Methods

Subjects
Detailed information on drug abuse was obtained. In order to ascertain that drug-induced activation of dopaminergic neurotransmission did not interfere with the experiments, all subjects had to produce a urine sample negative for THC, cocaine, morphine, amphetamine, metamphetamine, PCP, methadone, barbiturates, benzodiazepines, and tricyclics and their metabolites (Rapid Check®, Craig Medical Inc., Vista, U.S.A.) before being considered for study inclusion.

General experimental procedure
In order to increase the credibility of the experiment the following procedures were adopted. (a) Explanation on how pain signals are generated in the muscle by infusion of hypertonic saline and that the analgesic cream penetrates the skin and blocks the pain signals where they are generated, i.e. in the muscle. (b) Subjects were told that the experiment followed an ‘open-trial’ or ‘open-label’ design, in which both the experimenter and the subject know which cream is administered. They were further told that this is common practice in early drug development before larger doubled-blinded trials are conducted. (c) Subjects were given a questionnaire after the manipulation session (adapted from (Borkovec and Nau, 1972)) and were told that the purpose of the questionnaire was to evaluate the effectiveness of the cream. This method has been used in previous placebo research (Watson et al., 2006).

Manipulation session
The study was explained in detail to the participants using scripted instructions. Subjects were told that they would receive two intramuscular infusions of hypertonic (5.5 %) saline, one after application of an analgesic cream and one after application of a control cream to control for the moisturizing effects of the analgesic cream. In fact, the two creams were identical and pain was surreptitiously reduced in the placebo condition by administering a lower concentration saline solution (2.6%). This ‘conditioning’ procedure enhances the effectiveness of placebos and has been successfully used in previous studies of placebo analgesia (Price et al., 1999; Wager et al., 2004; Bingel et al., 2006). In the manipulation session, all subjects underwent first the infusion that was paired with the control cream. For the second infusion, paired with the ‘analgesic cream’, the lower concentration saline solution was used.

Hypertonic saline infusion
After insertion of a 25 Gauge needle into the anterior tibialis muscle of the left or right leg (randomized and counter-balanced across subjects), the respective cream was applied to a 5 cm x 10 cm area around the needle and covered with a Tegaderm® patch. After 20 minutes (‘to allow absorption of the cream’), the hypertonic saline infusion was commenced. Each infusion started with a bolus (30 ml/h) during minute 1, followed by a constant infusion (10 ml/h) during minutes 2-6, a second bolus was given at minute 7 (30 ml/h), followed by a constant infusion of 10 ml/h during minutes 8-20. We have shown previously that this infusion protocol produces moderate to strong pain in healthy young males reliably (Wood et al., 2007). After a short break, a 25 Gauge needle was inserted in the anterior tibialis muscle of the contralateral leg, the other cream was applied in the
same way as before and the infusion was started after the 20-min absorption period.

Assessment of personality traits
We assessed traits for which strong evidence from tracer studies, biochemical challenge tests, or genetic studies exists that they are related to dopaminergic neurotransmission (Carver and White, 1994; Menza et al., 1995; Kaasinen et al., 2001; Suhara et al., 2001; Kaasinen et al., 2004; Cohen et al., 2005; Netter, 2006).

Voxel-based morphometry (VBM) of MRI data
Standard image analysis procedures for gray matter density were applied. Each image underwent automated correction for intensity non-uniformity and intensity standardization by normalizing gray-level intensities to a common scale (Sled et al., 1998). The corresponding MRI volumes were spatially normalized to a widely used T1-weighted MRI template in stereotaxic space, the Montreal Neurological Institute/International Consortium for Brain Mapping (MNI/ICBM) 152 standard, to adjust for differences in total brain volume and brain orientation. Linear transformations were used to align individual images to the template in order to preserve cerebral asymmetries (Collins et al., 1994).

Classification of brain tissue into gray matter, white matter, cerebrospinal fluid (CSF) and background was performed by means of an advanced neural net classifier (Zijdenbos et al. 2002, labeling each voxel based on the MRI signal. Voxels with an effect of insufficient image resolution leading to a mixing of different tissue types were corrected with a partial volume estimation algorithm, which classified a voxel continuously rather than discretely (Kim et al., 2005). Tissue classification was used to extract each tissue type as a binary map; the numbers of voxels in a tissue type provided the total volume of the tissue itself within the binary map. After tissue classification, the skull, brainstem, cerebellum, and dura were removed from further analysis.

Data smoothing was used to convert the binary data into a range of continuous data and to reduce the effect of individual variation in the exact location of gyri and sulci (Watkins et al., 2001). Images were smoothed with an isotropic Gaussian kernel of 10 mm FWHM in order to reduce false positives occurring at smaller smoothing values (Salmond et al., 2002). These smoothed images were used as the 3D-maps for gray matter density, measured from the intensity of the image within each voxel.

Results
Personality traits
Average scores (SD) were as follows. Novelty seeking: 23.6 (6.0), harm avoidance: 9.2 (7.3), BAS drive: 12.2 (2.5), BAS fun seeking: 13.6 (2.0), BAS reward responsiveness: 17.9 (1.8). The average scores in our sample were a bit higher (and accordingly, lower for harm avoidance) than published normative data (Cloninger et al., 1993; Carver and White, 1994), maybe because our subjects were younger (mean age of 22 years versus 34 years). Novelty seeking seems indeed to be age dependent (Cloninger, 1994). Alternatively, there could be a selection bias regarding subjects that volunteer for pain studies.
Principal component analysis with Varimax rotation was used for data reduction of the five assessed personality traits. All variables loaded onto one component (harm avoidance loaded negatively, in accordance with previous literature showing an inverse relationship with dopaminergic neurotransmission) (Table S1), confirming that they are measuring related traits. Each individual’s factor score of this component was used for subsequent analyses as the ‘dopamine-related trait variable’.

Table S1
Component Matrix\(^{(a)}\)

<table>
<thead>
<tr>
<th>Component</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCI_NS</td>
<td>.820</td>
</tr>
<tr>
<td>TCI_HA</td>
<td>-.745</td>
</tr>
<tr>
<td>BAS_Drive</td>
<td>.855</td>
</tr>
<tr>
<td>BAS_Fun</td>
<td>.887</td>
</tr>
<tr>
<td>BAS_RR</td>
<td>.437</td>
</tr>
</tbody>
</table>

Extraction Method: Principal Component Analysis
\(^{(a)}\) 1 component extracted

Table S2: Correlations between individual personality traits and placebo analgesic response

<table>
<thead>
<tr>
<th>Personality Trait</th>
<th>Placebo analgesic response r (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Novelty seeking (TCI)</td>
<td>0.53 (0.01)</td>
</tr>
<tr>
<td>Harm avoidance (TCI)</td>
<td>0.33 (0.13)</td>
</tr>
<tr>
<td>Behavioral drive (BAS)</td>
<td>0.52 (0.014)</td>
</tr>
<tr>
<td>Fun seeking (BAS)</td>
<td>0.62 (0.002)</td>
</tr>
<tr>
<td>Reward responsiveness (BAS)</td>
<td>0.29 (0.2)</td>
</tr>
</tbody>
</table>

Correlations were computed using Pearson’s Correlation Coefficient. TCI, Temperament and Character Inventory; BAS, Behavioral Appetitive System.

References


