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Low Startle Magnitude May Be a Behavioral Marker of Vulnerability to Cocaine Addiction

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Abstract

Cocaine addicted men have low startle magnitude persisting during prolonged abstinence. Low startle rats show greater cocaine self-administration than high startle rats. Low startle may be a marker of a vulnerability to heightened cocaine-related behaviors in rats and similarly may be a marker of vulnerability to cocaine addiction in humans.

Keywords

Cocaine; Acoustic Startle; Dopamine; Self-administration; Novelty Preference

The acoustic startle response is a short-latency reflex to a sudden auditory stimulus that is mediated by a simple neuronal pathway in the brainstem and spinal cord (Lee et al., 1996). Men with a history of cocaine addiction have markedly diminished acoustic startle magnitudes (Corcoran et al., 2011; Efferen et al., 2000), an effect that persists for a full year after cessation of cocaine (Corcoran et al., 2011). Although chronic cocaine intake could reduce startle magnitude, chronic cocaine has not led to a consistent decrease in acoustic startle magnitude in rats (Adams et al., 2001; Mansbach et al., 1994; Murphy et al., 2001; Mutschler and Miczek, 1998) or rhesus monkeys (Kirkland et al., 2009). Startle is extremely stable when retested in humans (Abel et al., 1998; Duncan et al., 2001) and rats (Pettersson et al., 2015), and is 60%–70% heritable in humans (Anokhin et al., 2003; Hasenkamp et al., 2010).

Because startle magnitude is a stable trait and because there is little evidence that chronic cocaine reduces startle magnitude in rats, it is possible that low startle in men precedes cocaine addiction and might even be a marker for a vulnerability to develop cocaine

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addiction. The purpose of the current study was to evaluate whether low acoustic startle magnitude in rats might predict animal behavior in the self-administration model of cocaine addiction (Ahmed 2010; Deroche-Gamonet et al., 2004; Koob, 1995; Wise, 1984), and novelty preference, which predicts cocaine self-administration (Belin et al., 2011).

Group housed adult male Sprague-Dawley rats (Charles-River, Raleigh, NC) were used for these studies. The Institutional Animal Care and Use Committees at Yerkes National Primate Research Center and Emory University approved all experiments and animal care procedures.

In each experiment acoustic startle magnitude was first measured in groups of rats on each of 3 days with a 48-hr interval between each test according to methods previously reported by our group (Meloni and Davis, 1999). Startle responses were evoked by 50-msec 95-decibel (dB) white-noise bursts delivered against background noise (60-dB wideband). Startle response magnitudes were quantified using an accelerometer (Model U321AO2; PCB Piezotronics) affixed to the bottom of each startle chamber. The rats' startle response displaced the accelerometer, producing a voltage output proportional to the cage movement. This output was amplified, digitized, and interfaced to a Macintosh computer. Startle magnitude was defined as the maximal peak-to-peak voltage that occurred during the first 200 msec after onset of the startle-eliciting white-noise burst. High and low startle rats were designated as those with baseline startle magnitudes in the highest and lowest quartiles. The remaining rats were not used.

In preparation for cocaine self-administration experiments, rats were fitted with jugular catheters in accord with published methods (Caine et al., 1993; Shahbazi et al., 2008). No rats contracted an infection or other illness that required them to be excluded from further procedures. In each experiment prior to cocaine self-administration low and high startle rats were trained in a dimly lit operant chamber to self-administer food during two daily 2-hr sessions by means of active lever pressing on a fixed ratio 1 schedule. All animals reached the criterion of obtaining 100 45-mg food pellets in the second 2-hr session. The two groups did not significantly differ in active or inactive lever pressing (see Figure 1a).

Cocaine self-administration testing occurred during subsequent daily 2-hr sessions. The session began with a 4-sec priming injection of 0.5 mg/kg of cocaine followed immediately by presentation of the active and inactive levers. Responses on the active lever resulted in a 4-sec infusion of 0.1 ml of 0.5 mg/kg of cocaine on a fixed ratio 1 schedule. This was followed by a 20-sec presentation of a cue light above the active lever signaling a time-out period during which responses on either lever had no effect.

In the first experiment 20 rats were tested for startle magnitude as described above and divided into low and high startle groups with $n=5$ in each group. They were then trained to self-administer food and then cocaine. Once self-administration of the 0.5-mg/kg concentration of cocaine was acquired, determined by 3 consecutive days of responding on the active lever with less than 10% variability around the mean, the concentration of cocaine was varied so that each rat, in an irregular and counterbalanced order, self-administered 0.0625, 0.125, 0.25, and 1.0 mg/kg cocaine concentrations. Each new concentration of

cocaine was presented on consecutive days until the number of reinforced responses was stabilized with less than 10% variability. The 3-day mean of stabilized responding for each cocaine concentration was used to determine the dose-response curve for high and low startle animals.

The drug naïve acoustic startle magnitudes of low and high startle rats were significantly different (means $\pm$ SEMs: Low: 1.1 $\pm$ 0.1; High: 3.6 $\pm$ 0.5; $t_{(8)} = 5.02$, $p < 0.002$). High and low startle rats did not differ in the number of days necessary to acquire operant responding for a food reinforcer or self-administration of cocaine. Low startle rats had higher rates of lever pressing at each of the various doses of cocaine (Figure 2). An overall analysis of variance with dose as a within-subjects factor and group as a between-subjects factor found a significant difference between the two groups ($F_{(1,8)} = 9.98$, $p < 0.01$). There was also a significant dose effect ($F_{(4,32)} = 40.22$, $p < 0.001$), and no group by dose interaction ($F_{(4,32)} = 2.24$). In summary, low startle rats self-administered more cocaine than high startle rats. The higher rates of bar pressing in the low startle rats at the various doses of cocaine were not simply because they pressed more in general. Bar press rates on the inactive lever at each dose did not differ statistically between low and high startle rats (see Figure 1b).

Low startle rats also had generally higher rates of active lever responses during the cued 20-sec time-out period following each cocaine delivery. Time-out bar press rates at each cocaine dose are shown in Figure 1c. Because this data set had significant non-homogeneity of variance between low and high startle groups ($F_{\max} > 9.2$, $df = 4$, $p < 0.05$), time-out responses were converted to log10 scores to normalize the data. ANOVA of these transformed scores found significantly higher scores in the low startle group ($F_{(1,8)} = 23.16$, $p < 0.001$) and an effect of dose ($F_{(4,32)} = 13.84$, $p < 0.001$) with a significant quadratic trend ($F_{(1,8)} = 74.20$, $p < 0.0001$). Because time-out responses typically covary with the number of reinforced lever presses, the number of time-out responses was divided by the number of reinforced responses for each rat at each dose. With this conversion, low startle rats still made significantly more time-out responses than high startle rats ($F_{(1,8)} = 9.95$, $p < 0.01$). In summary, when time-out lever pressing was used as a measure of cocaine related behavior, low startle rats had higher cocaine related behavior during time-out periods than high startle rats.

Another 56 rats were tested for startle magnitude and divided into low and high quartile startle groups with $n=14$ in each group that significantly differed in drug naïve acoustic startle (means, $\pm$ SEMs: Low: 1.2 $\pm$ 0.05; High: 3.3 $\pm$ 0.2; $t_{(26)} = 12.80$, $p < 0.001$). Novelty preference was tested in a single preference session. Rats were placed in a central hallway with access to two adjacent compartments blocked. After 5 min rats were confined to either compartment A or B for 25 min, counterbalanced across rats. After this period rats were allowed to freely explore the familiar compartment, the central hallway, and the unfamiliar compartment for 15 min. Preference scores were computed as: $[\text{time in unfamiliar compartment} / (\text{time in familiar} + \text{time in unfamiliar})] * 100$. The low startle rats displayed a greater level of novelty preference than high startle rats (means, $\pm$ SEMs: Low: 66.7 $\pm$ 4.5; High: 42.6 $\pm$ 5.2; $t_{(26)} = 3.55$, $p < 0.001$). This was based on the amount of time

in the unfamiliar compartment (Low: 470.2 $\pm$ 40.1 vs. High: 305.5 $\pm$ 36.0) vs. amount of time in the familiar compartment (Low: 229.1 $\pm$ 30.6 vs. High: 436.0 $\pm$ 52.2).

This study was a reverse translational investigation based on our results in cocaine-addicted men who have abnormally low acoustic startle magnitudes that fail to normalize after one year of abstinence (Corcoran et al., 2011; Efferen et al., 2000). Our hypothesis was that low acoustic startle magnitude would predict animal behavior in models of cocaine addiction. Collectively, our data are supportive of this hypothesis and are in conceptual accord with our human data..

Cocaine self-administration in animals is a well-accepted model of cocaine addiction in humans (Ahmed 2010; Deroche-Gamonet et al., 2004; Koob, 1995; Wise, 1984). Our present data indicate that low startle rats exhibited greater cocaine self-administration compared to high startle rats. We also report that low startle rats exhibited increased bar pressing on the active lever during signaled time-out periods when cocaine was unavailable. This behavior is conceptualized as persistent drug seeking and as a model of compulsive drug seeking in cocaine addicted humans (Deroche-Gamonet, et al., 2004; Belin et al., 2011). Finally, we found that low startle rats had enhanced novelty preference compared to high startle rats. High novelty preference is predictive of addiction-like behaviors as measured by the Deroche-Gamonet model, specifically inability to refrain from drug seeking and resistance to shock-induced punishment of cocaine taking (Deroche-Gamonet et al., 2004; Belin et al., 2008; Belin et al., 2011).

Limitations of this study include the modest sample size in the cocaine self-administration experiments, which must render our findings preliminary. We did not specifically test for behaviors that model anxiety, so cannot rule out low anxiety in the low startle group as a contributor to our results.

What might account for an association between low startle and vulnerability to animal behavioral models of cocaine addiction and, by extension, vulnerability to cocaine addiction in humans? A plethora of animal and human studies indicate that addiction is a complex of impulsivity and compulsivity behaviors that are reliant on circuitry involving the ventral tegmental area, striatum including the nucleus accumbens, prefrontal cortex, extended amygdala, among other areas, and are heavily reliant on modulations of dopamine signaling, plus interactions with glutamate, norepinephrine, and corticotropin-releasing factor (Koob and Volkow, 2010; 2016). Relevant to our report, human cocaine addicts have blunted dopamine responsiveness (Volkow et al. 1997; Martinez et al., 2007) and reductions in dopamine D2 receptors that persist for months of abstinence (Volkow et al., 1999).

There is partial overlap between the neurochemistry mediating addiction and startle. In rats, acoustic startle is modulated by a variety of different neurotransmitters, often in complex ways; however, dopamine figures prominently. Startle magnitude is dose-dependently increased by cocaine, which effect is blocked by the D2 antagonist haloperidol, but not by norepinephrine or serotonin antagonists (Davis, 1985). Startle magnitude is enhanced by drugs that increase dopamine signaling (Davis, 1980), and especially by dopamine D1 receptor activation, but this appears to be dependent on a complex cooperative interaction

between dopamine D1 and D2 receptors because the effect can be blocked by antagonists of either dopamine D1 or D2 receptor subtypes (Meloni and Davis 1999). Additionally, startle is modulated by glutamate acting on both NMDA and AMPA receptors in the brainstem and spinal cord (Spiera and Davis, 1988; Boulis et al., 1990; Miserendino and Davis, 1993), and is also modulated by serotonin, norepinephrine, GABA, and corticotropin-releasing factor (Davis, 1980; Johansson et al., 1995; Liang et al., 1992; Meloni and Davis, 2000). Thus it is not yet clear which neurotransmitters are implicated in our findings linking low startle and animal behaviors in models of cocaine addiction.

Novelty preference is associated with motivation to obtain cocaine, cocaine seeking, and reinstatement of cocaine seeking (Belin et al. 2011). Novelty preference is blocked by a D1 antagonist at a low dose that does not impair locomotor activity (Bardo et al., 1993), by low doses of apomorphine that reduce dopamine signaling (Bardo et al., 1990), and by lesions that reduced nucleus accumbens dopamine (Pierce et al., 1990), implying that intact dopamine activity is necessary for expression of this behavior. Thus the potential mechanism linking high novelty preference with low startle remains unclear.

Koob and Le Moal (2001) hypothesized that individuals with genetically determined vulnerability to develop addiction may have reward system abnormalities that predate drug addiction. The high heritability of startle magnitude (Anokhin et al., 2003; Hasenkamp et al., 2010) indicates that this trait has a significant genetic component. Low startle may be a marker for an abnormal hedonic set point leading to vulnerability to addiction in humans, and similarly leading to vulnerability to cocaine-seeking behaviors in the rat experiments we have described. However, this is extremely speculative. Future work is needed to replicate this work and discover the molecular underpinnings of our findings.

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Figure 1.

(a) Presses on active and inactive levers on Days 1 and 2 of training for food. Low and high startle rats did not differ. (b) Presses on active and inactive levers at each cocaine dose. Low startle rats exceeded high startle rats on the active lever ($F_{(1,8)} = 9.98$, $p < 0.01$). (c) Presses on active lever during cued time-outs. Low startle rats exceeded high startle rats in ANOVA on log10 transformed data ($F_{(1,8)} = 23.16$, $p < 0.001$).

Figure 2.

Dose Response Curve. Low acoustic startle rats ($n = 5$) achieved more cocaine reinforced responses (infusions/2 hrs) than high startle rats ($N = 5$; $F_{(1,8)} = 9.98$, $p < 0.01$). Values are Means $\pm$ SEM