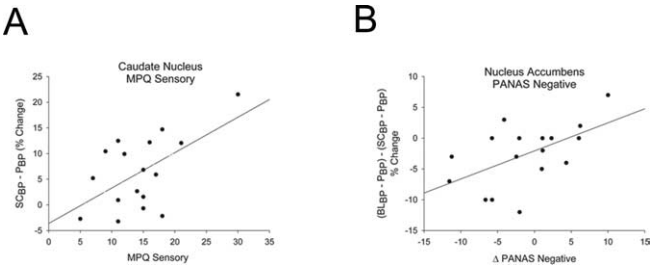

Figure 2. Correlations between activation of DA D_2 neurotransmission during pain and the volunteer's subjective ratings. ***A***, Significant correlation between the pain-specific activation of dorsal caudate DA D_2 neurotransmission (percentage reductions in D_2 receptor BP from saline control to pain) ($SC_{BP} - P_{BP}$ % Change) and MPQ sensory subscale scores ratings ($r = 0.57$; $p < 0.05$). ***B***, Significant correlation between the activation of nucleus accumbens DA D_2 neurotransmission during nonspecific elements of pain stress [(baseline − pain) − (saline control − pain subtraction), shown as ($BL_{BP} - P_{BP}$) − ($SC_{BP} - P_{BP}$) on the figure] and enhancements in the negative affective state of the volunteers during pain as measured by the PANAS negative affect subscale ratings (Δ PANAS Negative) ($r = 0.64$; $p < 0.01$).

DA D_2 system activation during pain stress, controlling for pain nonspecific components

In the studies above, we could not examine the contribution of elements not specific to pain (e.g., directed attention to ratings, pain expectation, and needle placement) when comparing the data with a baseline study without intervention. In a second series, a larger sample of 17 subjects was studied under identical conditions of sustained pain stress, administered 45 min after tracer administration but using a saline control state. The latter involved the infusion of nonpainful isotonic saline and every 15 s ratings of pain in a manner identical to that of the actual pain challenge with expectation that pain may take place. A 15 min resting period (baseline, no pain expectation) preceded the pain stress challenge.

Changes in DA receptor availability from baseline (needle in place, no pain expectation) to pain stress states were observed in two large, bilateral clusters of the basal ganglia, incorporating both dorsal caudate and putamen and the right nucleus accumbens (left cluster, peak coordinates, −15, 14, 15; cluster size, 3174 mm^3; $z = 9.20$; $p < 0.0001$ after correction for multiple comparisons; right cluster peak coordinates, 16, 19, 6; cluster size, 4054 mm^3; $z = 9.47$; $p < 0.0001$ after correction for multiple comparisons). These findings replicated those obtained in experiment 1 but using a fixed baseline (pain order and a single scanning period).

Subtraction of saline control and pain stress data (DA responses controlling for pain nonspecific elements, such as ratings and pain expectation) showed gains in DA activity that were exclusively restricted to the dorsal caudate (bilaterally, left, −15, 4, 20; $z = 6.6$; $p < 0.0001$; right, 17, 2, 20; $z = 6.9$; $p < 0.0001$) and putamen (bilaterally, left, −26, 4, 8; $z = 6.1$; $p < 0.0001$; right, 27, 11, 9; $z = 7.5$; $p < 0.0001$) (Fig. 1). The average percentage reductions in DA D_2 receptor availability from saline control to pain stress conditions were 11% in left and right caudate and 6 and 12% in the left and right putamen, respectively. No significant DA activation was observed in the nucleus accumbens.

For the regions identified here, positive correlations were obtained between DA D_2 system activation in the right caudate and right putamen and MPQ total scores (r values = 0.62 and 0.63, respectively; $p < 0.01$) and MPQ sensory (r values = 0.57 and 0.53; $p < 0.05$), and MPQ pain affect (r values = 0.66 and 0.67; $p < 0.005$) subscale scores (Fig. 2*A*). No significant correlations between regional DA D_2 system activation and changes in PANAS negative affect scores were obtained.

DA D_2/D_3 activation in the nucleus accumbens and the subjective experience of pain stress

We then examined the relevance of nucleus accumbens DA D_2/D_3 activity to the subjective experience of pain stress, by comparing the data from baseline pain stress and saline control pain stress analyses in the larger sample of 17 subjects. The difference between those two contrasts delineated an area of DA activity localized in the right nucleus accumbens (extending into the ventral area of the caudate, contralateral to pain; coordinates, 12, 16, 0; cluster size, 954 mm^3; $z = 7.1$; $p < 0.0001$ after correction for multiple comparisons) (Fig. 1) that could not be accounted for by the perception of pain itself. Activation of DA D_2/D_3 neurotransmission in this region was positively correlated with pain-associated increases in PANAS ratings of negative affect ($r = 0.64$; $p < 0.01$) (Fig. 2*B*) and fear ($r = 0.61$; $p < 0.01$). No significant correlations were obtained between nucleus accumbens DA D_2/D_3 system activation and pain-specific ratings.

Baseline DA D_2 receptor availability and pain psychophysics

Resting levels of DA D_2 receptor BP in the putamen have been associated with cold and heat pain thresholds in healthy volunteer samples (Hagelberg et al., 2002; Pertovaara et al., 2004; Martikainen et al., 2005). We examined whether similar relationships between baseline DA D_2 binding measures of pain sensitivity would be obtained in the 25 subjects scanned. VAS pain intensity ratings to the standardized 150 μl of bolus of hypertonic saline that initiated the pain challenge (acute pain sensitivity) were positively correlated with right putamen DA D_2 BP ($r = 0.44$; $p < 0.05$). The total infusion of hypertonic saline required for pain maintenance, a measure of sustained pain tolerance, was negatively correlated with DA D_2 BP in the same region ($r = -0.42$; $p < 0.05$). These data are then consistent with that published by others in both the directionality and localization on this effect.

Discussion

The present work demonstrates the involvement of DA receptor-mediated neurotransmission in the human responses to sustained pain stress, as measured by [^{11}C]raclopride and PET. This radiotracer labels D_2 receptors in the dorsal basal ganglia, whereas in ventral regions, approximately one-third of its bind-

ing may reflect D_3 receptor populations (Seeman et al., 2006). We ascertained the activation of DA release in dorsal (nigrostriatal) and ventral (mesolimbic) basal ganglia circuits. Furthermore, the gain in DA release was positively correlated with sensory and pain affect ratings and with the negative affect experienced by the volunteers. Nigrostriatal DA D_2 system activation was associated exclusively with ratings of sensory and affective qualities of the pain, whereas mesolimbic DA D_2/D_3 activity was related to the increase in negative affective state and fear ratings of the volunteers during the challenge.

Dopaminergic neurons in the mesolimbic system increase their firing and the release of DA during the receipt and anticipation of rewards (Robbins and Everitt, 1996; Waelti et al., 2001; Berridge and Robinson, 2003). This mechanism is thought to underlie the reinforcing effects of both natural rewards and drugs of abuse and to mediate incentive-motivational mechanisms in goal-directed behaviors (Wise, 2004; Schultz, 2006). However, an emerging preclinical literature indicates that mesolimbic DA is also involved in responses to aversive stimuli (Thierry et al., 1976; Louilot et al., 1986; Horvitz, 2000). This has led to the hypothesis that this neurotransmitter system is more generally involved in encoding and responding to salient stimuli, regardless of their valence. Initial neuroimaging studies in humans seem to support this view. For example, dorsal and ventral basal ganglia blood flow, as measured with functional MRI, increases during gambling tasks when there is anticipation of both monetary gain and loss (Knutson et al., 2000) and in response to the presence of nonrewarding, infrequent, and therefore more salient, distractors (Zink et al., 2003). Activation of DA D_2/D_3 neurotransmission in the ventral striatum has also been shown during a psychological stress task using molecular imaging techniques similar to those of the present work (Pruessner et al., 2004).

We observed DA release and DA D_2/D_3 receptor activation in the ventral basal ganglia (nucleus accumbens and ventral portion of the caudate nucleus) in analyses when the receptor availability in the pain stress state was compared with a baseline state. Conversely, these effects were no longer significant in comparisons in which the control state incorporated the expectation of pain receipt, suggesting that anticipatory elements accounted for a substantial proportion of DA activation in this region. This is consistent with the involvement of the mesolimbic dopaminergic system in anticipatory responses, albeit typically studied in the context of predicted reward (for review, see Wise, 2004; Schultz, 2006). More relevant to the understanding of individual variations in the experience of pain stress, the gains in nucleus accumbens DA activity were positively associated with the enhancement in negative affect and fear ratings during pain stress. This finding is an important step in understanding the dissociation between physical and emotional responses to pain stress in humans and the known interindividual variations in ratings of pain and emotional state in clinical samples (Giesecke et al., 2003; Harris et al., 2005).

These data contrast with that of some animal models, whereby the inactivation of the nucleus accumbens core by the administration of an analgesic agent has been associated with hyperalgesic responses to Formalin (Magnusson and Martin, 2002). In the rodent, evidence of DA release in the nucleus accumbens has been shown for prolonged, but not brief, pain challenges (Louilot et al., 1986; Schmidt et al., 2002), an effect that was associated with reductions in pain behavior. However, these reductions in pain behavior were blocked by an opioid receptor antagonist, suggesting their mediation by the activation of downstream endogenous opioid systems (Gear et al., 1999; Schmidt et al., 2002). This interpretation is supported by microdialysis studies in rodents demonstrating the release of endogenous opioid peptides in the nucleus accumbens by drugs activating DA neurotransmission (Olive et al., 2001).

The second circuit implicated in pain-induced DA release, the nigrostriatal pathway, has been typically involved in motor functions, particularly the initiation of motor responses. However, dorsal areas of the striatum also receive projections from somatosensory cortex (Haber et al., 2000). Initial reports have described increases in acute thermal pain sensitivity after the destruction of substantia nigra neuronal bodies and reductions during its stimulation and enhancement in striatal DA release (Lin et al., 1981). DA-depleting neurotoxins have also been shown to reduce morphine- and amphetamine-induced analgesia in the Formalin rodent model. Increases in pain thresholds have also been described after DA D_2 receptor agonist administration, systemically or directly in the dorsal striatum (Altier and Stewart, 1999; Magnusson and Fisher, 2000). However, the opposite effects have also been shown in some animal models (e.g., visceral pain, deafferentization pain), in which the systemic and intracerebroventricular administration of DA D_2 antagonists was associated with antinociceptive effects (Lyerly et al., 1988; Frussa-Filho et al., 1996; Weizman et al., 2003). These results were also similar to those of human clinical trials with DA D_2 receptor antagonists (Fields, 1989; Zitman et al., 1991). Finally, higher pain thresholds have been described in DA D_2 receptor knock-out mice for some pain models (Mansikka et al., 2005), as well as enhancements of morphine-induced analgesia (King et al., 2001). The existing literature is therefore conflicting and further confounded by the known interactions between DA and opioid circuits (Chen et al., 1993; Unterwald, 2001; Zubieta et al., 2003a).

The present work demonstrates gains in DA neurotransmission in dorsal (nigrostriatal) basal ganglia terminal fields during a painful state in humans. As was observed with the ventral, mesolimbic regions, increases in DA activity in these areas were associated with a more pronounced subjective experience. However, in the case of the dorsal caudate and putamen, DA activation was exclusively correlated the individual ratings of the sensory and unpleasant qualities of the pain, as defined by the McGill Pain Questionnaire word descriptors, and not with the emotional state of the volunteers during the challenge.

Last, we replicate findings by another group describing negative relationships between basal DA D_2 receptor availability in the putamen and phasic and tonic pain thresholds in healthy volunteers (Hagelberg et al., 2002; Pertovaara et al., 2004; Martikainen et al., 2005). Consistent with these data, we observed a positive correlation between putamen DA D_2 receptor BP measures and ratings of pain intensity to a standard, acute pain stimulus and negatively with a measure of sustained pain tolerance in the pain model used. That same group has reported reductions in presynaptic dopamine turnover, as measured with [^{11}C]fluorodopa and PET, in patients diagnosed with idiopathic mouth-burning syndrome (Jääskeläinen et al., 2001). However, these findings were not replicated in a study of atypical facial pain patients (Hagelberg et al., 2003).

This report is the first demonstration of DA D_2 system activation in response to pain stress in humans, involving both dorsal and ventral basal ganglia regions, albeit with different psychophysical implications. Dorsal caudate and putamen DA activity were associated with the subjective description of pain severity. Examination of individual variations in DA function in persistent pain conditions, which are both frequent and oftentimes disabling and difficult to treat, appears justified in view of the

present findings. These data also indicate that DA mechanisms may be critical to explain the increasingly recognized individual differences in pain report in clinical samples (Harris et al., 2005) and, by extension, their analgesic requirements. At a more immediately practical level, it also points to the possible utility of pharmaceuticals interacting with these receptors in the treatment of pain. These are broadly available agents that may open new avenues of treatment for persistent painful conditions.

The gain in DA release in mesolimbic, nucleus accumbens terminal fields, conversely, was not specific to the physical experience of pain stress. Variations in nucleus accumbens DA activity in humans corresponded to more prominent negative emotional states during the experience of pain stress. Negative emotional states, in turn, have been associated with both more distress and poorer clinical outcomes in clinical samples, including comorbidity with other disorders (Sullivan et al., 2005; Compton and Volkow, 2006). In this regard, the baseline concentration of DA D_2/D_3 receptors and gains in DA activity in the nucleus accumbens have been associated with the reinforcing effects of drugs of abuse (Volkow et al., 2004). Ventral basal ganglia DA is also thought to mediate the effects of stress to increase the likelihood of initiation and recurrence of drugs abuse (Robinson and Berridge, 2003). Variations in risk for drug abuse after initial exposures could then be mediated by individual differences in the response of this neurotransmitter system to pain, itself a physical and emotional stressor. Substantial comorbidity also exists between clinical pain syndromes and mood disorders, the neurobiology of which has been very poorly explored. Here we show that ventral basal ganglia DA D_2/D_3 system function may represent an important point of interaction between the neurobiologies of emotion, reward, and pain regulation.

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