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# Acute nicotine disrupts consolidation of contextual fear extinction and alters long-term memory-associated hippocampal kinase activity

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ABSTRACT

Previous research has shown that acute nicotine, an agonist of nAChRs, impaired fear extinction. However, the effects of acute nicotine on consolidation of contextual fear extinction memories and associated cell signaling cascades are unknown. Therefore, we examined the effects of acute nicotine injections before (pre-extinction) and after (post-extinction) contextual fear extinction on behavior and the phosphorylation of dorsal and ventral hippocampal ERK1/2 and JNK1 and protein levels on the 1st and 3rd day of extinction. Our results showed that acute nicotine administered prior to extinction sessions downregulated the phosphorylated forms of ERK1/2 in the ventral hippocampus, but not dorsal hippocampus, and JNK1 in both dorsal and ventral hippocampus on the 3rd extinction day. These effects were absent on the 1st day of extinction. We also showed that acute nicotine administered immediately and 30 min, but not 6 h, following extinction impaired contextual fear extinction suggesting that acute nicotine disrupts consolidation of contextual fear extinction memories. Finally, acute nicotine injections immediately after extinction sessions upregulated the phosphorylated forms of ERK1/2 in the ventral hippocampus, but did not affect JNK1. These results show that acute nicotine impairs contextual fear extinction potentially by altering molecular processes responsible for the consolidation of extinction memories.

## 1. Introduction

Nicotinic acetylcholine receptors (nAChRs) regulate a variety of cell signaling cascades that are important for various behavioral processes such as long-term memory formation (see Kutlu & Gould, 2016 for a review). In the hippocampus, nAChRs gate calcium ($Ca^{2+}$) and sodium into the cell (Wonnacott, 1997). By modulating $Ca^{2+}$ influx, activation of nAChRs leads to activation of hippocampal cell signaling cascades through the increase in cyclic adenosine monophosphate (cAMP) and phosphorylation of various kinases, including kinases within the family of mitogen-activated protein kinases (MAPKs; Dajas-Bailador, Soliakov, & Wonnacott, 2002; Nuutinen, Barik, Jones, & Wonnacott, 2007). For example, nicotine, an agonist of nAChRs, has been shown to alter the phosphorylation state of extracellular signal-regulated kinases 1 and 2 (ERK1/2; Gould et al., 2014; Neugebauer, Henehan, Hales, & Picciotto, 2011; Valjent, Hervé, Girault, & Caboche, 2004). Phosphorylation of ERK1/2 in the hippocampus is required for long-term potentiation (LTP; Coogan, O'Leary, & O'Connor, 1999; Winder et al., 1999), a process that may underlie long-term memory formation (Bliss & Collingridge, 1993), and consolidation of fear memories (Trifilieff et al., 2006). Importantly, Gould et al. (2014) showed that acute nicotine administration prior to contextual fear conditioning shifted the learning-associated ERK1/2 activity in the hippocampus and enhanced hippocampus-dependent fear learning. This suggests that nicotine may enhance consolidation of fear memories by altering phosphorylation patterns of hippocampal ERK1/2.

Another MAPK family kinase that plays an important role in long-term memory formation is c-jun N-terminal kinase 1 (JNK1). Although JNK1 seems to have a minimal role in baseline LTP (Li et al., 2007), there is evidence showing that JNK1 is required for hippocampal long-term depression (LTD; Curran, Murray, & O'Connor, 2003; Li et al., 2007), a process responsible for the reversal of LTP. In addition, Leach, Kenney, and Gould (2015) showed that JNK1 knockout (KO) mice, under baseline conditions, expressed similar levels of contextual fear conditioning as wild-type (WT) littermates, but unlike WT mice, KO mice did not show stronger learning with an increased number of training trials. This suggests JNK1 may modulate the strength of memories. There is also evidence from our lab showing that hippocampal JNK1 phosphorylation is required for the acute nicotine-induced enhancement of contextual fear conditioning (Kenney, Florian, Portugal, Abel, & Gould, 2010; Leach, Kenney, & Gould, 2016). For example, acute nicotine upregulated the JNK1 mRNA in the hippocampus

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during the nicotine enhancement of contextual fear conditioning (Kenney et al., 2010). Moreover, the acute nicotine-induced enhancement of contextual fear conditioning was absent in JNK1 KO mice (Leach et al., 2016). These results show that JNK1 may be critical for the nicotinic modulation of long-term memory formation.

In contrast to the enhancing effects of acute nicotine on contextual fear conditioning, we recently showed that acute nicotine administered prior to extinction impaired contextual fear extinction (Kutlu & Gould, 2014) and this effect was dependent on high-affinity α4β2 nAChRs (Kutlu, Holliday, & Gould, 2016). Fear extinction is a process whereby new inhibitory memories are created, which results in a reduction of learned responses (Myers & Davis, 2007). Both hippocampal LTP (de Carvalho Myskiw, Benetti, & Izquierdo, 2013) and LTD (Dalton, Wang, Floresco, & Phillips, 2008; Dalton, Wu, Wang, Floresco, & Phillips, 2012; Kim et al., 2007; Lin, Lee, & Gean, 2003) have been implicated in successful extinction learning. In addition, ERK1/2 and JNK1 have been shown to play important roles in LTP and LTD, respectively. Accordingly, there is evidence showing that both ERK1/2 and JNK1 are also necessary for extinction learning (Bevilaqua et al., 2007; Fischer et al., 2007; Matsuda et al., 2010). Therefore, one possibility is that ERK1/2 and JNK1 modulate consolidation of extinction memories by mediating plasticity similar to that measured with LTP and LTD.

Our results showing that nicotine administered prior to each extinction session impaired contextual fear extinction suggest that nicotine may alter encoding of extinction memories. However, processes triggered by nicotine may still be active when encoding of extinction memories transitions to consolidation after the extinction session ends. Therefore, it is not clear if the impairment of contextual extinction is a result of disrupted encoding or consolidation of long-term extinction memories. Moreover, the effects of acute nicotine on hippocampal kinases-associated memory consolidation processes during contextual fear extinction are unknown. In the present study, we investigated the effects of acute nicotine administration prior and immediately following each extinction session on hippocampal levels of phosphorylated ERK1/2 and JNK1. Second, in order to test our hypothesis that acute nicotine alters memory consolidation processes, we examined the effects of acute nicotine on consolidation of extinction memories by administering nicotine within and outside the memory consolidation window, a 6 h temporal window where newly acquired memories are stabilized into long-term memory storage (Dudai, 2004; Katche, Cammarota, & Medina, 2013). Given that our previous studies showed that the dorsal hippocampus (dHPC) mainly controlled the effects of nicotine on fear acquisition (Gould et al., 2014; Kenney et al., 2010) whereas the ventral hippocampus (vHPC) mainly modulated the effects of nicotine on contextual fear extinction (Kutlu, Tumolo, Holliday, Garret, & Gould, 2016), we investigated these two sub-regions separately.

Therefore, we examined the effects of acute nicotine injections before (pre-extinction) and after (post-extinction) contextual fear extinction on behavior and the phosphorylation of dorsal and ventral hippocampal ERK1/2 and JNK1 and protein levels on the 1st and 3rd day of extinction. Our results showed that acute nicotine administered prior to extinction sessions downregulated the phosphorylated forms of ERK1/2 in the ventral hippocampus, but not dorsal hippocampus, and JNK1 in both dorsal and ventral hippocampus on the 3rd extinction day. These effects were absent on the 1st day of extinction. We also showed that acute nicotine administered immediately and 30 min, but not 6 h, following extinction impaired contextual fear extinction suggesting that acute nicotine disrupts consolidation of contextual fear extinction memories. Finally, acute nicotine injections immediately after extinction sessions upregulated the phosphorylated forms of ERK1/2 in the ventral hippocampus, but did not affect JNK1.

## 2. Method

### 2.1. Subjects

Subjects were adult male C57BL/6J mice (8–10 weeks old, Jackson Laboratory, Bar Harbor, ME) that were group-housed and maintained in a 12 h light/dark cycle. All mice had access to food and water *ad libitum*. Mice were given a week of acclimation when they arrived at our animal colony. In addition, mice were tagged with a marking solution 24 h prior to training. Training and retention test occurred between 9:00 am and 7:00 pm. Behavioral procedures used in this study were approved by the Temple University Institutional Animal Care and Use Committee.

### 2.2. Apparatus

All behavioral experiments took place in four identical chambers (18.8 × 20 × 18.3 cm), which were composed of Plexiglas and placed in sound attenuating boxes (MED Associates). Ventilation fans that produced a background noise (65 dB) were mounted in the back of each behavioral chamber. The chamber floors were metal grids (0.20 cm in diameter and 1.0 cm apart) connected to a shock generator. The stimuli were controlled by an IBM-PC compatible computer running MED-PC software.

### 2.3. Drug

For the initial behavioral experiments, nicotine hydrogen tartrate salt (0.18 mg/kg freebase, Sigma) dissolved in 0.9% physiological saline (saline) or saline alone was injected intraperitoneally (i.p.) immediately, 30 min, or 6 h following each extinction session. Injection volumes were 10 mL/kg as in previous studies (e.g., Kutlu & Gould, 2014). The 0.18 mg/kg dose of acute nicotine was chosen based on our previous reports showing impaired contextual fear extinction at this dose (Kutlu & Gould, 2014; Kutlu, Holliday, et al., 2016). For the subsequent western blotting experiments, the same dose of nicotine or saline was administered i.p. 2–4 min prior to each extinction session for "pre-extinction" experiments; whereas nicotine was administered immediately after each extinction session for the "post-extinction" experiments.

### 2.4. Behavioral procedures

Freezing behavior, which was defined as the absence of voluntary movement except respiration (Davis, James, Siegel, & Gould, 2005), was employed as the dependent variable. A time sampling method was used in which subjects were observed every 10 s for 1 s and scored as active or freezing. Subjects were trained in contextual fear conditioning. Specifically, mice were placed in the fear conditioning chambers and following a 120 s baseline period, they received 2 conditioned stimulus (CS, 30-s white noise, 85 dB)-unconditioned stimulus (US; a 2-s, 0.57-mA foot-shock) pairings wherein the CS and the US coterminated. Twenty-four hours later mice were re-introduced to the fear conditioning chambers to assess for their freezing responses to the context (Retention Test). The duration for both fear conditioning and the retention test was 5 min and 30 s. For the next 5 days, mice were given contextual fear extinction where they were placed in the chambers for 5 min and their freezing behavior was scored. For the behavioral experiments examining the effects of acute nicotine on consolidation of contextual fear extinction, injections were administered immediately, 30 min, or 6 h after each extinction session starting from the retention test. These time points were chosen as immediate injections and injections at 30 min time-point would lay within the memory consolidation window whereas injections at 6 h time-point would lay outside the consolidation window and serve as a control (Dudai, 2004; Katche et al., 2013).