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The serotonin transporter gene and startle response during nicotine deprivation☆

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ABSTRACT

Affective startle probe methodology was used to examine the effects of nicotine administration and deprivation on emotional processes among individuals carrying at least one s allele versus those with the l/l genotype of the 5-Hydroxytryptamine (Serotonin) Transporter Linked Polymorphic Region, *5-HTTLPR* in the promoter region of the serotonin transporter gene [solute ligand carrier family 6 member A4 (*SLC6A4*) or SERT]. Smokers ($n = 84$) completed four laboratory sessions crossing deprivation (12-h deprived vs. non-deprived) with nicotine spray (nicotine vs. placebo). Participants viewed affective pictures (positive, negative, neutral) while acoustic startle probes were administered. We found that smokers with the l/l genotype showed significantly greater suppression of the startle response when provided with nicotine vs. placebo than those with the s/s or s/l genotypes. The results suggest that l/l smokers, who may have higher levels of the serotonin transporter and more rapid synaptic serotonin clearance, experience substantial reduction in activation of the defensive system when exposed to nicotine.

1. Introduction

Several neurotransmitters have been implicated in the effects of short-term nicotine administration and deprivation, including serotonin (5-HT; Benowitz, 2008). Serotonin is involved in the regulation of many brain functions, including sleep, cognition, sensory perception, motor activity, temperature regulation, mood, appetite, sexual behavior, and hormone secretion (Murphy et al., 2008). The diversity of the physiologic function of 5-HT is likely due to the fact that it orchestrates the activity and interaction of several other neurotransmission systems (Lesch and Mossner, 1998). Studies have shown that 5-HT function has broad developmental effects, promoting differentiation of serotonergic as well as glutaminergic neurons (Pezawas et al., 2005). Additionally, 5-HT levels are considered to be critical for the emotional development of the brain and can modify neural connections implicated in an increased risk for mood and anxiety-related disorders (Gross and Hen, 2004; Gaspar et al., 2003).

Within the central 5-HT system, the serotonin transporter (5-HTT) is thought to regulate the magnitude and duration of serotonergic signaling by governing the reuptake of serotonin from the synaptic junction. The transporter is encoded by a single gene, the solute ligand carrier family 6 (neurotransmitter transporter, serotonin) member A4 (*SLC6A4*), on chromosome 17q11.1-q12, the promoter region of which contains a 44-bp insertion/deletion polymorphism, also known as the *5-HTTLPR*. Research suggests that the long (l) allele of this polymorphic region is more than twice as active as the short (s) allele (Heils et al., 1996; Lesch et al., 1996), resulting in increased transcription of the serotonin transporter, and the potential for increased reuptake of serotonin from the synaptic cleft, and overall lower levels of serotonin in the synapse (Murphy et al., 2001), though not all studies have reported this functional relationship in humans (Shioe et al., 2003; Willeit et al., 2000).

The *5-HTTLPR* has been linked to alcohol abuse, schizophrenia, depression, bipolar disorder, and smoking, though with inconsistent results (Serretti et al., 2006). Based on the literature, it seems that individuals with extremely high or extremely low serotonin levels may be at risk for affective disorders, alcoholism, anxiety disorders, and personality characteristics such as neuroticism (Lesch and Mossner, 1998; Serretti et al., 2006; Gonda et al., 2009; Murphy et al., 2008).

☆ The following IAPS slides were used: positive, 4220, 4652, 4658, 4659, 4660, 4670, 5621, 5629, 8030, 8370, 8490, and 8500; neutral, 7000, 7010, 7020, 7030, 7040, 7050, 7060, 7080, 7090, 7100, 7150, and 7170; negative, 3010, 3060, 3100, 3120, 3130, 3150, 3170, 3500, 6230, 6350, 6560, and 9410. The subjects were asked to view each slide and then rate the slide on affective valence (positive, negative), arousal (low, high), and craving (low, high) by selecting a box on a Likert scale ranging from 1 to 9. Results are presented in Cinciripini et al. (2006).

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A recent meta-analysis (Munafo et al., 2004) examining the roles of many candidate genes and smoking behaviors suggested that individuals with at least one copy of the s allele of the *5-HTTLPR* were less likely than those without to successfully quit smoking after behavioral treatment. Another study reported that individuals with at least one s allele and high levels of neuroticism were more nicotine dependent and more likely to report smoking to reduce negative affect than those with the l/l genotype and individuals with low levels of neuroticism (Hu et al., 2000). On the other hand, at least three studies have reported a positive association between the l allele of the *5-HTTLPR* and smoking (versus never smoking; Ishikawa et al., 1999; Kremer et al., 2005; Arinami et al., 1999), or no relationship at all (David et al., 2007; Trummer et al., 2006; Gonda et al., 2009; Rasmussen et al., 2009). Another study demonstrated that smokers exhibited higher 5-HT transporter availability in the brainstem than non-smokers (Staley et al., 2001), an area in which 5-HT transporter function may be altered in individuals with mood disorders (Willeit et al., 2000).

Given conflicting data associating the serotonin system with the regulation of emotion as well as the well-documented association between negative affect and smoking (Copeland et al., 2009; Leventhal et al., 2009), it may be that the gene exerts an effect on smoking behavior in complex ways that are not completely understood but which at some level effect neurobiological pathways shared by both nicotine and serotonin. Differences in behavioral phenotypes associated with smoking may be partly related to the effects of the gene on serotonin transport (and availability) which in turn, affect the activation/suppression of very fundamental neurobiological mechanisms related to processing of emotional information. These emotional processing systems and structures are likely to be very primitive in the sense that they are phylogenetically very old and are activated by motivationally relevant stimuli that predispose the organism towards appetitive (approach) or defensive (avoidance) behavior. A paradigm such as affective picture modulation using the acoustic startle response (ASR) may provide a sensitive measure of the functional impact the *5-HTTLPR* genotype on brain mechanisms associated with appetitive and defensive systems, particularly in response to events that have clear motivational relevance to the smoker: nicotine administration and deprivation.

The startle (eye blink) response is a defensive reflex, which occurs during the presentation of an unexpected auditory stimulus (probe). Its strength is measured by the electromyographic (EMG) changes in the orbicularis oculi region of the eye and is highly sensitive to ambient emotional cues. It is well established that the magnitude of the blink (EMG) response to the startling acoustic probe is enhanced when subjects view unpleasant or negative emotional pictures in comparison to pleasant and neutral pictures (e.g., Cook et al., 1992; Vrana et al., 1988), whereas the response is reduced during viewing of positive as opposed to negative or neutral emotional cues (Cuthbert et al., 1996). Startle potentiation by unpleasant stimuli is thought to reflect activation of the aversive/defensive motivational system, while startle inhibition by positive emotional cues may reflect activation of appetitive or approach motivational processes (Lang et al., 1990, 1992). The neural circuitry underlying the startle response may involve several structures also associated with nicotine administration and withdrawal, such as the prefrontal cortex, hippocampus, amygdala, and the nucleus accumbens (Swerdlow and Geyer, 1999; Hare et al., 2005; Phan et al., 2005; Stein et al., 1998).

A handful of studies have in fact investigated the cognitive-affective mechanisms associated with the *5-HTTLPR*. Beevers et al. (2007) reported that depressed inpatients with at least one short allele exhibited more cognitive bias for anxiety-related words than those with two long alleles. Additionally, other studies have suggested that short allele carriers may more intensely process fear-related emotional stimuli (Bertolino et al., 2005; Surguladze et al., 2008; Osinsky et al., 2008), while Fox et al. (2009) reported that individuals with the l/l genotype evidenced bias towards positive pictures and avoidance of threat related images. A study with adolescents revealed a linear relation between presumed serotonin availability (based on genotype) and the magnitude of attention towards angry and happy faces (Perez-Edgar et al., 2010). These studies were done with relatively small samples that are more typical of cognitive neuroscience studies rather than the traditionally larger genetic studies.

We are aware of only one study, not conducted in smokers, in which the effects of the *5-HTTLPR* was studied specifically in relation to the human startle response. That study showed that *5-HTTLPR* short allele carriers exhibited heightened startle responses compared to those with the l/l genotype (Brocke et al., 2006). However, the authors were unable to confirm their hypothesis that the s carriers would exhibit heightened fear-potentiated startle responses in comparison to those with the l/l genotype, as they found no interaction with picture content. Other studies have suggested that the manipulation of serotonin levels using pharmacological treatment may potentiate (Liechti et al., 2001; Hayward et al., 2005), attenuate (Murphy et al., 2006), or have no effect at all (Phillips et al., 2000; Quednow et al., 2004) on the startle response.

1.1. Hypothesis

In this study, acoustic startle probe methodology was used to examine the effects of nicotine administration and deprivation on emotional processes associated with drive and motivation (i.e., activation of the defensive system using the startle probe) with respect to genotype at the *5-HTTLPR* of the serotonin transporter gene, *SLC6A4*. The individuals in this study were a sub-sample of a larger study addressing the effects of deprivation and nicotine administration on the acoustic startle response more generally (Cinciripini et al., 2006). Specifically, our aim was to directly test the relationship between the three *5-HTTLPR* genotypes (i.e., s/s, s/l, and l/l), overnight nicotine deprivation, acute nicotine administration, and activation of the defensive system using the startle probe.

We hypothesized that the startle response in individuals with the *5-HTTLPR* l/l genotype would be notably suppressed in response to acute nicotine administration for overnight nicotine deprived smokers relative to non-deprived smokers. We further predicted that this suppression would be most pronounced when these individuals were exposed to negative emotional cues compared to positive or neutral cues.

2. Method

2.1. Participants

Smokers were recruited from the Houston metropolitan area using newspaper advertisements and flyers. They were paid $125 for attending 4 laboratory sessions and an additional five dollars each session for arriving promptly. Smokers who were between the ages of 18 and 59, fluent in English, smoked 10 or more cigarettes per day, had an expired carbon monoxide level greater than 8 ppm (or saliva cotinine >30 ng/ml), and reported no uncontrolled medical illness were included in the study. Individuals were excluded if they were taking psychotropic or narcotic medication, met criteria for a current psychiatric disorder, reported hearing loss, were using tobacco products other than cigarettes, or were involved in current smoking cessation activity. Smokers were not recruited based on their interest in quitting and no cessation treatment was provided. A total of 113 participants contributed to the main study (Cinciripini et al., 2006). A sub-sample of 84 individuals, restricted to African American and Caucasians smokers, provided genotypic data for the current analyses.

2.2. Design

This study used a within-subjects design. Smokers attended four 90-min laboratory sessions scheduled approximately 3 days apart. The four sessions provided a complete crossing of two pre-laboratory smoking deprivation conditions (12-h