# Stony Brook University

# Age Differences in Loneliness:

# Neural Correlates, Neurogenetics and Functional Connectivity

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**Alexandra D'Agostino**

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Abstract of the Dissertation

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Loneliness, defined as the subjective experience of social isolation, has been linked to poor health outcomes (e.g. depression, cardiovascular disease) and is prevalent among older adult populations. To improve upon current treatments, the interaction between genetic and environmental risk factors for loneliness and their effect on the brain must be better understood. The present study investigated the brain basis of loneliness in younger (mean age = 20.4) and older adults (mean age = 62.9). We used functional magnetic resonance imaging (fMRI) and an emotional picture task to address this question. fMRI data were collected on a 3T Siemens Trio Scanner, with functional whole-brain images acquired using a gradient echo T2*-weighted EPI scan (TR = 2.5 s; TE = 30 ms; flip angle = 90; FOV = 256mm). 99 subjects (49 older, 50 younger) viewed pleasant and unpleasant social and non-social images in the scanner followed by completion of questionnaires including an objective measure of loneliness, the Social

Network Index, and a subjective measure of loneliness, the UCLA loneliness scale. Saliva samples were collected for genotyping analysis of single nucleotide polymorphisms (SNPs) in the oxytocin receptor gene (*OXTR*) that are associated with pair bonding and social behaviors. Questionnaire results indicated that older adults were significantly less lonely, anxious, shy, and depressed and had more frequent social contacts compared to younger adults. Eye tracking data demonstrated that older adults spent significantly more dwell time on faces while viewing both pleasant and unpleasant social images. Furthermore, older adults showed significantly greater activation in the fusiform gyrus during viewing of pleasant social images, even when controlling for differences in loneliness and gaze ($p < 0.05$, FWE corrected). These results support the positivity effect, which is a tendency among older adults to attend preferentially to positive information to increase their emotional satisfaction. Secondly, our genetic analyses demonstrated that individuals with the A/G genotype of the *OXTR* rs53576 SNP scored significantly higher on the shyness scale compared to G/G subjects ($p < 0.005$). Additionally, functional connectivity analyses indicated increased connectivity between the hypothalamus and a cluster in the limbic lobe for individuals with the rs53576A genotype during viewing of unpleasant social pictures. These results suggest that rs53576A individuals show a heightened response to negative social information. Taken together, our results highlight the influence of age, genetic polymorphisms and neural circuits on social behavior.

*For Patrick and Lily*

## Table of Contents

## List of Figures

## List of Tables

## List of Abbreviations

(ASDs) - autism spectrum disorders

(AVP) - arginine vasopressin

*(AVPR1A*) - arginine vasopressin receptor 1A gene

(BDI) - Beck Depression Index

(CG) - complicated grief

(dACG) - dorsal anterior cingulate gyrus

(EPI) - echoplanar imaging

(ERQ) - Emotion Regulation Questionnaire

(fMRI) – functional magnetic resonance imaging

(FWE) - family wise error

(gPPI) – generalized psychophysiological interaction

(IAPS) - International Affective Picture System

(IAs) - interest areas

(IFG) - inferior frontal gyrus

(MITE) - Mind in the Eyes

(mPFC) - medial prefrontal cortex

(NAcc) - nucleus accumbens

(NCG) - non-complicated grief

(OTKO) - oxytocin knockout

(OTRKO) - oxytocin receptor knockout

(*OXTR*) - oxytocin receptor gene

(PET) - positron emission tomography

(PiL) - Purpose in Life

(ROIs) - regions of interest

(SCAN) - Social, Cognitive and Affective Neuroscience

(SNI) - Social Network Index

(SNPs) - single nucleotide polymorphisms

(SST) - Socioemotional Selectivity Theory

(STAI) - State-Trait Anxiety Inventory

(STG) - superior temporal gyrus

(SVCs) - Small volume corrections

(TMS) - transcranial magnetic stimulation

(ToM) - Theory of Mind

(VTA) - ventral tegmental area

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## Introduction

As a result of increased life expectancy, elderly populations are expanding in many developed countries; the expected lifespan for babies born since the turn of the 21st century is predicted to be 100 in countries such as Japan, Canada and the U.S (Christensen, Doblhammer, Rau, & Vaupel, 2009). Old age is commonly associated with shrinking social networks and there is a great deal of interest in the effects of social isolation and loneliness on mental and physical health. Frequent loneliness affects 5 to 15 percent of people over 65 and up to 50 percent of people over the age of 80 (Pinquart & Sorensen, 2001). Environmental risk factors for loneliness include bereavement (Peplau, 1982), living alone and deteriorating health (Savikko, Routasalo, Tilvis, Strandberg, & Pitkälä, 2005). In addition to poor physical health (Hawkley, Thisted, Masi, & Cacioppo, 2010; Luanaigh & Lawlor, 2008; Tomaka, Thompson, & Palacios, 2006), loneliness is associated with cognitive decline (Shankar, Hamer, McMunn, & Steptoe, 2013), depression (Alpass & Neville, 2003), and both anatomical (Kanai et al., 2012) and functional brain differences (J. T. Cacioppo, Norris, Decety, Monteleone, & Nusbaum, 2009; S. Cacioppo, Balogh, & Cacioppo, 2015; Kanai et al., 2012).

Importantly, loneliness is usually concomitant with other social deficits. For instance, it correlates negatively with social network size (Stokes, 1985). To fully understand the neural underpinnings of loneliness, circuits that govern other social behaviors and traits such as attachment, social approach, theory of mind (ToM), social craving and loneliness must also be considered. Behavioral neuroscience studies in

animal models provide a solid foundation for mapping of these circuits. For example, research in prairie voles has investigated pair bonding behavior and the neuroendocrine mechanisms that regulate it. Of special interest are recent optogenetic studies in rodents that have illuminated specific neural circuits necessary for social behaviors. For example, excitation of projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAcc) leads to increased social interactions in mice (Gunaydin et al., 2014). While these animal models are indispensible for studying the neural circuitry of social behavior, they cannot model more abstract concepts such as empathy and ToM, defined as the ability to attribute mental states to oneself and others (Sodian & Kristen, 2010). These concepts have instead been studied in human neuroimaging experiments, which have identified not only how these social abilities are affected in individuals with disorders such as autism but also how those behavioral deficits are related to brain structure and function. Here, we will compare the behavioral neuroscience literature in animal models to the human neuroimaging and genetic literature in order to create a clearer picture of the neural circuitry governing more complex social behaviors. Finally, this discussion of social circuitry will be placed in the context of aging and its effects on cognition and social and emotional processing.

## Social Behaviors: Neural Circuitry and Genetics

### *Methods for Studying Social Behaviors*

Although more abstract social emotions, such as loneliness and empathy, are complex and often difficult to study in animal models, other aspects of social behaviors have been successfully operationalized in studies using a behavioral neuroscience approach. Examples include pair bonding in voles, social separation in rats, and attachment between mothers and offspring. In the human neuroimaging literature, attachment, loneliness, social rejection, and ToM have been studied extensively. The development of new research techniques, such as optogenetic approaches in mice and functional connectivity in humans, allows researchers to better elucidate neural circuits of social behaviors and to determine how well animal models can be mapped onto human brain circuitry.

One of the challenges to studying neural circuitry lies in identifying the directionality of projections between brain regions. A second difficulty involves understanding how activation of specific subregions drives behavior. Studying neural circuitry with traditional techniques such as lesions or electrode implantation is difficult due to their imprecision and lack of temporal control. Optogenetic approaches can overcome these limitations by first targeting specific subsets of cells via viral vectors and then switching them on and off with light-controlled temporal precision. Specifically, optogenetics involves the expression of opsins such as channelrhodopsins within the cell membrane (Deisseroth et al., 2006). Once these opsins are expressed, light stimulation via optic fibers causes these cells to depolarize (Deisseroth et al., 2006) or

hyperpolarize, in the case of halorhodopsins and archaerhodopsins (Schobert & Lanyi, 1982; Uegaki, Sugiyama, & Mukohata, 1991). With this powerful technique, specific neural circuits can be targeted and switched on and off in awake, behaving animals to observe the effect on behavior.

While optogenetic techniques allow for precise identification of projections between brain areas and their behavioral output, there are limits to extending animal research to human behavior. While behaviors such as anxiety employ much of the same neural circuitry in humans and rodents (Phelps & LeDoux, 2005), humans experience a wider range of emotions such as jealousy and regret that are impossible to study in animals because we do not know their mental state. These limitations underscore the need for human fMRI studies in the social neuroscience literature. One relatively new analysis technique that uses fMRI data to study neural circuits is functional connectivity. Functional connectivity measures the extent to which the activity in two spatially remote brain areas is temporally correlated (Friston, 2011; Friston, Frith, Liddle, & Frackowiak, 1993). A limitation of functional connectivity is that it measures correlation and not causation. Effective connectivity, by contrast, can overcome this limitation by measuring the influence that one neural system exerts on another (Friston, 2011; Friston, Frith, & Frackowiak, 1993). The following sections will discuss animal models of specific social behaviors alongside related human neuroimaging studies to synthesize the current literature and create a comprehensive view of the neural circuits involved in social behaviors and reward processing (Figure 1).

*Neuroendocrine Regulation of Attachment*

Social attachment is crucial for survival in many species and is unique from other social interactions in its selectivity and long-lasting duration (Insel & Young, 2001). Two classic examples of social attachment include pair bonding and mother-infant attachment. Pair-bonding has been studied extensively in prairie voles because they exhibit all three criteria for monogamy; an exclusive mating relationship, co-parenting and preferred association with one opposite-sex partner (Barrett & Young, 2015). Furthermore, prairie voles maintain these monogamous behaviors in the laboratory environment. Mother-infant attachment varies widely across species but is generally defined by the amount of time mothers spend with their young. Rat models have been integral in the study of mother-infant bonding because mothers show a very distinct onset of maternal behaviors (Insel & Young, 2001).

Neuroendocrine mechanisms regulating social attachment have been studied extensively, particularly in rodent models. Oxytocin and vasopressin are two neuropeptides essential for social behaviors. They differ in structure by only two amino acids and are synthesized in the hypothalamus and released from the posterior pituitary (Insel, 1997). As will be discussed below, oxytocin and vasopressin have unique contributions to not only mother-infant attachment but to other social behaviors as well.

Oxytocin has been identified as a key hormone and neuropeptide important not only during childbirth (Fuchs, Fuchs, Husslein, Soloff, & Fernstrom, 1982) but also for maternal bonding behaviors (Galbally, Lewis, IJzendoorn, & Permezel, 2011; Kendrick et al., 1997) and affiliation (Witt, Winslow, & Insel, 1992). Oxytocin has been shown to

be essential for mother-infant bonding; oxytocin knockout (OTKO) and oxytocin receptor knockout (OTRKO) mice emitted fewer ultrasonic vocalizations when separated from their mothers and OTKO mice took longer to reunite with their mothers (Barrett & Young, 2015). Aside from these maternal functions, however, oxytocin also has anxiolytic properties and plays a role in many different social behaviors (Uvnäs-Moberg, Arn, & Magnusson, 2005). A study in male rats demonstrated that chronic injections of oxytocin significantly increased social, non-sexual behaviors (Witt et al., 1992). Oxytocin is also important for partner preference formation. Specifically, monogamous female prairie voles had a higher density of oxytocin receptors within the NAcc compared to nonmonogamous vole species and blocking these receptors led to a reduction in partner preference formation (Young, Lim, Gingrich, & Insel, 2001).

Human studies have found that mothers with secure attachment to their child showed activation in the ventral striatum, which contains the NAcc, and in oxytocin-related hypothalamic and pituitary regions. This activation was accompanied by increased peripheral oxytocin levels while viewing their child's face (Strathearn, Fonagy, Amico, & Montague, 2009). In a related study, separate attachment styles in adults (secure, anxious, and avoidant) were correlated with different patterns of activation during social feedback about task performance (Vrtička, Andersson, Grandjean, Sander, & Vuilleumier, 2008). Specifically, positive feedback correlated with activation in the striatum and VTA, although this activation was reduced in avoidant subjects (Vrtička et al., 2008). Negative feedback was associated with left amygdala activation while also correlating positively with anxious attachment subjects (Vrtička et al., 2008). These

results indicate that attachment style is associated with differential brain response and oxytocin release to social stimuli.

While the present study will focus on oxytocin, arginine vasopressin (AVP) is another neuropeptide that, when acting through the V1aR receptor subtype, is important for pair bonding in rodents (Young & Wang, 2004) as well as humans (Walum et al., 2008). Research in monogamous prairie voles indicates that vasopressin acts in the ventral pallidum and lateral septum of males to facilitate attachment and pair bonding (Young et al., 2001; Young & Wang, 2004). In keeping with the importance of vasopressin in male attachment, Walum and colleagues found that men carrying the RS3 334 allele of the arginine vasopressin receptor 1A (*AVPR1A*) gene were less likely to be married and scored lower on the partner bonding scale compared to men not carrying the RS3 334 allele (Walum et al., 2008). A second study investigated whether common genetic variants of the *AVPR1A* gene were related to success of social integration but did not find any significant correlation, although the authors suggest that this may be due to lack of statistical power (Chang et al., 2014). More research is needed to understand how genetic polymorphisms in the *AVPR1A* gene correspond with social functioning.

*Social Approach*

Social approach is another aspect of social behavior necessary for building and maintaining social bonds. In recent years, optogenetic research in rodents has highlighted the neural circuits for social approach. Dopaminergic projections from the VTA to areas including the NAcc and medial prefrontal cortex (mPFC) are a main part of

the reward circuit (see Figure 1) and code for the salience of both social and non-social stimuli (Berridge & Robinson, 1998; Bromberg-Martin, Matsumoto, & Hikosaka, 2010; Russo & Nestler, 2013; Wise, 2000). In support of this, Gunaydin and colleagues (2014) found that optogenetic stimulation of the VTA-NAcc circuit led to an increase in social interactions in mice. Interestingly, they also discovered that while activation of the VTA-mPFC projection had no effect on social behavior, it did lead to increased anxiety as evidenced by a conditioned place aversion. These studies strongly suggest that projections from the VTA to the NAcc are involved with processing of social rewards.

Human research has also investigated the neural correlates of social approach. Schilbach and colleagues conducted a study which exposed subjects to virtual faces that looked directly at the subject or at a third person with either a socially relevant expression or an arbitrary expression (Schilbach et al., 2006). In the direct gaze condition, subjects showed increased activation in the anterior medial prefrontal cortex while increased activation was seen in the precuneus during a third person gaze (Schilbach et al., 2006). Additionally, socially relevant expressions were positively correlated with activation in the ventral medial prefrontal cortex while arbitrary expressions were associated with increased activation in the middle temporal gyrus (Schilbach et al., 2006). Interestingly, a second study found that placement along the shy-bold continuum predicted neural responses to familiar versus stranger faces (Beaton et al., 2008). Specifically, they found that compared with bold adults, shy individuals showed increased amygdala activation in response to stranger faces and increased left amygdala activation in response to familiar faces (Beaton et al., 2008). Compared to shy adults, bold adults exhibited increased NAcc and subcallosal cortex

activation to both familiar and stranger faces (Beaton et al., 2008). A related fMRI study by Richey and colleagues (2012; J. A. Richey et al., 2014) found that people with autism spectrum disorders (ASDs) or social anxiety disorder show decreased NAcc activation to social reward anticipation. Furthermore, they found that activation of the vmPFC was increased in subjects with ASD in response to certain non-social rewards. Areas of activation in these studies of social approach show a great deal of overlap with reward circuitry (Figure 1). Furthermore, personality appears to be correlated with activation in these areas, suggesting individual differences in the reward response to socially relevant stimuli.

*Mirror Neurons and Theory of Mind*

Electrophysiological studies in macaques have identified mirror neurons in area F5 of the ventral premotor cortex (Matelli, Luppino, & Rizzolatti, 1985). Mirror neurons fire action potentials both when a certain action is performed (such as grasping for an object) and when that same action is being observed (Gallese, Fadiga, Fogassi, & Rizzolatti, 1996; Rizzolatti, Fadiga, Gallese, & Fogassi, 1996). Mirror neurons were first demonstrated in humans using transcranial magnetic stimulation (TMS); subjects had similar patterns of motor evoked potentials during observation of an action compared to performance of the action (Fadiga, Fogassi, Pavesi, & Rizzolatti, 1995). Since that initial study, specific brain regions encompassing the mirror neuron network have been identified, including the left superior temporal sulcus (BA 21), left inferior parietal lobule (BA 40) and the anterior part of Broca's region (BA 45) (Rizzolatti, Fadiga, Matelli, et al., 1996).

While a definitive function of mirror neurons is still uncertain, there are several studies suggesting that mirror neurons are important for normal social functioning, particularly with regards to empathy and ToM, defined as the ability to attribute different mental states to oneself and others (Sodian & Kristen, 2010). For instance, a positron emission tomography (PET) study in healthy adults found that the right inferior frontal cortex was more active during assessment of facial emotions (Nakamura et al., 1999). A second study found that autistic individuals showed decreased activation in several putative mirror neuron regions (BA 44/45, superior temporal gyrus (STG), right insula and the left amygdala) while performing the mind in the eyes (MITE) task (Frith & Frith, 2000). During the MITE, subjects are instructed to identify an emotion based only on the eye region of the face. In keeping with these findings, a study of autistic children found that they fail to employ ToM (Baron-Cohen, Leslie, & Frith, 1985). Specifically, the children in the study were shown a series of pictures in which one character places a marble in a basket and then leaves the room. While she is gone, a second character moves the marble from the basket to a box. The children were then asked to state where the first character would initially look for her marble. While normal children recognized that she would first look in the basket, the majority of autistic children stated that she would look in the box, suggesting that they had difficulty understanding the first character's mental state (Baron-Cohen et al., 1985). These studies suggest that mirror neurons are important for ToM and should be considered a part of social circuitry more generally.

*Social Craving*

Craving for social contact is an important factor in initiating social behaviors. Studies of drug craving have shown a positive correlation between the intensity of craving for drugs such as cocaine and alcohol and activation in the dorsal and ventral striatum, respectively (Braus et al., 2001; Sinha et al., 2005). One social equivalent of drug craving could be yearning for a lost loved one. To that end, O'Connor and colleagues recruited subjects experiencing complicated grief (CG), in which an individual experiences prolonged, unyielding grief and found that these individuals showed increased NAcc activity while viewing grief related words (O'Connor et al., 2008). Importantly, NAcc activity correlated positively with yearning for the deceased in both CG and non-complicated grief (NCG) subjects, which may represent a form of social craving (O'Connor et al., 2008).

Feelings of love can also be considered to be a form of social craving. Studies of maternal and romantic love found that both types led to increased activation in the VTA, caudate, striatum, middle insula and anterior cingulate (Bartels & Zeki, 2004; Fisher, Aron, & Brown, 2005). A more recent study of long-term romantic love between married couples found activation in many of the same regions related to reward processing (including the VTA and dorsal striatum) while subjects viewed pictures of their partners (Acevedo, Aron, Fisher, & Brown, 2011). However, areas that are typically associated only with maternal love were also activated such as the globus pallidus, thalamus, insular cortex and anterior and posterior cingulate (Acevedo et al., 2011). Furthermore, romantic and maternal love was also associated with decreased activation in the amygdala (Bartels & Zeki, 2004). The amygdala's role in fear, anxiety and social

behavior has been shown repeatedly in human studies (LeDoux, 2003) and also plays a role in psychopathology. For instance, studies have shown that patients with anxiety show greater amygdala activation to emotional faces compared to controls (Etkin & Wager, 2007; M. Stein, Simmons, Feinstein, & Paulus, 2007; M. B. Stein, Goldin, Sareen, Zorrilla, & Brown, 2002). Romantic and maternal love was also associated with deactivation in the middle prefrontal, inferior parietal and middle temporal cortices as well as the posterior cingulate cortex, which are areas associated with cognition and negative emotions (Bartels & Zeki, 2004). These findings suggest that putative social forms of craving, such as grief and love, may increase activation in reward regions while downregulating activation in brain areas associated with anxiety and unpleasant emotions.

*Loneliness and Social Isolation*

Loneliness can be defined as the subjective experience of a negative emotional state related to unfulfilled intimate and social needs (Peplau, 1982). It has also been suggested that loneliness can be predicted by the difference in desired and achieved levels of social contact (Perlman & Peplau, 1981). As such, there is no animal literature that specifically addresses loneliness. However, studies in which animals are randomly assigned to different social living conditions (group housed, pair housed, single housed) are often used to model depression and other psychiatric disorders and to investigate environmental factors. It is important to emphasize, however, that social isolation is not always accompanied by feelings of loneliness. In fact, different species show different responses to being placed in social isolation. For example, titi monkeys are

monogamous and show an increase in plasma cortisol during social isolation while polygynous squirrel monkeys show no differences in plasma cortisol as a result of social isolation (Mendoza & Mason, 1986).

Gross anatomical differences exist between socially isolated individuals and healthy controls. Rabbits that were socially isolated from their mothers showed increased synaptic density in the infralimbic cortex, an area involved with learning and emotion (Ovtscharoff Jr & Braun, 2001). In macaques, increased social network size was positively correlated with increased grey matter area within the inferior temporal gyrus and the rostral prefrontal cortex (Sallet et al., 2011). Loneliness in humans is associated with reduced grey matter in the left posterior superior temporal sulcus, which is an area important for social perception (Kanai et al., 2012).

In humans, subjective loneliness has also been associated with changes in the neural processing of social and emotional stimuli (J. T. Cacioppo et al., 2009). Cacioppo and colleagues found that loneliness was associated with reduced activation in the ventral striatum to viewing of pleasant social images (J. T. Cacioppo et al., 2009). Interestingly, lonely individuals showed greater activation in the ventral striatum to pleasant, non-social images compared to non-lonely subjects. As the ventral striatum is a region known for its role in reward (de la Fuente-Fernández et al., 2002; Delgado, Nystrom, Fissell, Noll, & Fiez, 2000; Schott et al., 2008; Schultz, Apicella, Scarnati, & Ljungberg, 1992) and motivation (Randall et al., 2012), these findings suggest that lonely individuals are less rewarded by pleasant social stimuli. Furthermore, a recent high density electroencephalogram (EEG) study analyzed brain microstates during a Stroop task to demonstrate that lonely individuals process negative social words more

quickly than non-lonely individuals (S. Cacioppo et al., 2015). These results support the evolutionary theory of loneliness, which posits that lonely individuals show hyper-vigilance to threatening social stimuli (Bangee, Harris, Bridges, Rotenberg, & Qualter, 2014; J. T. Cacioppo, Cacioppo, Capitanio, & Cole, 2015).

Twin studies have identified loneliness as a heritable trait, with 48% of the observed variance in adults due to genetic factors (Boomsma, Willemsen, Dolan, Hawkley, & Cacioppo, 2005). Genetic polymorphisms in several candidate genes have been associated with loneliness including *CHRNA4*, *BDNF*, *MTFHR* (see Goossens et al. (2015) for a review). One well-studied gene with regards to loneliness, and the focus of the current work, is the oxytocin receptor gene (*OXTR*). A study of 195 Chinese Han family autism trios investigated four different SNPs in the *OXTR* gene (Wu et al., 2005). Two of the four SNPs tested (rs2254298A and rs53576A) were found to correlate significantly with autism (Wu et al., 2005). Based on the connection between *OXTR* polymorphisms and autism, Lucht and colleagues investigated the effect of *OXTR* polymorphisms on loneliness and positive and negative affect (Lucht et al., 2009). Their main finding was that *OXTR* rs53576 A/A was associated with significantly lower positive affect in male adults (Lucht et al., 2009). *OXTR* rs2254298 A/A also related to lower positive affect in both genders, but only on a trend level. Importantly, social loneliness was found to be higher in adults with the rs53576 A/A genotype but only on a trend level, which may be due to a low sample size (Lucht et al., 2009). The rs53576A genotype, considered the risk allele, has also been linked to deficits in empathy (Rodrigues, Saslow, Garcia, John, & Keltner, 2009) and attachment (Costa et al., 2009).

Aside from these social impairments, *OXTR* risk alleles are also associated with

differences in brain connectivity, particularly in hypothalamic-limbic circuits that regulate emotion. A study by Tost and colleagues (2010) found that individuals with the *OXTR* rs53576A allele showed increased structural connectivity between the hypothalamus and the dorsal anterior cingulate gyrus (dACG) and amygdala as well as increased functional connectivity between the hypothalamus and amygdala. A separate study by Tost and colleagues (Tost et al., 2011) found that the rs2254298A carriers also showed increased structural coupling between the hypothalamus and dACG along with decreased hypothalamic grey matter volume. Similarly, Inoue and colleagues (2010) observed increased bilateral amygdala volume in rs2254298A allele carriers. While these findings underscore the effects of *OXTR* gene polymorphisms on social behavior and brain structure and function, additional research is needed to fully understand the mechanism of action of these risk alleles.

Effects of Aging on Social and Emotional Processing

Deleterious effects of age on cognition (Salthouse, 1996) and memory (Prull, Gabrieli, & Bunge, 2000) are widely recognized. In the past several decades, however, the aging literature has begun to focus on how emotion regulation and social processing change with age. Of particular interest is the positivity effect, which is the observation that with age comes a preference to attend to and remember positive over negative information (Mather & Carstensen, 2005). The positivity effect (also referred to as the positivity bias in studies where participants are given a choice of stimuli) has been demonstrated across many different modalities. For instance, a dot-probe study demonstrated that older adults were faster at responding to a dot when it appeared behind a positive face relative to younger adults (Carstensen & Mikels, 2005). In a related study, increased activation in the amygdala of older adults was observed during viewing of positive pictures (Mather et al., 2004). While older adults show a positivity bias, younger adults seem to focus more on negative stimuli. For instance, when researching features of a new car, younger adults spent significantly more time focusing on its negative aspects (Mather, Knight, & McCaffrey, 2005). Further, when viewing negative faces, younger adults showed increased activation of the left amygdala (Iidaka et al., 2002). Together, these findings support the idea of a positivity effect among older adults.

Aging is often accompanied by decreases in social network (Cornwell, Laumann, & Schumm, 2008). This reduction in social contacts may be due in part to the socioemotional selectivity theory (SST), which posits that when people perceive the time they have left in their lives to be diminishing, their social behavior changes to focus

more on their emotionally close partners and less on expanding their social networks (Carstensen, 2006). Importantly, the SST is not due to age directly but rather perceived future time. Although typical of older adults, younger adults faced with a time constraint, such as an upcoming move across the country, will adopt goals similar to those of older adults that include focusing more on emotionally close relationships.

These age differences in social preference may also be associated with differences in brain response to social stimuli. For instance, when completing the MITE task described previously, both younger and older adults were found to recruit mirror neuron areas (posterior superior temporal sulcus and temporo-parietal junction) (Castelli et al., 2010). Older adults, however, showed bilateral activation of components of the mirror neuron system that are also located in lingual areas, including the precentral gyrus, inferior frontal gyrus (IFG), the STG and the claustrum. Increased activation in these areas suggests that older adults may rely more on the word choices at the bottom of the screen to determine the correct emotion (Castelli et al., 2010). These findings are consistent with the idea of the cognitive control model, which purports that with age, increased emotional regulation reduces amygdala activation to negative stimuli (Mather, 2012). Other fMRI data indicate age differences in recruitment of the fusiform gyrus in response to faces (Burianová, Lee, Grady, & Moscovitch, 2013). Specifically, during a face matching task, older adults did not recruit the left fusiform gyrus and did not show functional connectivity between the left and right fusiform gyrus. However, older adults did show functional connectivity between the right fusiform gyrus and left orbitofrontal cortex that was correlated with increased face matching, suggesting that compensatory networks play a part in face processing as we age

(Burianová et al., 2013). In sum, older adults show differences in brain activation in areas related to social processing that are accompanied by behavioral differences such as investing in a few close relationships and focusing more on positive information.

Overview of Dissertation

The present work sought to investigate the neural correlates of loneliness in younger and older adults using human neuroimaging techniques. To accomplish this, we utilized an fMRI task that presented both pleasant and unpleasant social and nonsocial pictures. Our primary goal was to determine whether loneliness correlates with brain response to social and emotional stimuli in younger and older adults. Based on findings from Cacioppo's (2009) prior study of loneliness, we hypothesized that younger lonely adults would show decreased activation in the ventral striatum to pleasant social images. Because older adults have been found to attend more to positive social stimuli and to value a few close relationships (Mather & Carstensen, 2005; Carstensen, 2006), we did not expect to find differences in ventral striatal activation between lonely and non-lonely older adults.

We also focused on several other regions of interest that were found by Cacioppo and colleagues (2009) to be related to loneliness including the insula, caudate, fusiform, STG and IFG. As mentioned previously, the insula and caudate have both been linked to social craving (Bartels & Zeki, 2004; Fisher, Aron, & Brown, 2005). The fusiform gyrus is an area activated during facial processing and recognition (Kanwisher, McDermott, & Chun, 1997; McCarthy, Puce, Gore, & Allison, 1997) and also in response to emotionally valenced social images (Geday, Gjedde, Boldsen, & Kupers, 2003). Furthermore, the fusiform gyrus has been shown to be differentially activated in younger and older adults (Burianová et al., 2013; Kensinger & Schacter, 2008). Both the STG and IFG are thought to be part of the mirror neuron system and

their recruitment during social processing has been shown to vary by age (Castelli et al., 2010). We predicted that activation in these areas would differ based on loneliness and age.

Our second aim was to determine whether certain polymorphisms of the *OXTR* gene would correlate with loneliness. Both the rs53576A and rs2254298A *OXTR* polymorphisms have been associated with autism (Wu et al., 2005) and in particular, the rs53576A risk allele is associated with reduced empathy (Rodrigues et al., 2009) and attachment (Costa et al., 2009). Here, we hypothesized that *OXTR* rs53576A and rs2254298A would be correlated with increased loneliness.

Lastly, functional connectivity analyses were performed to address our final goal of investigating neurogenetic circuits underlying loneliness. Based on prior research (Tost et al., 2010; Tost et al., 2011), we hypothesized that both rs53576A and rs2254298A *OXTR* carriers would show increased functional connectivity between the hypothalamus, dACG and amygdala during the task. In sum, the present work expands upon previous fMRI studies of loneliness by inclusion of an older adult population and the integration of genetic factors and circuit level activation.

## Materials and Methods

Participants and Questionnaires

Subjects were 49 older adults (mean age of 62.88 ± 6.15) and 50 younger adults (mean age of 20.36 ± 2.00). All participants were prescreened to exclude for any history of psychiatric diagnoses, use of mood altering or psychoactive medication, infectious disease symptoms, major medical conditions, and history of head trauma. All participants gave informed, written consent prior to the experiment and were compensated at a rate of $20 per hour.

Following their participation in several fMRI tasks, subjects provided saliva samples for genetic analysis and completed a set of questionnaires in addition to demographic and health information. The following questionnaires were collected to assess both subjective and objective aspects of social connectedness (see Appendix for questionnaire forms). The UCLA loneliness scale (Russell, 1996) is a measure of subjective loneliness. The Social Network Index (Cohen, Doyle, Skoner, Rabin, & Gwaltney, 1997) asks subjects to report the number of people they interact and communicate with on a regular basis. The results of the Social Network Index (SNI) generate two measures: the number of people in the social network with which the subject has regular contact and the number of social roles in which the subject has frequent contact with at least one person. The UCLA loneliness scale and the SNI were included to assess subjective and objective loneliness, respectively. The Shyness Scale (Hopko, Stowell, Jones, Armento, & Cheek, 2005) was included as a questionnaire of interest based on research indicating that shyness is correlated with loneliness and

difficulties in social situations (Zimbardo, Pilkonis, & Norwood, 1977). The Purpose in Life (PiL) Questionnaire (Boyle, Barnes, Buchman, & Bennett, 2009) is a measure of the degree to which a person feels that they have purpose in their life. Purpose in life was chosen as a covariate of interest based on data indicating an age-related decrease in purpose in life (Pinquart, 2002). Furthermore, purpose in life is positively associated with social integration and a higher quality of social contacts (Pinquart, 2002).

Several questionnaires were also collected to control for other behavioral traits that may confound our measure of loneliness. Specifically, the State-Trait Anxiety Inventory (STAI) measures the subject's current level of anxiety as well as their characteristic anxiety level (Spielberger, Gorsuch, & Lushene, 1968). The Emotion Regulation Questionnaire (ERQ) assesses how well subjects are able to regulate their positive and negative emotions and is broken up into two components, expressive suppression and cognitive reappraisal (Gross & John, 2003). The Beck Depression Index (BDI) is a widely used measure of the severity of depression (Beck, Steer, & Brown, 1996).

fMRI Image Acquisition

All fMRI scans were conducted at the Social, Cognitive and Affective Neuroscience (SCAN) Center at Stony Brook University. Imaging data were acquired on a 3 Tesla Siemens TrioTim scanner (Siemens Medical, Erlangen, Germany) with a 12-channel head coil. High-resolution volume scans using 3D MPRAGE were collected on all subjects. During analysis, the functional scans for each participant were registered to the brain volume generated by the MPRAGE scan. Functional images were acquired

using a gradient echo T2*-weighted echoplanar imaging (EPI) scan with an axial-oblique orientation and a flip angle of 90°, repetition time (TR) = 2.5 s, echo time (TE) = 30 ms, 34 slices, 4 mm thick with no gap, and a field of view (FOV) of 256 mm. Several seconds of dummy volumes were collected initially to allow time for magnetic saturation.

fMRI Task Design

The fMRI task presented social and emotional stimuli to participants and was modeled after Cacioppo's study of loneliness (J. T. Cacioppo et al., 2009). Stimuli were presented in a counterbalanced block design consisting of social and nonsocial images of varying pleasantness (Figure 2). The task was divided into two runs, each lasting 5 minutes and 33 seconds. Social images contained two people while nonsocial images contained no people. There were four different conditions (pleasant social, pleasant nonsocial, unpleasant social, unpleasant nonsocial), with each condition presented four times. The images were selected from the International Affective Picture System (IAPS) (Lang, Bradley, & Cuthbert, 2008) and chosen to ensure that the normative arousal ratings (Grühn & Scheibe, 2008) were matched across all 4 conditions to avoid any confounds due to differences in arousal. Additionally, normative valence ratings were matched between valence groups (i.e. pleasant social and pleasant nonsocial) to ensure that images were equally pleasant or unpleasant. The pleasant social picture set had a mean valence rating of 7.26 (± 0.31) and mean arousal of 5.24 (± 0.47). The pleasant nonsocial picture set had a mean valence of 7.27 (± 0.38) and mean arousal of 5.27 (± 0.47). The unpleasant social picture set had a mean valence of 2.70 (± 0.46) and mean arousal of 5.23 (± 0.80). The unpleasant nonsocial picture set had a mean

valence of 2.70 (± 0.26) and mean arousal of 5.28 (± 0.68). Images were presented to the participant through an optical mirror attached to the head coil. The pictures were presented in blocks of 4 images, with each image presented for 5 seconds. Subjects were instructed to freely view the images while always keeping their eyes and attention on the image. Subjects were also asked to rate the valence of each picture using an fMRI compatible button box according to the following scale: positive (index finger), neutral (middle finger) and negative (ring finger). Each intertrial interval consisted of a black crosshair against a grey background and was presented for 20 seconds. The task also utilized eye-tracking software (Eyelink; SR Research, ON, Canada) to assess age differences in attention and to determine if individual differences in gaze fixation during social and nonsocial images affected brain response.

Eye Tracking Analysis

An Eyelink 1000 eye-tracker was used to sample eye position at 1000 Hz. Prior to the start of the experiment, a calibration screen was presented and each subject was asked to follow a black dot on the screen with their eyes. This calibration served to match eye position to particular locations around the screen. Calibrations were considered acceptable if the average error was under 0.49 degrees and the maximum error was below 0.99 degrees.

Data analysis was performed offline using the DataViewer application (SR Research, ON, Canada). This software package measured the number, duration and location of all fixations to assess how much time was spent looking at different parts of each image. Interest areas (IAs) for the social images were defined as the faces in the

image and cumulative fixation duration (dwell time) within these IAs was computed. Dwell time on faces was summed across images within a block and then across blocks and expressed as a percentage of total dwell time. Percentages were then averaged across subjects to generate group mean data.

fMRI Data Analysis

Analysis of imaging data was conducted using standard preprocessing procedures in SPM8 (Wellcome Department of Cognitive Neurology, London, UK). Motion correction was first performed using the ArtRepair toolbox (Mazaika, Whitfield-Gabrieli, Reiss, & Glover, 2007) to remove volumes that contained motion artifacts of more than 1mm/TR and replace them with interpolated data from the volumes that came before and after the removed volume. If more than 20% of volumes needed repair, the data were discarded. Slice timing correction was performed followed by realignment to correct for motion. Coregistration was then performed followed by segmentation. Finally, images were normalized to the standard Montreal neurological Institute space and then smoothed with a Gaussian kernel of 8mm FWHM.

First level, single-subject analyses were run using a file for each subject that specified the onset and duration of images in each category (social pleasant, social unpleasant, non-social pleasant, non-social unpleasant and fixation). Second level, random effects analyses were performed to examine differences in brain activation between the different conditions (e.g. activation that is greater in the pleasant social condition compared to the pleasant non-social condition). These contrasts were set up for each subject at the first level of analysis. Regression analyses were also performed

to correlate brain activation to social and nonsocial images with scores on loneliness, shyness, SNI and PiL questionnaires. For all regression analyses, a family wise error (FWE) rate of 0.05 was used. The xjView toolbox was used for anatomical labeling of activated clusters (http://www.alivelearn.net/xjview). Small volume corrections (SVCs) were also performed to focus on a priori areas of interest that were based on previous studies of loneliness.

To analyze functional connectivity, a generalized psychophysiological interaction (gPPI) toolbox was used (McLaren, Ries, Xu, & Johnson, 2012). Unlike PPI analyses, the gPPI analysis allows for comparison of more than two experimental conditions and reduces the likelihood of false positives and negatives (McLaren et al., 2012). Functional EPI scans were used in the gPPI analysis. Seed regions for this analysis were selected a priori based on previous studies of loneliness, *OXTR* polymorphisms and aging. Specifically, the hypothalamus was defined as a seed region based on the finding that individuals with the *OXTR* rs53576A allele showed a structural correlation between the hypothalamus and the dACG (Tost et al., 2010). The fusiform gyrus was also selected as a seed region based on results showing that fusiform recruitment differed based on age and the valence of the information being encoded (Burianová et al., 2013; J. T. Cacioppo et al., 2009). The left fusiform gyrus was defined based on group maps for the whole brain analysis of the pleasant social greater than pleasant nonsocial contrast.

Several regions of interest (ROIs) were selected based on findings from Cacioppo's (2009) study of loneliness to be used for SVC. Specifically, for the pleasant social greater than pleasant nonsocial contrast the NAcc, fusiform gyri and left insula

were selected. Secondly, for the unpleasant social greater than unpleasant nonsocial contrast the STG, right caudate, and right IFG were selected. The right caudate and fusiform gyri were defined anatomically using the AAL atlas in WFUPickatlas (Maldjian, Laurienti, Kraft, & Burdette, 2003). The NAcc, left insula, right IFG and STG were defined anatomically using the IBASPM71 atlas in WFUPickatlas (Maldjian et al., 2003).

Saliva Sampling

After the scanning session ended, saliva samples were collected from participants using Oragene saliva kits (DNA Genotek, Inc., Ottawa, Ontario, Canada). Participants were instructed to spit into 2 tubes to collect a total of 4 ml of saliva. Samples were then stored at -20°C until the time of analysis.

DNA Isolation and Genotyping of OXTR

DNA was extracted from saliva collected with Oragene® Discover saliva DNA self-collection kits (DNA Genotek, Inc.) and stored at -20$^{o}$C prior to processing. Crude DNA isolation was done with PT-L2P Purifier reagent (DNA Genotek Inc.). DNA was subsequently phenol/chloroform extracted, centrifuged in Phase Lock Gel Light 2 ml Tubes (5 PRIME GmbH) and ethanol precipitated. Concentration and purity of the DNA were determined on a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific, DE). Samples were stored at -20°C until use. Genotyping of *OXTR* according to single-nucleotide polymorphisms (SNPs) rs2254298 and rs53576 was performed on a LightScanner instrument (Idaho Technology, currently BioFire Diagnostics, LLC.) by High Resolution Melting (HRM) analysis, a sensitive and specific method for the

detection of SNPs (Wittwer, Reed, Gundry, Vandersteen, & Pryor, 2003). As a first step, PCR was carried out in 10 ul total volume in Bio-Rad 96-Well PCR plates (HSP-9665, white well/black shell) under mineral oil with 15 ng of template DNA in a Mastercycler PCR device (Eppendorf, Germany) using LightScanner Master Mix (BioFire Defense) and the following primers at 500 nM final concentration: OR1_L 5'-GAAGAAGCCCCGCAAACTG-3' and OR1_R 5'-GCCCCTTTCAGGAAACCATC-3' (53 bp product with rs2254298); OR2_L 5'-GCACAGCATTCATGGAAAGGA-3' and OR2_R 5'-TCCCTGTTTCTGTGGGACTGA-3' (74 bp with rs53576). Primers were designed with Primer 3 (Rozen & Skaletsky, 2000) such that corresponding amplicons did not include any additional common SNPs (checked with UCSC Genome Browser). Plates were covered with TempPlate Sealing Film (USA Scientific) and cycled as following: initial enzyme heat activation at 95$^{o}$C for 2 min, 41 cycles of 95$^{o}$C for 20 sec, 64$^{o}$C (with rs2254298 primers) or 65$^{o}$C (with rs53576 primers) for 20 sec, and 72$^{o}$C for 7 sec, followed by a final 30 sec extension at 72$^{o}$C, a DNA denaturation at 95$^{o}$C for 30 sec and DNA renaturation/hold at 27$^{o}$C. At this point a plate was analyzed by HRM. Because SNPs under consideration were of A/G type, PCR fragments that included them demonstrated distinct melting profiles indicative of G/G, A/G, and A/A genotypes. The specificity and robustness of PCR were further confirmed by agarose gel electrophoresis. The Hardy-Weinberg equilibrium was tested with both SNPs using the $\chi^2$-test.

## Results

Questionnaire Scores

The average age of the younger cohort was 20.4 (n = 50; *SD* = 2.00) while the older cohort had a mean age of 62.9 (n = 49; *SD* = 6.15). Scores on the UCLA loneliness scale, shyness scale, BDI, expressive suppression scale and State and Trait Anxiety inventory were significantly lower in the older group compared to the younger group (see Table 1). Interestingly, although social network size did not differ between younger and older adults, older adults had significantly more high contact individuals in their networks (Table 1). No significant gender differences were found for any of the questionnaires.

Valence Ratings

Pleasant social images were given an average valence rating of 0.9 (*SD* = 0.19) while pleasant nonsocial images were rated 0.69 (*SD* = 0.27) on average (Figure 3). For the unpleasant images, social images were given an average rating of -0.75 (*SD* = 0.18) while nonsocial images received an average rating of -0.84 (*SD* = 0.21). There was a main effect of valence ($F(1, 75) = 2130.352$, $p < 0.001$), with pleasant pictures (*M* = 0.782, *SE* = 0.022) rated more positively than unpleasant pictures (*M* = -0.793, *SE* = 0.02). There was also a main effect of social condition ($F(1, 75) = 68.386$, $p < 0.001$), indicating that nonsocial stimuli were rated more negatively (*M* = -0.084, *SE* = 0.018) than social stimuli (*M* = 0.073, *SE* = 0.012). Additionally, older adults rated unpleasant

social images significantly less negative compared to younger adults ($F(1, 83) = 5.064$, $p = 0.027$; Figure 3).

Eye Tracking Data

Eye tracking data indicate that overall, subjects spent significantly more dwell time on pleasant faces (66%) than unpleasant faces (57%) ($t(55) = 8.63$, $p < 0.001$). Eye tracking data also show that older adults spent significantly more time viewing faces during both the pleasant and unpleasant social blocks compared to younger adults (see Figure 4 and Figure 5). These results were significant for unpleasant picture blocks ($F(1, 54) = 12.584$, $p = 0.001$) and pleasant picture blocks ($F(1, 54) = 4.804$, $p = 0.033$) when controlling for age-related differences in state and trait anxiety, shyness and loneliness. No significant differences in eye tracking were found when comparing across loneliness, shyness, PiL or SNI scores. However, a strong positive correlation between PiL score and preference for viewing pleasant faces was found. The Pearson's correlation was 0.323 with a p value of 0.024 (Figure 6). Additionally, subjects that rated unpleasant social images more negatively scored higher on the shyness scale (Figure 7).

fMRI Results

*Age differences*

A one-sample t-test was performed to compare brain activation to pleasant social images in younger and older adults. Whole brain analysis for the pleasant social greater than pleasant nonsocial contrast indicated that older adults had significantly more

activation in the right fusiform gyrus compared to younger adults (Figure 8). The whole brain analysis for the same contrast was still significant even when controlling for loneliness (Figure 9). To control for the fact that older adults spent significantly more time viewing positive faces, a separate analysis was run with a subset of subjects that had eye tracking data (N = 56) to control for the percent time spent viewing pleasant faces. A SVC for the fusiform gyrus indicated that activation in this area was still significantly greater for the pleasant social greater than pleasant nonsocial contrast in older adults even when controlling for age differences in viewing of faces (Figure 10). Whole brain analysis for the pleasant nonsocial greater than fixation condition revealed that younger adults showed greater activation in the right and left cerebellum lobes (Figure 11). Similarly, whole brain analysis for the unpleasant nonsocial greater than fixation contrast indicated that younger adults had greater activation in the right cerebellum (Figure 12). Lastly, whole brain analysis for the unpleasant social greater than fixation contrast indicated increased activation in the right limbic lobe for younger adults compared to older adults (Figure 13).

*Social connectedness (loneliness, shyness and social network)*

A whole-brain regression analysis of the pleasant social minus pleasant nonsocial contrast with UCLA loneliness scores as a covariate of interest did not reveal any significant areas of activation. Results remained insignificant even when controlling for state and trait anxiety, depression, cognitive reappraisal, and expressive suppression. The same analyses were also run separately with shyness scores and SNI scores (high

contact and number of people), although these did not reveal any significant areas of activation.

These analyses were repeated for all other contrasts (pleasant social greater than fixation, pleasant nonsocial greater than fixation, unpleasant social greater than unpleasant nonsocial, unpleasant social greater than fixation, unpleasant nonsocial greater than fixation) but results did not reveal any significant areas of activation. SVCs were performed with our a priori ROIs but these also did not yield significant results. Finally, to exactly replicate the conditions of Cacioppo's (2009) study, we also ran all of the same contrasts with only the younger female subjects but there were no regions significantly correlated with loneliness.

*Age X Social Connectedness Interaction*

A SVC for the left insula indicated an age by loneliness interaction for the pleasant social greater than pleasant nonsocial contrast while controlling for BDI, STAI and ERQ scores. Specifically, younger adults higher in loneliness showed increased insula activation to pleasant social images while lonely older adults showed less activation in this region (Figure 14). A SVC for the right caudate showed an age by loneliness interaction for the unpleasant social greater than fixation contrast while controlling for BDI, STAI and ERQ scores. Specifically, younger adults higher in loneliness showed increased activation in the caudate to unpleasant social pictures compared to older adults (Figure 15). A similar result was found for the same contrast with an age by shyness interaction; younger adults scoring higher on the shyness scale showed

increased activation in the right caudate compared to older adults ($p < 0.05$, FWE corrected).

*Functional connectivity*

Functional connectivity results for the unpleasant social greater than fixation contrast indicated an allele-dependent increase in functional connectivity between the hypothalamus (seed region) and a cluster in the left limbic lobe for the rs53576 A/G and A/A genotypes (Figure 19) while controlling for age, gender, and behavioral measures. No significant differences in functional connectivity were found for any of our seed regions as a function of loneliness.

Genetic Results

For the *OXTR* rs53576 polymorphism, 47% of subjects were identified as G/G, 37% as A/G and 16% as A/A (Table 2). With regards to the *OXTR* rs2254298 polymorphism, 70% of subjects were identified as G/G, 26% as A/G and 4% as A/A (see Table 2). No significant deviation from the Hardy-Weinberg equilibrium was observed for either SNP ($p > 0.05$). No gender differences were found for rs53576 ($\chi2=0.362$; df=2, p=0.834) and rs2254298 ($\chi2=1.620$; df=2, p=0.445). A main effect of *OXTR* rs2254298 genotype on valence ratings of unpleasant social pictures was observed ($F(2, 80) = 4.306$, $p = 0.017$). A bonferroni post hoc test indicated that individuals with the A/A genotype rated unpleasant social pictures significantly more negative compared to A/G ($p = 0.013$; Figure 16) and G/G ($p = 0.029$; Figure 16). A significant main effect of *OXTR* rs53576 genotype on shyness scores was observed ($F(2, 90) = 5.133$, $p = 0.008$). A bonferroni

post hoc test indicated that individuals with the G/G genotype scored significantly lower on the shyness scale compared to A/G individuals ($p$ = 0.007; Figure 17). Furthermore, a whole-brain regression analysis (controlling for BDI, STAI and ERQ scores) indicated that individuals with the G/G genotype showed a positive correlation between loneliness and activation in the right thalamus in response to unpleasant nonsocial images (Figure 18).

## Discussion

Prior research has identified differences in brain activation and genetic polymorphisms that are related to self-reported levels of loneliness. The present study sought to extend the study of loneliness and its genetic and neural correlates to both older and younger adult populations. Here, I present evidence to suggest that older adults are not only less lonely than younger adults but also show key differences in attention and brain response to social and emotional images.

Self-report questionnaires indicated that older adults were less lonely, shy, anxious, and depressed compared to the younger cohort. A lack of scores at the higher end of the loneliness scale for older adults may reflect age-related decreases in desired social contact and an increased interest in emotionally close partners as predicted by the SST (Carstensen, 2006). In support of this idea, we found that although the number of people in the social network did not differ by age, older adults had significantly more social roles in which they had regular contact with others (at least once every two weeks). Differences in questionnaire scores across age may also be related to a selection bias in our subject pool. Older adults who participated in our study may have been more outgoing and social compared to their peers whereas our younger cohort consisted of college students who participated in the study as part of a requirement for course credit, although they did have a choice of different assignments or studies and thus their participation in our experiment was strictly voluntary.

While older adults in our study reported fewer feelings of loneliness, they also showed differences in how they attended to both pleasant and unpleasant social pictures. Specifically, older adults spent significantly more dwell time on faces in

unpleasant images and to a lesser extent on faces in pleasant images as well. Our findings partly agree with previous studies; a positivity effect in older adults has been observed such that recall of positive images is improved relative to recall of negative images (Charles, Mather, & Carstensen, 2003). Interestingly, however, increased attention to unpleasant faces was still significantly greater in older adults in our study even when controlling for differences in anxiety, shyness and loneliness. These findings suggest clear age differences in how adults attend to unpleasant social stimuli. Dot probe (Isaacowitz, Wadlinger, Goren, & Wilson, 2006a; Mather & Carstensen, 2003) and eye tracking (Isaacowitz, Wadlinger, Goren, & Wilson, 2006b) studies have both shown that older adults tend to look away from negative faces and toward positive ones. One explanation for our seemingly contradictory findings is that in previous studies subjects were given an option of which stimulus to focus on (happy face versus a neutral face) whereas in the present study subjects were instructed to view a single image at a time. Because of this study design, our findings might point to a general preference for viewing faces among older adults rather than a preference for viewing negative stimuli.

Our fMRI results indicated age differences in responses to pleasant social and nonsocial images. Specifically, older adults showed increased activation in the right fusiform gyrus to pleasant social images, which remained significant even when controlling for the age differences in loneliness and attention to faces discussed above. Similarly, a previous study found increased functional connectivity between the right fusiform and orbitofrontal cortex among older adults during a face matching task (Burianová et al., 2013). This increased connectivity was thought to be a compensatory

mechanism for decreased connectivity between the left and right fusiform gyri in older adults (Burianová et al., 2013). Our finding of increased activation in the right fusiform might be a result of compensatory circuits related to aging. Alternatively, increased activation of the right fusiform in older adults may suggest increased sensitivity to positive social stimuli which would support the positivity effect that has been observed in older adults (Charles et al., 2003). Interestingly, younger adults in our study showed increased activation of the cerebellum to nonsocial images regardless of valence. Apart from its role in motor function, the cerebellum is involved with attention (Allen, Buxton, Wong, & Courchesne, 1997) and emotion (Parvizi, Anderson, Martin, Damasio, & Damasio, 2001; Turner et al., 2007), suggesting that younger adults may be more responsive to nonsocial images.

Age differences in brain response to unpleasant social stimuli were also observed. Additional whole brain analyses revealed that younger adults showed increased activation of the right limbic lobe to unpleasant social images. The limbic lobe is an area associated with emotion, particularly in response to aversive stimuli (Liberzon et al., 2000; Taylor, Liberzon, & Koeppe, 2000). In agreement with our findings, previous studies have also shown similar age differences in response to negative emotional stimuli; younger adults showed significantly more activity in the left amygdala to negative faces relative to older adults (Iidaka et al., 2002). Our results suggest that younger adults have an increased fear response to unpleasant social images relative to older adults.

Our hypothesis of decreased ventral striatal activation to pleasant social images in lonely younger adults was not supported by our study. In fact, loneliness was not

correlated with brain activity for any of our contrasts, even when replicating Cacioppo's (2009) study by including only young female subjects. Considering that UCLA scores were similar in our study and in Cacioppo's (2009), we can speculate that differences in the IAPS image set, task design and data analysis may have contributed to our differing results. However, we did observe interactions between age and loneliness that modulated brain response to social stimuli. Specifically, greater activation in the left insula was observed during the pleasant social greater than pleasant nonsocial contrast in younger adults higher in loneliness while older adults higher in loneliness showed reduced activity in this area. The insula is an area known for its role in craving; a study of maternal and romantic love found that both types led to increased activation in the middle insula (Bartels & Zeki, 2004). Increased insula activation to pleasant social images coupled with higher loneliness scores among our younger subjects suggests that younger lonely adults may crave social interaction.

An age by loneliness interaction was also observed for the unpleasant social greater than fixation contrast. Younger adults higher in loneliness showed increased activation in the right caudate compared to older adults scoring high in loneliness. Our results contradict Cacioppo's (2009) finding of decreased activity in the right caudate to unpleasant social images in lonely younger individuals, although we did see that same pattern in our older subjects. Activity in the caudate is related to social reward (Izuma, Saito, & Sadato, 2008) and response to negative emotional stimuli (Carretié et al., 2009). Thus, our findings suggest that lonely younger adults are more responsive to unpleasant social stimuli compared to lonely older adults, who may have down-

regulation in this region as a result of repeated negative social interactions, as has been theorized previously (J. T. Cacioppo et al., 2009).

Results of our genetic analyses indicated differences in both behavior and neural activation across different *OXTR* genotypes. Specifically, subjects with the G/G genotype of the rs53576 polymorphism scored significantly lower on the shyness scale compared to A/G subjects. These findings agree with previous studies showing that the A/G rs53576 genotype is associated with a reduction in empathy (Rodrigues et al., 2009), positive affect (Lucht et al., 2009) and attachment (Costa et al., 2009).
Our imaging results indicated a loneliness by genotype interaction for the unpleasant nonsocial greater than fixation contrast. Specifically, subjects with the rs53576 G/G genotype who scored higher on the loneliness scale showed increased activation in the thalamus during viewing of unpleasant nonsocial pictures. Conversely, a previous study found a reduction in thalamic activation to pictures of angry faces stimuli following administration of intranasal oxytocin (Domes et al., 2007). Because the rs53576 G/G genotype is associated with higher levels of emotion-regulation (Rodrigues et al., 2009), we would expect that those with the G/G genotype would show reduced thalamic activation to negative stimuli. Thus, our findings suggest that loneliness may modify the effect of *OXTR* rs53576 genotype on brain response to negative stimuli.

For the rs2254298 *OXTR* polymorphism, we found that subjects with the A/A genotype rated unpleasant social images as significantly less negative compared to both A/G and G/G genotype individuals. Previous studies of the rs2264298 genotype and behavioral traits have been mostly inconclusive. Lucht and colleagues (2009) found that the A/A genotype for the rs2254298 *OXTR* polymorphism showed a decrease in

positive affect but only on a trend level. By contrast, they found that G/G genotype individuals showed lower social loneliness scores. It is also important to note that our study included only 4 individuals with the A/A genotype. As such, additional research is needed to determine how rs2254298 *OXTR* polymorphisms related to loneliness, affect and brain response.

As mentioned previously, the rs53576A genotype is associated with increased functional connectivity between the hypothalamus and the amygdala (Tost et al., 2010). Here, we extended these findings by demonstrating an increase in functional connectivity between the hypothalamus and a cluster in the limbic lobe in subjects with the rs53576A genotype in response to unpleasant social stimuli. The rs53576A *OXTR* risk allele has previously been associated with reduced empathy (Rodrigues et al., 2009), positive affect (Lucht et al., 2009) and attachment (Costa et al., 2009). Our data indicate an increase in connectivity between the hypothalamus to limbic areas in individuals with the *OXTR* risk allele that suggests a heightened response to negative social information.

Overall, the results of the present study support the positivity effect by demonstrating that older adults were significantly less lonely, spent more time viewing faces and showed greater fusiform activation to pleasant faces relative to younger adults. Interestingly, younger lonely adults showed greater activation in the insula to pleasant social pictures, which may represent increased social craving in these individuals. Our genetic findings expanded upon previous studies by demonstrating that subjects with the rs53576A risk allele were more shy and showed increased functional connectivity between the hypothalamus and limbic lobe, suggesting an increased

response to negative social stimuli. Taken together, our results highlight interactions between genes, neural circuits and social behavior.

Limitations and Future Directions

Our study contained several limitations with regards to its subject pool and experimental design. First, all of our subjects fell into two discrete age groups (18 to 28 and 55 to 81); inclusion of a wider age range would allow us to analyze how social networks and subjective loneliness change across different life stages. Our data were also limited by a lack of scores at the high end of the loneliness scale. To control for this, subjects should be screened prior to participating in the study to ensure a larger spread of loneliness scores. Another caveat to interpreting our results is that our image set consisted of pictures that featured both people and background scenery. This could be improved upon by including face-only stimuli to ensure that all subjects are looking at the same features of the stimulus. Additionally, while the social images in our study featured two people to clearly indicate a social interaction, further experiments should include images with a single person as well as pictures with groups of people in order to assess brain response to different social contexts.

While the present work focused on *OXTR* polymorphisms, there are many other candidate genes that may be related to loneliness. In addition to *AVPR1A*, which was discussed previously, recent studies in older adults have identified several new genes that are linked to loneliness. One such gene is *MTHFR*, which encodes methylenetetrahydrofolate reductase, an enzyme important for catalyzing 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate (Lan et al., 2012). 5-

methyltetrahydrofolate is a cosubstrate for homocysteine remethylation to methionine, which is an important process as increased levels of homocysteine contribute to vascular disease (W.-C. Tsai et al., 2000). Lan and colleagues studied 323 male subjects over the age of 60 and found that the C/C genotype of the C677T polymorphism *MTHFR* was associated with increased loneliness when controlling for age, education, cognition, and depression (Lan et al., 2012). A second gene that has recently been linked to loneliness is *CHRNA4*, which encodes the neuronal nicotinic acetylcholine receptor alpha-4 subunit (S. J. Tsai et al., 2012). Nicotinic acetylcholine receptors are distributed in areas of the brain related to depression such as the basal ganglia, VTA and amygdala and they also serve to regulate the release of dopamine, which is important for reward processing and motivation (Albuquerque, Pereira, Alkondon, & Rogers, 2009). Tsai and colleagues found that in males, individuals homozygous for the C/C genotype of the *CHRNA4* rs1044396 polymorphism had an increased incidence of depression and loneliness (S. J. Tsai et al., 2012). Future research should investigate these genes further to establish their link to loneliness.

Another important consideration when measuring brain activation across different age groups are anatomical and vascular differences that may affect the fMRI signal. For instance, there is evidence of anatomical differences in older adults including sulcal widening and reduced grey matter volume that can affect automatic spatial normalization (Crinion et al., 2007; Samanez-Larkin & D'Esposito, 2008). Older brains also show differences in vasculature, including thickening of the vascular basement membrane and thinning of the endothelium (Kalaria, 1996). These vascular changes may affect coupling between the neural activity and the BOLD signal, which is modeled

by the hemodynamic response (HDR) function in fMRI. The effect of these age differences has been demonstrated in several empirical studies. Taoka and colleagues (1998) ran an fMRI study that demonstrated age differences in the HDR; older subjects had a slower signal rise in the motor cortex during a hand-squeezing task. Furthermore, a study of functional connectivity during a Stroop task found that older adults showed less functional connectivity between the seed region and areas within the same circuit (Geerligs, Maurits, Renken, & Lorist, 2014). Conversely, older adults had increased connectivity between the seed region and areas outside of that circuit. Together, these findings suggest less specificity among functional networks in older adults (Geerligs et al., 2014).

One way of controlling for these age-related differences in the BOLD signal is to derive an HDR individually for each subject by having them complete a simple motor or visual task (Samanez-Larkin & D'Esposito, 2008). To account for differences in brain morphology, the experimental design could utilize optimal normalization algorithms or use a template brain specific to that particular population (Samanez-Larkin & D'Esposito, 2008). Future studies may benefit from employing some of these techniques to control for age-related changes in the BOLD signal and in brain morphology.

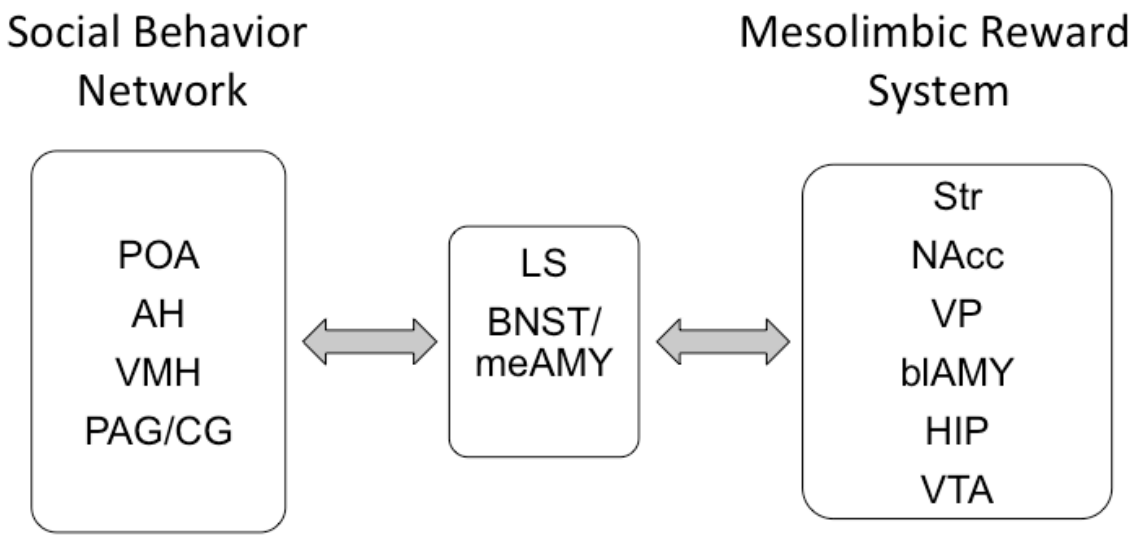



Figure 1. Neural circuits of social behavior and reward. Connections between nodes in these circuits have been determined primarily through neurochemical, developmental, tract-tracing and lesion/stimulation studies in rodents. The LS and BNST/meAMY are areas that are involved with both social behaviors and reward. Arrows represent anatomical connections between brain regions. AH, anterior hypothalamus; blAMY, basolateral amygdala; BNST/meAMY, bed nucleus of the stria terminalis/medial amygdala; HIP, hippocampus; LS, lateral septum; NAcc, nucleus accumbens; PAG/CG, periaqueductal gray/central gray; POA, preoptic area; Str, striatum; VMH, ventromedial hypothalamus; VP, ventral pallidum; VTA, ventral tegmental area. Modified from O'Connell and Hofmann, 2011 (Figure 1).

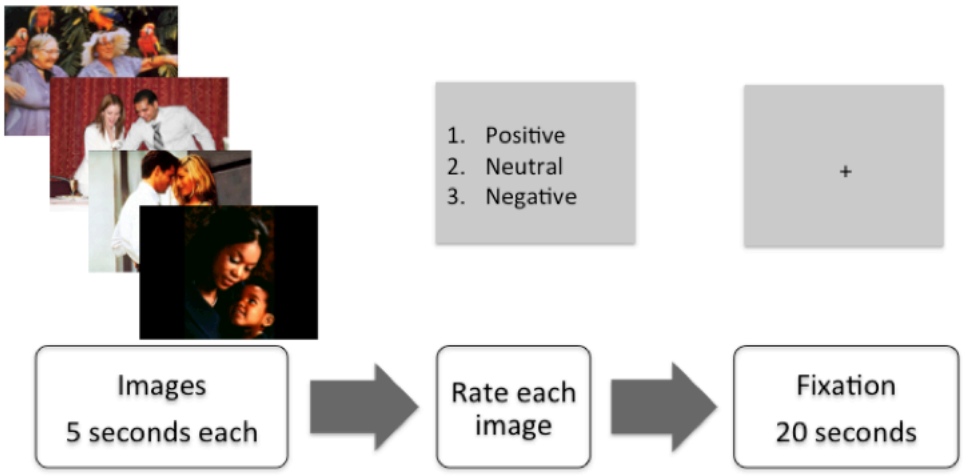



Figure 2. fMRI task paradigm. Blocks of 4 images (presented for 5 seconds each) were displayed while subjects were asked to rate each image as indicated above. A fixation cross was presented for 20 seconds following each block.

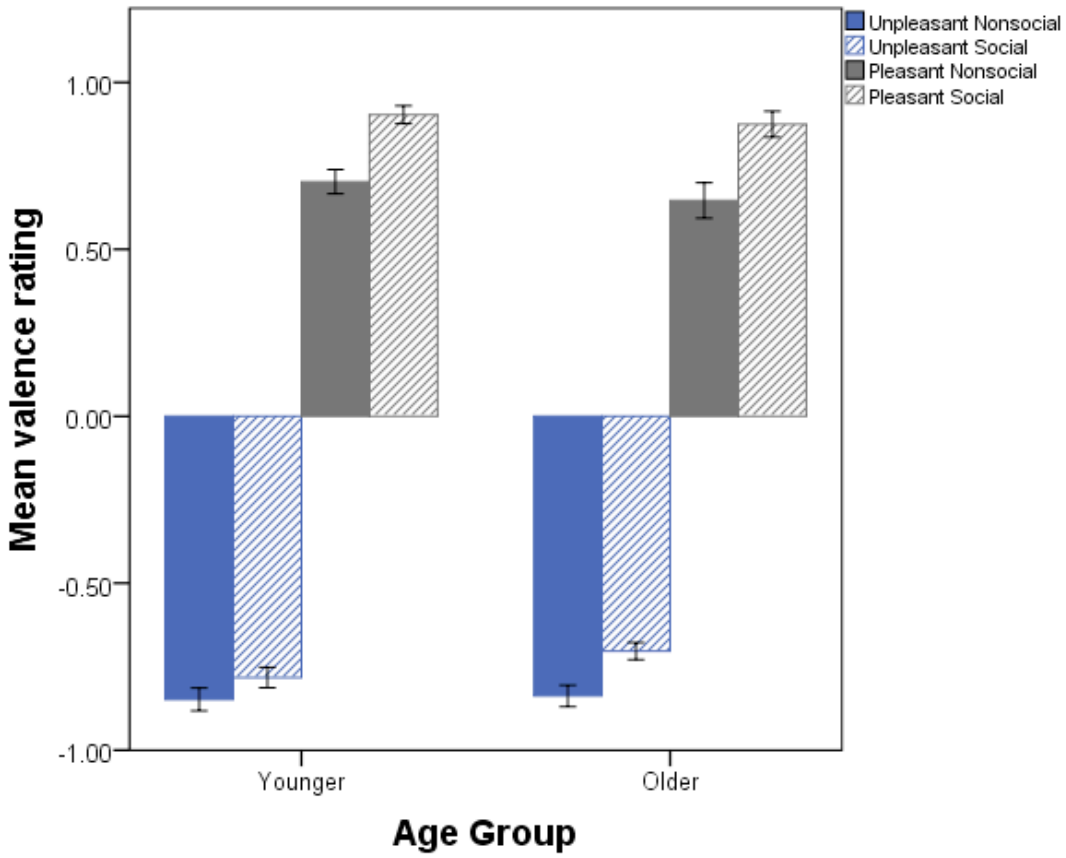



Figure 3. Mean valence ratings across social condition and pleasantness. A main effect of valence ($p < 0.001$) as well as social type was observed ($p < 0.001$). Older adults rated unpleasant social images significantly less negative compared to younger adults ($F(1, 83) = 5.064$, $p = 0.027$). Error bars indicate 1 *SE*.

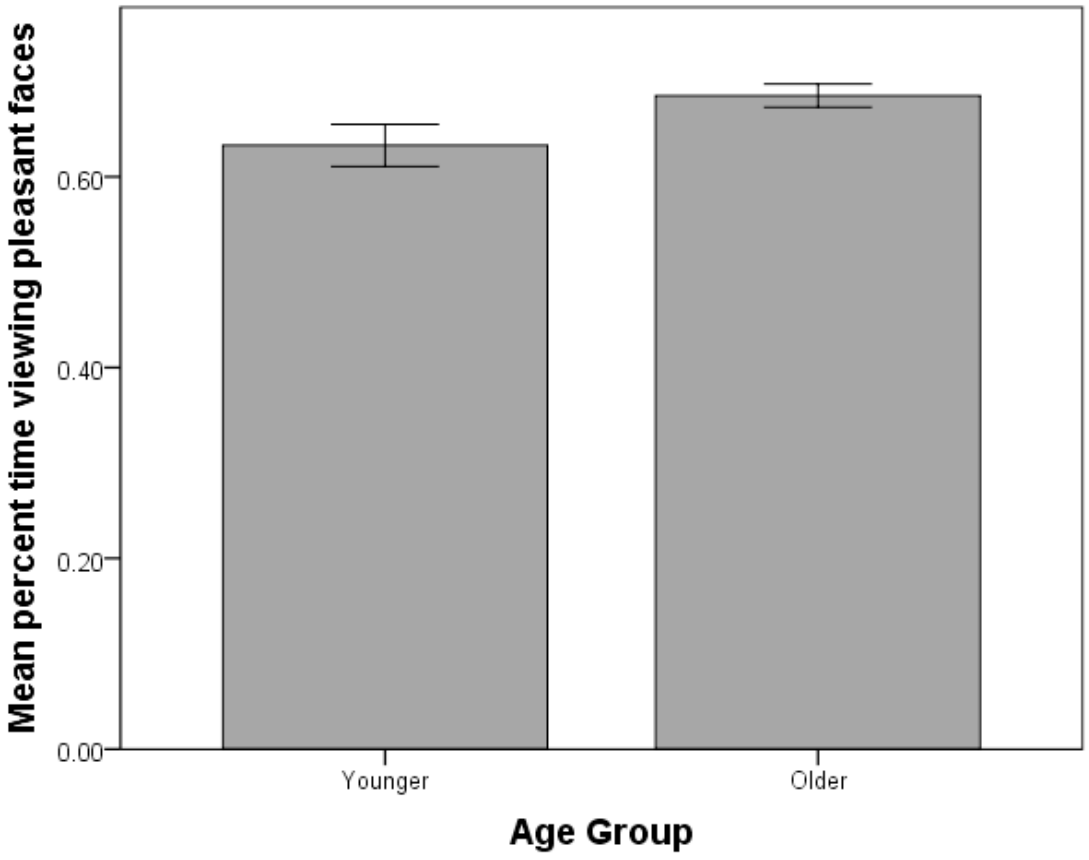



Figure 4. Average percent time viewing faces during the pleasant social condition as a function of age group. Older adults spent significantly more time viewing pleasant faces ($F(1, 54) = 4.819$, $p = 0.032$). Error bars indicate 1 *SE*.

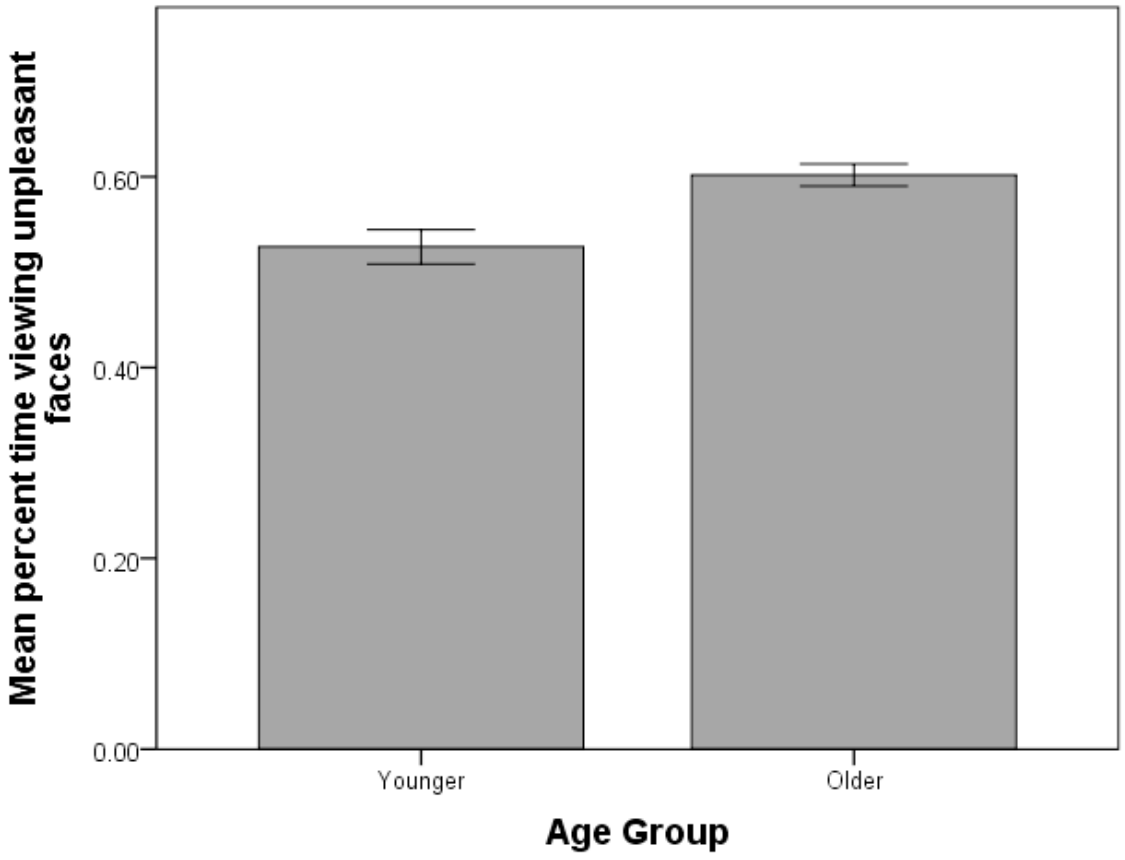



Figure 5. Average percent time viewing faces during the unpleasant social condition as a function of age group. Older adults spent significantly more time viewing unpleasant faces ($F(1, 54) = 13.442$, $p = 0.001$). Error bars indicate 1 *SE*.

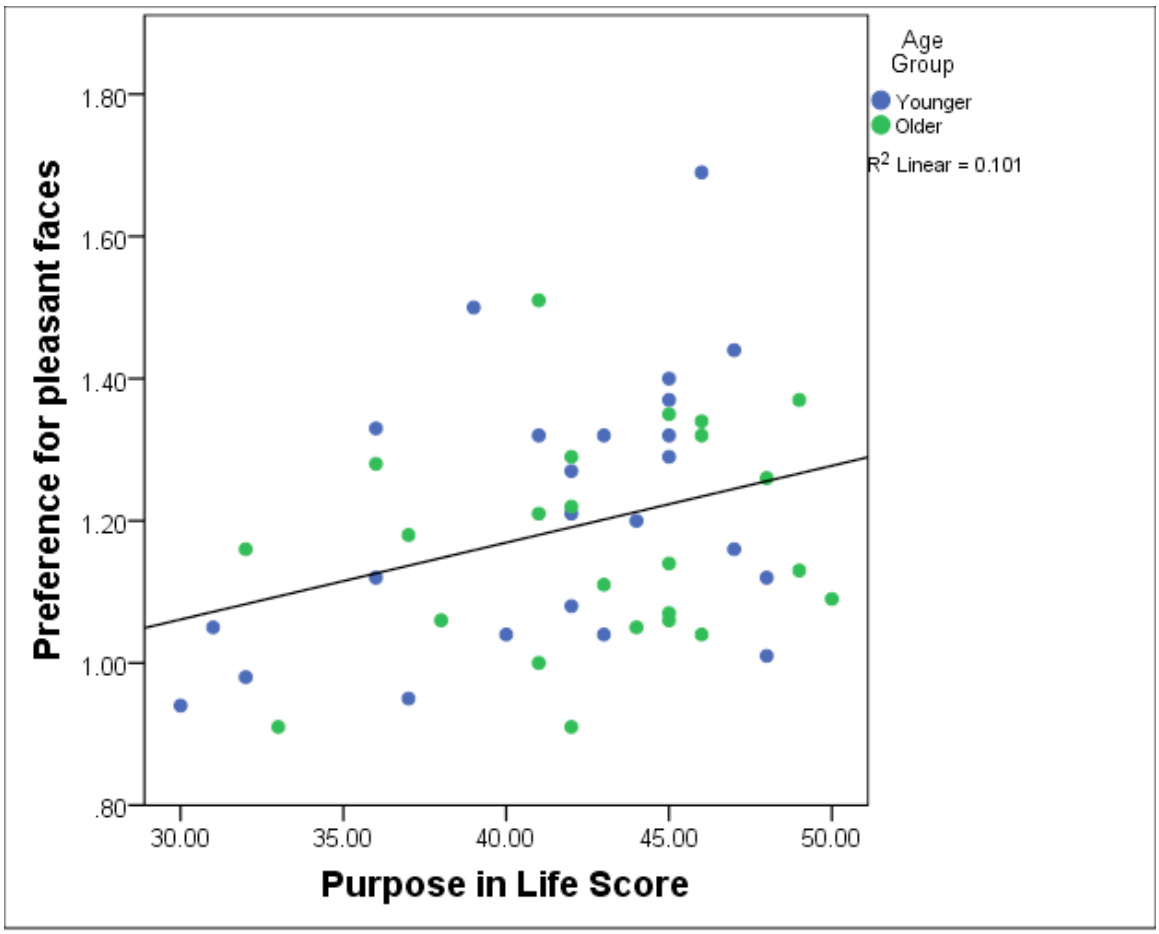



Figure 6. Preference for pleasant faces correlates with purpose in life. Purpose in life was positively correlated with a preference for viewing pleasant faces over unpleasant faces ($r(49) = 0.323$, $p = 0.024$).

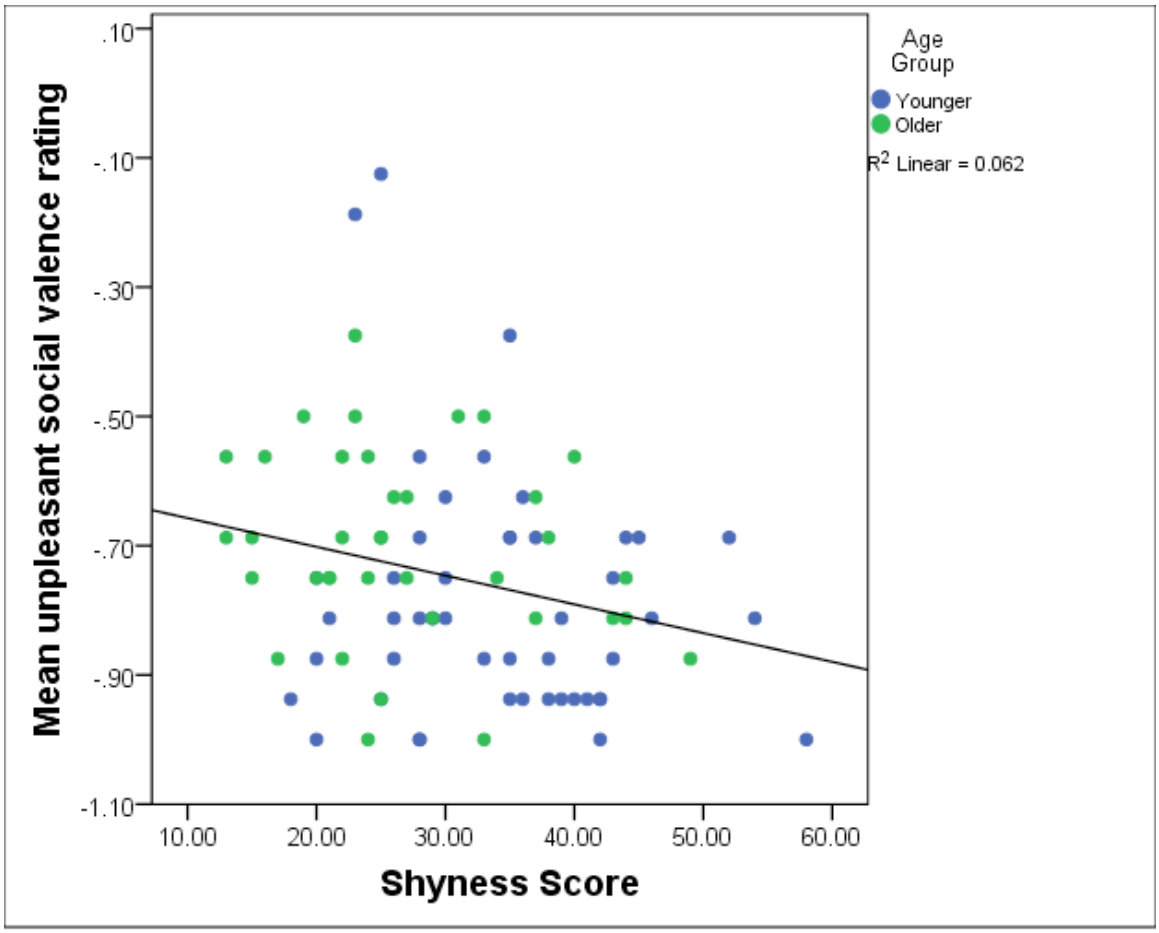
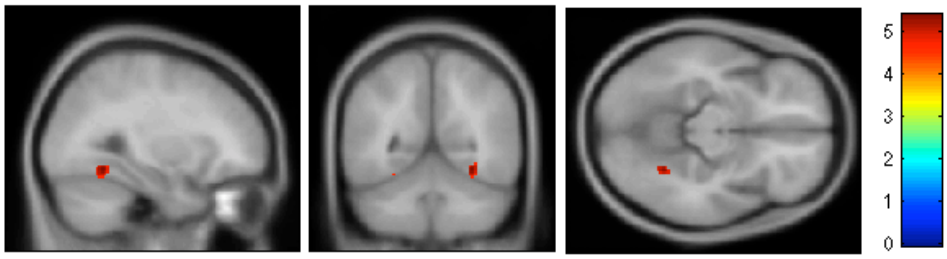
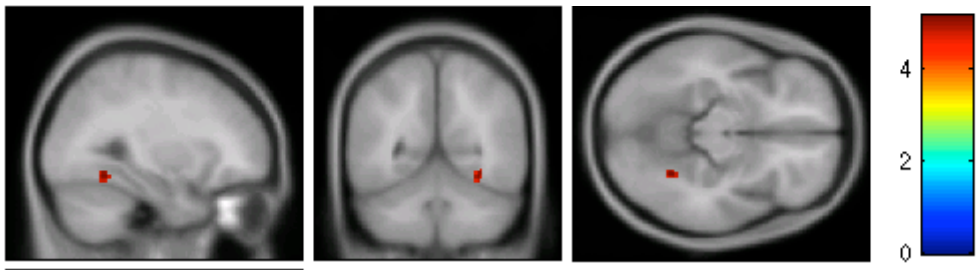



Figure 7. Valence scores correlate with shyness. Individuals scoring higher on the shyness scale rated unpleasant social pictures as significantly more negative ($r(85) = -0.248$, $p = 0.022$).



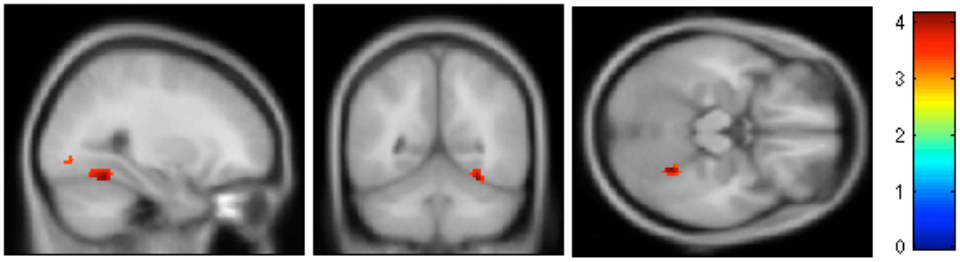

| *Pleasant Social – Pleasant Nonsocial Contrast* | | | |
|---|---|---|---|
| Region | Cluster Size | T | MNI Coordinates (x, y, z) |
| Right fusiform | 56 | 5.39 | 30, -54, -12 |

Figure 8. Whole brain analysis of age differences for the pleasant social greater than pleasant nonsocial contrast. Older adults showed significantly more activation in the right fusiform gyrus compared to younger adults ($p < 0.05$ FWE corrected).



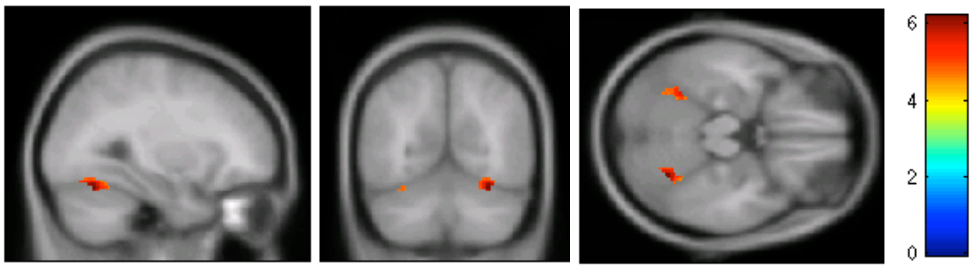

| *Pleasant Social – Pleasant Nonsocial Contrast* | | | |
|---|---|---|---|
| Region | Cluster Size | T | MNI Coordinates (x, y, z) |
| Right fusiform | 34 | 5.15 | 30, -54, -12 |

Figure 9. Whole brain analysis of age differences for the pleasant social greater than pleasant nonsocial contrast when controlling for differences in loneliness. Older adults showed significantly more activation in the right fusiform gyrus compared to younger adults ($p < 0.05$, FWE corrected).



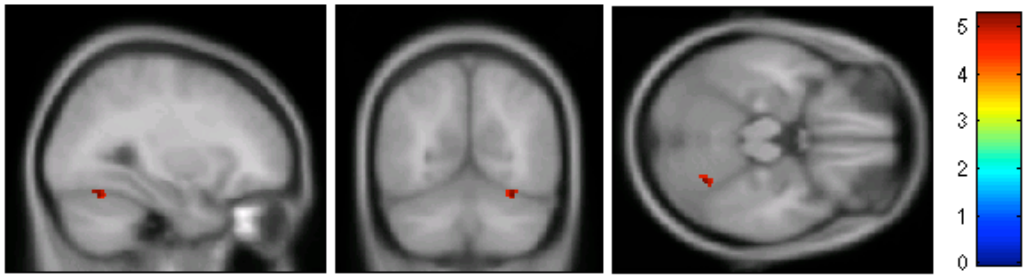

| *Pleasant Social – Pleasant Nonsocial Contrast* | | | |
|---|---|---|---|
| Region | Cluster Size | T | MNI Coordinates (x, y, z) |
| Right fusiform | 104 | 4.16 | 28, -54, -18 |
| | | 3.91 | 26, -60, -14 |

Figure 10. SVC analysis of age differences for the pleasant social greater than pleasant nonsocial contrast while controlling for differences in gaze. In a subset of subjects (N = 56) with eye tracking data, a SVC for the fusiform gyrus indicated increased activation in older adults relative to younger adults ($p < 0.05$, FWE corrected).



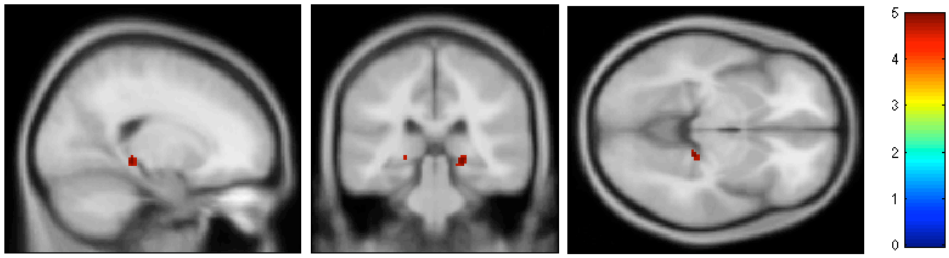

| *Pleasant Nonsocial – Fixation Contrast* | | | |
|---|---|---|---|
| Region | Cluster Size | T | MNI Coordinates (x, y, z) |
| Right cerebellum | 136 | 6.20 | 30, -62, -20 |
| Left cerebellum | 65 | 5.54 | -30, -54, -20 |
| | | 4.74 | -20, -54, -20 |

Figure 11. Whole brain analysis of age differences for the pleasant nonsocial greater than fixation contrast. Younger adults showed more activation in the right and left cerebellum compared to older adults ($p < 0.05$, FWE corrected).




| *Unpleasant Nonsocial – Fixation Contrast* | | | |
|---|---|---|---|
| Region | Cluster Size | T | MNI Coordinates (x, y, z) |
| Right cerebellum | 32 | 5.27 | 30, -62, -20 |

Figure 12. Whole brain analysis of age differences for the unpleasant nonsocial greater than fixation contrast. Younger adults showed more activation in the right cerebellum compared to older adults ($p < 0.05$, FWE corrected).




| *Unpleasant Social – Fixation Contrast* | | | |
|---|---|---|---|
| Region | Cluster Size | T | MNI Coordinates (x, y, z) |
| Right limbic lobe | 25 | 4.90 | 20, -32, -6 |

Figure 13. Age differences in brain activation to the unpleasant social greater than fixation contrast. Younger adults showed more activation in the right limbic lobe compared to older adults ($p < 0.05$, FWE corrected).

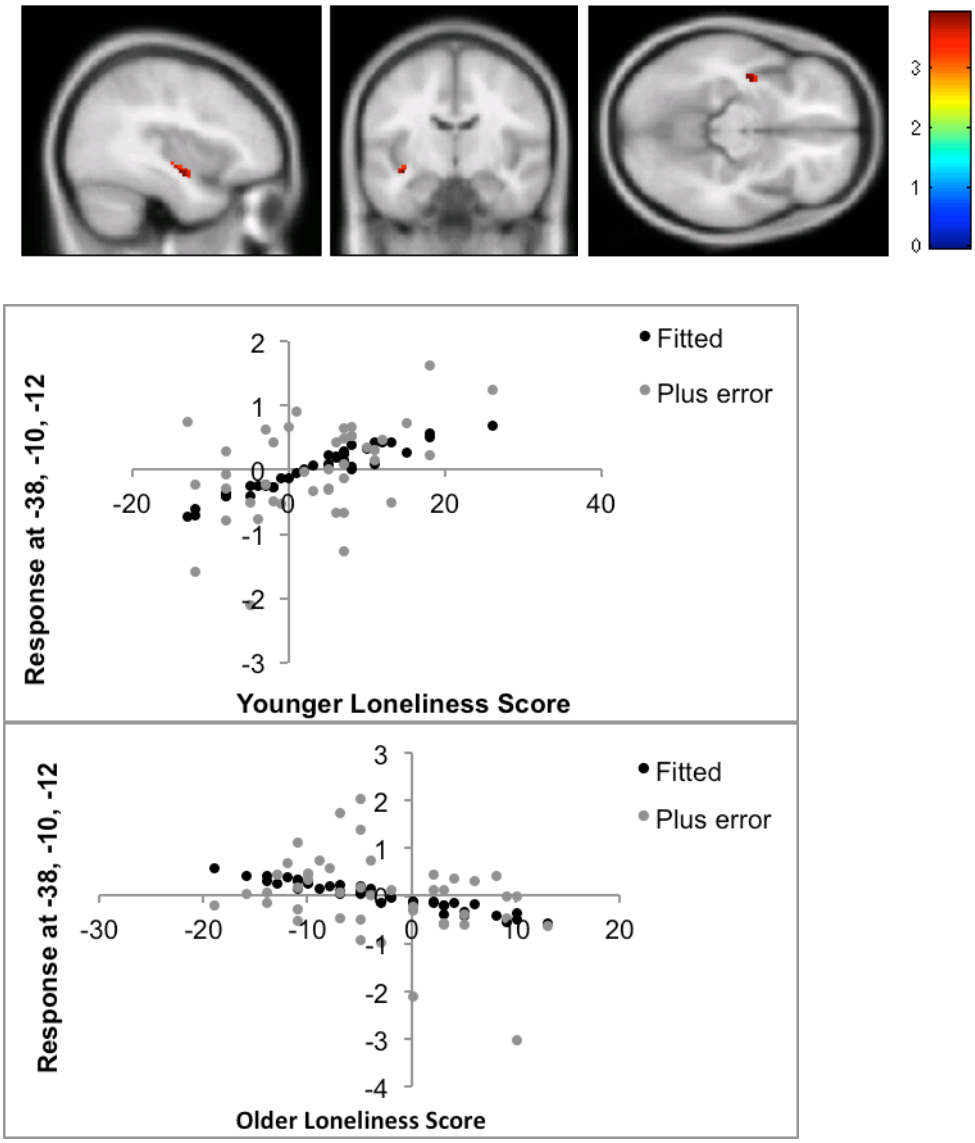
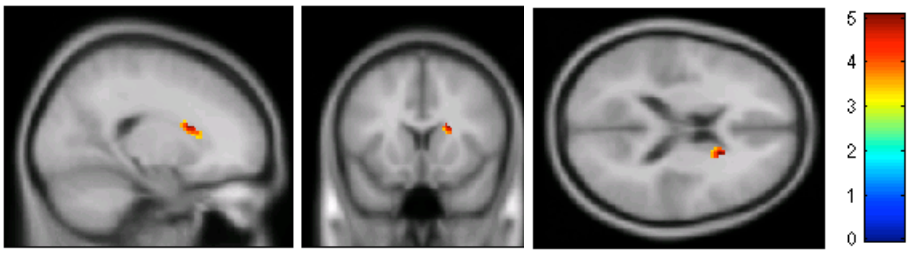
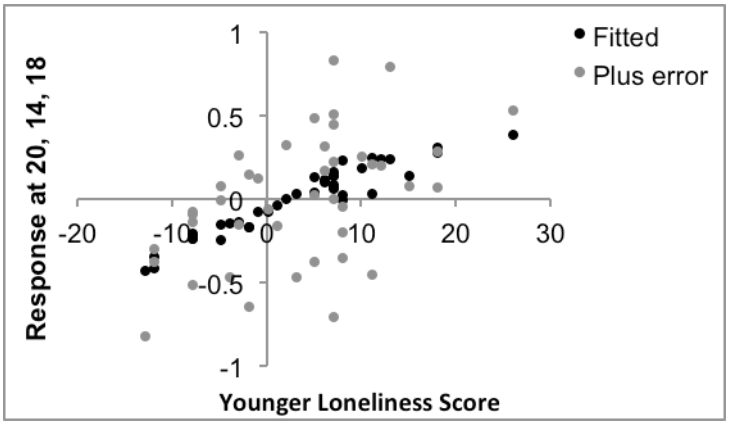



Figure 14. Age by loneliness interaction for the pleasant social greater than pleasant nonsocial contrast. An ROI analysis with the left insula indicated that younger adults with greater loneliness scores showed increased insula activation to pleasant social images while older adults with higher loneliness scores showed less activation in this region ($p < 0.05$, FWE corrected).



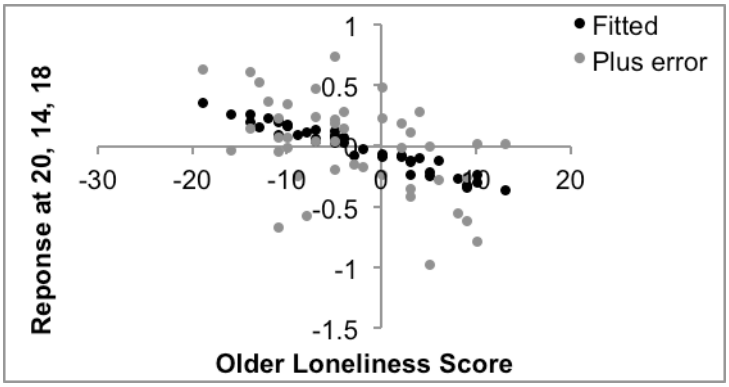




Figure 15. Age by loneliness interaction for the unpleasant social greater than fixation contrast. Younger adults higher in loneliness showed increased activation in the caudate to unpleasant social pictures compared to older adults ($p < 0.05$, FWE corrected).

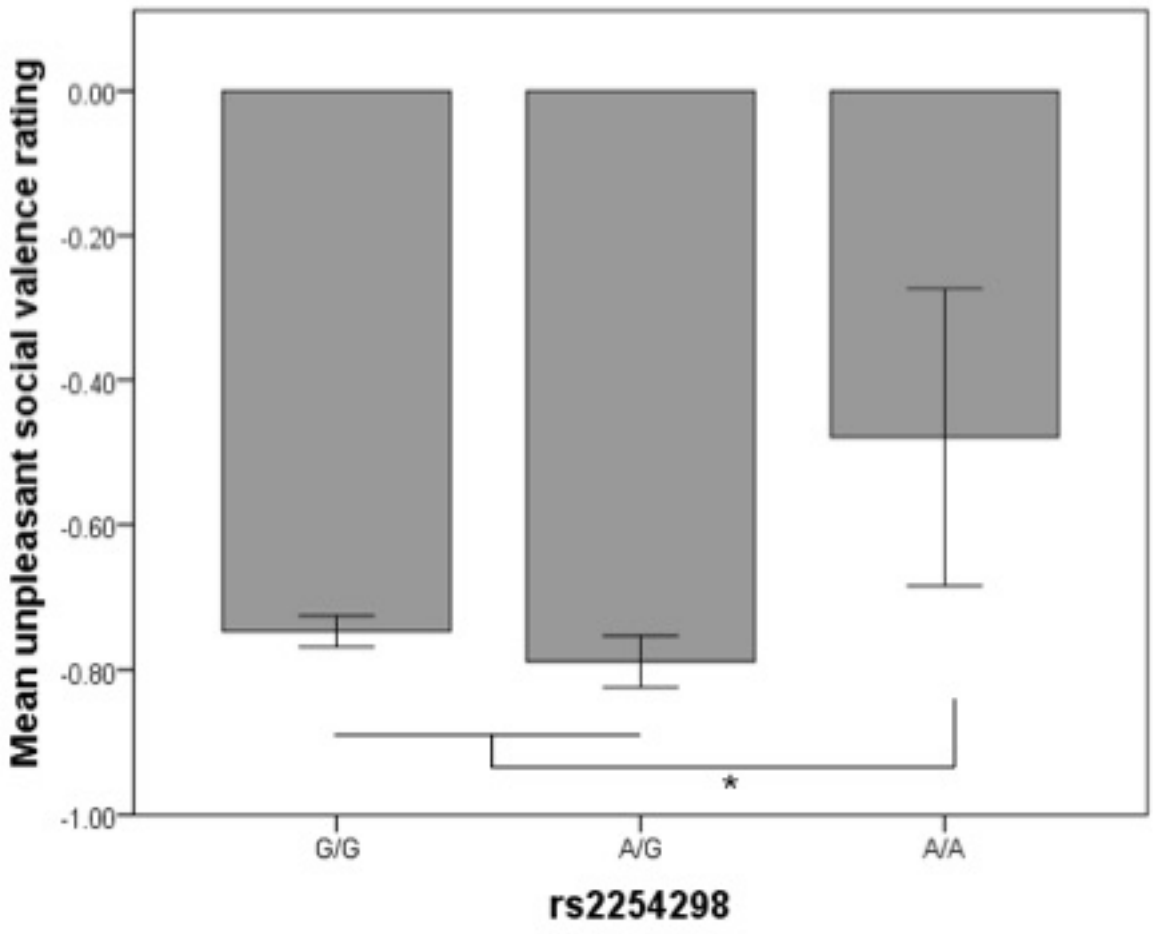



Figure 16. *OXTR* rs2254298 SNPs and valence ratings. Subjects with the A/A genotype rated unpleasant social images significantly less negative compared to G/G and A/G genotypes ($p < 0.05$).

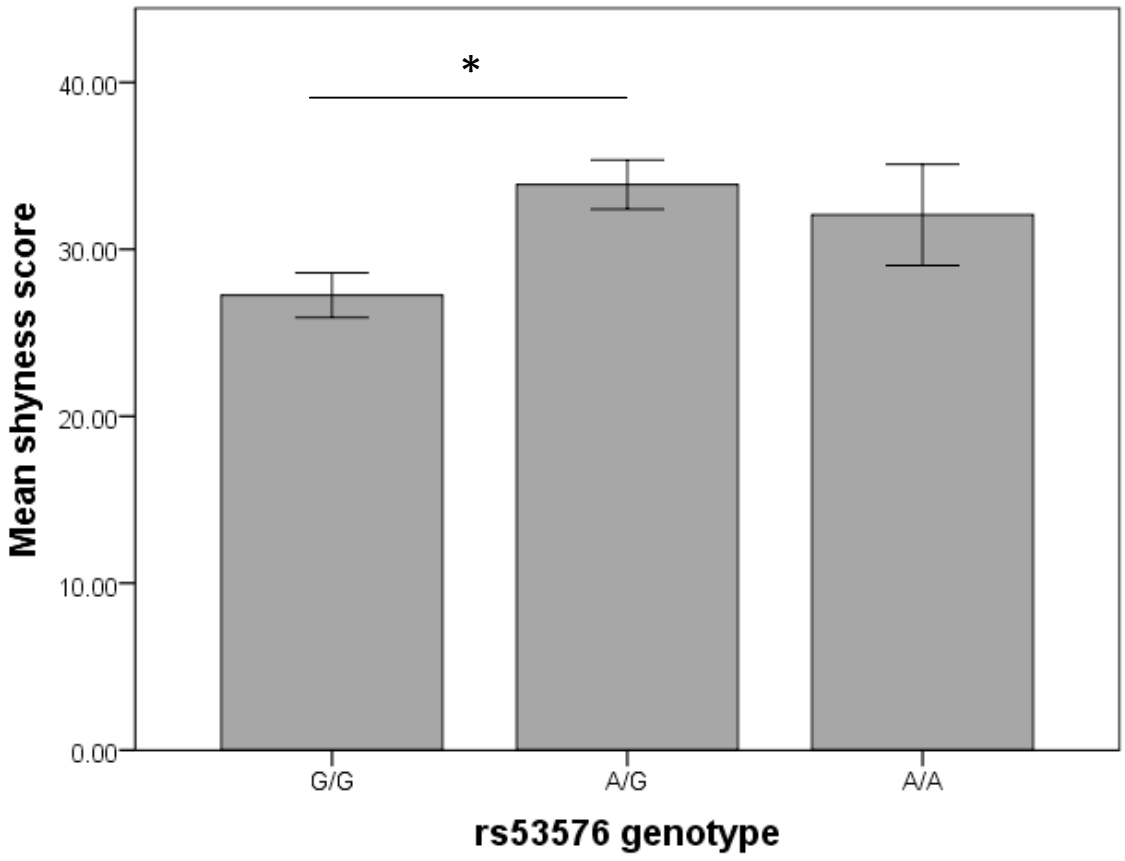



Figure 17. *OXTR* rs53576 SNPs and shyness scores. Subjects with the G/G genotype scored significantly lower on the shyness scale compared to A/G subjects (p = 0.007).

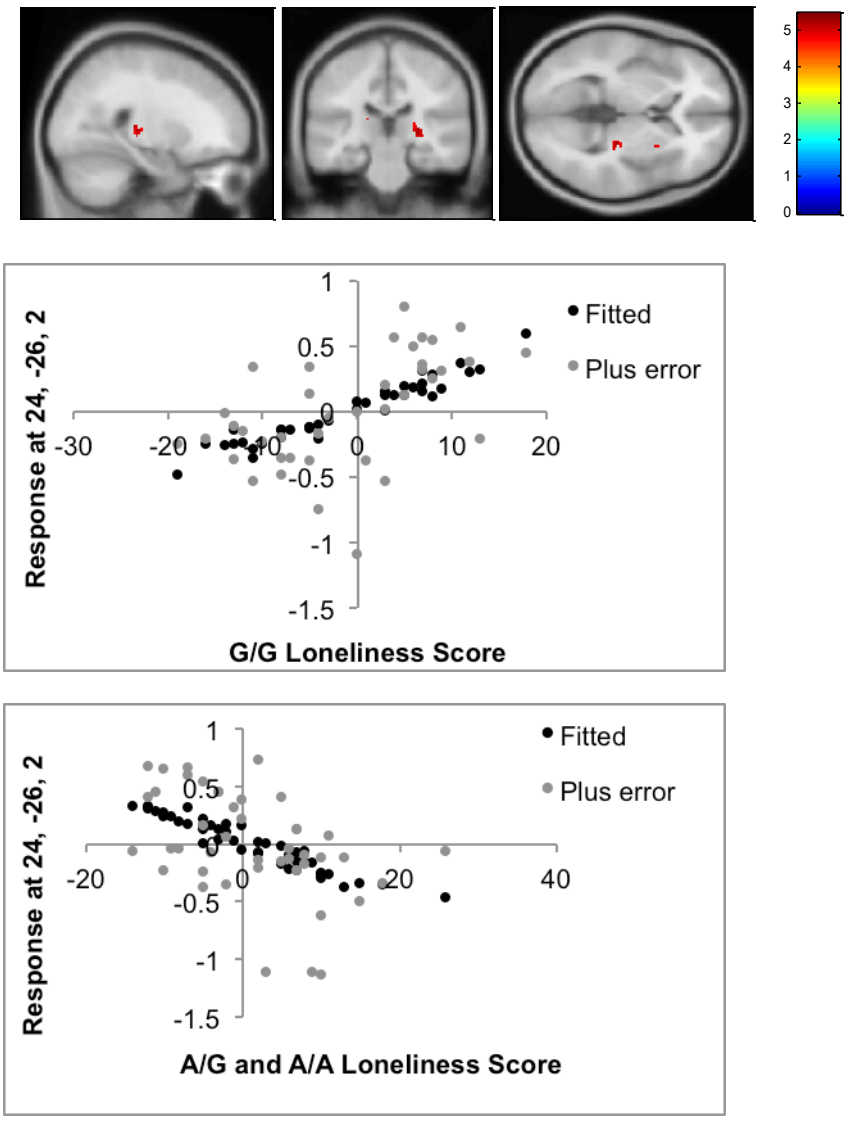
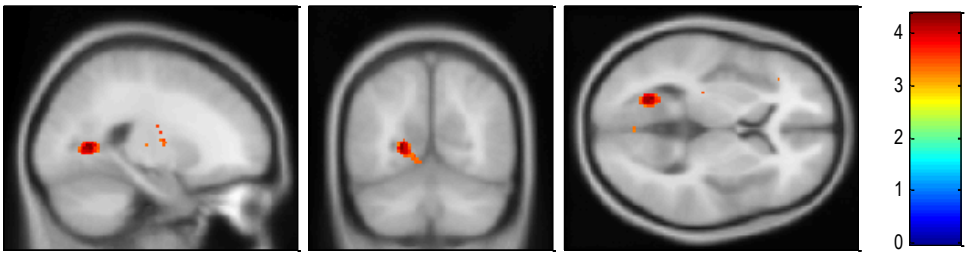



Figure 18. Loneliness by *OXTR* rs53576 genotype interaction. Individuals with the G/G genotype showed a positive correlation between loneliness and activity in the right thalamus in response to unpleasant nonsocial images relative to fixation ($p < 0.05$, FWE corrected).




| *Unpleasant Social > Fixation Contrast* | | | |
|---|---|---|---|
| Region | Cluster Size | T | MNI Coordinates (x, y, z) |
| Left limbic lobe | 328 | 4.36 | -22, -62, 4 |

Figure 19. Increased functional connectivity for *OXTR* rs53576A. Carriers of rs53576A showed increased functional connectivity between the hypothalamus and a cluster in the limbic lobe during viewing of unpleasant social pictures relative to fixation. The cluster contained posterior cingulate, lingual, occipital and anterior cerebellar regions ($p < 0.05$, FWE corrected at the cluster level).

| Questionnaire | | Age Groups | | *t*-test | | |
|---|---|---|---|---|---|---|
| | | Younger | Older | *t*-value | df | *p* |
| UCLA Loneliness** | Mean | **43.58** | **37.6** | 3.53 | 97 | 0.001 |
| | SD | 8.92 | 7.92 | | | |
| SNI People | Mean | **17.77** | **18.54** | -0.386 | 87 | 0.7 |
| | SD | 8.71 | 10.12 | | | |
| SNI High Contact * | Mean | **6.47** | **7.06** | -2.124 | 81.328 | 0.037 |
| | SD | 1.06 | 1.62 | | | |
| Shyness** | Mean | **34.02** | **26.65** | 4.033 | 97 | 0 |
| | SD | 9.17 | 9 | | | |
| STAI State* | Mean | **34.55** | **29.56** | 2.604 | 95 | 0.011 |
| | SD | 10.74 | 7.87 | | | |
| STAI Trait** | Mean | **39.74** | **32.22** | 4.149 | 97 | 0 |
| | SD | 10.25 | 7.54 | | | |
| BDI* | Mean | **9.44** | **6.25** | 2.198 | 79.519 | 0.031 |
| | SD | 8.82 | 5.15 | | | |
| ERQ Cognitive Reappraisal | Mean | **30.84** | **32.49** | -1.184 | 97 | 0.239 |
| | SD | 7.75 | 5.98 | | | |
| ERQ Expressive Suppression** | Mean | **15.26** | **12.02** | 3.188 | 97 | 0.002 |
| | SD | 5.35 | 4.74 | | | |
| PiL | Mean | **40.98** | **41.68** | -0.579 | 86 | 0.564 |
| | SD | 5.42 | 5.93 | | | |

Table 1. Age differences in behavioral data. Older adults scored significantly lower on measures of loneliness, shyness, depression, expressive suppression and state and trait anxiety. Older adults also had significantly more people in their social networks with whom they interacted frequently. Asterisk indicates that $p < 0.05$, two asterisks indicate that $p < 0.01$.

| **rs2254298** | | | **rs53576** | | |
|---|---|---|---|---|---|
| Genotype | N | Frequency | Genotype | N | Frequency |
| G/G | 67 | 70% | G/G | 44 | 47% |
| A/G | 25 | 26% | A/G | 34 | 37% |
| A/A | 4 | 4% | A/A | 15 | 16% |

Table 2. Genotype numbers and frequencies for the rs2252498 and rs53576 *OXTR* polymorphisms. All frequencies were within the Hardy-Weinberg equilibrium ($p > 0.05$).

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## Appendix: Questionnaires

*UCLA Loneliness Scale*

The following statements describe how people sometimes feel. For each statement, please indicate how often you feel the way described.

Answer choices: 1. Never 2. Rarely 3. Sometimes 4. Always

1. How often do you feel that you are "in tune" with the people around you?
2. How often do you feel that you lack companionship?
3. How often do you feel that there is no one you can turn to?
4. How often do you feel alone?
5. How often do you feel part of a group of friends?
6. How often do you feel that you have a lot in common with the people around you?
7. How often do you feel that you are no longer close to anyone?
8. How often do you feel that your interests and ideas are not shared by those around you?
9. How often do you feel outgoing and friendly?
10. How often do you feel close to people?
11. How often do you feel left out?
12. How often do you feel that your relationships with others are not meaningful?
13. How often do you feel that no one really knows you well?
14. How often do you feel isolated from others?
15. How often do you feel you can find companionship when you want it?
16. How often do you feel that there are people who really understand you?
17. How often do you feel shy?
18. How often do you feel that people are around you but not with you?
19. How often do you feel that there are people you can talk to?
20. How often do you feel that there are people you can turn to?

*Social Network Index*

This questionnaire is concerned with how many people you see or talk to on a regular basis including family, friends, workmates, neighbors, etc. Please read and answer each question carefully. Answer follow-up questions where appropriate.

1. Which of the following best describes your marital status?
    a. Currently married & living together, or living with someone in a marital-like relationship
    b. Never married & never lived with someone in a marital-like relationship
    c. Separated
    d. Divorced or formerly lived with someone in a marital-like relationship
    e. Widowed
2. How many children do you have (If you don't have any children, check '0')
3. How many of your children do you see or talk to on the phone at least once every 2 weeks?
4. Are either of your parents living? (If neither is living, check '0')
5. Do you see or talk on the phone to either of your parents at least once every 2 weeks?
6. Are either of your in-laws (or partner's parents) living? (If you have none, choose 'not applicable')
7. Do you see or talk on the phone to either of your partner's parents at least once every 2 weeks?
8. How many other relatives (other than your spouse, parents & children) do you feel close to? (If not applicable, choose '0')
9. How many of these relatives do you see or talk to on the phone at least once every 2 weeks?
10. How many close friends do you have? (meaning people that you feel at ease with, can talk to about private matters, and can call on for help)
11. How many of these friends do you see or talk to at least once every 2 weeks?
12. Do you belong to a church, temple, or other religious group?
13. How many members of your church or religious group do you talk to at least once every 2 weeks? (This includes at group meetings and services.)
14. Do you attend any classes (school, university, technical training, or adult education) on a regular basis?
15. How many fellow students or teachers do you talk to at least once every 2 weeks? (This includes at class meetings.)

16. Are you currently employed either full or part-time?
17. How many people do you supervise?
18. How many people at work (other than those you supervise) do you talk to at least once every 2 weeks?
19. How many of your neighbors do you visit or talk to at least once every 2 weeks?
20. Are you currently involved in regular volunteer work?
    1. How many people involved in this volunteer work do you talk to about volunteering related issues at least once every 2 weeks?
    2. Do you belong to any groups in which you talk to one or more members of the group about group-related issues at least once every 2 weeks? Examples include social clubs, recreational groups, trade unions, commercial groups, professional organizations, groups concerned with children like the PTA or Boy Scouts, groups concerned with community service, etc.
    3. Consider those groups in which you talk to a fellow group member at least once every 2 weeks. Please provide the following information for each such group: the name or type of group and the total number of members in that group that you talk to at least once every 2 weeks.

*Shyness Scale*

Please read each item carefully and decide to what extent it is characteristic of your feelings and behavior. Fill in the blank next to each item by choosing a number from the scale printed below.

Answer choices: 1. Highly uncharacteristic or untrue, strongly disagree 2. Uncharacteristic 3. Neutral 4. Characteristic 5. Very characteristic or true, strongly agree

1. I feel tense when I'm with people I don't know well.
2. I am socially somewhat awkward.
3. I do not find it difficult to ask other people for information.
4. I am often uncomfortable at parties and other social gatherings.
5. When in a group of people, I have trouble thinking of the right things to talk about.
6. It does not take me long to overcome my shyness in a new situation.
7. It is hard for me to act natural when I am meeting new people.
8. I feel nervous when speaking to someone in authority.
9. I have no doubts about my social competence
10. I have trouble looking someone right in the eye.
11. I feel inhibited in social situations.
12. I do not find it hard to talk to strangers.
13. I am more shy with members of the opposite sex

*Purpose in Life*

For the questions below, please indicate how much you agree or disagree with the statement.

Answer choices: 1. Completely disagree 2. Somewhat disagree 3. Neither agree or disagree 4. Somewhat agree 5.Completely agree

1. I feel good when I think of what I've done in the past and what I hope to do in the future.
2. I live life one day at a time and don't really think about the future.
3. I tend to focus on the present, because the future nearly always brings me problems.
4. I have a sense of direction and purpose in life.
5. My daily activities often seem trivial and unimportant to me.
6. I used to set goals for myself, but that now seems like a waste of time.
7. I enjoy making plans for the future and working them to a reality.
8. I am an active person in carrying out the plans I set for myself.
9. Some people wander aimlessly through life, but I am not one of them.
10. I sometimes feel as if I've done all there is to do in life.

*STAI – State*

A number of statements which people have used to describe themselves are given in the following questions. Read each statement and then choose the appropriate answer to indicate how you feel right now, that is, at this moment. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present feelings best.

Answer choices 1. Not at all 2. Somewhat 3. Moderately so 4. Very much so

4. I feel calm
5. I feel secure
6. I am tense
7. I feel strained
8. I feel at ease
9. I feel upset
10. I am presently worrying over possible misfortunes
11. I feel satisfied
12. I feel frightened
13. I feel comfortable
14. I feel self-confident
15. I feel nervous
16. I am jittery
17. I feel indecisive
18. I am relaxed
19. I feel content
20. I am worried
21. I feel confused
22. I feel steady
23. I feel pleasant

*STAI – Trait*

A number of statements which people have used to describe themselves are given in the following questions. Read each statement and then choose the appropriate answer to indicate how you generally feel. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present feelings best.

Answer choices: 1. Almost never 2. Sometimes 3. Often 4. Almost always

1. I feel pleasant
2. I feel nervous and restless
3. I feel satisfied with myself
4. I wish I could be as happy as others seem to be
5. I feel like a failure
6. I feel rested
7. I am “calm, cool, and collected”
8. I feel that difficulties are piling up so that I cannot overcome them
9. I worry too much over something that really doesn’t matter
10. I am happy
11. I have disturbing thoughts
12. I lack self-confidence
13. I feel secure
14. I make decisions easily
15. I feel inadequate
16. I am content
17. Some unimportant thought runs through my mind and bothers me
18. I take disappointments so keenly that I can’t put them out of my mind
19. I am a steady person
20. I get in a state of tension or turmoil as I think over my recent concerns and interests

*Emotion Regulation Questionnaire*

We would like to ask you some questions about your emotional life, in particular, how you control (that is, regulate and manage) your emotions. The following questions involve two distinct aspects of your emotional life. One is your emotional experience, or what you feel like inside. The other is your emotional expression, or how you show your emotions in the way you talk, gesture, or behave. Although some of the following questions may seem similar to one another, they differ in important ways.

Answer choices 1. Strongly disagree 2. 3. 4. Neutral 5. 6. 7. Strongly agree

1. When I want to feel more *positive* emotion (such as joy or amusement), *I change what I'm thinking about.*
2. I keep my emotions to myself.
3. When I want to feel less *negative* emotion (such as sadness or anger), *I change what I'm thinking about.*
4. When I am feeling *positive* emotions, I am careful not to express them.
5. When I'm faced with a stressful situation, I make myself *think about it* in a way that helps me stay calm.
6. I control my emotions by *not expressing them*.
7. When I want to feel more *positive* emotion, *I change the way I'm thinking about the situation*.
8. I control my emotions by *changing the way I think* about the situation I'm in.
9. When I am feeling *negative* emotions, I make sure not to express them.
10. When I want to feel less *negative* emotion, I *change the way I'm thinking* about the situation.

*Beck Depression Inventory*

This questionnaire consists of 21 groups of statements. Please read each group of statements carefully, and then pick out the one statement in each group that best describes the way you have been feeling during the past two weeks, including today. If several statements in the group seem to apply equally well, choose the highest choice for that group.

1. Sadness
0 I do not feel sad.
1 I feel sad much of the time.
2 I am sad all the time.
3 I am so sad and unhappy that I can't stand it.

2. Pessimism
0 I am not discouraged about my future.
1 I feel more discouraged about my future than I used to be.
2 I do not expect things to work out for me.
3 I feel my future is hopeless and will only get worse.

3. Past Failure
0 I do not feel like a failure.
1 I feel I have failed more than I should have.
2 As I look back, I see a lot of failures.
3 I feel I am a total failure as a person.

4. Loss of Pleasure
0 I get as much pleasure as I ever did from the things I enjoy.
1 I don't enjoy things as much as I used to.
2 I get very little pleasure from the things I used to enjoy.
3 I can’t get any pleasure from the things I used to enjoy.

5. Guilty Feelings
0 I don't feel particularly guilty
1 I feel guilty over many things I have done or should have done.
2 I feel quite guilty most of the time.
3 I feel guilty all of the time.

6. Punishment Feelings
0 I don't feel I am being punished.
1 I feel I may be punished.
2 I expect to be punished.
3 I feel I am being punished.

7. Self-Dislike
0 I feel the same about myself as ever.
1 I have lost confidence in myself.
2 I am disappointed in myself.
3 I dislike myself.

8. Self-Criticalness
0 I don't criticize or blame myself more than usual.
1 I am more critical of myself than I used to be.
2 I criticize myself for all of my faults.
3 I dislike myself.

9. Suicidal Thoughts or Wishes
0 I don't have any thoughts of killing myself.
1 I have thoughts of killing myself, but I would not carry them out.
2 I would like to kill myself.
3 I would kill myself if I had the chance.

10. Crying
0 I don't cry any more than I used to.
1 I cry more than I used to.
2 I cry over every little thing.
3 I feel like crying, but I can’t.

11. Agitation
0 I am no more restless or wound up than usual.
1 I feel more restless or wound up than usual.
2 I am so restless or agitated that I have to keep moving or doing something.
3 It’s hard to get interested in anything.

12. Loss of Interest
0 I have not lost interest in other people or activities.
1 I am less interested in other people or things than before.
2 I have lost most of my interest in other people or things.
3 It’s hard to get interested in anything.

13. Indecisiveness
0 I make decisions about as well as I ever could.
1 I find it more difficult to make decisions than usual.
2 I have much greater difficulty in making decisions than I used to.
3 I have trouble making any decisions.

14. Worthlessness
0 I do not feel I am worthless.
1 I don't consider myself as worthwhile and useful as I used to.
2 I feel more worthless as compared to other people.
3 I feel utterly worthless.

15. Loss of Energy
0 I have as much energy as ever.
1 I have less energy than I used to have.
2 I don't have enough energy to do very much.
3 I don't have enough energy to do anything.

16. Changes in Sleeping Pattern
0 I have not experienced any change in my sleeping pattern.
1a I sleep somewhat more than usual.
1a I sleep somewhat less than usual.
2a I sleep a lot more than usual.
2b I sleep a lot less than usual.
3a I sleep most of the day.
3b I wake up 1-2 hours early and can't get back to sleep.

17. Irritability
0 I am no more irritable than usual.
1 I am more irritable than usual.
2 I am much more irritable than usual.
3 I am irritable all the time.

18. Changes in Appetite
0 I have not experienced any change in my appetite.
1a My appetite is somewhat less than usual.
1b My appetite is somewhat greater than usual.
2a My appetite is much less than before.
2b My appetite is much greater than usual.
3a I have no appetite at all.
3a I crave for food all the time.

19. Concentration Difficulty
0 I can concentrate as well as ever.
1 I can't concentrate as well as usual.
2 It's hard to keep my mind on anything for very long.
3 I find I can't concentrate on anything.

20. Tiredness or Fatigue
0 I am no more tired or fatigued than usual.
1 I get more tired or fatigued more easily than usual.
2 I am too tired or fatigued to do a lot of the things I used to do.
3 I am too tired or fatigued to do most of the things I used to do.

21. Loss of Interest in Sex
0 I have not noticed any recent change in my interest in sex.
1 I am less interested in sex than I used to be.
2 I am much less interested in sex now.
3 I have lost interest