Published in final edited form as:

Behav Neurosci. 2007 October ; 121(5): 1119–1124. doi:10.1037/0735-7044.121.5.1119.

Extracellular Signal-Regulated Kinase 1/2 Involvement in the Enhancement of Contextual Fear Conditioning by Nicotine

Jonathan D. Raybuck and Thomas J. Gould

Department of Psychology, Temple University.

Abstract

Contextual fear conditioning is enhanced by nicotine, but the cellular mechanisms underlying this effect are unknown. Extracellular signal regulated kinase 1/2 (ERK 1/2) has been shown to play an integral role in the formation of contextual fear memories. As such, it is possible that ERK 1/2 is involved in the enhancement of contextual fear conditioning by nicotine. To determine whether ERK 1/2 plays a role in this enhancement, a dose of SL327 (a selective, systemic ERK 1/2 inhibitor) that is subthreshold for inhibiting contextual fear conditioning was coadministered with nicotine prior to training, testing, or both training and testing of contextual fear conditioning in C57BL/6 mice. When administered prior to training, this subthreshold dose of SL327 attenuated the enhancement of contextual fear conditioning by nicotine to levels similar to those of vehicle-treated animals. When administered prior to testing, the subthreshold dose of SL327 did not significantly alter conditioning. These results suggest that activation of ERK 1/2 by nicotine during acquisition leads to an enhancement of contextual fear conditioning.

Keywords

nicotine; contextual fear conditioning; ERK 1/2; learning; acetylcholine

Nicotine enhances contextual fear conditioning (Davis & Gould, 2006; Davis, Porter, & Gould, 2005; Feiro & Gould, 2005; Gould & Higgins, 2003; Gould & Wehner, 1999) but not cued fear conditioning (Gould, Feiro, & Moore, 2004). Recent studies suggest that this effect is mediated by $\alpha 4\beta 2$ nicotinic acetylcholine receptors (nAChRs; Davis & Gould, 2006; Wehner et al, 2004). Little is known of the cellular mechanisms that underlie the effect of nicotine on contextual fear conditioning. In contrast, the cellular mechanisms that underlie contextual fear conditioning are well characterized. For example, extracellular signal-regulated kinase 1/2 (ERK 1/2) is among several intracellular signaling proteins that are implicated in contextual fear conditioning (Atkins, Selcher, Petraitis, Traskos, & Sweatt, 1998). Research indicating that nicotine administration, like conditioning, increases ERK 1/2 activity (Valjent, Pages, Herve, Girault, & Caboche, 2004) suggests that this second messenger may be involved in the enhancement of contextual fear conditioning by nicotine.

Numerous studies have shown that ERK 1/2 activation is necessary for the formation of long-term memories of contextual fear conditioning (for review see Sweatt, 2001). ERK 1/2 is activated after training (Ahi, Radulovic, & Spiess, 2004; Atkins et al., 1998; Sananbenesi, Fischer, Schrick, Spiess, & Radulovic, 2002, 2003; Selcher, Atkins, Traskos, Paylor, & Sweatt, 1999), and inhibition of mitogen-activated protein kinase kinase (MEK), the immediate

upstream activator of ERK 1/2, produces deficits in contextual fear conditioning (Ahi et al., 2004; Atkins et al., 1998; Sananbenesi et al., 2002; Selcher et al., 1999). Genetic knockout studies have also implicated ERK 1/2 in fear conditioning; MEK dominant negative mice, which have decreased ERK 1/2 activity, show deficits in contextual fear conditioning (Shalin, Zirrgiebel, Honsa, Julien, Miller, Kaplan, & Sweatt, 2004).

Although the involvement of ERK 1/2 in the effects of nicotine on contextual fear conditioning has not been investigated until now, nicotine has been shown to activate ERK 1/2 in multiple brain areas (Brunzell, Russell, & Picciotto, 2003), including the hippocampus (Valjent et al., 2004), an area shown to be integral to contextual fear conditioning (Logue, Paylor, & Wehner, 1997; Philips & Ledoux, 1992). Studies of synaptic plasticity further suggest that ERK 1/2 may be involved in the effects of nicotine. Hippocampal long-term potentiation (LTP), which has long been regarded as a representative model of the cellular and molecular processes underlying learning (Bliss & Collingridge, 1993; Izquierdo & McGaugh, 2000), activates ERK 1/2 (English & Sweatt, 1996), and inhibition of ERK 1/2 activation impairs LTP induction (Atkins et al., 1998; Coogan, O'Leary, & O'Connor, 1999; English & Sweatt, 1997; Impey, Obrietan, et al., 1998; Winder et al., 1999). Just as nicotine enhances contextual fear conditioning (Gould & Wehner, 1999), nicotine facilitates the induction of LTP (Fujii, Ji, Morita, & Sumikawa, 1999) and can even induce hippocampal LTP (Matsuyama, Matsumoto, Enomoto, & Nishizaki, 2000). Additionally, inhibition of ERK 1/2 disrupts nicotine-facilitated LTP (Wang, Chen, Zhu, & Chen, 2001). These results suggest that nicotine-enhanced activation of ERK 1/2 may facilitate synaptic plasticity and could mediate the effects of nicotine on contextual fear conditioning.

The present experiments investigate the role of ERK 1/2 in nicotine enhancement of contextual fear conditioning. If nicotine enhances contextual fear conditioning through increases in ERK 1/2 activation, then this enhancement should be susceptible to interference by a dose of an ERK 1/2 inhibitor that is subthreshold for inhibiting contextual fear conditioning. To test this, SL327, a selective, systemic inhibitor of ERK 1/2 activation, was coadministered with nicotine at training, testing, or both training and testing.

Eight- to 12-week-old male C57BL/6J mice, obtained from the Jackson Laboratory (Bar Harbor, ME), were maintained on a 12-hr light–dark cycle with lights on at 7:00 a.m. Mice were housed in groups of 4 with ad libitum access to food and water. All procedures were approved by the Temple University Institutional Animal Care and Use Committee.

Mice were trained and tested in four identical Med Associates (St. Albans, VT) conditioning chambers housed in sound-attenuating cubicles. Ventilation fans provided a 69-dB background noise. The conditioning chambers consisted of stainless steel sides; Plexiglas front, back, and ceiling panels; and a grid floor composed of 18 stainless steel bars connected to a wiring harness that delivered a 0.57-mA unconditioned stimulus (US) foot shock. The conditioned stimulus (CS) was an 85-dB white noise delivered by speakers mounted on the left side of each chamber. The conditioning program was run with Med Associates software.

SL327—a selective systemic MEK 1 inhibitor (Atkins et al., 1998) structurally analogous to U0126 (Favata et al., 1998) that does not have significant effects on PKA, PKC, or CaMKII (Selcher et al., 1999)—and (-)-nicotine hydrogen tartrate salt were purchased from Sigma-Aldrich (St. Louis, MO). Injections were counterbalanced across training and testing such that each mouse received two injections prior to training and two injections prior to testing: SL327 or vehicle, and nicotine or saline. SL327 at 5 and 10 mg/kg (10 ml/kg injection volume), dissolved in a 10% Cremaphor (Sigma-Aldrich) and 90% saline solution, was administered by intraperitoneal injection 20 min prior to training and testing in order to establish a subthreshold dose for pairing with nicotine. Prior research demonstrated that 10 mg/kg of SL327

significantly inhibits hippocampal ERK activity (Selcher et al., 1999). The timing of SL327 administration was based on peak ERK 1/2 inhibition time (Selcher et al., 1999), ERK 1/2 activation times subsequent to contextual fear conditioning (Sananbenesi et al., 2002), and preliminary behavioral pharmacology experiments in our lab. Nicotine tartrate (0.25 mg/kg equivalent to 0.09 mg/kg freebase) was dissolved in saline and administered by intraperitoneal injection (10 ml/kg injection volume) 5 min prior to training and testing. The dose of nicotine, administration route, and injection time were based on prior studies (Gould & Higgins, 2003; Petersen, Norris, & Thompson, 1984).

All procedures were conducted during the mice's light cycle. Only contextual fear was tested because nicotine enhances contextual but not cued fear conditioning (Gould et al., 2004; Gould & Higgins, 2003). In addition, contextual fear conditioning shows greater disruption by ERK 1/2 inhibition than cued fear conditioning (Selcher et al., 1999). For conditioning, baseline freezing was assessed during the first 120 s, and then mice received two paired presentations, separated by 120 s of a CS (30-s, 85-dB white noise) paired with a coterminating US (2-s, 0.57-mA footshock). The training session was terminated 30 s following the second CS-US presentation. Testing occurred 24 hr later, during which mice were placed in the conditioning chambers and contextual fear conditioning was assessed over 5 min. For a detailed description of the training and testing procedures, see Gould & Lommock (2003).

Fear was quantified as percent freezing, calculated by dividing observed freezing by the total observations during that period (Gould & Wehner, 1999). Percent freezing data were analyzed with SPSS version 13. Data sets were tested for significance with analyses of variance (ANOVAs). Homogeneity of variance was tested with Levene's statistic (Cohen, 2001). Tukey's post hoc tests of statistical significance ($p < .05$; Cohen, 2001) were conducted when data sets had equal variance. Data sets with unequal variance were tested with Games-Howell post hoc tests (Maxwell & Delaney, 2003). In addition to contextual freezing, baseline freezing was analyzed to assess general drug effects.

In Experiment 1, a dose of SL327 subthreshold for disrupting contextual fear conditioning was determined. Mice received SL327 (0 [vehicle], 5 or 10 mg/kg) 20 min prior to contextual fear conditioning, and saline 5 min prior to conditioning to match later experiments. An ANOVA indicated a significant difference among groups for contextual freezing, $F(2, 35) = 5.72$, $p < .05$ (see Figure 1A), and no difference for baseline freezing, $F(2, 35) = 0.00$, $p > .05$. Games-Howell post hoc tests revealed that mice that received 10 mg/kg of SL327 show significantly less contextual fear conditioning than mice in the vehicle group. Mice receiving 5 mg/kg SL327 did not significantly differ from vehicle. Thus, the 10-mg/kg dose of SL327 disrupted contextual fear conditioning, whereas the 5-mg/kg dose was subthreshold for disrupting contextual fear conditioning.

In Experiment 2, the role of ERK 1/2 in the enhancement of contextual fear conditioning by nicotine was investigated. The subthreshold dose of SL327 (5 mg/kg) established in Experiment 1 was administered 20 min prior to training; vehicle was administered 20 min prior to testing. Nicotine (0.09 mg/kg) or saline was administered 5 min prior to both training and testing. Thus, mice received vehicle/saline, vehicle/nicotine, SL327/nicotine, or SL327/saline at training and vehicle/saline or vehicle/nicotine at testing. An ANOVA showed a significant effect of drug treatment on contextual fear conditioning, $F(3, 60) = 6.71$, $p < .05$ (see Figure 1B), but not baseline freezing, $F(3, 60) = 1.00$, $p > .05$. Tukey's post hoc comparisons revealed that the vehicle/nicotine group demonstrated significantly higher levels of contextual fear conditioning than all other drug treatments. Notably, the SL327/nicotine group demonstrated significantly lower levels of contextual fear conditioning than the vehicle/nicotine group but levels of contextual fear conditioning that were not different from the vehicle/saline group. These results demonstrate that a subthreshold dose of SL327 administered prior to training of

contextual fear conditioning attenuates the enhancement of contextual fear conditioning by nicotine.

The effects of SL327 both at training and testing and at testing alone on nicotinic enhancement of contextual fear conditioning were also examined. Coadministration of SL327 and nicotine at training and testing yielded results identical to coadministration prior to training alone; ANOVAs indicated a significant effect of drug condition on contextual fear conditioning, $F(3, 76) = 4.05, p < .05$ (see Figure 1C), but not baseline freezing, $F(3, 76) = 1.45, p > .05$. Tukey's post hoc comparisons indicated that the vehicle/nicotine group demonstrated greater levels of contextual fear conditioning than all other groups. In addition the SL327/nicotine group displayed no enhancement compared with the vehicle/saline group. These results indicate that attenuation of nicotine-enhanced contextual fear conditioning by SL327 at training only was not due to a state-dependent effect, because state-dependent learning theory would predict that learning occurring in one state (SL327 and nicotine) would not be retrieved in another state (vehicle and nicotine), but learning and retrieval occurring during the same state (SL327 and nicotine) would not be disrupted. Because the effects of SL327 on enhancement of contextual fear conditioning by nicotine are consistent regardless of SL327 treatment at testing, it is unlikely that the effects of SL327 on nicotine-enhanced memories are due to state-dependent effects.

In contrast to the effects of coadministration of SL327 and nicotine at training only, coadministration of SL327 with nicotine at testing did not significantly alter nicotine enhancement of contextual fear conditioning. ANOVAs showed a significant effect of drug condition on contextual fear, $F(3, 75) = 7.16, p < .05$ (see Figure 1D). Tukey's post hoc comparisons revealed that the vehicle/nicotine and SL327/nicotine groups demonstrated higher levels of contextual fear than did the vehicle/saline group. Additionally, there was no significant effect of SL327 on retrieval at testing, because the SL327/saline group was not statistically different from the vehicle/saline group. There was no significant effect of drug condition on baseline freezing, $F(3, 75) = 0.32, p > .05$. These results indicate that there is no significant effect of a subthreshold dose of SL327 on retrieval of nicotine-enhanced contextual fear conditioning.

The present experiment extends previous reports (Atkins et al., 1998; Selcher et al., 1999) indicating that ERK 1/2 is involved in contextual fear conditioning by demonstrating that the enhancement of contextual fear conditioning by nicotine is blocked by a dose of the ERK 1/2 inhibitor SL327 that is subthreshold for disrupting contextual fear conditioning. This effect was seen when SL327 was administered at training but not testing. Thus, these results suggest that the enhancement of contextual fear conditioning by nicotine is mediated by ERK 1/2 activation at training. However, the mechanisms through which nicotine may increase ERK 1/2 activation during learning are still unknown.

Nicotine has been shown to activate ERK 1/2 in cultures of PC12h cells (Nakayama, Numakawa, Ikeuchi, & Hatanaka, 2001), SH-SY5Y cells (Dajas-Bailador, Soliakov, & Wonnacott, 2002), cultured hippocampal neurons (Dajas-Bailador et al., 2002), and multiple brain regions (Brunzell et al., 2003; Valjent et al., 2004). Furthermore, Hu, Liu, Chang, and Berg (2002) demonstrated that nicotine induces activation of CREB and upregulation of cFOS in hippocampal neurons, and that activation of CREB by nicotine was dependent upon ERK 1/2 phosphorylation. Recent evidence indicates that hippocampal CREB activity is essential for the formation of long-term memory (Florian, Mons, & Roullet, 2006; Graves, Dalvi, Lucki, Blendy, & Abel, 2002; Impey, Smith, et al., 1998; Wood, Kaplan, Park, Blanchard, Oliveira, Lombardi, & Abel, 2005). Thus, nicotine can increase the activity of signaling cascades underlying learning-related plasticity, but how nicotine is coupled to these cascades is unclear.

There are at least three routes by which nicotine could act to enhance learning-related ERK 1/2 activity: (a) direct activation of the ERK 1/2 signaling cascade, (b) facilitation of ERK activity mediated by *N*-methyl-D-aspartate receptors (NMDARs), and (c) stimulation of the release of neurotransmitters that can activate ERK 1/2. Nicotinic receptors can gate Ca⁺⁺ influx (Barrantes, Westwick, & Wonnacott, 1994; Seguela, Wadiche, Dineley-Miller, Dani, & Patrick, 1993), an event that can activate intracellular signaling molecules such as ERK 1/2 (Rosen, Ginty, Weber, & Greenberg, 1994). Thus, during contextual fear conditioning, nicotine-mediated calcium influx through nAChRs could boost ERK 1/2 activation and enhance learning.

Nicotine-mediated effects at nAChRs may also interact with NMDAR-mediated effects to enhance ERK 1/2 signaling and contextual fear conditioning. Multiple lines of evidence support this possibility. First, nAChRs and NMDARs co-localize on the same neurons, suggesting that processes mediated by each receptor could interact during learning (Risso et al., 2004). Second, nAChRs and NMDARs have been shown to critically interact during acquisition of contextual fear conditioning (Gould & Lewis, 2005); similar results have been found for other types of learning (Ciamei, Averson, Cestari, & Castellano, 2002; Levin, Bettegowda, Weaver, & Christopher, 1998). Finally, nAChRs and NMDARs have been shown to interact during synaptic plasticity. Specifically, nicotine enhances NMDAR-dependent LTP (Sawada, Ohno-Shosaku, & Yamamoto, 1994; Yamazaki, Jia, Niu, & Sumikawa, 2006). The mechanisms through which nicotine could interact with NMDARs are unknown, but at least three possibilities exist: nAChR activation could contribute to NMDAR activation, nAChR activation could boost NMDAR signaling, and nicotine could stimulate glutamate release.

NMDARs are critically involved in contextual fear conditioning (Bardgett et al., 2003; Csernansky et al., 2005; Gould & Lewis, 2005; Gould, McCarthy, & Keith, 2002); activation of NMDARs requires conjunctive release of glutamate with postsynaptic depolarization (Nowak, Bregestovski, Ascher, Herbert, & Prochiantz, 1984). Nicotine-mediated nAChR gating of cations could contribute to the depolarization necessary for NMDAR activation. In addition, NMDARs, like nAChRs, gate calcium (Riedel, Platt, & Micheau, 2003); thus, as discussed earlier, nicotine-induced activation of nAChRs could contribute to activation of cell-signaling cascades involved in contextual fear conditioning that are activated by NMDARs. Finally, nicotine can stimulate the release of glutamate (Fedele, Varnier, Ansaldo, & Raiteri, 1998; McGehee, Heath, Gelber, Devay, & Role, 1995; Radcliffe, Fisher, Gray, & Dani, 1999). Increased levels of glutamate could facilitate NMDAR activation, which could lead to ERK 1/2 activation during contextual fear conditioning.

Additionally, nicotine has been shown to modulate the release of other neurotransmitters. Acetylcholine, norepinephrine, dopamine, and 5-hydroxytryptamine are all released in response to nicotine administration (Grady et al., 2002; Grady, Marks & Collins, 1994; Rao, Correa, Adams, Santori, & Sacaan, 2003; Singer et al., 2004). These neurotransmitters have also been implicated in contextual fear conditioning (Ji, Wang, & Li, 2003; Melik, Babar-Melik, Ozgunen, & Binokay, 2000; Pezze, Heidbreder, Feldon, & Murphy, 2001; Roberts, Krucker, Levy, Slanina, Sutcliffe, & Hedlund, 2004; Soares, Fornari, & Oliveira, 2006) and are associated with activation of ERK 1/2 (Berkeley & Levey, 2003; Chen, Nguyen, Pike, & Russo-Neustadt, 2007; Errico, Crozier, Plummer, & Cowen, 2001; Runyan & Dash, 2004). Ergo, nicotine-stimulated neurotransmitter release could enhance contextual fear conditioning through activation or enhancement of ERK 1/2 activity.

In summary, the present findings are the first to suggest that nicotine enhances contextual fear conditioning by increasing ERK 1/2 activity at training. The mechanisms by which nicotine could be coupled to ERK 1/2 activity are as yet unknown. However, recent evidence indicates that nicotine could enhance ERK 1/2 activation through at least three mechanisms: direct

activation of the ERK 1/2 signaling cascade via calcium influx, interaction with NMDARs, and enhancement of the release of neurotransmitters that effect learning-related processes and increase ERK 1/2 activation. In future studies we will examine the mechanisms through which nicotine enhances ERK 1/2 activation during contextual fear conditioning.

Acknowledgments

Support for this study was provided by National Institute on Drug Abuse (NIDA) Grant DA017949. Jonathan D. Raybuck was supported by NIDA Training Grant T32DA07237.

References

- Ahi J, Radulovic J, Spiess J. The role of hippocampal signaling cascades in consolidation of fear memory. *Behavioral Brain Research* 2004;149:17–31.
- Atkins C, Selcher J, Petraitis J, Traskos J, Sweatt J. The MAPK cascade is required for mammalian associative learning. *Nature Neuroscience* 1998;1:602–609.
- Bardgett ME, Boeckman R, Krochmal D, Fernando H, Ahrens R, Csernansky JG. NMDA receptor blockade and hippocampal neuronal loss impair fear conditioning and position habit reversal in C57Bl/6 mice. *Brain Research Bulletin* 2003;60(1–2):131–142. [PubMed: 12725901]
- Barrantes GE, Westwick J, Wonnacott S. Nicotinic acetylcholine receptors in primary cultures of hippocampal neurons: Pharmacology and Ca^{++} permeability. *Biochemical Society Transcripts* 1994;22(3):294S.
- Berkeley JL, Levey AI. Cell-specific extracellular signal-regulated kinase activation by multiple G protein-coupled receptor families in hippocampus. *Molecular Pharmacology* 2003;63:128–135. [PubMed: 12488545]
- Bliss TV, Collingridge GL. A synaptic model of memory: Long-term potentiation in the hippocampus. *Nature* 1993 January 7;361:31–39. [PubMed: 8421494]
- Brunzell DH, Russell DS, Picciotto MR. In vivo nicotine treatment regulates mesocorticolimbic CREB and ERK signaling in C57Bl/6J mice. *Journal of Neurochemistry* 2003;84:1431–1441. [PubMed: 12614343]
- Chen MJ, Nguyen TV, Pike CJ, Russo-Neustadt AA. Norepinephrine induces BDNF and activates the PI-3K and MAPK cascades in embryonic hippocampal neurons. *Cell Signaling* 2007;19:114–128.
- Ciamei A, Averson M, Cestari V, Castellano C. Effects of MK-801 and nicotine combination on memory consolidation in CD1 mice. *Psychopharmacology* 2002;154:126–130. [PubMed: 11314674]
- Cohen, BH. Explaining psychological statistics. Vol. 2nd ed. New York: Wiley; 2001.
- Coogan AN, O'Leary DM, O'Connor JJ. P42/44 MAP kinase inhibitor PD098059 attenuates multiple forms of synaptic plasticity in rat dentate gyrus in vitro. *Journal of Neurophysiology* 1999;81:103–110. [PubMed: 9914271]
- Csernansky JG, Martin M, Shah R, Bertchume A, Colvin J, Dong H. Cholinesterase inhibitors ameliorate behavioral deficits induced by MK-801 in mice. *Neuropsychopharmacology* 2005;30:2135–2143. [PubMed: 15956997]
- Dajas-Bailador FA, Soliakov L, Wonnacott S. Intracellular Ca^{2+} signals evoked by stimulation of nicotinic acetylcholine receptors in SH-SY5Y cells: Contribution of voltage-operated Ca^{2+} channels and Ca^{2+} stores. *Journal of Neurochemistry* 2002;80:520–530. [PubMed: 11905997]
- Davis JA, Gould TJ. The effects of DHBE and MLA on nicotine-induced enhancement of contextual fear conditioning in C57Bl/6 mice. *Psychopharmacology (Berlin)* 2006;184:345–352. [PubMed: 15988571]
- Davis JA, Porter J, Gould TJ. Nicotine enhances both foreground and background contextual fear conditioning. *Neuroscience Letters* 2005;394:202–205. [PubMed: 16260086]
- English JD, Sweatt JD. Activation of p42 mitogen-activated protein kinase in hippocampal long term potentiation. *Journal of Biological Chemistry* 1996;271:24329–24332. [PubMed: 8798683]
- English JD, Sweatt JD. A requirement for the mitogen-activated protein kinase cascade in hippocampal long term potentiation. *Journal of Biological Chemistry* 1997;272:19103–19106. [PubMed: 9235897]

- Errico M, Crozier RA, Plummer MR, Cowen DS. 5-HT(7) receptors activate the mitogen activated protein kinase extracellular signal related kinase in cultured rat hippocampal neurons. *Neuroscience* 2001;102:361–367. [PubMed: 11166122]
- Favata MF, Horiuchi KY, Manos EJ, Daulerio AJ, Stradley DA, Feeser WS, et al. Identification of a novel inhibitor of mitogen-activated protein kinase kinase. *Journal of Biological Chemistry* 1998;273:18623–18632. [PubMed: 9660836]
- Fedele E, Varnier G, Ansaldo MA, Raiteri M. Nicotine administration stimulates the in vivo N-methyl-D-aspartate receptor/nitric oxide/cyclic GMP pathway in rat hippocampus through glutamate release. *British Journal of Pharmacology* 1998;125:1042–1048. [PubMed: 9846643]
- Feiro O, Gould TJ. The interactive effects of nicotinic and muscarinic cholinergic receptor inhibition on fear conditioning in young and aged C57BL/6 mice. *Pharmacology, Biochemistry, and Behavior* 2005;80:251–262.
- Florian C, Mons N, Rouillet P. CREB antisense oligode-oxynucleotide administration into the dorsal hippocampal CA3 region impairs long- but not short-term spatial memory in mice. *Learning & Memory* 2006;13:465–472. [PubMed: 16882863]
- Fujii S, Ji Z, Morita N, Sumikawa K. Acute and chronic nicotine exposure differentially facilitate the induction of LTP. *Brain Research* 1999;846:137–143. [PubMed: 10536221]
- Gould TJ, Feiro O, Moore D. Nicotine enhances trace cued fear conditioning but not delay cued fear conditioning in C57BL/6 mice. *Behavioral Brain Research* 2004;155:167–173.
- Gould TJ, Higgins JS. Nicotine enhances contextual fear conditioning in C57BL/6J mice at 1 and 7 days post-training. *Neurobiology of Learning and Memory* 2003;80:147–157. [PubMed: 12932430]
- Gould TJ, Lewis MC. Coantagonism of glutamate receptors and nicotinic acetylcholinergic receptors disrupts fear conditioning and latent inhibition of fear conditioning. *Learning Memory* 2005;12:389–398. [PubMed: 16077017]
- Gould TJ, Lommock JA. Nicotine enhances contextual fear conditioning and ameliorates ethanol-induced deficits in contextual fear conditioning. *Behavioral Neuroscience* 2003;117:1276–1282. [PubMed: 14674846]
- Gould TJ, McCarthy MM, Keith RA. MK-801 disrupts acquisition of contextual fear conditioning but enhances memory consolidation of cued fear conditioning. *Behavioural Pharmacology* 2002;13:287–294. [PubMed: 12218509]
- Gould TJ, Wehner JM. Nicotine enhancement of contextual fear conditioning. *Behavioral Brain Research* 1999;102:31–39.
- Grady SR, Marks MJ, Collins AC. Desensitization of nicotine-stimulated [3H]dopamine release from mouse striatal synaptosomes. *Journal of Neurochemistry* 1994;62:1390–1398. [PubMed: 8133269]
- Grady SR, Murphy KL, Cao J, Marks MJ, McIntosh JM, Collins AC. Characterization of nicotinic agonist-induced [(3)H]dopamine release from synaptosomes prepared from four mouse brain regions. *Journal Pharmacology and Experimental Therapeutics* 2002;301:651–660.
- Graves L, Dalvi A, Lucki I, Blendy JA, Abel T. Behavioral analysis of CREB alphas mutation on a B6/129 F1 hybrid background. *Hippocampus* 2002;12:18–26. [PubMed: 11918283]
- Hu M, Liu QS, Chang KT, Berg DK. Nicotinic regulation of CREB activation in hippocampal neurons by glutamatergic and nonglutamatergic pathways. *Molecular and Cellular Neurosciences* 2002;21:616–625. [PubMed: 12504594]
- Impey S, Obrietan K, Wong ST, Poser S, Yano S, Wayman G, et al. Cross talk between ERK and PKA is required for Ca²⁺ stimulation of CREB-dependent transcription and ERK nuclear translocation. *Neuron* 1998;21:869–883. [PubMed: 9808472]
- Impey S, Smith DM, Obrietan K, Donahue R, Wade C, Storm DR. Stimulation of cAMP response element (CRE)-mediated transcription during contextual learning. *Nature Neuroscience* 1998;1:595–601.
- Izquierdo I, McGaugh JL. Behavioural pharmacology and its contribution to the molecular basis of memory consolidation. *Behavioural Pharmacology* 2000;11(7–8):517–534. [PubMed: 11198125]
- Ji JZ, Wang XM, Li BM. Deficit in long-term contextual fear memory induced by blockade of beta-adrenoceptors in hippocampal CA1 region. *European Journal of Neuroscience* 2003;17:1947–1952. [PubMed: 12752794]

- Levin ED, Bettegowda C, Weaver T, Christopher NC. Nicotine-dizocilpine interactions and working and reference memory performance of rats in the radial-arm maze. *Pharmacology, Biochemistry and Behavior* 1998;61:335–340.
- Logue SF, Paylor R, Wehner JM. Hippocampal lesions cause learning deficits in inbred mice in the Morris water maze and conditioned-fear task. *Behavioral Neuroscience* 1997;111:104–113. [PubMed: 9109628]
- Matsuyama S, Matsumoto A, Enomoto T, Nishizaki T. Activation of nicotinic acetylcholine receptors induces long-term potentiation in vivo in the intact mouse dentate gyrus. *European Journal of Neuroscience* 2000;12:3741–3747. [PubMed: 11029644]
- Maxwell, SE.; Delaney, HD. Designing experiments and analyzing data: A model comparison perspective. Vol. 2nd ed. Mahwah, NJ: Erlbaum; 2003.
- McGehee DS, Heath MJ, Gelber S, Devay P, Role LW. Nicotine enhancement of fast excitatory synaptic transmission in CNS by presynaptic receptors. *Science* 1995 September 22;269:1692–1696. [PubMed: 7569895]
- Melik E, Babar-Melik E, Ozgunen T, Binokay S. Median raphe nucleus mediates forming long-term but not short-term contextual fear conditioning in rats. *Behavioral Brain Research* 2000;112:145–150.
- Nakayama H, Numakawa T, Ikeuchi T, Hatanaka H. Nicotine-induced phosphorylation of extracellular signal-regulated protein kinase and CREB in PC12h cells. *Journal of Neurochemistry* 2001;79:489–498. [PubMed: 11701752]
- Nowak L, Bregestovski P, Ascher P, Herbert A, Prochiantz A. Magnesium gates glutamate-activated channels in mouse central neurones. *Nature* 1984 February 2;307:462–465. [PubMed: 6320006]
- Petersen DR, Norris KJ, Thompson JA. A comparative study of the disposition of nicotine and its metabolites in three inbred strains of mice. *Drug Metabolism and Disposition* 1984;12:725–731. [PubMed: 6150822]
- Pezze MA, Heidbreder CA, Feldon J, Murphy CA. Selective responding of nucleus accumbens core and shell dopamine to aversively conditioned contextual and discrete stimuli. *Neuroscience* 2001;108:91–102. [PubMed: 11738134]
- Phillips RG, LeDoux JE. Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behavioral Neuroscience* 1992;106:274–285. [PubMed: 1590953]
- Radcliffe KA, Fisher JL, Gray R, Dani JA. Nicotinic modulation of glutamate and GABA synaptic transmission of hippocampal neurons. *Annals of the New York Academy of the Sciences* 1999;868:591–610.
- Rao TS, Correa LD, Adams P, Santori EM, Sacaan AI. Pharmacological characterization of dopamine, norepinephrine and serotonin release in the rat prefrontal cortex by neuronal nicotinic acetylcholine receptor agonists. *Brain Research* 2003;990:203–208. [PubMed: 14568345]
- Riedel G, Platt B, Micheau J. Glutamate receptor function in learning and memory. *Behavioral Brain Research* 2003 March 18;140:1–47.
- Risso F, Grilli M, Parodi M, Bado M, Raiteri M, Marchi M. Nicotine exerts a permissive role on NMDA receptor function in hippocampal noradrenergic terminals. *Neuropharmacology* 2004;47:65–71. [PubMed: 15165834]
- Roberts AJ, Krucker T, Levy CL, Slanina KA, Sutcliffe JG, Hedlund PB. Mice lacking 5-HT receptors show specific impairments in contextual learning. *European Journal of Neuroscience* 2004;19:1913–1922. [PubMed: 15078565]
- Rosen LB, Ginty DD, Weber MJ, Greenberg ME. Membrane depolarization and calcium influx stimulate MEK and MAP kinase via activation of Ras. *Neuron* 1994;12:1207–1221. [PubMed: 8011335]
- Runyan JD, Dash PK. Intra-medial prefrontal administration of SCH-23390 attenuates ERK phosphorylation and long-term memory for trace fear conditioning in rats. *Neurobiology of Learning and Memory* 2004;82:65–70. [PubMed: 15341790]
- Sananbenesi F, Fischer A, Schrick C, Spiess J, Radulovic J. Phosphorylation of hippocampal Erk-1/2, Elk-1, and p90-Rsk-1 during contextual fear conditioning: Interactions between Erk-1/2 and Elk-1. *Molecular and Cellular Neurosciences* 2002;21:463–476. [PubMed: 12498787]
- Sananbenesi F, Fischer A, Schrick C, Spiess J, Radulovic J. Mitogen-activated protein kinase signaling in the hippocampus and its modulation by corticotropin-releasing factor receptor 2: A possible link between stress and fear memory. *Neuropharmacology* 2003;44:1089–1099. [PubMed: 12763101]

- Sawada S, Ohno-Shosaku T, Yamamoto C. Augmenting action of nicotine on population spikes in the dentate gyrus of the guinea pig. *Neuroscience Research* 1994;20:317–322. [PubMed: 7870385]
- Seguela P, Wadiche J, Dineley-Miller K, Dani JA, Patrick JW. Molecular cloning, functional properties, and distribution of rat brain $\alpha 7$: A nicotinic cation channel highly permeable to calcium. *Journal of Neuroscience* 1993;13:596–604. [PubMed: 7678857]
- Selcher JC, Atkins CM, Trzaskos JM, Paylor R, Sweatt JD. A necessity for MAP kinase activation in mammalian spatial learning. *Learning & Memory* 1999;6:478–490. [PubMed: 10541468]
- Shalin S, Zirrgiebel U, Honsa K, Julien J, Miller F, Kaplan D, Sweatt J. Neuronal MEK is important for normal fear conditioning in mice. *Journal of Neuroscience Research* 2004;75:760–770. [PubMed: 14994337]
- Singer S, Rossi S, Verzosa S, Hashim A, Lonow R, Cooper T, et al. Nicotine-induced changes in neurotransmitter levels in brain areas associated with cognitive function. *Neurochemical Research* 2004;29:1779–1792. [PubMed: 15453274]
- Soares JC, Fornari RV, Oliveira MG. Role of muscarinic M1 receptors in inhibitory avoidance and contextual fear conditioning. *Neurobiology of Learning and Memory* 2006;86:188–196. [PubMed: 16647280]
- Sweatt JD. The neuronal MAP kinase cascade: A biochemical signal integration system subserving synaptic plasticity and memory. *Journal of Neurochemistry* 2001;76:1–10. [PubMed: 11145972]
- Valjent E, Pages C, Herve D, Girault JA, Caboche J. Addictive and non-addictive drugs induce distinct and specific patterns of ERK activation in mouse brain. *European Journal of Neuroscience* 2004;19:1826–1836. [PubMed: 15078556]
- Wang J, Chen YB, Zhu XN, Chen RZ. Activation of p42/44 mitogen-activated protein kinase pathway in long-term potentiation induced by nicotine in hippocampal CA1 region in rats. *Acta Pharmacologia Sinica* 2001;22:685–690.
- Wehner JM, Keller JJ, Keller AB, Picciotto MR, Paylor R, Booker TK, et al. Role of neuronal nicotinic receptors in the effects of nicotine and ethanol on contextual fear conditioning. *Neuroscience* 2004;129:11–24. [PubMed: 15489024]
- Winder DG, Martin KC, Muzzio IA, Rohrer D, Chruscinski A, Kobilka B, Kandel ER. ERK plays a regulatory role in induction of LTP by theta frequency stimulation and its modulation by beta-adrenergic receptors. *Neuron* 1999;24:715–726. [PubMed: 10595521]
- Wood MA, Kaplan MP, Park A, Blanchard EJ, Oliveira AM, Lombardi TL, Abel T. Transgenic mice expressing a truncated form of CREB-binding protein (CBP) exhibit deficits in hippocampal synaptic plasticity and memory storage. *Learning & Memory* 2005;12:111–119. [PubMed: 15805310]
- Yamazaki Y, Jia Y, Niu R, Sumikawa K. Nicotine exposure in vivo induces long-lasting enhancement of NMDA receptor-mediated currents in the hippocampus. *European Journal of Neuroscience* 2006;23:1819–1828. [PubMed: 16623839]

Figure 1. Effects of SL327 on nicotine-enhanced contextual fear conditioning

(A) Administration of SL327, a selective inhibitor of extracellular signal-regulated kinase (ERK) 1/2, blocks contextual fear conditioning at a dose of 10 mg/kg but not at a dose of 5 mg/kg. Therefore, SL327 is subthreshold for the disruption of contextual fear conditioning at a dose of 5 mg/kg. (B) Subthreshold inhibition of ERK 1/2 at training of contextual fear conditioning blocks the enhancement of contextual fear conditioning by nicotine (0.09 mg/kg). SL327 (5 mg/kg) was administered only prior to training; prior to testing, mice received a matched vehicle injection. (C) Subthreshold inhibition of ERK 1/2 at training and testing of contextual fear conditioning blocks the enhancement of contextual fear conditioning by nicotine (0.09 mg/kg). (D) Subthreshold inhibition of ERK 1/2 at testing has no significant

effect on nicotine-enhanced contextual fear conditioning. SL327 (5 mg/kg) administered only prior to testing; prior to training, mice received a matched vehicle injection. Asterisk (*) indicates significant difference from vehicle control groups. Data are mean ($\pm$ *SEM*).