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Abstract

The size of human social networks significantly exceeds the network that can be maintained by social grooming or touching in other primates. It has been proposed that endogenous opioid release following social laughter would provide a neurochemical pathway supporting long-term relationships in humans (Dunbar, 2012) yet this hypothesis currently lacks direct neurophysiological support. We used positron emission tomography (PET) and μ-opioid-receptor (MOR) specific ligand [11C]carfentanil to quantify laughter-induced endogenous opioid release in 12 healthy males. Before the social laughter scan, the subjects watched with their close friends laughter-inducing comedy clips for 30 min. Before the baseline scan, subjects spent 30 min alone in the testing room. Social laughter increased pleasurable sensations and triggered endogenous opioid release in thalamus, caudate nucleus, and anterior insula. In addition, baseline MOR availability in the cingulate and orbitofrontal cortices was associated with the rate of social laughter. In a behavioral control experiment, pain threshold – a proxy of endogenous opioidergic activation – was elevated significantly more in both male and female volunteers after watching laughter-inducing comedy vs. non-laughter inducing drama in groups. Modulation of the opioidergic activity by social laughter may be an important neurochemical pathway that supports formation, reinforcement, and maintenance of social bonds between humans.

Significance statement

Social contacts are of prime importance to humans. The size of human social networks significantly exceeds the network that can be maintained by social grooming in other primates. Here we used positron emission tomography to show that endogenous opioid release following social laughter may provide a neurochemical mechanism supporting long-term relationships in humans. Participants were scanned twice; following 30-minute social laughter session, and after spending 30 minutes alone in the testing room (baseline). Endogenous opioid release was stronger following laughter versus baseline scan. Opioid receptor density in the frontal cortex predicted social laughter rates, Modulation of the opioidergic activity by social laughter may be an important neurochemical mechanism reinforcing and maintaining social bonds between humans.

Introduction

Humans and other primates use social touching or grooming for reinforcing social structures (Dunbar and Shultz, 2010; Suvilehto et al., 2015). Because blockade of opioid receptors stimulates grooming and social behaviour in primates (Meller et al., 1980; Fabre-Nys et al., 1982) it has been proposed that touching-dependent modulation of the μ-opioid-receptor (MOR) system might support maintenance of social bonds. Yet, because the size of human social networks exceeds the network that can be maintained by dyadic social touching (Dunbar, 2012), it has been proposed that other means such as social laughter, have evolved to release endogenous opioids system just as grooming does. Because social laughter could allow simultaneous opioid release among all the members of an interacting group, it might play a critical role in enabling humans to live in exceptionally large social networks (Dunbar, 2012). However, the neurochemical basis of human social laughter remain poorly understood.

The μ-opioid receptors (MORs) mediate the effects of endogenous and exogenous opioids contributing to the rewarding effects of food and drugs (Henriksen and Willoch, 2008; Nummenmaa and Tuominen, in press). Endogenous opioids modulate prosocial behaviour in polygamous rodents (Panksepp et al., 1980) and MOR-gene-knockout mice pups display deficits in attachment behaviour (Moles et al., 2004). Rhesus infants carrying a gain-of-function OPRM1 77G allele experience increased reward from maternal contact and display increased measures of attachment (Barr et al., 2008) while in humans, A118G polymorphism of the OPRM1 is associated with enhanced dispositional and neural sensitivity to social rejection (Way et al., 2009). In line with these findings, elevated cerebral MOR availability is associated with secure social-attachment behavior in humans (Nummenmaa et al., 2015). Finally, opioid receptor antagonists increase the frequency of grooming (Fabre-Nys et al., 1982) and grooming solicitations (Keverne et al., 1989) in primates, adding to the evidence that specifically the opioid system may underlie social bonding.

Grooming-based social bonding imposes severe constraints on the maximum possible size of social groups (Dunbar, 1991). Consequently, ecological pressures demanding larger group sizes have led to evolution of more effective mechanisms for facilitating social bonding (Dunbar, 2008). Laughter is a universally-recognized expression of positive social emotion, occurring most frequently in human social interactions (Sauter et al., 2010; Scott et al., 2015) but also present in nonhuman primates (Preuschoft, 1992; Ross et al., 2009).

Humans volitionally use laughter as an expression of prosociality, possibly for bonding purposes (Scott et al., 2015) and shared sense of humour is indeed a strong predictor of affiliation and altruism (Curry and Dunbar, 2013). Because laughter is highly contagious (Provine, 2004) it would allow MOR responses to spread throughout the interacting group to increase the effectiveness of this type of 'vocal grooming' (Dunbar, 2012).

Here we tested directly whether social laughter results in cerebral opioid release, as quantified with *in vivo* positron emission tomography (PET). Measures of MOR availability were acquired with the MOR-specific ligand [^{11}C]carfentanil during two separate sessions: social laughter and a neutral baseline condition. Under this experimental design, endogenous opioid release would be manifested as lower [^{11}C]carfentanil binding potential (BP_{ND}) in the social laughter vs. baseline condition. Building on animal research on grooming-dependent MOR activation, we expected to see significantly increased MOR activation in the social laughter condition.

Materials methods

Subjects and self-reports

The study protocol was approved by the ethics board of the Hospital District of Southwest Finland, and the study was conducted in accordance with the Declaration of Helsinki. Twelve healthy male adults (age range 20–32, M_{age} = 22.9 years, SD_{age} = 3.26) volunteered for the study. Only young males were scanned, because age and sex influence both MOR availability and the capacity to engage the MOR system (Gabilondo et al., 1995; Zubieta et al., 1999). In addition to standard PET and MRI exclusion criteria, subjects were excluded if they met any of the following criteria: poor compliance, smoking, excessive alcohol consumption (> 8 units/week), use of illicit drugs, current medication affecting the central nervous system, or a history of or current neurological or psychiatric disease confirmed using the structured clinical interview for DSM-IV, medical history, and blood tests. Subjects were compensated for their time and travel costs, and they signed ethics-committee-approved informed-consent forms. Subjects reported their mood (sleepiness, happiness, tension, irritability, pain, pleasure, calmness, and amusement) using a visual analogue scale (VAS; range 0–100). Responses were recorded at the beginning (0 min; immediately following the laughter / baseline manipulation), in the middle (27 min) and in the end (51 min) of the PET scans (see below).

Social Laughter Manipulation

Each subject underwent two PET scans on the same day: *a social laughter* scan and a neutral baseline scan. Before the social laughter scan, the subject and his two close friends watched pre-selected comedy clips together for 30 min in a private room. Subjects were seated in front of a data projector screen, and given access to a computer with a pre-selected playlist of short, amusing comedy clips on YouTube. They were then asked to watch the clips similarly as they would be watching TV. A similar protocol has been previously validated to reliably trigger social laughter (Dunbar et al., 2012). Social-laughter manipulation was performed before rather than during the PET scan to avoid laughing-related gross head and other bodily movements during data acquisition. Before the baseline scan, the subjects spent 30 min alone in the preparation room without contacts with other individuals.

During pre-scan laughter manipulation, the sounds of social laughter were recorded with Olympus Digital Recorder VN-711PC. The recorder was placed in the middle of the room and the microphone was aimed at the subjects. Subsequently, frequency of laughter bursts was coded from the recordings. Two laughter bursts were considered as separate if they were separated by at least 3 s of silence. Nonspecific sounds, such as humming, coughing, or amused speaking were not considered as laughs. Because this laughter measure did not differentiate between laughter of the scanned subject vs. the two accompanying subject, the subjects also reported, on a scale ranging from 0 to 100, how much they thought they laughed throughout the session.

PET Data Acquisition and Analysis

PET scans immediately followed the laughter and baseline conditions. During the PET acquisition, the subject was lying in the PET scanner wearing hospital clothes and covered with light blankets. The lights in the scanner room were dimmed. Social laughter and baseline conditions were separated by a 2-h break to allow for tracer decay. The order of the scans was counterbalanced across subjects.

Data were acquired with Philips Ingenuity PET-MR scanner at Turku PET Centre. Radiotracer production has been described previously (Hirvonen et al., 2009; Karlsson et al., 2015). After intravenous (M = 250 MBq, SD = 28 MBq) radioligand (mean injected mass 0.34 µg) bolus-injection, radioactivity in the brain was measured with the PET camera for 51 min

with in-plane resolution of 3.75 mm. The subjects were lying in a supine position throughout the studies. Data were corrected for dead-time, decay and measured photon attenuation and dynamic PET-scans were reconstructed with MRP reconstruction method (Alenius & Ruotsalainen, 1997). High-resolution (1 mm^3) anatomical MR reference images were acquired using a T1-weighted sequence (TR 25 ms, TE 4.6 ms, flip angle 30°, scan time 376 s). To correct for head motion, dynamic PET images were first realigned frame-to-frame. The individual T1-weighted MR images were coregistered to the summation images calculated from the realigned frames. Reference regions were drawn manually on MRI images using PMOD 3.4 software (PMOD Technologies Ltd., Zurich, Switzerland). Receptor availability was expressed in terms of BP_{ND}, which is the ratio of specific to non-displaceable binding in the brain. We used the occipital cortex as the reference region, which is known to be practically devoid of MOR (Hiller and Fan, 1996). BP_{ND} was calculated for each voxel using the simplified reference tissue model (SRTM) with reference-tissue time activity curves (TACs) as input data (Gunn et al., 1997). This outcome measure is not confounded by blood flow or tracer transport (Sander et al., 2014). The subject-wise parametric BP_{ND} images were normalized to the MNI space using the T1-weighted MR images, and smoothed with a Gaussian kernel of 8-mm FWHM.

The effects of social laughter on MOR availability were then assessed in SPM12 (http://www.fil.ion.ucl.ac.uk/spm/) using a repeated measures *t* test. Statistical threshold was set at $p < 0.05$, FDR corrected at cluster level. Effect size maps (Cohen's *d*) were also computed to allow visual inspection of the magnitude of the observed BP_{ND} changes. Linear regression analysis was further used to test whether baseline MOR availability would be associated with social laughter rate in the laughter manipulation. In a complementary approach, we also computed and visualised these associations in anatomical regions of interest (ROIs) generated in key components of the reward circuit and regions critical for socioemotional processing (thalamus, ventral striatum, dorsal caudate, amygdala, insula, orbitofrontal cortex, anterior, medial and posterior cingulate cortices) delineated using the AAL (Tzourio-Mazoyer et al., 2002) and Anatomy toolboxes (Eickhoff et al., 2005). Striatal divisions were performed manually as in (Katsyri et al., 2013).

Results

Viewing the comedy clips successfully elicited group laughter, with a mean rate of 1.04 (*SD* = 0.60) laughter bursts per minute. The objective group laughter rate also correlated

significantly with subjects' self-reported laughter rate, M = 61.83, SD = 21.40 r_s = 0.57, p = 0.05. Self-reports revealed that social laughter led to increased experience of amusement and calmness at the beginning (ps < 0.05) but not at the mid- or endpoints of the scans (**Fig. 1**). Whole-brain analysis of the PET data revealed that social laughter increased endogenous opioid release (as reflected by decreased [^{11}C]carfentanil BP_{ND}s) in several regions including thalamus, caudate nucleus and putamen, and insular, cingulate and frontal cortices. Opposite effects were observed in middle cingulate cortices (see **Fig. 2** for effect size and t-contrast maps).

Because MOR tone is known to be positively associated with self-reported sociability, we next tested whether baseline MOR availability would predict the amount of social laughter in the pre-experimental manipulation. Full-volume analyses (**Fig. 3**) revealed widespread positive associations, most profoundly observed in orbitofrontal and cingulate cortices as well as in the ventral striatum. Analysis based on the self-reported laughter scores yielded essentially a similar pattern of results (data not shown). Results from anatomical region-of-interest (ROI) analyses (**Fig. 4**) paralleled those of the full-volume analyses, with significant associations (r^2s > 0.38, ps < 0.05) observed in anterior, middle and posterior cingulate cortices, orbitofrontal cortex and ventral striatum.

As we scanned only males and used a low-level baseline condition (spending 30 min alone in the control room), the data do not conclusively show that social laughter per se would have triggered the observed endogenous opioid release, and that this would occur in both sexes. To control for these effects, we reanalyzed our previous data (Dunbar et al., 2012) on the effects of social laughter on pain thresholds (as a proxy for endogenous opioid release). In this study, male and female subjects viewed either live comedy or drama, and performed a "wall-sit" exercise as a pain threshold assay one hour before and immediately after the comedy/drama shows. In this task the subjects lean against a wall with legs at right angles until it becomes too painful, and they collapse to ground. The reanalysis revealed that pain threshold was significantly higher in the (laughter-inducing) comedy vs. drama condition, $t(29)$ = 2.54, p = 0.017, suggesting that laughter and not mere social presence of others is necessary for endogenous opioid release. Importantly, there was no difference between pain threshold change between males and females, p = 0.93 (M_{males} = 49.3 ± 16.0 s, $M_{females}$ = 47.4 ± 10.3 s).

Because laughter and subjective experience of pleasure were not directly recorded in the above study, we also ran a new control experiment (19 females, 24 males) where we measured laughter rates and acquired subjective ratings of amusement from male and female subjects while they viewed 30-min medleys of comedy movies versus neutral movie clips similarly as in the main experiment. Laughter rate, $F(1,39) = 14.83$, $p < 0.001$, $\eta_p^2 = 0.28$, and subjective experience of amusement, $F(1,39) = 7.62$, $p < 0.01$, $\eta_p^2 = 0.16$, was significantly higher in the comedy versus neutral movie conditions, but there were no differences between male and female subjects ($p > 0.47$).

Discussion

Our results demonstrate for the first time that social laughter is associated with MOR system activation and that baseline MOR availability specifically predicts the amount of experimentally induced social laughter. Laughter triggered endogenous opioid release in brain regions involved in processing of rewards and arousal (thalamus, caudate nucleus) but also in the insular cortices that have been associated with interoceptive, gustatory, and nociceptive processing (Wicker et al., 2003; Critchley et al., 2004; Singer et al., 2004). Our results show that social laughter triggers endogenous opioid release, and this could provide a powerful way for modulating social bonds in groups. Taken together, these data suggest that the opioid system plays a key role in mammalian prosocial communication and possibly also in social bonding, in addition to the well-known oxytocin and vasopressin systems (Young et al., 2001).

Social laughter increased positive mood and calmness, in accordance with the anxiolytic effects of exogenous opioid agonists (Colasanti et al., 2011). Laughter and positive facial expressions are important prosocial signals in humans (Scott et al., 2015) and also in non-human primates: Macaques and chimpanzees use a quiet smile-like gesture to appease aggressive conspecifics, whereas relaxed open-mouth vocalizations are associated with both play behavior and pair formation (Preuschoft, 1992; Waller and Dunbar, 2005). Prior studies have found that laughter involves engagement of both affective and reward networks in the brain (Wild et al., 2003). Thus, we propose that laughter-evoked, coordinated MOR activity in these systems could constitute a candidate neurochemical mechanism underlying incentive motivation towards bonding. The present results also accord with the general role of the endogenous opioids in prosociality. For example, opioid antagonist naltrexone increases self-reported pain ratings and unpleasant experiences when seeing others in pain

(Rutgen et al., 2015b). Placebo analgesia mediated by opioidergic system (Pecina and Zubieta, 2015) reduces negative emotional experiences when seeing others in distress, which is also reflected as attenuated brain responses to seeing others experiencing pain (Rutgen et al., 2015a; Rutgen et al., 2015b).

Our results parallel those stemming from pharmacological manipulation studies on the opioidergic basis of social grooming in nonhuman primates (Fabre-Nys et al., 1982; Graves et al., 2002). It is thus possible that human social laughter could support similar social functions as grooming does in humans and other primates, that is reducing tension (c.f. self-report data) and anxiety-related behaviours (Graves et al., 2002), also including establishing and maintaining social structures (Dunbar and Shultz, 2010; Suvilehto et al., 2015). In accordance with these findings, significant laughter-induced MOR activation was also observed in the anterior insula. The unmyeliated C-tactile fibers project to the insula but not to the primary somatosensory cortices (Olausson et al., 2002), and this tactile system responding to slow, pleasurable stroking may provide the sensory pathway for emotional and affiliative touching. Along with prior functional imaging studies showing insula activation while subjects listen to vocal laughter bursts (Sander and Scheich, 2005), our data support the claim that social laughter may engage the same affective-sensory circuits as does physical grooming, being consistent with Darwin's original proposal that laughter is a kind of 'tickling of the mind' (Darwin, 1872).

Surprisingly, we also observed decreased laughter-triggered opioid activity in the cortical midline regions. Although such observations are not uncommon in studies with [^{11}C]carfentanil (Hsu et al., 2013), their interpretation is not straightforward. Decrease in BP_{ND} may result from externalization or conformational changes in the receptors, but whether it is caused by increased or decreased endogenous opioid tone cannot be determined within the current design.

Unlike grooming, social laughter allows engagement of the MOR-dependent bonding mechanism *among all members* of an interacting group. Laughter is highly contagious, and in an appropriate pleasurable context simply hearing the sound of laughing may be sufficient to triggering laughter (Provine, 2004). This in turn may engage the MOR system of all individuals in the group hearing the laughter. Consequently, laughter imposes less severe timing constraints on social bonding compared with grooming, and it could thus play a critical role in enabling humans to live in exceptionally large social networks with numerous

affiliative and non-reproductive relationships (Dunbar, 2012). Such laughter-based bonding mechanisms are functional very early on during human development; spontaneous laughter and smiles are the very first prosocial gestures infants direct towards their caregivers. As the occurrence of infants smiles is strongly related to the caregiver's positive emotions (Mireault et al., 2015) laughter and smiling are thought to reflect coordinated bonding behavior within the infant–caregiver dyad. Due to the spreading of the opioidergic response across listeners, social laughter likely plays a major role in early socialization of the infant to their family and their proximal social networks, as well as directly underpinning the mother-infant bond. However, we stress that our data strictly pertains only to the laughter-induced opioid release and even though this parallels with the well-known effects of opioids on social bonding in other primates, further work is required to establish causal relationships between laughter-induced opioid release and social boding.

Baseline MOR availability predicts the rate of social laughter

Baseline MOR availability predicted linearly the rate of social laughter during the experimental laughter manipulation. This effect was observed in the key nodes of the reward circuit (amygdala and ventral striatum) and frontal and cingulate cortices supporting socioemotional functions. These results provide direct evidence for the hypothesis that MOR availability is associated with sociability, as previously indicated by correlational PET studies (Nummenmaa et al., 2015). Importantly, full-volume analysis also revealed association between laughter rate and MOR availability in amygdala. The role of amygdala in nonverbal emotional processing is well established (Sander and Scheich, 2005), and functional neuroimaging studies have shown that amygdala is involved in involuntary (emotional) but not voluntary laughter (Wild et al., 2003; Scott et al., 2015). High availability of endogenous MORs may enhance the calming effects of endogenously released opioid peptides acting on the MOR during social laughter, thus promoting reinforcement of attachment bonds. Genetically determined individual differences in opioidergic neurotransmission influence social behavior (Moles et al., 2004; Barr et al., 2008; Way et al., 2009). It is consequently likely that genetically determined MOR expression significantly contributes to this effect, nonetheless neuroplastic changes in the MOR system following repeated exposures to pleasurable social interaction likely play a role too.

Humans have an urgent need to feel belonging to groups and a bulk of studies have shown that both large social networks and the availability of social support are associated

with beneficial effects for somatic health in humans (Broadhead et al., 1983; Liu and Newschaffer, 2011; Holt-Lunstad et al., 2015), but also with infant survival in monkeys (Silk et al., 2003). Prior studies also suggest that such laughter-dependent effects on somatic health occur already at the level of the immune system: Social laughter releases immunoenhancers (such as β-endorphins), but it also increases the activity of natural killer cells (lymphocytes) and lowers cortisol levels in blood circulation (Berk et al., 1989). Because both the tendency to avoid intimate social interactions (Nummenmaa et al., 2015) and mood disorders are associated with lower regional availability of MOR (Kennedy et al., 2006), the present data show that laughter may not only be an important mechanism for maintaining social relationships, but also an effective behavioural coping mechanism against stressful situations.

Limitations

It must be noted that the observed BP_{ND} changes may also reflect receptor internalization or altered conformation, rather than occupancy by endogenous neurotransmitter. Our outcome measure (BP_{ND}) cannot directly specify which interpretation is most appropriate. Moreover, because we scanned only males we do not know whether our results translate directly to females. Yet our behavioral control data, showing elevated pain threshold when watching comedy vs. drama clips together with friends, suggest that laughter likely triggers similar opioid release also in female subjects. To maximize statistical power our study used a highly natural laughter manipulation coupled with a low-level baseline condition (spending 30 minutes alone before the scan); thus it can be questioned whether our results would be specific to social laughter versus mere social presence of others. However, our behavioural control data suggests that only social laughter rather than mere social presence of other individuals (or pleasure, see Dunbar et al., 2012) leads to endogenous opioid release. Finally, due to limited sample size this first *in vivo* demonstration of laughter-induced opioid release should be considered as preliminary and interpreted with some caution.

Conclusions

We conclude that baseline level and modulation of the μ-opioid system by social laughter could be an important neurochemical mechanism reinforcing and maintaining social bonds between humans. These data accord with animal studies (Keverne et al., 1989) in suggesting that the calming effects of endogenous opioidergic activity during social

interactions constitute a key mechanism promoting intragroup affiliation and bonding. Social touching and grooming are important means for bonding in humans and other primates (Dunbar and Shultz, 2010; Suvilehto et al., 2015), however, the present findings underline the central role of nonverbal communication and laughter in modulating interpersonal bonds and making large social networks possible.

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Figure Captions

Fig 1. Self-reported amusement and calmness before, during, and after the PET scans in the laughter and baseline conditions. Asterisks denote significant between-conditions differences. Note that the first time point ("Before") was recorded immediately following the laughter / baseline manipulation and before entering the PET scanner.

Fig 2. Brain regions showing increased (hot colors) and decreased (cool colors) endogenous opioid release during the social laughter versus baseline conditions. Top row shows unthresholded effect size maps, bottom row T-contrast maps thresholded at $p < 0.05$, FDR corrected at the cluster level. Colourbars denote the d / T statistic ranges.

Fig 3. Brain regions showing significant association between baseline MOR availability and social laughter rate (laughs per minute). The data are thresholded at $p < 0.05$, FDR corrected at cluster level.

Fig 4. Association between baseline MOR availability and social laughter rate (laughs per minute) in orbitofrontal cortex (OFC), ventral striatum (vSTR), and anterior (ACC) and middle (MCC) cingulate cortices (all *p*s < 0.05). Cook's distances < .57 for all observations suggests that no single data point or their removal significantly drives the correlations.