Neurobiology of Learning and Memory 128 (2016) 110–116

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

Rapid Communication

# Cognitive control deficits during mecamylamine-precipitated withdrawal in mice: Possible links to frontostriatal BDNF imbalance

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ARTICLE INFO

*Article history:*
Received 15 July 2015
Revised 2 December 2015
Accepted 10 January 2016
Available online 13 January 2016

*Keywords:*
Nicotine withdrawal
Addiction
Cognitive flexibility
BDNF
Mice

ABSTRACT

Nicotine is a major psychoactive and addictive component of tobacco. Although cessation of tobacco use produces various somatic and affective symptoms, withdrawal-related cognitive deficits are considered to be a critical symptom that predict relapse. Therefore, delineating the cognitive mechanisms of nicotine withdrawal may likely provide gainful insights into the neurobiology of nicotine addiction. The present study was designed to examine the effects of nicotine withdrawal induced by mecamylamine, a non-specific nicotinic receptor (nAChR) antagonist, on cognitive control processes in mice using an operant strategy switching task. Brain-derived neurotrophic factor (BDNF) modulates synaptic transmission in frontostriatal circuits, and these circuits are critical for executive functions. Thus, we examined the effects of mecamylamine-precipitated nicotine withdrawal on prefrontal and striatal BDNF protein expression. Mice undergoing precipitated nicotine withdrawal required more trials to attain strategy switching criterion as compared to the controls. Error analysis indicated that impaired performance in these animals was mostly related to their inability to execute the new strategy. The striatal/prefrontal BDNF ratios robustly increased following precipitated nicotine withdrawal. Moreover, higher BDNF ratios were associated with longer task acquisition. Collectively, our findings illustrate that mecamylamine-induced nicotine withdrawal disrupts cognitive control processes and that these changes are possibly linked to perturbations in frontostriatal BDNF signaling.



Nicotine addiction is a global health problem and smoking-related illness reigns atop the causes of preventable death worldwide. Even though chronic nicotine exerts very little positive effects on mood and motor/cognitive performance as opposed to other drugs of abuse (Epping-Jordan, Watkins, Koob, & Markou, 1998; Risner & Goldberg, 1983), smokers continue to consume cigarettes presumably to alleviate unpleasant withdrawal-related physiological/affective symptoms and to restore activity in brain reward pathways (Johnson, Hollander, & Kenny, 2008; Miyata & Yanagita, 2001). Despite the availability of treatments for smoking cessation that mostly focus on normalizing the reward function and motivational/affective components of nicotine addiction, relapse to smoking after quit attempts still remains very high (Gonzales et al., 2006; Hughes, Peters, & Naud, 2008). Because of the extensive overlap between cognitive and reward-/motivation-related brain processes, chronic drug-induced neuroadaptive changes and possible interactions between these processes are proposed to underlie compulsive drug use (Everitt et al., 2008; Gould, 2010). Moreover, nicotine withdrawal-related cognitive deficits are hypothesized to predict relapse (Ashare, Falcone, & Lerman, 2014). Therefore, delineation of cognitive mechanisms that determine higher rates of relapse during nicotine withdrawal is likely to provide gainful insights into the neurobiology of nicotine addiction.

Loss of cognitive control in drug addicts is primarily manifested as the inability to change responding to stimuli previously associated with drug stimulus or reward, and deficits in cognitive flexibility are critical in triggering drug craving and relapse (Stalnaker, Takahashi, Roesch, & Schoenbaum, 2009; Volkow et al., 2010). Although the effects of nicotine withdrawal on reward/motivation and contextual learning are well studied, how it affects cognitive flexibility and what cellular mechanisms are responsible for the effects are not known. Brain-derived neurotrophic factor (BDNF) plays an important role in activity-dependent regulation of synaptic function, cognition, affect and conditioned reward (Chao, 2003; Lu, Christian, & Lu, 2008; Nestler & Carlezon, 2006). BDNF gene polymorphism has been linked to nicotine dependence (Lee, Anastasia, Hempstead, Lee, & Blendy, 2015). Moreover, frontostriatal circuits involving discrete regions of the prefrontal cortex (PFC) and dorsal striatum are implicated in decision-making (Balleine, Delgado, & Hikosaka,

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http://dx.doi.org/10.1016/j.nlm.2016.01.003
1074-7427/

2007; Ragozzino, 2007) and BDNF regulates corticostriatal synaptic plasticity and cognitive flexibility by activating its cognate receptor, tyrosine kinase B (trkB) (D'Amore, Tracy, & Parikh, 2013; Jia, Gall, & Lynch, 2010). The present study was designed to assess the effects of nicotine withdrawal on cognitive control processes using an operant strategy switching paradigm in mice. Moreover, we also determined whether alterations in strategy-based decision processes during nicotine withdrawal are tied to changes in prefrontal and striatal BDNF protein levels.

*Subjects*. Male C57BL/6J mice (8–10 weeks; 20–25 g) were purchased from Jackson Laboratories (Bar Harbor, ME). Animals were individually housed in a temperature/humidity-controlled environment with a 12-h light/dark cycle (07:00 lights on). Mice were progressively water-restricted to 5 min of water/day. Operant training was conducted 7 days/week between 9:00 and 16:00 h. Food pellets (PMI LabDiet) were available *ad libitum* during the experiment. All experimental procedures were authorized by the Institutional Care and Use Committee (IACUC) of Temple University and complied with regulations from the National Institute of Health.

*Operant training procedure*. Mice were trained on an operant cognitive flexibility task using standard mouse chambers (Med Associates) as described previously in our studies (Cole, Poole, Guzman, Gould, & Parikh, 2015; Ortega, Tracy, Gould, & Parikh, 2013; D'Amore et al., 2013). Briefly, animals were autoshaped on a FR-1 schedule of reinforcement to acquire lever press responses and subsequent reinforcement of reward (10 μl of 0.066% saccharin solution). The animals were then advanced to the pretraining phase. A session began with the illumination of houselight. After an inter-trial interval (ITI) of 9 ± 3 s, a lever (either left or right) was presented and remained active for 10 s or until a lever press response occurred. Lever presentations were completely randomized with no more than 5 activations from the same side. To control for any novelty effects associated with the visual stimulus during later phases of training, trials were randomly associated with unpredictably occurring visual cues (presented only in 50% of trials) that involved illumination of the panel light above the lever. A lever press on the cued trials co-terminated both the visual cue and the lever. Animals that reached pretraining criterion (30 rewards and <20% omissions) were then implanted with mini-osmotic pumps for chronic drug administration (see "Chronic nicotine administration, induction of withdrawal and experimental design").

After recovery and following retention of performance, the animals were held on the pretraining phase for two weeks. Mice then progressed to the visual discrimination phase which required making a correct choice by responding to the lever paired with the visual cue light. A trial started with the illumination of a 7 s visual cue either from the left or right panel (pseudorandomized sequence across trials), followed by the presentation of both levers 2 s later. Both the stimulus light and levers co-terminated together. A lever press response on the cued lever was scored as a "correct response" and was followed by reward (sweetened water) delivery. Responses on the incorrect lever (errors) were not rewarded and resulted in a "time out" (punishment) period characterized by a 10 s extinguishing of the house light. Punishment on incorrect responses was introduced to discourage indiscriminate responding to levers. Following the completion of the punishment phase, the house light was turned "on" and the ITI (9 ± 3 s) was reinstated. Failure to respond to any of the levers resulted in omissions. Animals were required to exhibit ⩾80% correct responses and <20% omissions for 3 consecutive days to attain criterion following which they were advanced to testing on strategy switching phase. The experimental parameters for strategy-shifting phase were identical to the previous stage except that the contingencies were altered in such a way that the animals were required to eliminate a visual cue-based strategy and adopt a new spatial response strategy to achieve rewards. Mice were required to press the correct lever (either left or right) to earn a reward irrespective of visual cue presentation, which remained random. Responding on an incorrect lever resulted in an incorrect response (set-shift error) and led to the initiation of the time out phase. Half of the animals were trained with the reverse set of rules. Performance criterion was defined as ⩾80% correct responses and <20% omissions for three consecutive sessions. Each behavioral session for both the visual discrimination phase and the strategy switching phase consisted of 30 trials/day.

The number of correct responses, errors, omissions, response latencies and reward retrieval latencies was obtained for each behavioral session. The total number of performed trials to criterion, errors to criterion and omissions were obtained for each training phase using the above described criteria. Response accuracies were calculated for each session using the formula: correct responses/(correct + incorrect responses) ∗ 100. Strategy shifting performance was characterized by distinguishing whether an incorrect response occurred due to the perseverance of a previously learned strategy or failure to acquire/maintain a new strategy. For strategy switching performance, errors were classified as perseverative, regressive and never-reinforced based on criterion reported in previous studies (Cole et al., 2015; D'Amore et al., 2013; Haluk & Floresco, 2009). A perseverative error occurred if the animal responded to the incorrect lever when the visual cue was illuminated above it on ⩾60% of trials within a session. This is indicative of perseverance to the previously learned strategy. Depending on the training performance in the preceding session, an error was scored as a regressive error if the animal made <60% incorrect responses on the cue-associated lever in subsequent sessions. At this point, the animals were making fewer errors and are considered to be inhibiting the previously learned strategy and executing the new strategy. Never-reinforced errors occur if an animal responded on the incorrect lever while the visual cue was presented from the opposite side. Both regressive and never-reinforced errors were categorized as "learning errors" as they reflected an index of the acquisition/execution of a new strategy.

*Chronic nicotine administration, induction of withdrawal and experimental design*. Mini-osmotic pumps (model 1004; DURECT Corporation, Cupertino, CA) were implanted subcutaneously under isoflurane anesthesia in mice to deliver either saline (control) or nicotine (18 mg/kg/day; free base) for 4 weeks (refer to Supplementary Materials for surgery details). After recovery and following retention of presurgery performance, the animals were held on the pretraining phase until 2 weeks and then progressed to the visual discrimination phase. As the acquisition of visual discrimination typically requires 4–7 training sessions, the animals were kept on this phase of the task for a maximum period of 1 week and then split into 4 treatment groups before the assessment of strategy switching performance (see Fig. 1 for Experimental Design).

Systemic administration of mecamylamine, a non-specific nAChR antagonist, in nicotine-treated rodents is a well-established model to produce somatic signs of nicotine withdrawal (Damaj, Kao, & Martin, 2003; Salas, Pieri, & De Biasi, 2004) as well as withdrawal-related decreases in brain reward function typically observed in abstinent smokers (Watkins, Stinus, Koob, & Markou, 2000; Miyata & Yanagita, 2001; Hilario, Turner, & Blendy, 2012). Another advantage of using this model is that induction of withdrawal symptoms could be specifically timed prior to the onset of behavioral sessions. Moreover, withdrawal symptoms could be induced over multiple behavioral sessions during the acquisition of strategy switching. Therefore, the mecamylamine-precipitated nicotine withdrawal model was used in our study. Starting on day 22, the nicotine withdrawal group received a daily subcuta-