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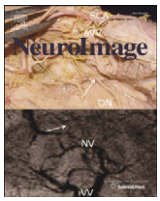

Amygdala response to smoking-cessation messages mediates the effects of serotonin transporter gene variation on quitting

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ABSTRACT

The amygdala is critically involved in detecting emotionally salient stimuli and in enhancing memory for emotional information. Growing evidence also suggests that the amygdala plays a crucial role in addiction, perhaps by strengthening associations between emotionally-charged drug cues and drug-seeking behavior. In the current study, by integrating functional MRI (fMRI), genetics, and outcome data from a large group of smokers who completed a smoking-cessation intervention and attempted to quit, we show that the amygdala also plays a role in quitting. Specifically, we demonstrate that the amygdala response to smoking-cessation messages in smokers trying to quit is a predictor of their post-intervention quitting outcome. We further show that the amygdala response is modulated by genetic variation in the serotonin transporter and mediates the impact of this genetic variation on quitting. These results point to a gene–brain–behavior pathway relevant to smoking cessation, and add to our understanding of the role of the amygdala in nicotine addiction.

Introduction

Cigarette smoking continues to be a leading preventable cause of morbidity and mortality in the U.S. (CDC, 2003, 2008). Many smokers attempt to quit but a majority relapse within 6 months (Quaak et al., 2009), highlighting a vital need for more effective interventions. Identification of brain predictors of relapse susceptibility could inform the design and selection of smoking-cessation interventions in the clinic, while also adding to our understanding of brain processes underlying nicotine addiction.

The goal of the current study was to examine the role of amygdala response to smoking-cessation messages in quitting as well as the genetic sources of variability in amygdala response. The amygdala plays a critical role in emotion processing, including response to emotionally salient stimuli and acquisition of emotional learning (LeDoux, 2000; Phelps, 2006). Emotionally salient information tends to be remembered better than emotionally neutral information (Cahill and McGaugh, 1995), and both animal and human studies suggest that the amygdala is required for this enhanced learning (McGaugh, 2004). For drug users, drug-related cues are emotionally arousing and are known to activate the amygdala (Childress et al., 1999; Franklin et al., 2007; Kilts et al., 2001). Therefore, drug-related information is more likely to be remembered by them and influence their behavior. Consistent with this view, growing evidence suggests that the amygdala is involved in drug addiction, by signaling the salience of drug-related cues and creating strong associations with drug-seeking behaviors (Robbins et al., 2008; See et al., 2003).

Previous neuroimaging studies in smokers have shown the link between amygdala response to smoking-related cues on the one side, and craving intensity (Franklin et al., 2011; Goudriaan et al., 2010) and relapse susceptibility (Janes et al., 2010) on the other side. In contrast, amygdala response to smoking-cessation messages has not yet been examined. We hypothesized that the amygdala may also play an important role in how smokers respond to smoking-cessation messages and how these messages influence their smoking behavior. Specifically, we expected that a greater amygdala response to smoking-cessation messages should be associated with enhanced memory of these messages, and therefore increased odds of quitting success. To test our hypothesis, we integrated functional MRI (fMRI) and outcome data from a large group of smokers who completed a smoking-cessation intervention, in order to examine whether the amygdala response to smoking-cessation messages predicted subsequent quitting outcome in these smokers.

We also investigated the genetic factors that may account for individual differences in amygdala response to smoking-cessation messages, using an imaging genetics approach (Bigos and Weinberger,

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2010; Hariri, 2009; Hariri et al., 2006). Growing evidence from imaging genetics suggests that amygdala reactivity to emotionally salient stimuli (e.g., angry faces) is modulated by genetic variation in the serotonin transporter (Hariri et al., 2002, 2005). Serotonin (or 5-hydroxytryptamine, 5-HT) is a major modulatory neurotransmitter in the mammalian brain, crucially involved in a range of brain processes, including stress response, arousal, motor activity, appetite, and mood (Jacobs and Azmitia, 1992)—but also cognitive control, cognitive flexibility, and decision making (Cools et al., 2008; Dayan and Huys, 2009; Kranz et al., 2010; Rogers, 2011). The serotonin transporter protein (5-HTT), responsible for reuptake of 5-HT from the synapse back into the presynaptic neuron, serves as a key regulator of 5-HT signaling.

Thus, in addition to testing if amygdala response to smoking-cessation messages predicted quitting, we also employed an imaging genetics approach to test whether two known functional polymorphisms in the 5-HTT gene (*SLC6A4*)–the 5-HTT-linked polymorphic region (5-HTTLPR) in the promoter (Heils et al., 1996; Lesch et al., 1996) and the serotonin transporter intron 2 (STin2) polymorphism (Fiskerstrand et al., 1999; Lesch et al., 1994)–modulated the amygdala response. The 5-HTTLPR is a 44-base pair insertion/deletion polymorphism in the promoter region, with the short allele (S) less efficiently transcribed than the long allele (L) (Heils et al., 1996; Lesch et al., 1996). In addition, the 5-HTTLPR includes an A→G single nucleotide substitution (rs25531), with the L_G allele being functionally equivalent to the S allele, leaving the L_A allele as the true high-transcription allele (Hu et al., 2006). The second functional polymorphism, STin2, is a 17-base pair insertion/deletion polymorphism in intron 2 of the 5-HTT gene *SLC6A4*, with the 12-repeat allele more efficiently transcribed than the 10-repeat allele (Fiskerstrand et al., 1999; Lesch et al., 1994). Based on previous evidence (Dannlowski et al., 2010; Hariri et al., 2002, 2005), we hypothesized that low-transcription alleles of the 5-HTTLPR/rs25531 and STin2 would be associated with a relatively increased amygdala response compared to high-transcription alleles.

Materials and methods

Subjects

Ninety-one smokers interested in quitting (44 females and 47 males, mean age = 37.5 years, mean number of cigarettes per day = 16.7) were recruited for the study (Chua et al., 2011), which included a web-based tailored smoking-cessation intervention developed at the University of Michigan's Center for Health Communications Research (Strecher et al., 2008). Smokers were eligible to participate if they smoked a minimum of 10 cigarettes per day and at least 100 cigarettes in their lifetime. Subjects were not enrolled in other smoking-cessation programs or taking pharmacological treatments for smoking cessation during study enrollment. All subjects were native English speakers, had normal vision and hearing, and had no history of head injury or mental illness. The study protocol was approved by the University of Michigan Medical School IRB and all subjects provided written informed consent.

Study design

The study involved 3 sessions plus a follow-up phone interview. In Session 1, subjects completed a baseline assessment of their smoking history and other health, demographic, and psychosocial characteristics relevant to smoking cessation. The responses were used to create tailored smoking-cessation messages for the subsequent intervention. In Session 2, subjects completed a Messages Task during functional MRI (fMRI). In Session 3, scheduled within one week of their fMRI session, subjects completed a web-based computer-tailored smoking-cessation intervention (Strecher et al., 2008) and started their quit attempt. All subjects also provided saliva for DNA extraction and genotyping, and received a 10-week supply of nicotine patches to help them quit. Four months after the intervention session, subjects were interviewed on the phone to determine their smoking-cessation outcome. The primary outcome measure was 7-day point-prevalence abstinence (cigarette free for the past 7 days), a metric that is highly correlated with both 24-hour point-prevalence abstinence and 30-day prolonged abstinence, as well as with physiological measures of smoking cessation (Velicer and Prochaska, 2004).

Messages Task

All subjects completed 5 runs of the Messages Task (Chua et al., 2011) during fMRI, with 2 blocks of each of the three types of messages (tailored smoking-cessation messages, untailored smoking-cessation messages, and neutral control messages) per run, 5 messages per block, for the total number of 150 messages. Each block lasted 24 s. The order of blocks in the Messages Task was pseudo-randomized within and across participants. Blocks were separated by fixations lasting 4–10 s (an average of 7 seconds). The messages were presented visually on the screen and simultaneously presented as an audio track. Subjects were instructed to pay attention to the messages but no response was required. Tailored smoking-cessation messages were created on the basis of the individual's responses during a baseline assessment and made references to this person's characteristics, experiences, and specific obstacles to quitting. Therefore, although of the same type, tailored smoking-cessation messages varied across subjects. Examples of tailored smoking-cessation messages include: *You want to quit because you are tired of spending your money on cigarettes; You feel like your friend will help you stay on track once you quit; You have a very strong urge to smoke when you first wake.* In contrast, untailored smoking-cessation messages were relevant to smokers in general and were identical for all subjects. Examples of untailored smoking-cessation messages include: *Many people quit with another person so they can support each other; Many people relapse due to stress, alcohol, and cravings; Most people need to try more than once to quit smoking for good.* Neutral control messages (i.e., world-knowledge messages not related to smoking) were also the same for all subjects. Examples of neutral messages include: *The longest duration of a solar eclipse was 7 minutes, 31 seconds; Bali attracts more tourists than any other Indonesian island; Global warming caused the recent collapse of an Antarctic ice shelf.*

Image acquisition

Scanning was performed on a 3T GE Signa Excite 2 scanner (Milwaukee, Wisconsin), beginning with a structural T1-overlay image (repetition time [TR] = 250 ms, echo time [TE] = 7 ms, flip angle [FA] = 75 degree, field of view [FOV] = 220 mm, 43 oblique axial slices, 256 × 256, slice thickness 3.0 mm). Functional scans were collected using a T2*-weighted spiral-in acquisition sequence (gradient echo, TR = 2000 ms, TE = 30 ms, FA = 90°, FOV = 220 mm, 64 × 64, slice thickness 3.0 mm) (Noll et al., 1998). High-resolution T1 scans were also obtained for precise anatomical localization (3D spoiled-gradient echo [3D-SPGR] with inversion recovery prep, time of inversion = 400 ms, TR = 9.0 ms, TE = 1.8 ms, FA = 15°, FOV = 260 mm, 128 slices, 256 × 256, 1.2 mm slice).

Image preprocessing

All functional scans were slice-time-corrected, motion-corrected, and realigned to the first scan using the MCFLIRT program (FSL Analysis Group, FMRIB, Oxford, UK). Subsequent processing was done using SPM (Wellcome Institute of Cognitive Neurology, London, UK). The T1-overlay was co-registered with a functional scan. The high-resolution 3D-SPGR image was co-registered to the T1-overlay