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Mice lacking the galanin gene show decreased sensitivity to nicotine conditioned place preference

Nichole M. Neugebauer, Robert M. Henehan, Claire A. Hales, Marina R. Picciotto *

Division of Molecular Psychiatry, Abraham Ribicoff Research Facilities, Connecticut Mental Health Center, Yale University School of Medicine, New Haven, CT, United States

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ABSTRACT

Previous work has indicated that the neuropeptide galanin decreases sensitivity to the rewarding effects of morphine and cocaine, but increases alcohol drinking. The aim of the current study was to examine the role of galanin signaling in nicotine reward by testing the effects of nicotine in mice lacking galanin peptide (GAL −/−) as compared to wild-type (GAL +/+) controls. Using an unbiased, three-chamber conditioned place preference (CPP) paradigm the dose–response function for nicotine CPP was tested in GAL −/− and GAL +/+ mice. Since activation of extracellular signal-related kinase (ERK2) is involved in the rewarding effects of several classes of drugs of abuse, we then measured the level of ERK2 phosphorylation in the nucleus accumbens shell (NACsh) and core (NACco) of GAL −/− and GAL +/+ mice following re-exposure to the CPP chamber previously paired with nicotine as a marker of mesolimbic system activation. Finally, we examined whether acute nicotine administration affects ERK2 activity in GAL −/− and GAL +/+ mice. GAL −/− mice required a higher dose of nicotine to induce a significant CPP compared to GAL +/+ mice. In the conditioning groups showing significant expression of nicotine CPP, only GAL +/+ mice showed ERK2 activation in the NACsh. This suggests that the nicotine CPP observed in GAL +/+ mice resulted in differential recruitment of ERK signaling in the NACsh compared to GAL −/− mice. In addition, no activation of ERK2 was observed following acute nicotine administration in either genotype. These data, along with prior results, suggest that galanin alters sensitivity to drugs of abuse differentially, with morphine, cocaine and amphetamine place preference suppressed, and nicotine and alcohol preference increased, by galanin signaling.

1. Introduction

There has been increasing interest in determining the mechanisms by which orexigenic neuropeptides modulate the neurochemical and behavioral responses to drugs of abuse. There is considerable evidence suggesting that neuropeptides involved in feeding can alter activity within the mesolimbic dopamine system, thereby affecting a number of motivated behaviors including those associated with the rewarding effects of drugs of abuse (Chung et al., 2009; Ericson and Ahlenius, 1999; Espana et al., 2010; LeSage et al., 2010; Maric et al., 2009; Sears et al., 2010). Among these neuropeptides, galanin is of interest because of its ability to modulate the rewarding effects of a number of classes of abused drugs, including opiates, psychostimulants and alcohol (reviewed in Picciotto et al., 2010).

Previous research has suggested that endogenous galanin inhibits the rewarding effects of morphine and cocaine as transgenic mice lacking the galanin peptide (GAL −/−) show increased sensitivity to the rewarding effects of these drugs in the conditioned place preference (CPP) paradigm compared to wild-type (GAL +/+) controls (Hawes et al., 2008; Narasimhaiah et al., 2009). Similarly, exogenous galanin administration (i.c.v.) decreases morphine-induced CPP in C57BL/6J mice (Zachariou et al., 2006). In contrast, galanin does not greatly alter cocaine-induced hyperactivity and does not change the dose–response function for cocaine self-administration, although GAL −/− mice are significantly more likely to be low responders for cocaine than GAL +/+ mice (Brabant et al., 2010). While the effect of galanin on amphetamine and alcohol reward have not been assessed directly, amphetamine-induced locomotor hyperactivity is decreased in transgenic mice over-expressing the galanin peptide (Kuteeva et al., 2005) and GAL −/− mice voluntarily consume less ethanol compared to GAL +/+ or galanin over-expressing mice (Karatayev et al., 2009, 2010).

The mesolimbic dopamine system has been implicated in the motivation to obtain drugs of abuse and natural rewards (for review see Baldo and Kelley, 2007; Everitt et al., 2008; Vucetic and Reyes, 2010) and there is evidence that galanin modulates activity of this pathway, possibly via indirect mechanisms (for review see Robinson and Brewer, 2008). Interestingly, it has recently been proposed that galanin regulates appetitive (i.e. food/drug-seeking) versus consummatory (i.e. food/drug-taking) aspects of reward, potentially through inhibition of the ascending mesolimbic dopamine system (McNamara

* Corresponding author. 34 Park Street, New Haven, CT 06508, United States. Tel.: +1 203 737 2041; fax: +1 203 737 2043.
E-mail address: marina.picciotto@yale.edu (M.R. Picciotto).

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and Robinson, 2010), suggesting that this may be a mechanism underlying galanin's effects on behaviors related to drug addiction.

Galanin exerts its action through G-protein coupled receptors (GalR1, GalR2, and GalR3), which stimulate several intracellular signaling pathways implicated in synaptic plasticity underlying reward-learning and addiction (Lang et al., 2007; Beninger and Gerdjikov, 2004; Russo et al., 2010). For example, activation of GalR1 or GalR2 results in G_i-mediated inhibition of cyclic AMP-responsive element binding (CREB) protein, which is critical for conditioned place preference to nicotine (Badie-Mahdavi et al., 2005; Brunzell et al., 2009). In addition, activation of GalR1 (through $G_{i/o}$-type G-proteins via $G\beta\gamma$-subunits) or GalR2 (through $G_{q/11}$-type G-proteins) can activate extracellular signal-related kinase (ERK; Lang et al., 2007). ERK activity, which can be evaluated by its phosphorylated state (P-ERK), is crucial for various aspects of learning and memory (Thomas and Huganir, 2004). This signaling pathway has also been implicated in the long-lasting neurobiological changes associated with administration of drugs of abuse (Girault et al., 2007). It has been shown previously that preference for a cocaine-paired environment is dependent on the ERK signaling pathway (Valjent et al., 2006). In addition, ERK activation is altered in mice administered nicotine through drinking water (Brunzell et al., 2003). Interestingly, galanin and the galanin receptor agonist galnon have been shown to decrease morphine- and cocaine-induced increases in ERK signaling (Picciotto, 2008).

The aim of the current study was to assess whether mice lacking the galanin peptide show an altered response to nicotine in the CPP paradigm. In addition, ERK activation in the nucleus accumbens shell and core (NACsh and NACco, respectively) was assessed in a subset of these mice following re-exposure to the CPP apparatus as a marker of plasticity in the mesolimbic system following repeated nicotine administration. In a separate group of mice, the effects of acute nicotine on ERK signaling in the nucleus accumbens were assessed to determine whether the altered ERK response was a direct effect of galanin signaling or was a response to the conditioned environment.

2. Materials and methods

2.1. Subjects

Congenic male galanin knockout (−/−) and wild type (+/+) mice on the 129 Ola/Hsd background were used for all experiments (Wynick et al., 1997). Mice used in these studies were generated using the following strategy to prevent any contribution of genetic drift to differences between congenic GAL −/− and GAL +/+ mice: GAL +/− mating pairs were used to generate GAL −/− or GAL +/+ mice and several breeding pairs of these homozygous mice were subsequently crossed to obtain experimental animals of the appropriate genotype (Narasimhaiah et al., 2009). Mice of the same genotype were housed together (2–5 per cage) in standard plastic mouse cages (Allentown Inc, Allentown, NJ USA) and had *ad libitum* access to chow (Harlan Teklad #2018) and water. Mice were allowed to habituate to the colony for at least 2 weeks before testing and were handled a minimum of 2 times a day for 2 days prior to experimental manipulation. All studies were conducted in accordance with the guidelines provided by the National Institutes of Health and were approved by the Yale Animal Care and Use Committee.

2.2. Drugs

Nicotine biatartrate salt was obtained from Sigma-Aldrich (Saint Louis, MO, USA) and was dissolved in 0.9% NaCl (saline). The pH was adjusted to 7.4 with NaOH. All injections were administered IP in a volume of 0.01 ml/g body weight. All doses are referred to with respect to the freebase.

2.3. Experiment 1: nicotine conditioned place preference

Behavioral experiments were conducted using modified three-chamber CPP boxes from Med Associates (ENV-256C Med Associates, Inc, St. Albans, VT, USA). Two conditioning chambers with retractable doors were separated by a smaller, grey neutral chamber with a grey Plexiglas floor. Both conditioning chambers had black walls. One conditioning chamber had a wire mesh floor and the other had a metal grid floor. Movement of each animal was recorded by photocell beam breaks and time spent in each chamber was recorded with Med-PC IV software.

Mice were transported to the testing room and allowed to habituate for at least 30 min prior to behavioral testing. Nicotine CPP was assessed in a similar manner to previous studies of morphine and cocaine CPP (Hawes et al., 2008; Narasimhaiah et al., 2009). On day 1 (pre-conditioning test), mice were placed in the center chamber and allowed to explore all 3 chambers for 15 min to determine baseline preference for each of the chambers. Mice that showed greater than 70% preference for any chamber were excluded from further testing n = 10 GAL −/− and n = 13 GAL +/+. Two conditioning sessions per day were conducted on days 2, 3, and 4. During the AM session (beginning at approximately 0900 h), mice were confined to one conditioning chamber for 30 min following saline injection. During the PM session (beginning at approximately 1300 h), mice were confined to the opposite conditioning chamber for 30 min following nicotine administration (0.05, 0.09, 0.18 or 0.36 mg/kg; n = 10–12/group). On day 5, the post-conditioning test was conducted. Mice were initially placed in the center chamber and allowed free access to all three chambers for a 15-min post-conditioning test. Animals were counterbalanced for drug-paired chamber based on genotype and baseline preference. The pre- and post-conditioning tests were conducted at an intermediate time between the AM and PM conditioning sessions (at approximately 1100 h). Data were collected as time spent in each chamber (seconds) during the pre- and post-conditioning test. In Fig. 1, the time spent in each of the chambers during the pre- (baseline) and post-conditioning test for each genotype is presented. These raw data were then used to calculate difference scores that reflect a change from baseline (post- minus pre-conditioning chamber), with positive numbers indicating an increase in time spent from baseline and negative numbers indicating a decrease in time spent from baseline for each genotype (Fig. 2). All data were analyzed using two-tailed, paired samples t-tests and level of significance was set at alpha < 0.05. Within 5 min following completion of the post-conditioning test, mice were sacrificed and brains were immediately frozen on dry ice and stored at −80 °C until western blot analysis.

2.4. Experiment 2: acute nicotine administration

The effect of acute nicotine administration on ERK phosphorylation was assessed. In order to habituate mice to the injection procedure, they were injected once a day for 3 days with saline. Mice received an injection of nicotine (0, 0.18 or 0.36 mg/kg, IP; n = 8/group) and brains were harvested 20 min later by rapid decapitation, immediately frozen using dry ice and stored at −80 °C until western blot analysis. Previous reports indicate the highest level of ERK activation following nicotine occurs at this time point in CD-1 mice (Valjent et al., 2004).

2.5. Western blot analysis for ERK phosphorylation

A random subset of the brains harvested following the post-conditioning test in Experiment 1 (n = 6–9/group) were used to determine levels of phosphorylated ERK (pERK) by western blotting as has been described (Hawes et al., 2008). Briefly, bilateral tissue punches (18 gauge, 1 mm thick slices) were taken from NACsh and

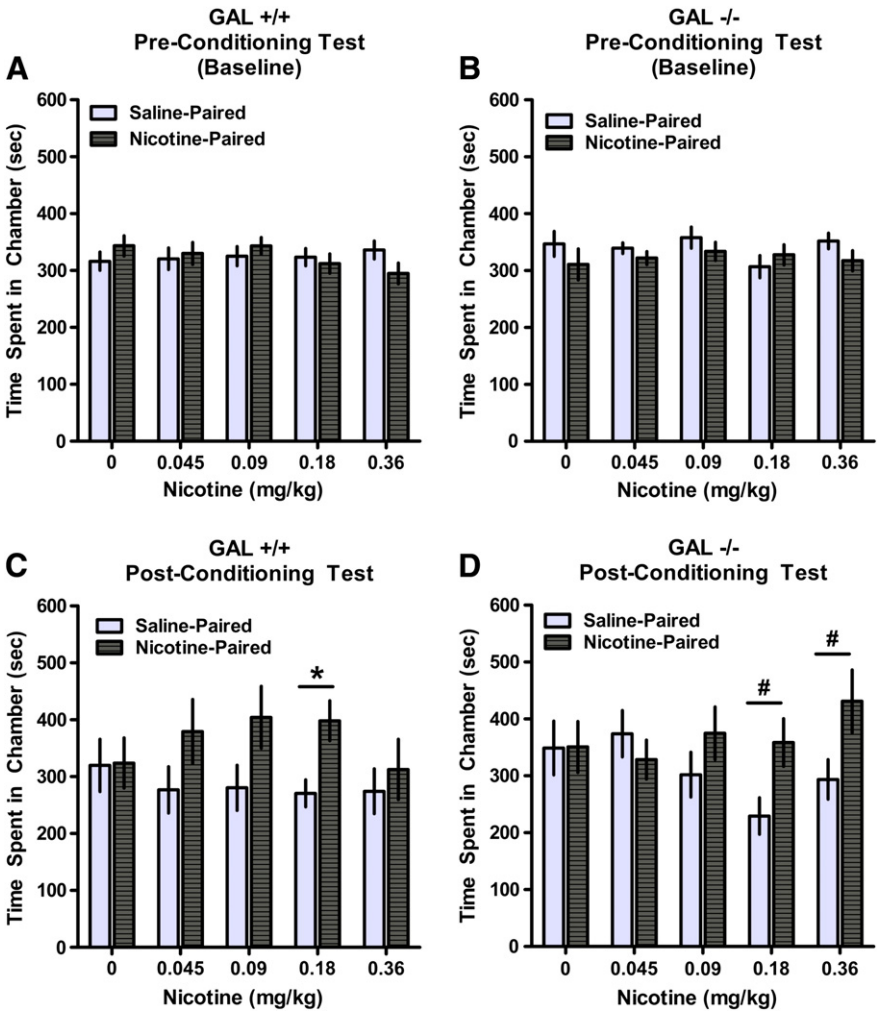

Fig. 1. Total time spent in the saline- and nicotine-paired compartment by each group of animals before and after conditioning. Data represent total time spent (seconds) in saline-paired or nicotine-paired chamber (mean ± SEM). Panels A and B: Total time spent in each chamber during the pre-conditioning test (baseline). Panels C and D: Total time (seconds) spent in each chamber during the post-conditioning test. $^{*}p<0.05$, $^{\#}p<0.07$.

NACco and cell lysis buffer (50 mM Tris, 1 mM EDTA, 1 mM EGTA, 1% SDS, and 1 mM PMSF) was added and immediately pulse sonicated for 5 s. Lowry reagents (Bio-Rad, Hercules, CA, USA) were used to determine protein concentrations according to manufacturer's instructions. For pERK and ERK immunoblots, 6 μg of protein for each sample was separated on a 10% poly-acrylamide gel and transferred to nitrocellulose membranes (Bio-Rad, Hercules, CA, USA). Blots were blocked in 5% Milk/Tris-buffered saline Tween-20 (TBST) for 1 h, washed with TBST, and incubated overnight in primary antibodies (ERK and pERK) diluted in TBST. Polyclonal antisera specific for p42/p44-MAPK (ERK 1/2) and the phosphorylated form of ERK 1/2 (Cell Signaling, Beverley, MA, USA) were used at a dilution of 1:1000 for both antibodies. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) immunoreactivity was used as an internal standard to control for protein loading. Following overnight incubation, blots were rinsed and incubated for 30 min at room temperature in anti-GAPDH monoclonal primary antibody (Advanced ImmunoChemical Inc., Long Beach, CA, USA) diluted 1:5000 in TBST. After washing three times for 5 min with TBST, blots were incubated with secondary antibodies for 1 h at room temp. The blots were incubated in IR Dye 800-conjugated anti-rabbit IgG (Rockland Inc., Gilbertsville, PA, USA) and Alexa fluor 680-conjugated anti-mouse IgG (Molecular Probes, Eugene, OR, USA) for 1 h at room temperature. Bands were visualized and quantified using the LI-COR Odyssey imager (LI-COR Biosciences, Lincoln, NE, USA) with Odyssey imaging software. The levels of ERK phosphorylation were determined by calculating the ratio of phosphorylated band intensity to total band intensity for ERK2 (p42ERK2). Data were normalized to the respective saline control to allow comparison across multiple blots by dividing each value by the saline group average and multiplying by 100 and were analyzed using two-tailed, independent samples t-tests. The level of significance was set at alpha<0.05.

3. Results

3.1. Experiment 1: nicotine conditioned place preference

No differences in chamber preference during the pre-conditioning test (baseline) were observed across genotypes in any of the treatment groups (Fig. 1A and B). There was a significant difference between time spent in the saline- and nicotine-paired chambers observed in GAL +/+ mice that were conditioned with the 0.18 mg/kg dose of nicotine ($t_{13}=2.32$; $p=0.04$). Interestingly, the dose response function is shifted to the left in GAL −/− mice as indicated by a trend towards significance following conditioning with the 0.18 mg/kg dose of nicotine ($t_{11}=1.99$; $p=0.07$), as well as the higher dose (0.36 mg/kg; $t_{11}=2.00$; $p=0.07$; Fig. 1C and D). Similarly, analysis of the difference scores in Fig. 2 indicated significant CPP in the GAL +/+ mice following conditioning with the 0.18 mg/kg dose of nicotine ($t_{13}=3.17$; $p=0.01$) and in the GAL −/− mice following conditioning with the

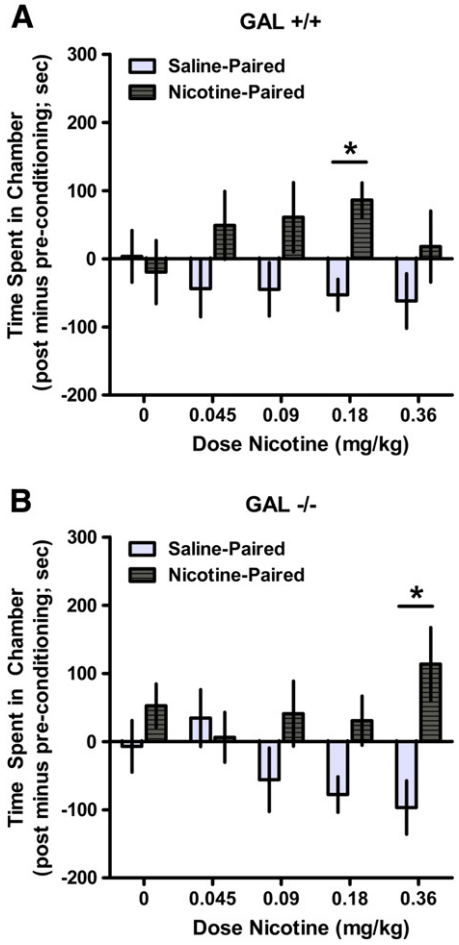

Fig. 2. Mice lacking the galanin peptide (GAL −/−) are less sensitive to the rewarding effects of nicotine. Data represent change from baseline expressed as the difference in time spent (seconds) between the pre- and post-conditioning test for each chamber (mean ± SEM). $^{*}p<0.05$.

0.36 mg/kg dose of nicotine ($t_{11}=2.46$; $p=0.03$). These results suggest that Gal −/− mice are less sensitive to the rewarding effects of nicotine compared to Gal +/+ mice. The results of measurements of ERK phosophorylation in the NACsh and NACco following re-exposure to the conditioning compartment are shown in Fig. 3. A statistically significant ($t_{14}=2.64$; $p<0.05$) increase in ERK phosphorylation in the nucleus accumbens shell following the post-conditioning test was observed in Gal +/+ mice conditioned with the 0.18 mg/kg dose of nicotine compared to saline control.

3.2. Experiment 2: acute nicotine administration

In order to determine whether the P-ERK signal was related to the rewarding effects of nicotine administered in the place preference context, or whether the pharmacological effects of nicotine were sufficient to induce ERK phosphorylation in these mice, the acute effects of nicotine on ERK phosphorylation were also assessed in Gal −/− and Gal +/+ mice. Acute nicotine administration (0.18, 0.36 mg/kg; IP) did not induce ERK phosphorylation in mice in either subregion of the NAC (Fig. 4).

4. Discussion

Several hypothalamic neuropeptides, including galanin, modulate the neurochemical and behavioral responses to drugs of abuse. Previous research has indicated that mice with constitutive knockout of the galanin peptide display increased sensitivity to the rewarding effects of morphine and cocaine, suggesting that endogenous galanin signaling opposes or inhibits the neurochemical actions of morphine and cocaine (Hawes et al., 2008; Narasimhaiah et al., 2009). In contrast, the results from the current study indicate that mice lacking the galanin peptide display decreased sensitivity to nicotine reward in the CPP paradigm, suggesting that galanin does not modulate drug-induced neurochemical changes similalry across all classes of abused drugs.

Although each drug of abuse has different initial effects on neurotransmission through interactions with specific receptors and subsequent sigaling pathways, these initally different mechanisms of action converge to increase mesolimbic and nigrostriatal dopamine transmission; however, it is important to keep in mind that each of these drug classes has unique effects on parts of the mesocortico-limbic circuitry that can modulate reward-related behavior (Pierce and Kumaresan, 2006). For example, systemic administration of nicotinic acetylcholine receptor antagonists and α-noradrenergic receptor decrease nicotine reward, but have little or no effect on cocaine reward (Sershen et al., 2010). In addition, it has been shown that antagonism of hippocampal nicotinic receptors and ablation of prefrontal cortical noradrenergic afferents inhibits morphine CPP (Rezayof et al., 2006; Ventura et al., 2005). Since galanin can modulate both acetylcholine and noradrenergic release in the hippocampus, it is possible that galanin differentially modulates the effects of drugs of abuse dependent on function of these neurotransmitters (Kehr et al., 2001; Elvander and Ogren, 2005).

An imbalance between cholinergic and dopaminergic signaling in the nucleus accumbens has been implicated in a variety of neurological disorders, likely because alterations in these neurotransmitter systems can impair the functioning of cortico-basal ganglia-thalamocortical loops (for review see Aosaki et al., 2010; Livingstone and Wonnacott, 2009). Cholinergic innervation of the ventral tegmental area and substantia nigra can influence the firing of dopaminergic neurons via nicotinic receptors on dopaminergic cell bodies, presynaptic glutamatergic terminals and GABAergic interneurons, resulting in modulation of dopamine release in target nuclei, such as the striatum (Mansvelder et al., 2002; Mena-Segovia et al., 2008). In addition, presynaptic nicotinic receptors on dopaminergic terminals and neurotransmission from striatal cholinergic interneurons in the striatum can affect dopamine release (Exley and Cragg, 2008). It has also been suggested that the cholinergic system may be involved in galanin-mediated modulation of dopamine signaling (Picciotto, 2008). Galanin administration into the paraventricular nucleus of the hypothalamus can decrease extracellular levels of acetylcholine and increase extracellular dopamine levels in NAC (Rada et al., 1998). Given the ability of acetylcholine release to alter dopamine dynamics and the important role of dopamine in both feeding and drug-related behaviors, it seems possible that galanin-induced alterations in cholinergic signaling could modulate drug-abuse related behaviors. Further research is needed to assess whether the basal levels of acetylcholine and dopamine are changed in GAL −/− mice, an effect that may result in differential responses to cocaine, morphine and nicotine reward. In addition, it will be important to determine the role of galanin in the reinforcing effects of morphine, alcohol, and nicotine using the self-administration paradigm as previous research has indicated galanin may modulate cocaine reward, but not reinforcement (Brabant et al., 2010; Narasimhaiah et al., 2009).

Exposure to a previously nicotine-paired chamber results in activation of CREB in several brain regions, including the VTA, NACsh, cingulate cortex and pedunculopontine tegmental nucleus and CREB activation is required in the NACsh for expression of nicotine CPP (Brunzell et al., 2009; Walters et al., 2003). One prominent regulator of CREB activity in neurons is ERK. Activation of the ERK signaling pathway in the NAC, which plays an important role in associative learning, is necessary for the expression of cocaine

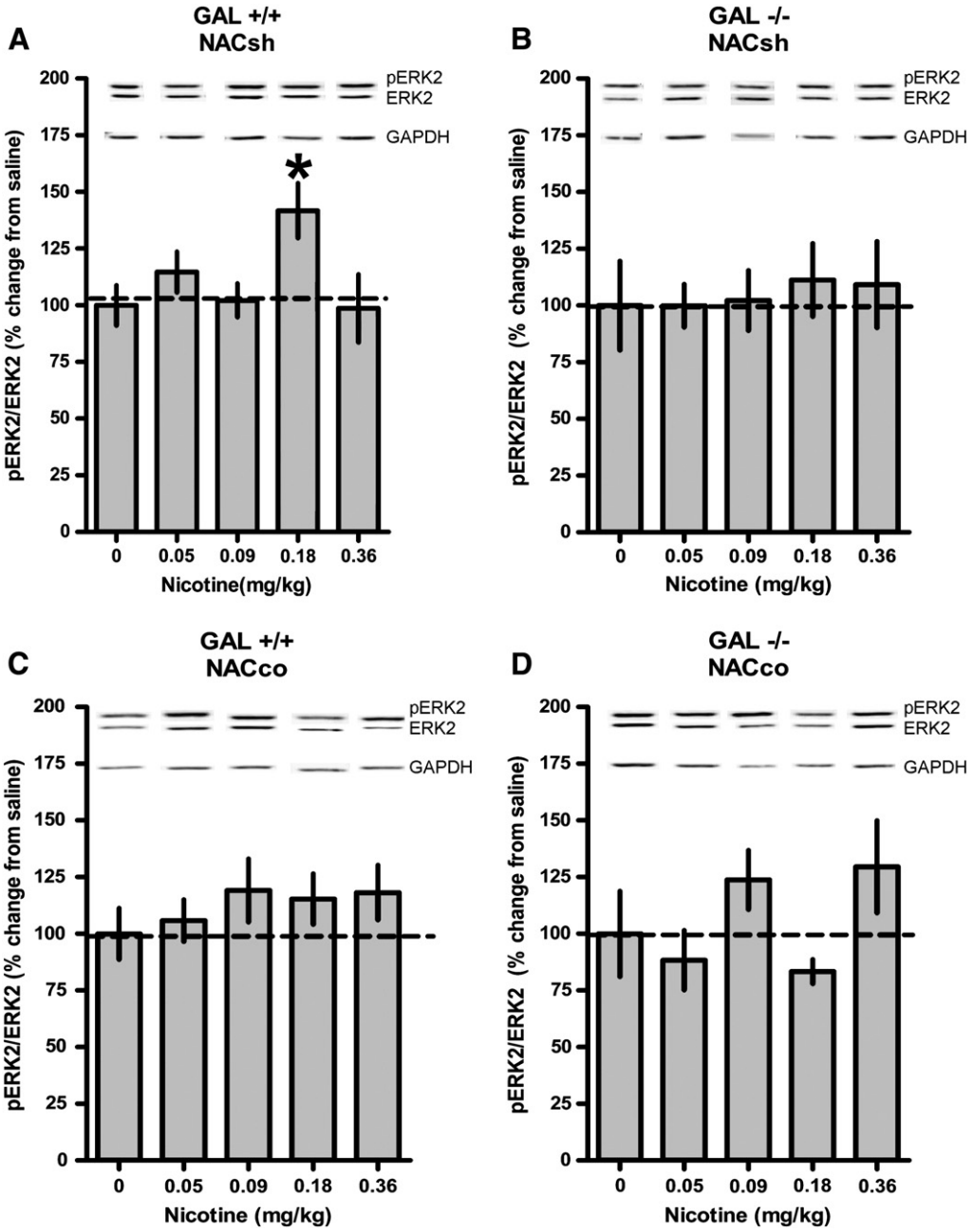

Fig. 3. Change in ERK activation following re-exposure to the CPP apparatus in NACsh and NACco. Data are expressed as mean ± SEM, relative to the respective saline control. *$p<0.05$.

and morphine reward (Valjent et al., 2006). While acute nicotine (0.4 mg/kg) has been reported to induce ERK activation in the NAC, similar to cocaine and morphine, it is unknown if ERK activation in the NAC is necessary for the expression of nicotine CPP (Valjent et al., 2004).

In contrast to previous studies using the CD-1 strain of mouse, acute nicotine administration did not alter ERK activation in the NAC. This may be due to strain differences since the GAL −/− and GAL +/+ mice were developed on the 129/OlaHsd background. Despite the lack of an acute increase in ERK phosphorylation following nicotine administration, both GAL −/− and GAL +/+ mice showed a significant nicotine CPP, raising the possibility that ERK activation occurs at a different time-point post nicotine administration in these mice compared to CD-1 mice (Valjent et al., 2004). In the current study, levels of ERK activation in the NACsh and NACco were determined in a random subset of mice from each conditioning group following behavioral assessment of nicotine CPP. GAL +/+ mice that exhibited significant nicotine (0.18 mg/kg) CPP showed increased ERK activation in the NACsh. However, no ERK activation was observed in the NAC of GAL −/− mice that exhibited significant nicotine (0.36 mg/kg) CPP, suggesting that ERK activation in NACsh may not be required for nicotine CPP in these mice or that the time-course of ERK activation was altered in these animals. While speculative, it is possible that the CREB activity in the NACsh, which is critical for nicotine CPP, is not regulated by ERK, but instead by another signaling cascade such as PKA or CaMKIV (Selcher et al., 2002). It is also possible that the ERK/CREB signaling pathway in other brain regions (not assessed in the current study) are critical for acquisition of nicotine CPP, such as the hippocampus.

In conclusion, the current results demonstrate that constitutive loss of the galanin peptide does not modulate the conditioned reinforcing effects of all classes of abused drugs uniformly, as assessed

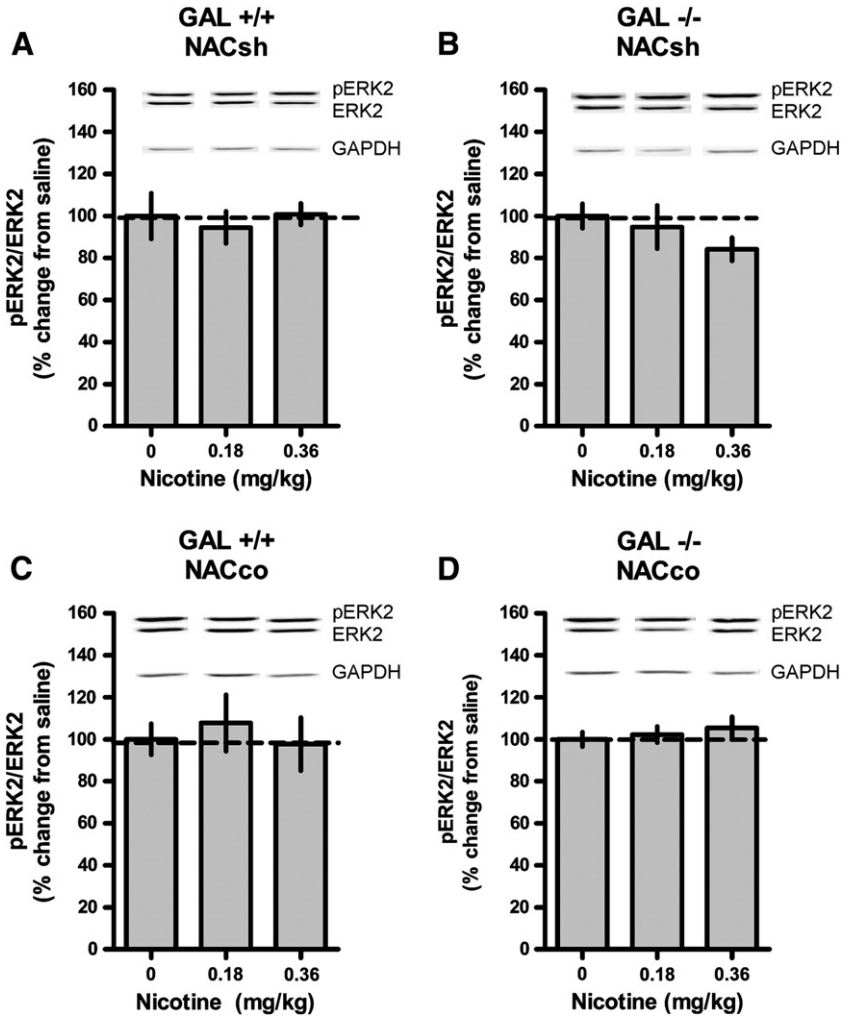

Fig. 4. Change in ERK activation following acute nicotine exposure in NACsh and NACco. Data are expressed as mean ± SEM, relative to the respective saline control. *$p<0.05$.

using the CPP paradigm. It is possible that compensatory mechanisms in these mice underlie the current results and further studies are needed to fully elucidate the role of galaninergic receptor signaling in drug-induced behaviors. Given the role of galanin, as well as other orexigenic neuropeptides, in motivated behavior for both food and drug-reward, galanin signaling remains a potential target for development of therapeutics to combat drug abuse.

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