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Dissociation of tolerance and nicotine withdrawal-associated deficits in contextual fear

Thomas J. Gould[a,*], Derek S. Wilkinson[a], Emre Yildirim[a], Julie A. Blendy[b], Michael D. Adoff[a]

[a]Department of Psychology, Weiss Hall, Neuroscience Program, Temple University, Philadelphia, PA 19122, USA
[b]Department of Pharmacology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

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ABSTRACT

Nicotine addiction is associated with the development of tolerance and the emergence of withdrawal symptoms upon cessation of chronic nicotine administration. Changes in cognition, including deficits in learning, are one of the most common withdrawal symptoms reported by smokers. However, the neural substrates of tolerance to the effects of nicotine on learning and the substrates of withdrawal deficits in learning are unknown, and in fact it is unclear whether a common mechanism is involved in both. The present study tested the hypothesis that tolerance and withdrawal are separate processes and that nicotinic acetylcholine receptor (nAChR) upregulation underlies changes in learning associated with withdrawal but not tolerance. C57BL/6 male mice were administered a dose of nicotine (3, 6.3, 12, or 24 mg/kg/d) chronically for varying days and tested for the onset of tolerance to the effects of nicotine on learning. Follow up experiments examined the number of days of chronic nicotine treatment required to produce withdrawal deficits in learning and a significant increase in [^{3}H] epibatidine binding in the hippocampus indicative of receptor upregulation. The results indicate that tolerance onset was influenced by dose of chronic nicotine, that tolerance occurred before withdrawal deficits in learning emerged, and that nAChR upregulation in the dorsal hippocampus was associated with withdrawal but not tolerance. This suggests that for the effects of nicotine on learning, tolerance and withdrawal involve different substrates. These findings are discussed in terms of implications for development of therapeutics that target symptoms of nicotine addiction and for theories of addiction.

1. Introduction

Adaptation in behavior and neural substrates occurs with chronic drug use; and for drugs of abuse, these adaptations may contribute to the formation and maintenance of addiction. The DSM-IV lists seven diagnostic criteria for addiction with a minimum of three present needed for a diagnosis of addiction (Association American Psychiatric, 2000). Two of

*Corresponding author. Fax: +1 215 204 5539.
E-mail address: tgould@temple.edu (T.J. Gould).

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the seven listed are tolerance and withdrawal symptoms. Because of the purported importance of tolerance and withdrawal in addiction, understanding the biological basis of these symptoms could advance treatment of addiction. Models and theories of addiction and drug action have proposed that tolerance and withdrawal are manifestations of the same phenomenon; however, these theories are not universally accepted as some have suggested that tolerance and withdrawal reflect separate processes.

The opponent process theory states that with drug abuse and addiction, a drug will initially produce a positive effect (the A process) but with continued use a countering effect (the B process) is generated to maintain homeostasis (Solomon and Corbit, 1973). As the B process reduces the desired effects of the A process, drug consumption may increase in an attempt to achieve the full A process; this has been suggested to be tolerance. In the absence of the drug and the associated A process, the B process will dominate resulting in a stronger negative effect, which has been proposed to be the mechanism underlying withdrawal (Poulos and Cappell, 1991). Thus, in this model tolerance and withdrawal would reflect the same process.

While the opponent process theory has appeal as it explains how drug-induced stress on homeostatic processes results in addiction phenotypes, there is a long history of work suggesting that tolerance and withdrawal may be separate processes rather than aspects of a single homeostatic process. If this is true, the opponent process theory would need to be amended. For example, Tatum et al. (1929) proposed that tolerance reflected a decrease in the depressant effects of morphine whereas withdrawal was related to the stimulant effects of morphine. Furthermore, in a review of morphine tolerance, Baker and Tiffany (1985) concluded that opponent or compensatory processes do not contribute to withdrawal symptoms. In both of these cases, tolerance and withdrawal would not reflect the same process.

Nicotine addiction is associated with the development of tolerance and the presence of withdrawal symptoms (Balfour, 1981; Gould and Leach, 2013; Jarvik and Henningfield, 1988; Paolini and De Biasi, 2011), and the effects of nicotine on cognitive processes are sensitive to both tolerance and withdrawal. In both humans and laboratory rodents, acute nicotine enhances learning and cognitive processes (Gould and Higgins, 2003; Gould and Wehner, 1999; Heishman et al., 2010; Kenney and Gould, 2008; Myers et al., 2008). With continued drug administration, tolerance for the cognitive enhancing effects of nicotine develops (Davis et al., 2005; Portugal et al., 2012a), and upon cessation of nicotine treatment, withdrawal-related deficits in learning and other cognitive measures emerge (Ashare et al., 2013; Davis et al., 2005; Heishman, 1999; Hendricks et al., 2006; Hughes et al., 1989; Jacobsen et al., 2005; Raybuck and Gould, 2009). This pattern of an initial enhancement, a decrease in enhancement with continued nicotine administration, and deficits in cognitive processes during abstinence appears to fit well within the opponent process theory of addiction but it remains to be determined if the neural substrates underlying tolerance to the pro-cognitive effects of nicotine are the same substrates underlying nicotine withdrawal disruption of cognition. Understanding the mechanisms that underlie tolerance and withdrawal-related changes in cognition is important because deficits in cognition during abstinence from tobacco are a major withdrawal symptom (Jacobsen et al., 2005; Kleinman et al., 1973; Mendrek et al., 2006; Snyder et al., 1989), and the severity of the cognitive deficits correlates with relapse rates (Patterson et al., 2010).

At the level of the receptor, chronic nicotine is associated with desensitization and upregulation (Hulihan-Giblin et al., 1990; Marks et al., 1983; Schwartz and Kellar, 1983; Sharp and Beyer, 1986). It has been proposed that the onset of desensitization is rapid whereas receptor upregulation occurs over a comparatively longer time period (Bullock et al., 1997; Collins et al., 1994; Ochoa et al., 1989). In addition, both desensitization and receptor upregulation have been proposed to contribute to tolerance for the somatic and locomotor effects of nicotine (Marks et al., 1983, 1985; Robinson et al., 2006, 2007); though other work suggested that there may be a dissociation of tolerance and receptor upregulation (Collins et al., 1990; McCallum et al., 2000).

In mice, nicotine acts directly in the dorsal hippocampus to enhance hippocampus-dependent learning and to produce withdrawal-related deficits in hippocampus-dependent learning after cessation of chronic administration (Davis and Gould, 2009; Kenney et al., 2012). The withdrawal-related deficits in hippocampus-dependent learning are associated with changes in dorsal hippocampal nicotinic acetylcholinergic receptor (nAChR) upregulation (Gould et al., 2012; Portugal et al., 2012b; Wilkinson et al., 2013) but it is unknown if nAChR upregulation underlies the observed tolerance to the cognitive enhancing effects of nicotine and whether tolerance and withdrawal-associated deficits in learning involve the same process.

The present study investigated whether behavioral tolerance to the effects of nicotine on hippocampus-dependent learning emerged at the same time point as withdrawal deficits in learning emerged and whether a significant change in nAChR upregulation was temporally associated with the development of tolerance and/or withdrawal. It was hypothesized that if tolerance and withdrawal-associated changes in hippocampus-dependent learning involve different neural mechanisms, then tolerance would emerge at a different time than withdrawal deficits and that nAChR upregulation would be temporally related to withdrawal but not tolerance. These experiments also investigated the influence of dose on the onset of tolerance to the cognitive enhancing effects of nicotine.

2. Results

2.1. *Tolerance*

To determine how dose affects onset of tolerance to the effects of nicotine on hippocampus-dependent learning, separate experiments were performed in which mice were implanted with osmotic minipumps that delivered chronic saline or nicotine (3, 6.3, 12, or 24 mg/kg/d) for up to 6 days depending on when tolerance emerged for each dose. There was a significant main effect day for 3 mg/kg/d nicotine ($n=9$–12 per group) on contextual freezing, $F(2, 61)=6.049$, $p<0.005$ (Fig. 1).