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SEX DIFFERENCES DURING HUMOR APPRECIATION IN CHILD SIBLING-PAIRS

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Abstract

The developmental origin of sex differences in adult brain function is poorly understood. Elucidating neural mechanisms underlying comparable cognitive functionality in both children and adults is required to address this gap. Humor appreciation represents a particularly relevant target for such developmental research because explanatory theories apply across the life span and underlying neurocircuitry shows sex differences in adults. As a positive mood state, humor is also of interest due to sex differences in rates of depression, a disorder afflicting twice as many women as men. In this study, we employed fMRI to investigate brain responses to funny versus positive (and neutral) video clips in 22 children ages 6 to 13 years, including 8 sibling pairs. Our data revealed increased activity to funny clips in bilateral temporo-occipital cortex, midbrain, and amygdala in girls. Conversely, we found heightened activation to positive clips in bilateral inferior parietal lobule, fusiform gyrus, inferior frontal gyrus, amygdala, and ventromedial prefrontal cortex in boys. Many of these effects persisted when looking at sibling-pairs only. We interpret such findings as reflecting the presence of early sex divergence in reward saliency / expectation and stimulus relevance attribution. These findings are discussed in the context of evolutionary and developmental theories of humor function.

Keywords

Humor Appreciation; Sex Differences; Reward; Children; fMRI

There is substantive evidence of sex differences in normal adult brain function, particularly as related to social cognitive affective processes (Domes et al., 2010; Harenski, Antonenko, Shane, & Kiehl, 2008; Krach et al., 2009; Proverbio, Adorni, Zani, & Trestianu, 2009; Schulte-Ruether, Markowitsch, Shah, Fink, & Piefke, 2008). However, the developmental origins of such sex differences remain poorly understood. This comes from the fact that very few functional magnetic resonance imaging (fMRI) investigations have examined sex differences in brain function at early developmental periods in children; those studies that have addressed this topic have mainly focused on basic language (Burman, Bitan, & Booth, 2008) or visual-spatial capacities (Clements-Stephens, Rimrodt, & Cutting, 2009; Kucian et al., 2007).

To bridge the critical knowledge gap related to developmental origins of adult sex differences in brain function, examination of comparable cognitive functionality and underlying neural mechanisms in both children and adults is required. Humor appreciation represents a particularly well-suited cognitive affective function for this purpose. First, there exists a large extant literature on the evolution and function of humor in the human species (Gervais & Wilson, 2005; Hurley, Dennett, & Adams, 2011; Wilbur & Campbell, 2011), of which some particularly emphasize sex differences as an important driving force. Second, sex differences related to humor processing have already been described in adults on both a behavioral as well as functional anatomical level (Azim, Mobbs, Jo, Menon, & Reiss, 2005; Kohn, Kellermann, Gur, Schneider, & Habel, 2011). Third, neurocircuitry underlying humor, a representative positive mood state (Gervais & Wilson, 2005), is likely to be susceptible to dysregulation in the course of mood disorders such as depression, a condition which is observed twice as frequently in women than in men (Nolen-Hoeksema, 1987). Moreover, we have recently shown that humor appreciation activates the same cognitive-affective brain circuits in children as has been observed in adults (Neely, Walter, Black, & Reiss, 2012). Altogether, these factors make humor appreciation an ideal candidate for the examination of putative developmental origins of sex differences in human social cognitive affective processes.

From an evolutionary perspective, humor appreciation in humans is thought to be conceptually linked with the expression of Duchenne laughter (Wild, Rodden, Grodd, & Ruch, 2003), a genetically predisposed (Caron, 2002; Provine, 2000) and inherently positive emotion eliciting (Panksepp, 1998) behavior in which “genuine” or “real” smiles are manifested. Derived from the primate relaxed open-mouth play face (Fry, 1994), such Duchenne laughter is primarily elicited in situations involving “nonserious social incongruity”, reflecting a sudden unexpected change in events within a safe social surrounding such as surprise in infants (i.e., a peek-a-boo face expressed by a parent) or tickling. These more basic forms of humor are also referred to as “protohumor”, and thought to serve as a fundamental social safety and play signal (Gervais & Wilson, 2005). In contrast, Duchenne laughter elicited by incongruity-based conceptual humor primarily in adults, is associated with “formal” attempts at inducing laughter (Gervais & Wilson, 2005). Both phenomena have putative self-regulatory as well as socially contagious emotional effects on others, and thus serve a myriad of functions in modern human society, ranging from enhanced individual physiology and psychology to dyadic and group-based interactions (Gervais & Wilson, 2005).

Based on fundamental evolutionary considerations, two recent theories of human humor have emerged. First, humor is argued to have evolved to ease and reinforce – through a feeling of mirth, amusement or positive affect – the detection of false inferences computed by the human brain in an attempt to anticipate the future (Hurley et al., 2011). Such a view is corroborated by psychological experiments linking humor to better individual emotional and physical health (e.g., resilience, stress response and immunological function), as well as to improved mood and cognition (Keltner & Bonanno, 1997; Martin, 2007; Neuhoff & Schaefer, 2002; Rosner, 2002; Wooten, 1996). Second, humor is proposed to act as a fitness indicator that provides females with information about underlying male mate quality, a mechanism linked to sexual selection theory (Wilbur & Campbell, 2011). This notion is supported by research showing that, in romantic contexts, females typically ‘evaluate’ while males ‘produce’ humorous offerings (Wilbur & Campbell, 2011). Furthermore, women’s evaluations of potential mates’ humor are predictive of their romantic interest, and preferences for and perceptions of humor by females are associated with preferences for and perceptions of male intelligence and warmth (Wilbur & Campbell, 2011). Given the fact that evolutionary theories of humor emphasize its vital role in modern human society,

understanding underlying brain substrates on a developmental level is of great interest to the scientific community.

Emerging functional magnetic resonance imaging (fMRI) data focused on the neural underpinnings of humor appreciation largely converge on the existence of two fundamental and dissociable mechanisms. These are (i) the resolution of conflict or incongruity through juxtaposition of mental states, and (ii) a positive feeling state related to mirth and/or reward (Azim et al., 2005; Bekinschtein, Davis, Rodd, & Owen, 2011; Franklin & Adams, 2011; Chan, Chou, Chen, & Liang, 2012; Chan et al., 2012; Goel & Dolan, 2001; Kohn et al., 2011; Mobbs, Greicius, Abdel-Azim, Menon, & Reiss, 2003; Moran, Wig, Adams, Janata, & Kelley, 2004; Neely et al., 2012; Samson, Hempelmann, Huber, & Zysset, 2009; Samson, Zysset, & Huber, 2008). Whereas the first mechanism has been shown to rely on temporo-occipito-parietal networks involved in theory of mind and semantic processing, the second mechanism has been linked with activity in mesocorticolimbic dopaminergic circuits. A recent study conducted with children in our laboratory (Neely et al., 2012) was able to differentiate neural responses to funny (humorous) *versus* positive (enjoyable/rewarding but not funny) video clips. The results of this study showed that activity in temporo-occipito-parietal areas as well as in midbrain (substantia nigra) significantly differentiated neural responses to funny *versus* positive movies. Thus, humor appreciation appears to involve neural computations additive to those recruited for basic positivity (reward) perception. These findings suggest that humor processing is a specific and well-differentiated higher-order human cognitive function.

Recently, researchers have begun to ask whether the neural substrates of humor appreciation in humans might differ as a function of two fundamental biological variables, namely sex (Azim et al., 2005; Kohn et al., 2011) and developmental stage (Neely et al., 2012). Whereas the assessment of sex differences might allow for the determination of biological factors influencing where and how a certain kind of information is processed within the human brain, developmental effects could provide important clues as to how the characteristics of such mechanisms arise. Extant findings suggest that (adult) females seem to appreciate humor (as opposed to neutral stimuli) by employing a greater degree of executive processing and language-based decoding, as well as by recruiting greater emotional and reward network response, the latter possibly related to less reward expectation (Azim et al., 2005; Kohn et al., 2011). Further, incongruity resolution and basic reward-related processing during humor appreciation (*versus* positivity perception) seem to be already functional during childhood (children ages 6 to 12 years; Neely et al., 2012).

The goal of the present study was to extend our knowledge regarding sex differences in humor appreciation to early development (i.e., childhood), and to compare obtained findings to adults (Azim et al., 2005; Kohn et al., 2011). In order to accomplish this goal, we recruited twenty-two typically developing children (ages 6 to 13; 13 females) to examine whether sex differences were already detectable in this age range. Moreover, out of the twenty-two participants, there were eight opposite-sex sibling-pairs, which allowed us to furthermore control for some genetic (at least 50%) as well as environmental influences (shared family context). As previously described, our main effects contrast compared brain activity to funny *versus* positive video clips (Neely et al., 2012), and not simply funny *versus* neutral stimuli (i.e., Azim et al., 2005; Kohn et al., 2011; Mobbs et al., 2003) as it is difficult to disentangle the relative effects of positive/rewarding stimulus characteristics from humorous characteristics if only the latter comparison is utilized.

The experimental task consisted of a video-viewing paradigm reported elsewhere (Neely et al., 2012). In short, participants were shown clips that were previously rated to be funny, positive, or neutral. Each stimulus category was presented 32 times within two successive

runs. Each trial started with a fixation cross (jittered between 500 and 13'000 ms), after which the clip started and played for its full duration (between 4'000 to 16'000 ms). Directly following each clip, participants had 3'000 ms to indicate by button press whether they liked or did not like the clip. Post response, the screen was replaced with a fixation cross on black background until the onset of the next trial (see Figure 1).

After the fMRI session, participants immediately saw all video clips again and provided ratings for each clip in terms of how funny (from 1= least funny to 8= most funny) and how enjoyable (from 1= least enjoyable to 8= most enjoyable) they experienced the stimuli. Behavioral ratings from one female and one male participant were unusable, leaving N= 20 (12 female and 8 male, from which 7 sibling-pairs were derived) for analyses incorporating these ratings.

METHODS

PARTICIPANTS

An initial pool of 34 participants underwent two functional MRI scans (each approximately 15 minutes in duration). Movement during scans was calculated based on the displacement and rotation required to realign functional scans at each time point with respect to a reference image. Participants with displaced scans that exceeded 21% of the total number of scans were excluded from further analysis (Neely et al., 2012), which concerned 8 individuals from whom fMRI scans were obtained. Data from an additional participant had to be removed from analysis because his brain mask did not include the temporal lobe (indicating that he moved significantly between the localizer scan and the functional scans), and data from another individual could not be used because scanning was aborted after one scanning session only due to discomfort. Finally, raw fMRI data from two individuals was lost due to a technical failure. This left us with fMRI scans from 22 typically developing children [13 female; mean age (SD): 10.0 (1.59) years; range 6.7 to 13.0 years; no significant age difference between the sexes: $t = 1.36$, $p = .19$] who were previously behaviorally and cognitively assessed, and who provided post-scan ratings of how humorous and enjoyable they found each clip. All participants were right-handed, had no clinical psychiatric symptoms or problems as indicated by parental responses to medical history-related questions during a phone screening, and were of average to gifted intelligence [as assessed by the Wechsler Abbreviated Scale of Intelligence (WASI)]. Participants and parents provided assent and consent, respectively, before participation. The Stanford University Panel on Human Subjects in Medical Research approved the study.

STIMULUS SELECTION

Because our participants included children with varied degrees of reading ability, we wanted to choose stimuli that did not depend solely on verbal humor, and therefore opted for movies depicting funny and non-funny (positive) scenes and events. To ensure that the chosen video clips would be funny (or not funny) to children participants, clips were pilot-tested with a matched sample of children – who did not participate in scanning; see Neely et al. (2012) for details. Finally selected funny video clips included scenes of people stumbling while skiing or running, a kid “swimming” on the floor, cars accidentally running in to things, animals performing amusing tricks, a kid being catapulted into the air from an inflatable couch, and many others. Positive video clips included scenes of dirt biking, gymnastics, dancing, snowboarding, a person jumping over a car, kids break-dancing, a performer playing music using water glasses, and many others. Neutral video clips included kids riding bicycles, kids singing in school plays, a child meeting Mickey Mouse, nature documentary style videos of animals, and many others.

EXPERIMENTAL TASK

The in-scanner task comprised a set of 96 short color video clips previously viewed and evaluated by age-matched controls to represent three categories: Funny, Positive, and Neutral. Each stimulus category included 32 video clips, 16 of which were presented in the first functional run, with the remainder in the second functional run (randomized and counterbalanced across subjects). Funny clips were rated as funny (and enjoyable), Positive clips were rated as enjoyable to watch but non-funny, and Neutral clips were not intended to evoke any emotional response.

Stimuli were presented at the center of a viewing screen above the head coil using a custom-built MR-compatible projection system and EPrime 2.0 software (<http://www.pstnet.com/eprime.cfm>). Participants lying in the scanner viewed stimuli via an angled mirror. The software program Optseq (<http://surfer.nmr.mgh.harvard.edu/optseq/>) was used to optimally randomize the order and spacing between stimuli in order to ensure orthogonality of stimulus conditions. Stimuli were divided into two runs so that participants would get a short break during the lengthy scan session. During the two runs (approximately 30 min total scan acquisition time), participants viewed all video clips and pressed a “Yes” button with their right index finger when they “liked” watching a video and a “No” button press with their right middle finger when they “did not like” watching a clip. This simple in-scanner task was utilized to ensure that participants were attending to the stimuli.

Briefly, each trial started with a fixation cross that was presented for 500 – 13000 ms (timing was based on optseq output) after which the video started and played for its full duration (4000 – 16000 ms). After the video played, the participant had 3000 ms to respond. Post-response, the screen was replaced with a fixation cross on black background until the onset of the next trial (see Figure 1a).

Participants were familiarized with the task before undergoing imaging by playing a short (approximately two minute duration) practice version of the task. Participants practiced in an MRI Simulator prior to imaging and re-practiced the task outside the scanner immediately prior to imaging. Stimuli used for the practice versions were different from those used in-scanner and featured different actors, activities, and scenes. Participants were encouraged to be honest in their assessments and were told that they were not expected to “like” all of the clips.

Post-imaging, participants were asked to re-watch all stimuli and provide more nuanced feedback by rating the clips using an ordinal interval scale to indicate (i) how funny they found the video (on a scale from 1=least funny to 8=most funny), and (ii) how enjoyable they found the video (on a scale from 1=least enjoyable to 8=most enjoyable).

fMRI ACQUISITION

Participants were scanned on a 3Tesla (GE Discovery MR750 scanner, Milwaukee, WI) MRI at Stanford’s Lucas Center for Neuroimaging using a custom-built single-channel birdcage head coil optimized for fMRI scans. Over two runs, a total of 914 whole-brain volumes were collected on 30 axial-oblique slices (4.0 mm thick, 1.0 mm skip) prescribed parallel to the intercommissural (AC-PC) line, using a T2*-weighted gradient echo spiral pulse sequence (Glover & Lai, 1998) sensitive to blood oxygen level-dependence (BOLD) contrast with the following acquisition parameters: Echo Time (TE) = 30.0 ms, repetition time (TR) = 2000 ms, flip angle = 80°, FOV = 22.0 cm, number of slices = 31, slice thickness = 4.0mm, acquisition matrix = 64×64, approximate voxel size = 3.4375 × 3.4375 × 4.0 mm. An automated high-order shimming method based on spiral acquisitions was used before acquisition of functional MRI scans in order to reduce blurring and signal loss arising from field inhomogeneities (Kim, Adalsteinsson, & Spielman, 2003). A high-resolution T1-

weighted three-dimensional inversion recovery spoiled gradient-recalled acquisition was acquired for co-registration with the following parameters: Echo Time (TE) = min full, repetition time (TR) = 8.1 s, flip angle = 15°, FOV = 224 cm, slice thickness = 1.6 mm, 124 slices in the coronal plane; matrix = 256 × 192. The images were reconstructed as a 256 × 256 × 124 matrix.

fMRI ANALYSIS

Statistical analyses were performed using Statistical Parametric Mapping software (SPM8; Wellcome Department of Cognitive Neurology, London, UK, <http://www.fil.ion.ucl.ac.uk/spm/software/spm8/>). Each subject's data was realigned and resliced, spatially smoothed (4mm full-width-half-max), motion corrected, co-registered to the individual's anatomical image and normalized to MNI space. In addition, we repaired movement and signal artifacts in our images using ArtRepair software (<http://cibsr.stanford.edu/tools/ArtRepair/ArtRepair.htm>; Glover, Mazaika, & Reiss), developed in house at the Center for Interdisciplinary Brain Sciences Research (CIBSR), and available for public download.

STATISTICAL ANALYSIS

After preprocessing, each individual subject was analyzed using the general linear model (Friston et al., 1995) implemented in SPM8. Individual subject data were high-pass filtered at 120 s, globally scaled and analyzed using a fixed-effects model comprising the three separate experimental conditions (rest was modeled implicitly). Group data were smoothed (4mm full-width half-max) and the individual subject contrast images were entered into a random-effects analysis. Unless otherwise noted, group results for each the two main effects contrasts (funny *versus* positive, and vice versa) are reported at a joint-expected probability threshold (Neely et al., 2012; Poline et al., 1997) with height (peak-level) threshold of $p < 0.001$ (uncorrected) and extent (cluster-level) threshold of $p < 0.05$ (FWE corrected). Sex differences were computed using a whole brain two-sample t-test approach, looking at the contrasts funny > positive and positive > funny by entering females and males as two different groups, and these contrasts were inclusively masked (at $p < .05$) with the main effects contrasts. Coordinates of activation are reported in MNI space.

RESULTS

BEHAVIOR

Results obtained from $N = 20$ and $N = 14$ (7 sibling-pairs) were nearly identical. Funny clips (humor) were rated as funnier than both positive and neutral clips [$N = 20$: t 's > 3.67, p 's = .002; $N = 14$: t 's > 3.22, p 's < .007], and funny and positive clips were rated as more enjoyable than neutral clips [$N = 20$: t 's > 8.81, p 's < .001; $N = 14$: t 's > 9.96, p 's < .001]. There was no significant difference in enjoyable ratings between funny and positive clip conditions [$N = 20$: $t = .80$, $p = .43$; $N = 14$: $t = 1.39$, $p = .19$; all paired samples t-tests] (see Figure 2a).

When assessing sex differences regarding behavioral post-scan ratings overall ($N = 20$), only one significant difference emerged: females ($N = 13$) rated positive clips as funnier than males' rating ($N = 9$) [$t = 2.24$, $p = .038$; independent samples t-test assuming equal variance]. For sibling pairs ($N = 14$), however, there were no sex differences [t 's < 1.29, p 's > .25; paired samples t-test] (see Figure 2b).

FMRI

To determine which brain regions were more active during funny *versus* positive clip processing, we computed the two main effects contrasts funny > positive and positive > funny, applying a joint-expected probability threshold with height (peak-level) $p < .001$ (uncorrected) and extent (cluster-level) of $p < .05$ (FWE corrected; Neely et al., 2012; Poline,

Worsley, Evans, & Friston, 1997). This revealed increased activity during funny clips in bilateral superior temporal gyrus (STG) including temporo-parietal junction (TPJ), posterior superior temporal sulcus (pSTS), and anterior STG; midbrain (substantia nigra), as well as right occipital cortex (see Figure 3a and Table 1). In turn, positive clips entailed heightened BOLD signal change in bilateral superior and inferior parietal lobule (SPL and IPL; BA 40), right medial temporal gyrus (MTG; BA 37), and right inferior frontal gyrus (BA 46; see Figure 3b and Table 1). We also computed the contrasts funny versus neutral and positive versus neutral. For these results, please refer to Table 1.

Subsequently, we conducted a conjunction analyses (inclusive masking procedure at $p < .05$) to determine if significant sex differences would be observed in the functional regions of interest (fROIs) noted above.

Initially, masking was carried out for the entire population ($N = 22$, 13 female) applying a whole brain two-sample t-test second level approach, and examining the contrasts funny > positive and positive > funny by entering females ($N = 13$) and males ($N = 9$) as two different groups. Results revealed that female participants overall displayed a stronger activation difference for funny *versus* positive clips in right supramarginal gyrus (SMG), left TPJ, right pSTS, right aSTG, midbrain (substantia nigra; see Figure 4a), and right occipital cortex (see Table 1). Conversely, male participants showed overall higher activity for positive *versus* funny clips in bilateral IPL (BA 40), right fusiform face area (BA 37), and right BA 46 (see Table 1). Because, according to previous literature, we also predicted sex differences during humor processing in the amygdala (Kohn et al., 2011; Mobbs et al., 2003; Mobbs, Hagan, Azim, Menon, & Reiss, 2005; Wild et al., 2003) and ventromedial prefrontal cortex (vmPFC; Goel & Dolan, 2001; Noonan, Mars, & Rushworth, 2011) on an *a priori* basis, we assessed BOLD signal change in those two brain areas as well. Although the amygdala and vmPFC were not highlighted in the funny > positive or positive > funny contrasts utilizing the entire sample ($n = 22$, see Table 1 and Figure 3), significant sex by condition interactions were observed in follow-up whole brain two-sample (male *versus* female) t-tests (left amygdala: peak voxel at $xyz = -30 -6 -18$; 5 voxels at $p < .001$ uncorrected; vmPFC: peak voxel at $xyz = 8 42 -8$; 60 voxels at $p < .001$ uncorrected; see Figure 4bc).

Subsequently, we assessed which of the sex effects observed in the entire sample ($N = 22$, 13 female) would persist if looking solely at sibling-pairs ($N = 16$, 8 female). To accomplish this aim, we utilized the funny > positive and positive > funny t-test (male *versus* female) contrasts in sibling-pairs only. Resulting activations were then inclusively masked (at $p < .05$) with the initial fROIs (see above). This analysis revealed persistent sex differences in sibling-pairs in the midbrain, bilateral TPJ and bilateral IPL. Furthermore, in the left amygdala, the sex difference remained significant when computing the whole brain two-sample t-test in a follow-up analysis (peak voxel at $xyz = -30 -6 -18$; 3 voxels at $p < .001$ uncorrected). In the vmPFC, a persistent sex difference could also be observed solely for sibling-pairs at a slightly lower statistical threshold (peak voxel at $xyz = 4 42 -10$; 21 voxels at $p < .01$ uncorrected).

As a final exploratory step, we determined how activity levels in fROIs displaying sex differences ($N = 22$) were related to one another. This was carried out by means of multiple regression analyses using extracted beta values from voxels of maximal activation for both the funny and positive conditions, which were then subtracted from each other to derive a difference score (funny *minus* positive). These analyses revealed a significant *positive* correlation between differential activity in the amygdala and midbrain [$\beta = .882$, $t = 3.33$, $p = .007$], as well as a trend towards a *negative* correlation between differential activity in the midbrain and vmPFC [$\beta = .614$, $t = 1.91$, $p = .083$] (see Figure 5ab).

DISCUSSION

The results of our study are the first to indicate that sex differences in human brain function related to humor already exist in young children. By integrating these new findings with the extant literature on humor appreciation, we can derive a more complete functional model describing how humor is processed within the human brain, in particular, in the context of the moderating effects of development and sex. We can also consider our results in relation to general (Gervais & Wilson, 2005) as well as more specific contemporary (Hurley et al., 2011; Wilbur & Campbell, 2011) evolutionary-based theories of humor.

In accordance with previously reported humor appreciation findings in adults (Azim et al., 2005; Bekinschtein, Davis, Rodd, & Owen, 2011; Franklin & Adams, 2011; Chan, Chou, Chen, & Liang, 2012; Chan et al., 2012; Goel & Dolan, 2001; Kohn et al., 2011; Mobbs, Greicius, Abdel-Azim, Menon, & Reiss, 2003; Moran, Wig, Adams, Janata, & Kelley, 2004; Neely et al., 2012; Samson, Hempelmann, Huber, & Zysset, 2009; Samson, Zysset, & Huber, 2008) and children (Neely et al., 2012), our results obtained from the contrast funny *versus* positive corroborate the notion that humor processing involves two fundamental, specific and dissociable mechanisms, namely (i) incongruity resolution and (ii) a positive affective feeling state.

Furthermore, our data for the first time disclose that sex differences in humor appreciation (Azim et al., 2005; Kohn et al., 2011) already exist in young children (ages 6 to 13). In the present investigation, such sex differences emerged because girls ($N = 22$, 13 female) displayed stronger differential processing of funny *versus* positive clips in bilateral temporo-occipital cortex, midbrain, and amygdala. This finding indicates that girls more readily engaged in incongruity resolution and experienced stronger mirth, positive feeling state and/or reward representation during humor appreciation (see i.e., Azim et al., 2005; Mobbs et al., 2003; Neely et al., 2012; Samson et al., 2009; Samson et al., 2008). In addition, our new results also imply that girls perceived humorous (as opposed to positive) information as intrinsically more salient. Such inference is based on current models of human amygdala function (Pessoa & Adolphs, 2010) that converge with the original relevance detector hypothesis (Sander, Grafman, & Zalla, 2003), postulating that the amygdala is most activated when processing information relevant to the goals, needs, beliefs and/or desires of an individual at a specific moment in time. In sum, sex differences observed during humor appreciation in children were characterized by stronger differential processing of funny *versus* positive clips in girls, suggesting greater reward sensitivity and stimulus salience.

Interestingly, our analyses for the first time also revealed increased BOLD signal change for positive *versus* funny clips. This new finding is likely related to the fact that the only other fMRI study directly contrasting these two conditions used a smaller sample size (Neely et al., 2012), and because all remaining previous fMRI humor appreciation investigations always contrasted humor to neutral control conditions (Azim et al., 2005; Bekinschtein, Davis, Rodd, & Owen, 2011; Franklin & Adams, 2011; Chan, Chou, Chen, & Liang, 2012; Chan et al., 2012; Goel & Dolan, 2001; Kohn et al., 2011; Mobbs, Greicius, Abdel-Azim, Menon, & Reiss, 2003; Moran, Wig, Adams, Janata, & Kelley, 2004; Neely et al., 2012; Samson, Hempelmann, Huber, & Zysset, 2009; Samson, Zysset, & Huber, 2008). In the present experiment, preferential processing of positive stimuli (relative to funny) was detected in bilateral superior and inferior parietal lobule (SPL and IPL; BA 40), right medial temporal gyrus (MTG; BA 37), and right inferior frontal gyrus (IFG; BA 46). These areas are known to be involved in various cognitive functions including attention, working memory, semantic processing, sensory association, and action recognition (Azim et al., 2005; Buccino, 2004; Colom, Jung, & Haier, 2007; Hargreaves et al., 2012; Naghavi & Nyberg, 2005). Moreover, parts of this network were previously shown to be implicated in

maintaining alternative / less probable (word) meanings, and in integrative processes requiring the computation of global coherence (Goel & Dolan, 2001). We therefore propose that in our study, heightened SPL/IPL, MTG and IFG activity reflects participant's intention to understand the underlying meaning of positive scenes, also involving the generation / anticipation of alternative outcomes (see below).

The above interpretation of SPL/IPL, MTG and IFG activation is further supported by the observation of overall stronger BOLD signal change to positive *versus* funny clips in the same areas in boys relative to girls (Table 1), and similar sex differences in the amygdala and ventromedial prefrontal cortex (vmPFC). As stated above, human amygdala function is most strongly linked to relevance detection (Pessoa & Adolphs, 2010; Sander et al., 2003). It therefore seems that for boys, positive (*versus* funny) information was more intrinsically salient, which is exactly the opposite pattern we observed in girls (see above). A possible explanation of this phenomenon could be derived from examining vmPFC activation, which displayed a strong positive *versus* funny activation difference in boys, but no such effect in girls. vmPFC activity has previously not only been associated with humor perception (Goel & Dolan, 2001), but also with reward-related decision-making in terms of outcome monitoring and particularly the representation of reward expectation (Noonan et al., 2011). Accordingly, we assume that in our study, boys watched positive clips with the strong anticipation of a funny "punch line" to arrive, reflected by high differential vmPFC activity. Overall, sex differences detected during the processing of positivity (as opposed to humor) in children were found driven by greater differential activity to positive *versus* funny clips in boys, most likely caused by strong reward anticipation and associated increased stimulus salience.

When integrating the above-mentioned findings on sex differences during humor appreciation in children (see Figure 6), two central functional components emerge, namely (i) reward sensitivity / expectation, and (ii) stimulus salience. Our results indicate that these functional components are computed via activity in three central brain areas, namely the midbrain (sensitivity), vmPFC (expectation), and amygdala (salience). In so doing, reward-related signal derived in the midbrain appears to play a key role, as differential (funny *versus* positive) activity extracted from the midbrain was positively correlated with activity in amygdala, but negatively with BOLD signal change in vmPFC (see Figure 5). In other words, the lower the reward expectation in the vmPFC, the higher reward sensitivity in the midbrain, and the stronger the saliency signal in the amygdala to funny (*versus* positive) clips (as observed in girls). Conversely, the higher the reward expectation in the vmPFC, the lower reward sensitivity in the midbrain, and the stronger the saliency signal in the amygdala to positive (*versus* funny) clips (as observed in boys).

Importantly, such distinct reward sensitivity and associated stimulus salience likely cannot be explained by distinct subjective feelings of funniness or enjoyment associated with funny or positive clip viewing, as post-scan ratings on these stimulus dimensions did not differ between boys and girls. In fact, the only behavioral sex difference we observed was an increased funny rating of positive movies in girls (N= 22). Consequently, differential brain activity for funny *versus* positive clips most likely reflects a sex bias for distinct processing mechanisms, rather than divergent subjective experience (Azim et al., 2005; Kohn et al., 2011).

From a developmental point of view, it is of great interest that sex differences in neural mechanisms underlying humor appreciation are already established early during development (ages 6 to 13). Consequently, the question arises as to whether this phenomenon is of a genetic and/or environmental origin. To address this issue, our data analysis included findings obtained from 8 opposite-sex sibling-pairs. This allowed us to

control for some (at least 50%) genetic and most environmental (shared family context) influence.

By examining sex differences during humor appreciation in opposite-sex sibling-pairs only (N= 16), most of our initial findings from the entire population (N= 22, 13 female) remained significant. More precisely, whereas increased funny *versus* positive clip processing was confirmed in bilateral temporal-occipital cortex, midbrain and amygdala in girls, activation difference for positive *versus* funny movies remained significant in bilateral IPL, amygdala, and vmPFC for boys. This suggests that the functioning of the central circuitry determining reward sensitivity / coding for reward expectation, as well as representing stimulus salience probably has some genetic (biological) underpinnings. However, given the fact that children have already been exposed to significant environmental influences by 6 to 13 years of age, we cannot be certain whether these early sex differences preferentially arise from genetic *versus* environmental factors. Yet, as is the case for most human cognitive traits (Monk, 2008), the answer will likely be that the interaction between both domains of influence significantly contributes to the observed sex differences.

Finally, the question remains of how our functional model of humor appreciation relates to the general (Gervais & Wilson, 2005), as well as the two more specific psychological (Hurley et al., 2011; Wilbur & Campbell, 2011) evolutionary theories mentioned at the beginning of this article.

From the available data and general considerations of humor evolution and function, it seems clear that humor appreciation is typically associated with a feeling of mirth, positive affect and/or reward, and that the latter most probably arises from incongruity resolution computed during the exposure to funny (but not positive) information (Gervais & Wilson, 2005; Hurley et al., 2011; Neely et al., 2012). However, such a relation between humor appreciation and reward seems plausible only if the actual reward transmitted by realizing the “punch line” arrives unexpectedly. Put differently, the rewarding properties of humor seem largest if the “anticipated future” constructed by our brain is most discrepant with the real outcome (reflecting a classic prediction error). And this, in turn, appears to be much more the case in females than in males – at least within contexts where it is evident that funny (*versus* positive and/or *versus* neutral) stimuli will be encountered at a certain stage. Furthermore, such mechanisms are already in place in children (ages 6 to 13) and even evident in opposite-sex sibling-pairs, which strongly suggests that biological, particularly genetic, as well as early environmental influences are pertinent.

A plausible explanation for genetic-biological factors influencing preferential humor appreciation in females might be linked to the findings reported by Wilbur and Campbell (2011) and associated with the sexual selection theory proposed by Charles Darwin (Darwin, 1981). To be able to objectively use humor as a fitness indicator during mate selection in an unbiased fashion, humor produced by males must be evaluated by females without any expectation in order to activate reward and theory of mind areas to a maximal degree. Such an account is corroborated by findings not only indicating that females activate parts of the reward system more strongly during reward *delivery* but men during reward *anticipation* (Dreher et al., 2007), but that the reward system in women is more reactive during the midfollicular phase, which represents part of the most fertile cycle time (Dreher et al., 2007). In other words, women’s reward system most readily represents reward *delivery* when the chances of becoming pregnant are the highest. Because humor appreciation mechanistically relies on incongruity resolution through reward delivery at the time of the “punch line”, the extant neuroimaging data supports the notion that mate selection by means of humor processing might be more effective in females than males because the female

brain, and particularly the reward circuit, is biologically better prepared to respond accordingly.

The novel findings presented here also have potential clinical relevance. Rates of depression increase markedly during adolescence, especially for girls (Kessler, Avenevoli, & Ries Merikangas, 2001), and by mid-adolescence the female:male ratio is approximately 2:1 for diagnostic-level depression (Angold, Costello, & Worthman, 1998; Hankin et al., 1998). Our findings of sex-based differences in activation within the vmPFC, midbrain, and amygdala suggest a phenomenon whereby, during the transition from childhood to adolescence, girls hold lower reward expectation whereas boys expect (and therefore may be more likely to seek out) reward. Thus, variation in reward activation in response to humor might also serve as a biomarker of risk for mood disturbance if in fact girls are less likely to expect to be rewarded as they move into adolescence. If such findings are confirmed by additional studies, the link between humor and reward circuitry may have implications for the many challenges adolescents face including peer-related social threat and the quest for individuation. Further understanding the sex-based differences in reward expectation may also have implications for addiction. While some neuroimaging evidence suggests that adolescence is associated with hypo-responsiveness to rewards, more recent findings point to the striatal system as hyper-responsive resulting in increased reward-seeking in adolescence (Galvan, 2010). In light of our findings that sex-based differences in brain circuitry related to reward processing are already well-established in childhood, future work could examine the extent to which differences in sensation-seeking and reward processing may be associated with risk for substance abuse and dependence in adolescence. Future studies should elaborate on sex differences during the processing of funny *versus* positive stimuli in children, adolescents and adults, and/or applying a longitudinal approach (Decety & Michalska, 2010), as well as employ genetic methods to better characterize the proposed mechanisms.

LIMITATIONS

We note several limitations of the present investigation.

First, the number of participants is relatively small, especially sibling pairs. This limitation is primarily due to the fact that we had to exclude a large number of data sets from group analyses due to excessive movement during scanning. Although the reported results reach commonly applied statistical thresholds, future studies in larger populations are needed for validation and generalization purposes.

Second, the age range of our participants was rather large (from 6.7 to 13.0 years). Even though there were no age differences between the sexes (see Methods), we still performed additional analyses to account for any potential age effects. When using age as an additional regressor of no interest in the whole-brain two-sample t-test analyses, we observed the same sex differences as reported in the original analyses (see Table 2). Furthermore, we did not find any influence of age on left amygdala and vmPFC activity to humor (no significant condition $\times$ age interactions [$p > .23$]). According to the above-described additional analyses, we are confident that there were no age effects in our sample that could have interfered with the reported sex differences. Related to age, our sample likely consisted of mostly pre-pubertal children. However, we cannot exclude the presence of individuals who had begun to show some pubertal changes, as we did not explicitly test for pubertal maturation. Because puberty onset may substantiate sex differences in humor processing related to mate selection and/or increased risk for depression (see Discussion), future investigations should more extensively compare sex differences during humor appreciation in pre-pubertal children and pubertal adolescents.

Finally, it should be mentioned here that we derived our interpretation of sex differences during humor appreciation in children from previously observed activation patterns in adults. Specifically, we argue that differential mesocorticolimbic activity reflects distinct reward magnitude and/or expectation representation, and that differential amygdala activation represents distinct stimulus salience attribution. Even though we are confident that these interpretations are supported by the data, studies in children are needed to more directly assess the proposed associations.

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REFERENCES

- Angold A, Costello EJ, Worthman CM. Puberty and depression: the roles of age, pubertal status and pubertal timing. *Psychological medicine*. 1998; 28(1):51–61. [PubMed: 9483683]
- Azim E, Mobbs D, Jo B, Menon V, Reiss AL. Sex differences in brain activation elicited by humor. *Proceedings of the National Academy of Sciences of the United States of America*. 2005; 102(45): 16496–16501. doi: 10.1073/pnas.0408456102. [PubMed: 16275931]
- Bekinschtein TA, Davis MH, Rodd JM, Owen AM. Why Clowns Taste Funny: The Relationship between Humor and Semantic Ambiguity. *Journal of Neuroscience*. 2011; 31(26):9665–9671. doi: 10.1523/jneurosci.5058-10.2011. [PubMed: 21715632]
- Buccino G. Neural substrates involved in executing, observing, and imagining actions. *Brain and Cognition*. 2004; 56(1):118–118.
- Burman DD, Bitan T, Booth JR. Sex differences in neural processing of language among children. *Neuropsychologia*. 2008; 46(5):1349–1362. doi: 10.1016/j.neuropsychologia.2007.12.021. [PubMed: 18262207]
- Caron JE. From ethology to aesthetics: Evolution as a theoretical paradigm for research on laughter, humor, and other comic phenomena. *Humor-International Journal of Humor Research*. 2002; 15(3): 245–281. doi: 10.1515/humr.2002.015.
- Chan Y-C, Chou T-L, Chen H-C, Liang K-C. Segregating the comprehension and elaboration processing of verbal jokes: An fMRI study. *Neuroimage*. 2012; 61(4):899–906. doi: 10.1016/j.neuroimage.2012.03.052. [PubMed: 22472220]
- Chan YC, Chou TL, Chen HC, Yeh YC, Lavalley JP, Liang KC, Chang KE. Towards a neural circuit model of verbal humor processing: An fMRI study of the neural substrates of incongruity detection and resolution. *Neuroimage*. 2012
- Clements-Stephens AM, Rimrodt SL, Cutting LE. Developmental sex differences in basic visuospatial processing: Differences in strategy use? *Neuroscience Letters*. 2009; 449(3):155–160. doi: 10.1016/j.neulet.2008.10.094. [PubMed: 19000742]
- Colom R, Jung RE, Haier RJ. General intelligence and memory span: Evidence for a common neuroanatomic framework. *Cognitive Neuropsychology*. 2007; 24(8):867–878. doi: 10.1080/02643290701781557. [PubMed: 18161499]
- Darwin, C. *The descent of man, and sexual selection in relation to sex*. Vol. Vols. 1 and 2. Princeton University Press; Princeton, NJ: 1981.
- Decety J, Michalska KJ. Neurodevelopmental changes in the circuits underlying empathy and sympathy from childhood to adulthood. *Developmental Science*. 2010; 13(6):886–899. doi: 10.1111/j.1467-7687.2009.00940.x. [PubMed: 20977559]

- Domes G, Schulze L, Boettger M, Grossmann A, Hauenstein K, Wirtz PH, Herpertz SC. The Neural Correlates of Sex Differences in Emotional Reactivity and Emotion Regulation. *Human Brain Mapping*. 2010; 31(5):758–769. doi: 10.1002/hbm.20903. [PubMed: 19957268]
- Dreher J-C, Schmidt PJ, Kohn P, Furman D, Rubinow D, Berman KF. Menstrual cycle phase modulates reward-related neural function in women. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104(7):2465–2470. doi: 10.1073/pnas.0605569104. [PubMed: 17267613]
- Franklin RG Jr, Adams RB Jr. The reward of a good joke: neural correlates of viewing dynamic displays of stand-up comedy. *Cognitive Affective & Behavioral Neuroscience*. 2011; 11(4):508–515. doi: 10.3758/s13415-011-0049-7.
- Friston KJ, Holmes AP, Poline JB, Grasby PJ, Williams SCR, Frackowiak RSJ, Turner R. ANALYSIS OF FMRI TIME-SERIES REVISITED. *Neuroimage*. 1995; 2(1):45–53. doi: 10.1006/nimg.1995.1007. [PubMed: 9343589]
- Fry WF. THE BIOLOGY OF HUMOR. *Humor-International Journal of Humor Research*. 1994; 7(2): 111–126. doi: 10.1515/humr.1994.7.2.111.
- Galvan A. Adolescent development of the reward system. *Frontiers in Human Neuroscience*. 2010; 4:6. doi: 10.3389/neuro.09.006.2010. [PubMed: 20179786]
- Gervais M, Wilson DS. The evolution and functions of laughter and humor: A synthetic approach. *Quarterly Review of Biology*. 2005; 80(4):395–430. doi: 10.1086/498281. [PubMed: 16519138]
- Glover GH, Lai S. Self-navigated spiral fMRI: Interleaved versus single-shot. *Magnetic Resonance in Medicine*. 1998; 39(3):361–368. doi: 10.1002/mrm.1910390305. [PubMed: 9498591]
- Glover, GH.; Mazaika, PK.; Reiss, AL. US2009103795-A1; US8055041-B2
- Goel V, Dolan RJ. The functional anatomy of humor: segregating cognitive and affective components. *Nature Neuroscience*. 2001; 4(3):237–238. doi: 10.1038/85076.
- Hankin BL, Abramson LY, Moffitt TE, Silva PA, McGee R, Angell KE. Development of depression from preadolescence to young adulthood: emerging gender differences in a 10-year longitudinal study. *Journal of abnormal psychology*. 1998; 107(1):128–140. [PubMed: 9505045]
- Harenski CL, Antonenko O, Shane MS, Kiehl KA. Gender differences in neural mechanisms underlying moral sensitivity. *Social Cognitive and Affective Neuroscience*. 2008; 3(4):313–321. doi: 10.1093/scan/nsn026. [PubMed: 19015084]
- Hargreaves IS, Leonard G, Pexman PM, Pittman DJ, Siakaluk PD, Goodyear BG. The neural correlates of the body-object interaction effect in semantic processing. *Frontiers in Human Neuroscience*. 2012; 6. doi: 2210.3389/fnhum.2012.00022.
- Hurley, MM.; Dennett, DC.; Adams, RB. Inside jokes: using humor to reverse-engineer the mind. MIT press; 2011.
- Keltner D, Bonanno GA. A study of laughter and dissociation: Distinct correlates of laughter and smiling during bereavement. *Journal of Personality and Social Psychology*. 1997; 73(4):687–702. doi: 10.1037/0022-3514.73.4.687. [PubMed: 9325589]
- Kessler RC, Avenevoli S, Ries Merikangas K. Mood disorders in children and adolescents: an epidemiologic perspective. *Biological psychiatry*. 2001; 49(12):1002–1014. [PubMed: 11430842]
- Kim DH, Adalsteinsson E, Spielman DM. Simple analytic variable density spiral design. *Magnetic Resonance in Medicine*. 2003; 50(1):214–219. doi: 10.1002/mrm.10493. [PubMed: 12815699]
- Kohn N, Kellermann T, Gur RC, Schneider F, Habel U. Gender differences in the neural correlates of humor processing: Implications for different processing modes. *Neuropsychologia*. 2011; 49(5): 888–897. doi: 10.1016/j.neuropsychologia.2011.02.010. [PubMed: 21320516]
- Krach S, Bluemel I, Marjoram D, Lataster T, Krabbendam L, Weber J, Kircher T. Are women better mindreaders? Sex differences in neural correlates of mentalizing detected with functional MRI. *Bmc Neuroscience*. 2009; 10. doi: 910.1186/1471-2202-10-9. [PubMed: 19208233]
- Kucian K, von Aster M, Loenneker T, Dietrich T, Mast FW, Martin E. Brain activation during mental rotation in school children and adults. *Journal of Neural Transmission*. 2007; 114(5):675–686. doi: 10.1007/s00702-006-0604-5. [PubMed: 17160371]
- Martin, RA. The psychology of humor. An integrative approach. Elsevier Academic Press; Burlington: 2007.

- Mobbs D, Greicius MD, Abdel-Azim E, Menon V, Reiss AL. Humor modulates the mesolimbic reward centers. *Neuron*. 2003; 40(5):1041–1048. doi: 10.1016/s0896-6273(03)00751-7. [PubMed: 14659102]
- Mobbs D, Hagan CC, Azim E, Menon V, Reiss AL. Personality predicts activity in reward and emotional regions associated with humor. *Proceedings of the National Academy of Sciences of the United States of America*. 2005; 102(45):16502–16506. doi: 10.1073/pnas.0408457102. [PubMed: 16275930]
- Monk CS. The development of emotion-related neural circuitry in health and psychopathology. [Review]. *Development and Psychopathology*. 2008; 20(4):1231–1250. doi: 10.1017/s095457940800059x. [PubMed: 18838040]
- Moran JM, Wig GS, Adams RB, Janata P, Kelley WM. Neural correlates of humor detection and appreciation. *Neuroimage*. 2004; 21(3):1055–1060. doi: 10.1016/j.neuroimage.2003.10.017. [PubMed: 15006673]
- Naghavi HR, Nyberg L. Common fronto-parietal activity in attention, memory, and consciousness: Shared demands on integration? *Consciousness and Cognition*. 2005; 14(2):390–425. doi: 10.1016/j.concog.2004.10.003. [PubMed: 15950889]
- Neely MN, Walter E, Black JM, Reiss AL. Neural Correlates of Humor Detection and Appreciation in Children. *Journal of Neuroscience*. 2012; 32(5):1784–1790. doi: 10.1523/jneurosci.4172-11.2012. [PubMed: 22302817]
- Neuhoff CC, Schaefer C. Effects of laughing, smiling, and howling on mood. *Psychological Reports*. 2002; 91(3):1079–1080. doi: 10.2466/pr0.91.8.1079-1080. [PubMed: 12585516]
- Nolen-Hoeksema S. Sex-differences in unipolar depression - evidence and theory. *Psychological Bulletin*. 1987; 101(2):259–282. doi: 10.1037//0033-2909.101.2.259. [PubMed: 3562707]
- Noonan MP, Mars RB, Rushworth MFS. Distinct Roles of Three Frontal Cortical Areas in Reward-Guided Behavior. *Journal of Neuroscience*. 2011; 31(40):14399–14412. doi: 10.1523/jneurosci.6456-10.2011. [PubMed: 21976525]
- Panksepp, J. *Affective Neuroscience: The Foundations of Human and Animal Emotions*. Oxford University Press; New York: 1998.
- Pessoa L, Adolphs R. Emotion processing and the amygdala: from a ‘low road’ to ‘many roads’ of evaluating biological significance. *Nature Reviews Neuroscience*. 2010; 11(11):773–782. doi: 10.1038/nrn2920.
- Poline JB, Worsley KJ, Evans AC, Friston KJ. Combining spatial extent and peak intensity to test for activations in functional imaging. *Neuroimage*. 1997; 5(2):83–96. doi: 10.1006/nimg.1996.0248. [PubMed: 9345540]
- Proverbio AM, Adorni R, Zani A, Trestianu L. Sex differences in the brain response to affective scenes with or without humans. *Neuropsychologia*. 2009; 47(12):2374–2388. doi: 10.1016/j.neuropsychologia.2008.10.030. [PubMed: 19061906]
- Provine, RR. *Laughter: A scientific investigation*. Viking; New York: 2000.
- Rosner F. Therapeutic efficacy of laughter in medicine. *Cancer Investigation*. 2002; 20(3):434–436. doi: 10.1081/cnv-120001187. [PubMed: 12025237]
- Samson AC, Hempelmann CF, Huber O, Zysset S. Neural substrates of incongruity-resolution and nonsense humor. *Neuropsychologia*. 2009; 47(4):1023–1033. doi: 10.1016/j.neuropsychologia.2008.10.028. [PubMed: 19046978]
- Samson AC, Zysset S, Huber O. Cognitive humor processing: Different logical mechanisms in nonverbal cartoons - an fMRI study. *Social Neuroscience*. 2008; 3(2):125–140. doi: 10.1080/17470910701745858. [PubMed: 18633854]
- Sander D, Grafman J, Zalla T. The human amygdala: an evolved system for relevance detection. *Reviews in the Neurosciences*. 2003; 14(4):303–316. [PubMed: 14640318]
- Schulte-Ruether M, Markowitsch HJ, Shah NJ, Fink GR, Piefke M. Gender differences in brain networks supporting empathy. *Neuroimage*. 2008; 42(1):393–403. doi: 10.1016/j.neuroimage.2008.04.180. [PubMed: 18514546]
- Wilbur CJ, Campbell L. Humor in Romantic Contexts: Do Men Participate and Women Evaluate? *Personality and Social Psychology Bulletin*. 2011; 37(7):918–929. doi: 10.1177/0146167211405343. [PubMed: 21521721]

- Wild B, Rodden FA, Grodd W, Ruch W. Neural correlates of laughter and humour. *Brain*. 2003; 126:2121–2138. doi: 10.1093/brain/awg226. [PubMed: 12902310]
- Wooten P. Humor: an antidote for stress. *Holistic nursing practice*. 1996; 10(2):49–56. [PubMed: 8550690]

Figure 1. Experimental paradigm explained by means of the depiction of one single trial
Each trial consisted of (i) a jittered fixation cross, (ii) clip presentation, (iii) agreeableness response, and (iv) a rest period. For more details, please refer to the introduction section.

Figure 2. Behavioral results

a) Post-scan ratings (N= 22) of funny (humor), positive and neutral clips on two scales measuring how funny and how enjoyable movies were experienced (y-axis). **b)** Post-scan ratings (N= 22) of the same clips on the same rating scales (y-axis) split by sex (13 female). Bars are shown $\pm$ 1 S.E.M., * = $p < .05$. For more information, please refer to the results section.

Figure 3. Brain activation maps of main effects

Statistical parametric maps of brain activations opposing **a)** funny *versus* positive and **b)** positive *versus* funny clips (N= 22), overlaid on a single-subject anatomical template brain. Statistical threshold for illustrative purposes was chosen at $p < .001$ (uncorrected at the peak) and $k = 20$.

Figure 4. Brain activation maps depicting sex differences

Top: Statistical parametric maps of brain activation (N= 22) displaying significant sex effects in **a)** midbrain (conjunction; $p < .001$ at the peak and $p < .05$ FWE corrected at the cluster level), as well as **b)** left amygdala and **c)** ventromedial prefrontal cortex (paired t-tests; $p < .001$ uncorrected at the cluster level). Activity is shown overlaid on a single-subject anatomical template brain. Bottom: Graphs illustrating activation in **a)** midbrain, **b)** left amygdala and **c)** ventromedial prefrontal cortex, derived from extracted raw activation (betas) at peak voxels, displaying significant sex (male *versus* female) $\times$ condition (funny *versus* humor) interactions. The y-axis corresponds to BOLD signal change in arbitrary units. vmPFC= ventromedial prefrontal cortex.

Figure 5. Correlations between differential (funny minus positive) brain activity
Raw activation (beta) values were extracted from **a)** the amygdala and midbrain, and **b)** midbrain and ventromedial prefrontal cortex (vmPFC).

Figure 6. Overview of brain areas that displayed a significant sex (male *versus* female) $\times$ condition (funny *versus* humor) interaction in children (N= 22)

Emphasized are the central components of this network (shown in orange) that displayed inter-correlated brain activity, including (i) ventromedial prefrontal cortex – vmPFC; reward expectation –, (ii) midbrain – reward sensitivity –, and (iii) amygdala – reward salience. AMY= amygdala, dIPFC= dorsolateral prefrontal cortex, IPL= inferior parietal lobule, MTG= medial temporal gyrus (BA 37), pSTS= posterior superior temporal sulcus, OCC= occipital cortex, TPJ= temporo-parietal junction, vmPFC= ventromedial prefrontal cortex.

Table 1

Brain activations for the four main effects contrasts (N= 22). IPL= inferior parietal lobule, STG= superior temporal gyrus, ITG= inferior temporal gyrus, TPJ= temporo-parietal-junction, MTG= medial temporal gyrus, pSTS= posterior superior temporal sulcus, SPL= superior parietal lobule, IPL= inferior parietal lobule, FFA= fusiform face area, IFG= inferior frontal gyrus, LPFC= lateral prefrontal cortex. Coordinates are given in MNI space.

Funny versus Neutral			
<i>p-Value</i>			
<i>FWE (cluster)</i>	<i>Voxels</i>	<i>xyz</i>	<i>Region</i>
< .001	3312	-44 -70 12	Left IPL / MTG
< .001	3396	62 -32 28	Right IPL
< .001	749	16 -24 -6	Midbrain / Thalamus
0.05	153	-10 -56 68	Left postcentral gyrus
0.036	168	16 -54 70	Right postcentral gyrus
Positive versus Neutral			
<i>p-Value</i>			
<i>FWE (cluster)</i>	<i>Voxel</i>	<i>xyz</i>	<i>Region</i>
< .001	2567	-56 -34 34	Left IPL
< .001	1692	-46 -68 2	Left ITG / MTG
< .001	3100	62 -28 28	Right IPL
< .001	1448	44 -58 -10	Right MTG
0.015	225	-12 -22 34	Cingulate Gyrus
0.017	220	48 4 0	Right Insula / STG
Funny versus Positive			
<i>p-Value</i>			
<i>FWE (cluster)</i>	<i>Voxel</i>	<i>xyz</i>	<i>Region</i>
< .001	1372	-54 -54 12	Left STG / TPJ
< .001	1684	50 -46 14	Right STG / TPJ
0.024	205	-4 -20 -8	Midbrain
< .001	605	-50 -28 -8	Left MTG / pSTS
0.025	202	10 -98 10	Right occipital cortex
Positive versus Funny			
<i>p-Value</i>			
<i>FWE (cluster)</i>	<i>Voxel</i>	<i>xyz</i>	<i>Region</i>
< .001	2491	-28 -68 54	Left IPL / SPL
< .001	10982	42 -52 56	Right IPL
0.03	193	56 -50 -14	Right MTG (FFA)
< .001	514	44 36 10	Right IFG (LPFC)

Table 2

Brain activations for the two sets of conjunction analyses (N= 22 and N= 16), determining in which of the functionally localized regions of interest (fROIs – see Table 1 *Funny versus Neutral* and vice versa) there was a significant sex effect. STG= superior temporal gyrus, TPJ= temporo-parietal-junction, MTG= medial temporal gyrus, pSTS= posterior superior temporal sulcus, SPL= superior parietal lobule, IPL= inferior parietal lobule, FFA= fusiform face area, IFG= inferior frontal gyrus, LPFC= lateral prefrontal cortex. Coordinates are given in MNI space.

Funny versus Positive × Sex in fROIs (N= 22)		
Voxel	xyz	Region
38	56 -48 24	Right SMG
164	-46 -66 16	Left MTG (TPJ)
65	-4 -20 -8	Midbrain
123	12 -98 12	Right occipital cortex
65	46 -32 0	Right STG (pSTS)
Positive versus Funny × Sex in fROIs (N= 22)		
Voxel	xyz	Region
886	54 -36 50	Right IPL
61	54 -50 -14	Right MTG (FFA)
90	-52 -38 54	Left IPL
140	44 36 12	Right IFG (LPFC)
Funny versus Positive × Sex in fROIs (N= 16)		
Voxel	xyz	Region
80	56 -48 24	Right SMG
183	-48 -66 16	Left MTG (TPJ)
95	-4 -20 -8	Midbrain
60	46 -32 0	Right STG (pSTS)
32	14 -96 12	Right occipital cortex
Positive versus Funny × Sex in fROIs (N= 16)		
Voxel	xyz	Region
30	-50 -38 56	Left IPL
238	54 -40 48	Right IPL