Received: 13 December 2019 | Revised: 17 April 2020 | Accepted: 4 May 2020

DOI: 10.1002/jnr.24649

RESEARCH ARTICLE

# The role of pituitary adenylyl cyclase activating polypeptide in affective signs of nicotine withdrawal

**Shiromani Nega** | **Paul Marquez** | **Abdul Hamid** | **Syed Muzzammil Ahmad** | **Kabirullah Lutfy**

Department of Pharmaceutical Sciences, College of Pharmacy, Western University of Health Sciences, Pomona, CA, USA

**Correspondence**
Kabirullah Lutfy, Department of Pharmaceutical Sciences, College of Pharmacy, Western University of Health Sciences, Pomona, CA 91766, USA.
Email: klutfy@westernu.edu

**Funding information**
Western University of Health Sciences, Grant/Award Number: Intramural Grant; Tobacco Related Disease Research Program, Grant/Award Number: TRDRP 24RT-0023

## Abstract

Recent evidence implicates endogenous pituitary adenylyl cyclase activating polypeptide (PACAP) in the aversive effect of nicotine. In the present study, we assessed if nicotine-induced conditioned place preference (CPP) or affective signs of nicotine withdrawal would be altered in the absence of PACAP and if there were any sex-related differences in these responses. Male and female mice lacking PACAP and their wild-type controls were tested for baseline place preference on day 1, received conditioning with saline or nicotine (1 mg/kg) on alternate days for 6 days and were then tested for CPP the next day. Mice were then exposed to four additional conditioning and were tested again for nicotine-induced CPP 24 hr later. Controls were conditioned with saline in both chambers and tested similarly. All mice were then, 96 hr later, challenged with mecamylamine (3 mg/kg), and tested for anxiety-like behaviors 30 min later. Mice were then, 2 hr later, forced to swim for 15 min and then tested for depression-like behaviors 24 hr later. Our results showed that male but not female mice lacking PACAP expressed a significant CPP that was comparable to their wild-type controls. In contrast, male but not female mice lacking PACAP exhibited reduced anxiety- and depression-like behaviors compared to their wild-type controls following the mecamylamine challenge. These results suggest that endogenous PACAP is involved in affective signs of nicotine withdrawal, but there is a sex-related difference in this response.

**KEYWORDS**

anxiety-like behaviors, conditioned place preference (CPP), depression-like behaviors, nicotine, PACAP knockout mouse, withdrawal

Shiromani Nega and Paul Marquez contributed equally to this study.

Edited by Alex Marshall. Reviewed by Cassandra D. Gipson, Balazs Gaszner, and Shafiqur Rahman.

The peer review history for this article is available at https://publons.com/publon/10.1002/jnr.24649.

  

## 1 | INTRODUCTION

Nicotine dependence is a major public health issue and chronic nicotine use remains one of the prime causes of preventable disease and death around the globe. It has been reported that nearly 50 million people in the United States continue to use one form of tobacco products (Fiore, Schroeder, & Baker, 2014). Repeated nicotine use can lead to addiction, characterized by uncontrollable drug use despite negative consequences. While about 70% of smokers want to quit, only a small percentage (7%) of them succeed. This is even more problematic if smokers simultaneously use other drugs, such as alcohol (Carmody, Brischetto, Matarazzo, O'Donnell, & Connor, 1985; DiFranza & Guerrera, 1990; Miller & Gold, 1998; Rimm, Chan, Stampfer, Colditz, & Willett, 1995), or suffer from neuropsychiatric disorders (Mackowick, Heishman, et al., 2012; Mackowick, Lynch, Weinberger, & George, 2012; Picciotto, Brunzell, & Caldarone, 2002; Picciotto & Zoli, 2002).

One of the major issues in treating drug addiction is the high rate of relapse (Hunt, Barnett, & Branch, 1971; Oslin, Liberto, O'Brien, Krois, & Norbeck, 1997). Abstinence from chronic use of tobacco products in humans induces withdrawal symptoms, characterized by restlessness, difficulty concentrating, irritability, anxiety, anhedonia, and weight gain (Hughes & Hatsukami, 1986). Anxiety- and depression-like behaviors develop following nicotine withdrawal in rodents as well (Bagosi et al., 2016). The affective signs associated with nicotine withdrawal serve as negative reinforcers and thus are the main precipitating factors for craving and relapse (Doherty, Kinnunen, Militello, & Garvey, 1995; George et al., 2007; Kenny & Markou, 2001). Therefore, further research is needed to fully characterize the neurobiological and molecular substrates of the negative affective states that develop following nicotine withdrawal.

Pituitary adenylyl cyclase activating polypeptide (PACAP), exits in two biologically active forms, PACAP-38 and PACAP-27, and belongs to the vasoactive intestinal peptide (VIP)/glucagon/growth hormone/secretin family with high homology to VIP, was originally reported to stimulate adenylyl cyclase in the pituitary cells (Miyata et al., 1989). The PACAP/PAC1 receptor system has been implicated in physiological responses as well as in neuropsychiatric disorders such as anxiety, schizophrenia, and depression (Ghatei et al., 1993; Reglodi, Kiss, Horvath, et al., 2012; Reglodi, Kiss, Szabadfi, et al., 2012; Reglodi, Tamas, Koppan, Szogyi, & Welke, 2012; Vaudry et al., 2009). In an earlier report, we found that the aversive effect of a higher dose of nicotine (1 mg/kg) was reduced in female mice lacking PACAP, whereas the rewarding action of a lower dose of nicotine (0.25 mg/kg) was not altered in the absence of PACAP (Tseng, Singh, Marquez, Hamid, & Lutfy, 2019). In the present study, we assessed the role of endogenous PACAP in nicotine-induced conditioned place preference (CPP) and dependence using both male and female mice. In particular, we determined the role of PACAP in anxiety- and depression-like behaviors that develop following nicotine withdrawal (Bagosi et al., 2016).

**Significance**

The present study determines the role of pituitary adenylyl cyclase activating polypeptide (PACAP) in nicotine reward and withdrawal precipitated by mecamylamine. We report here that male, but not female, mice lacking PACAP exhibited a comparable conditioned place preference following nicotine conditioning to their wild-type controls. However, male mice lacking PACAP compared to wild-type mice expressed reduced anxiety-like and depression-like behaviors following a mecamylamine challenge, suggesting that endogenous PACAP is involved in affective signs of nicotine withdrawal, but that this effect is sex dependent.

## 2 | MATERIALS AND EXPERIMENTAL METHODS

### 2.1 | Experimental subjects

A total of 30 male and 28 female mice lacking PACAP and their wild-type littermates/age- and sex-matched controls, fully backcrossed on a C57BL/6 mouse background strain, between the ages of 4 and 6 months, bred in-house from heterozygous breeding pairs were used throughout. Pups were weaned and genotyped at the age of 21–24 days. A standard polymerase chain reaction protocol was followed using samples obtained from ear snips. The PACAP mice have been described previously (Colwell et al., 2004). The original heterozygous breeding pairs were generously provided by Dr. James Waschek (UCLA, CA, USA). Mice were maintained in a humidity- and temperature-controlled room with unlimited access to regular laboratory chow and water except during each conditioning and test session. All experiments were carried out during the light phase of a 12-hr light/12-hr dark cycle and approved by the Institutional Animal Care and Use Committee at Western University of Health Sciences (Pomona, CA, USA).

### 2.2 | Experimental design and procedures

We used both male and female mice in all experiments. Each experiment was repeated at least two times with two to five naïve mice per cohort for each genotype and each sex. Mice were randomly assigned to saline or nicotine group and received conditioning in a counterbalanced manner. Power analysis was used to calculate sample size.

### 2.3 | The role of PACAP in nicotine-induced CPP

We used a three-compartment place conditioning apparatus and an unbiased place conditioning paradigm and assessed the role of PACAP

in nicotine-induced CPP. We also examined if there is any sex-related difference in this response. To this end, male ($n$ = 8 per genotype) and female (7 per genotype) mice lacking PACAP and their wild-type controls were brought to the test room, habituated for 1 hr and then tested for baseline place preference on day 1 (D1). On the following day, mice were brought to the test room, habituated for 1 hr and then treated with nicotine (1 mg/kg) or saline and confined to the drug-paired (DPCh) or vehicle-paired chamber (VPCh), respectively, for 15 min. We used this higher dose of nicotine because our pilot studies showed that the lower dose of nicotine (0.25 mg/kg) did not induce a significant CPP in male mice using this alternate-day nicotine conditioning paradigm (see Supplemental data, Figure S1). The following day, mice received the alternate treatment and were confined to the opposite chamber for 15 min. Control mice ($n$ = 7 per genotype and sex) were conditioned with saline in both chambers. This once-daily conditioning continued for 6 days (three nicotine and three saline conditionings for the drug group or six saline sessions for the control group). Mice were tested for place preference 24 hr after the last conditioning. Mice then received additional conditioning (two saline and two nicotine or four saline sessions for the drug and saline control groups, respectively) and tested for CPP again to assess if further conditioning would result in a different response between mice of the two genotypes. On the pre- and postconditioning test days, each mouse was placed in the central neutral chamber of the place conditioning apparatus and allowed to freely explore the conditioning chambers. The amount of time that mice spent in each chamber was recorded for 15 min using a video-camera located above the place conditioning chambers. The videos were analyzed using TopScan Version 2.00 (CleverSystem, Reston, VA, USA) by an experimenter who was blind to the experimental procedure and genotype as well as treatment. A significant increase in the amount of time that mice spent in the DPCh versus VPCh was considered a significant CPP.

## 2.4 | The role of PACAP in negative affective signs of mecamylamine-precipitated withdrawal

### 2.4.1 | The role of PACAP in anxiety-like behaviors following nicotine withdrawal

We used the elevated plus maze (EPM) and determined the role of endogenous PACAP in anxiety-like behaviors that develop following nicotine withdrawal. Our EPM apparatus consisted of two open (25 × 5 cm) and two closed (25 × 5 cm and 15 cm height) arms elevated 55 cm above the floor. To determine the role of PACAP in anxiety-like behaviors, male and female mice lacking PACAP and their wild-type littermates/age- and sex-matched controls were conditioned with nicotine/saline or saline alone and tested for CPP, as described above. Mice were then tested for anxiety-like behaviors 96 hr after the last CPP test. We selected this time point because there are low levels of abstinence withdrawal (Isola, Vogelsberg, Wemlinger, Neff, & Hadjiconstantinou, 1999; Semba, Wakuta, Maeda, & Suhara, 2004), and thus we primarily measure the negative affective states associated with mecamylamine-precipitated nicotine withdrawal. On the test day, mice were brought to the test room and allowed to acclimatize for 1 hr. Mice were then injected with mecamylamine (3 mg/kg) and tested for anxiety-like behaviors 30 min later. Each mouse was placed on the center of the maze, facing one of the open arms. An aerial video-camera attached to the ceiling with a clear view of the EPM was used to record the amount of time that mice spent on each arm and analyzed using the TopScan Version 2.00 (CleverSystem, Reston, VA, USA). All videos were analyzed by an experimenter who was blind to the experimental procedure and genotype as well as treatment. A significant decrease in the amount of time that mice spent on the open arms compared to their respective controls was considered as an increase in the level of anxiety-like behaviors.

### 2.4.2 | The role of PACAP in depression-like behaviors following nicotine withdrawal

We used the forced swim test (FST) to measure depression-like behaviors in male and female mice lacking PACAP and their wild-type controls following nicotine withdrawal. To determine the role of PACAP in depression-like behaviors following nicotine withdrawal, 2 hr after the EPM test, mice were forced to swim (32°C water) for 15 min. Mice were then tested for immobility time in the FST the following day. On the test day, mice were brought to the test room and habituated for 1 hr and then placed in the water to swim for 6 min. Given that regardless of the treatment all mice are mobile during the first 2 min of the test session, the amount of time that mice remained immobile during the last 4 min of the test period was analyzed using the Swim Scan (CleverSystem, Reston, VA, USA) by an experimenter who was blind to the experimental procedure and genotype as well as treatment. This is in accordance with previous studies (Can et al., 2011; Can, Grahame, & Gould, 2012; David, Renard, Jolliet, Hascoet, & Bourin, 2003; Petit-Demouliere, Chenu, & Bourin, 2005; Porsolt, 1979; Porsolt, Bertin, & Jalfre, 1977). A significant increase in the amount of that mice remained immobile compared to their respective controls was considered as an increase in the level of depression-like behaviors.

## 2.5 | Materials

Nicotine and mecamylamine were purchased from MP Biomedicals, Inc. (Solon, OH, USA) and Sigma/Aldrich (St. Louis, MO), respectively. Each drug was dissolved in normal saline (sterilized 0.9% sodium chloride in deionized water) and injected subcutaneously (nicotine) or intraperitoneally (mecamylamine).

## 2.6 | Data analysis

Data are presented as the mean (±*SEM*) of the amount of time that mice spent in the place conditioning chambers, on the open and

closed arms of the EPM or remained immobile in the FST. The data were analyzed using three-way repeated measures analysis of variance (ANOVA) or two-way ANOVA, whichever appropriate using GraphPad Prism 8 (GraphPad Software, San Diego, CA). The Fisher's LSD post hoc test was used to reveal the significant difference between groups. A $p < 0.05$ was considered significant.

## 3 | RESULTS

### 3.1 | Nicotine induced CPP in both male and female wild-type mice but was only reduced in female mice lacking PACAP

We used the place conditioning paradigm, a model of place preference and aversion (Bardo & Bevins, 2000), to determine the role PACAP in the rewarding action of nicotine in male and female mice lacking PACAP and their wild-type controls. Figure 1a shows the amount of time that male mice lacking PACAP [PACAP (−/−)] and their wild-type controls [PACAP (+/+)] spent in the DPCh and VPCh on the preconditioning (D1) and postconditioning (D8) test days. Three-way ANOVA of the data yielded no significant effect of time ($F_{(1,56)} = 3.46$; $p = 0.068$), no significant effect of genotype ($F_{(1,56)} = 2.15$; $n = 8$ per genotype; $p = 0.148$), as well as no significant interaction between time and genotype ($F_{(1,56)} = 0.11$; $p = 0.741$), genotype and context ($F_{(1,56)} = 0.02$; $p = 0.883$) or time, context and genotype ($F_{(1,56)} = 0.01$; $p = 0.826$). However, there was a significant effect of context ($F_{(1,56)} = 10.71$; $p = 0.002$) as well as a significant interaction between time and context ($F_{(1,56)} = 11.70$; $p = 0.001$). The post hoc test revealed that mice lacking PACAP (right panel) as well as their wild-type littermates/controls (left panel) spent more time in the DPCh compared to the VPCh on the postconditioning but not preconditioning test day, suggesting that mice of both genotypes expressed a significant CPP response. However, there was no significant difference in the CPP response between mice of the two genotypes, suggesting that the rewarding effect of nicotine was unaltered in the absence of PACAP in male mice. The CPP response was reduced in mice of both genotypes following the second set of conditionings and a subsequent CPP test (data not shown).

Unlike male mice, the rewarding effect of nicotine was blunted in female mice lacking PACAP compared to their wild-type controls (Figure 1b). Three-way ANOVA revealed a significant effect of genotype ($F_{(1,48)} = 5.46$; $n = 7$ per genotype; $p < 0.024$) and a significant interaction between genotype and context ($F_{(1,48)} = 7.91$; $p < 0.007$) as well as a trend toward an interaction between genotype, time, and context ($F_{(1,48)} = 3.93$; $p = 0.053$) but no significant effect of time ($F_{(1,48)} = 0.28$; $p = 0.599$), no significant effect of context ($F_{(1,48)} = 2.40$; $p = 0.128$), and no significant interaction between time and genotype ($F_{(1,48)} = 0.16$; $p = 0.691$) and time and context ($F_{(1,48)} = 1.45$; $p = 0.235$). The post hoc test revealed a significant CPP in wild-type mice (Figure 1b; left panel) but this response was blunted in mice lacking PACAP (Figure 1b; right panel), suggesting that the rewarding effect of nicotine was reduced in the absence of PACAP in female mice. The absence of the CPP response in PACAP-deficient mice was not due to a lack of response to nicotine in these mice because the drug suppressed motor activity in both wild-type and knockout mice during the conditionings (Figure S2, Supplemental Data). As observed in male mice, the CPP response was decreased in mice of both genotypes following the second set of conditionings (data not shown).

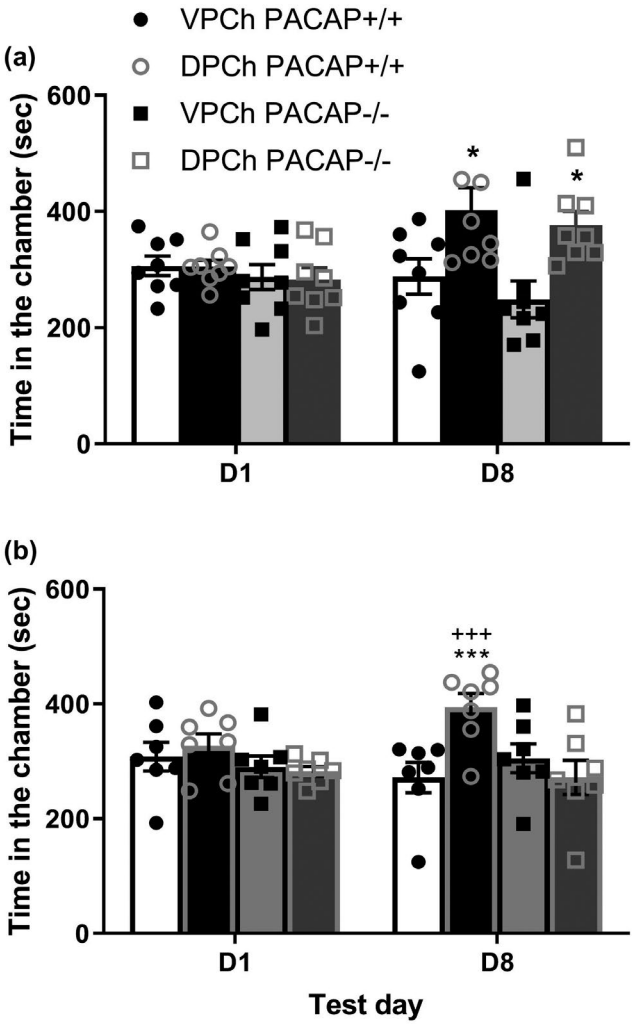


**FIGURE 1** Nicotine conditioning induced a significant CPP in male (a) mice lacking PACAP [PACAP (−/−)] and their wild-type controls [PACAP (+/+)] but this response was blunted in female PACAP (−/−) compared to PACAP (+/+) mice (b). Mice were tested for baseline place preference on day 1 (D1), conditioned with saline/nicotine (1 mg/kg, s.c.; $n = 8$ male and 7 female mice per genotype) or nicotine/saline on alternate days for 6 days and then tested for postconditioning place preference on day 8 (D8). Data represent the amount of time that mice spent in the drug-paired chamber (DPCh) versus vehicle-paired chamber (VPCh) on each test day and analyzed by three-way ANOVA. $^{*}p < 0.05$, $^{***}p < 0.001$ compared to their respective VPCh on day 8; $^{+++}p < 0.001$ versus DPCh in female mice lacking PACAP

Figure 2 shows the amount of time that mice lacking PACAP (right panels) and their wild-type controls (left panels) spent in the

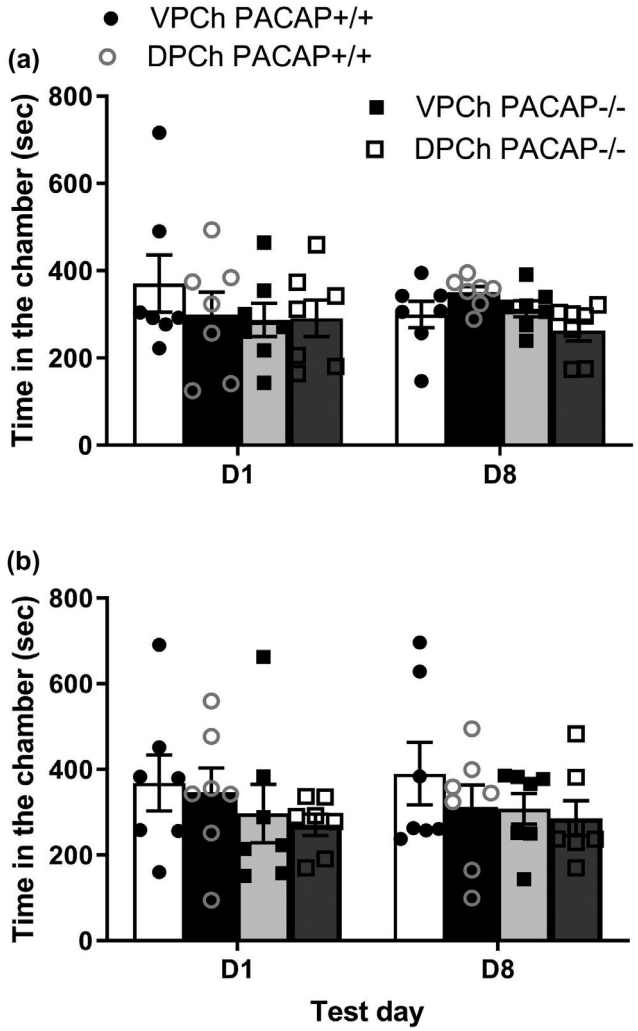


**FIGURE 2** Saline conditioning failed to induce CPP in male (a) or female (b) mice lacking PACAP [PACAP (−/−)] and their wild-type controls [PACAP (+/+)]. Mice were tested for baseline place preference on day 1 (D1), conditioned with saline/saline ($n$ = 7 mice per genotype and for each sex) on alternate days for 6 days and then tested for postconditioning place preference on day 8 (D8). Data represent the amount of time that mice spent in the drug-paired chamber (DPCh) versus vehicle-paired chamber (VPCh) on each test day and analyzed by three-way ANOVA

conditioning chambers on the CPP test day following conditioning with saline in both chambers. As can be observed, neither male (Figure 2a) nor female (Figure 2b) mice of either genotype expressed CPP after conditioning with saline in both chambers.

### 3.2 | Anxiety-like behaviors following mecamylamine-precipitated nicotine withdrawal were attenuated in male but not female PACAP-deficient mice

One of the affective signs of nicotine withdrawal is the expression of anxiety (Hughes, Hatsukami, Mitchell, & Dahlgren, 1986). EPM is widely used as a rodent model of anxiety-like behaviors (Lister, 1990). EPM has also been used to assess anxiety-like behaviors following nicotine withdrawal in mice (Bagosi et al., 2016). We used the EPM and determined the role of endogenous PACAP in anxiety-like behaviors that develop following nicotine withdrawal. We measured only affective signs of withdrawal in this study because we waited several days after cessation of nicotine and by that time, there is minimal, if any, somatic signs of withdrawal. However, negative affective states, such as anxiety and depression, are still evident and that may be the cause for craving and relapse. Figure 3 shows the amount of time that male mice lacking PACAP and their wild-type controls spent on the open and closed arms of EPM. Two-way ANOVA of the data on the open arms (Figure 3a) revealed no significant effect of genotype ($F_{(1,26)}$ = 2.19; $n$ = 7–8 mice of each sex per genotype and treatment, $p$ = 0.151) and no interaction between

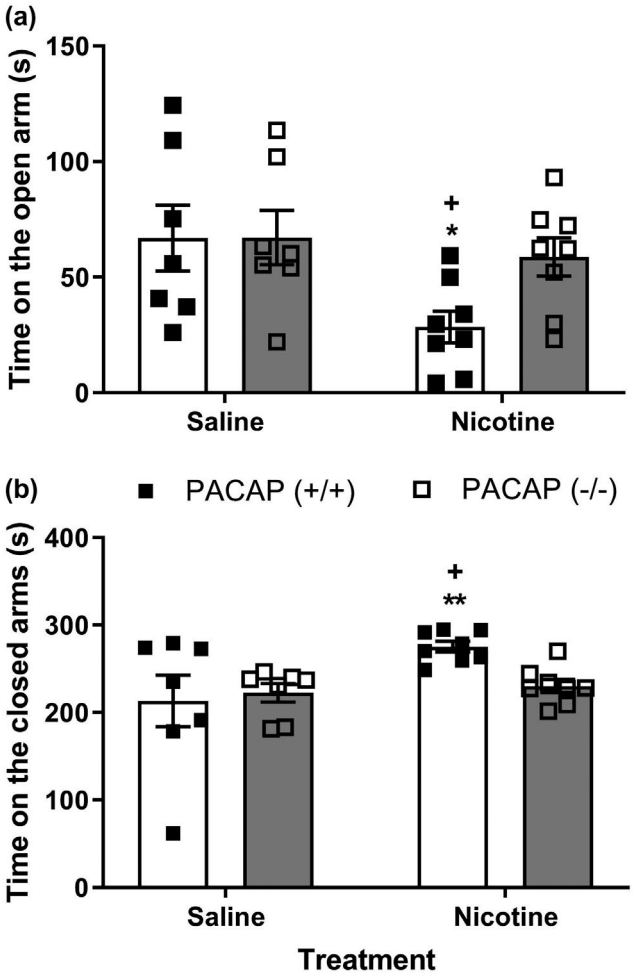


**FIGURE 3** Anxiety-like behaviors in male mice lacking PACAP [PACAP (−/−)] and their wild-type controls [PACAP (+/+)] conditioned with nicotine ($n$ = 8 mice per genotype) or saline ($n$ = 7 mice per genotype). Mice were tested for anxiety-like behaviors 30 min following a challenge dose of mecamylamine (3 mg/kg) in the elevated plus maze (EPM). Data represent the amount of time that mice spent on the open (a) and closed (b) arms of the EPM and analyzed by two-way ANOVA. *$p$ < 0.05, **$p$ < 0.01 versus wild-type mice conditioned with saline; +$p$ < 0.05 versus PACAP-deficient mice

genotype and treatment ($F_{(1,26)}$ = 2.12; $p$ = 0.157). Yet, there was a significant effect of treatment ($F_{(1,26)}$ = 5.15; $p$ = 0.031). Further analysis of the data showed that male wild-type mice conditioned with nicotine and challenged with mecamylamine before the EPM test spent a significantly ($p < 0.05$) lesser amount of time on the open arms compared to its wild-type saline-conditioned control mice as well as versus PACAP-deficient mice conditioned with nicotine (Figure 3a). Correspondingly, there was a significant effect of treatment ($F_{(1,26)}$ = 5.07; $p$ = 0.033) when the amount of time that mice spent in the closed arms was analyzed. The post hoc analysis of the data revealed that wild-type mice conditioned with nicotine spent more time on the closed arms compared to their saline pretreated wild-type controls ($p < 0.01$) as well as versus PACAP-deficient mice ($p < 0.05$) pretreated with nicotine (Figure 3b). In contrast, we did not observe any significant difference in anxiety-like behaviors in female mice of the two genotypes regardless of whether they were conditioned with nicotine or saline (Figure 4). Taken together, these results suggest that PACAP may be involved in anxiety-like behaviors that develop following nicotine withdrawal but there is a sex–PACAP interaction in this regard.

### 3.3 | Depression-like behaviors following nicotine withdrawal were reduced in male but not female mice lacking PACAP

Depression is another negative affective state that develops following nicotine withdrawal (Hughes et al., 1986). The immobility time in the FST is widely used as an animal model of despair, depression-like behaviors and, more recently, as a model of stress coping behavior (Commons, Cholanians, Babb, & Ehlinger, 2017; Petit-Demouliere et al., 2005). This model is also used to determine the development of depression-like behaviors following nicotine withdrawal (Bagosi et al., 2016). We used FST to determine the role of PACAP in depression-like behaviors following nicotine withdrawal. Figure 5 shows the amount of time that male mice lacking PACAP and their wild-type controls remained immobile during the last 4 min of the test session. Two-way ANOVA revealed no significant effect of genotype ($F_{(1,26)}$ = 0.63; $n$ = 7–8 mice of each sex per genotype and per treatment, $p$ = 0.435) but there was a significant interaction between genotype and treatment ($F_{(1,26)}$ = 5.03; $p < 0.034$) and trend toward a significant effect of treatment ($F_{(1,26)}$ = 4.09; $p$ = 0.053). The post hoc analysis of the data showed that male mice lacking PACAP conditioned with nicotine and underwent mecamylamine-precipitated nicotine withdrawal showed a significant decrease in the immobility time compared to their wild-type controls as well as their PACAP-deficient control mice (Figure 4a; $p < 0.05$). In contrast, female mice lacking PACAP and their controls were not different from each other regardless of whether they were conditioned with nicotine or saline (Figure 4b). These results suggest that male but not female PACAP-deficient mice treated with nicotine had a decreased level of depression-like behaviors following nicotine withdrawal compared to their wild-type controls.

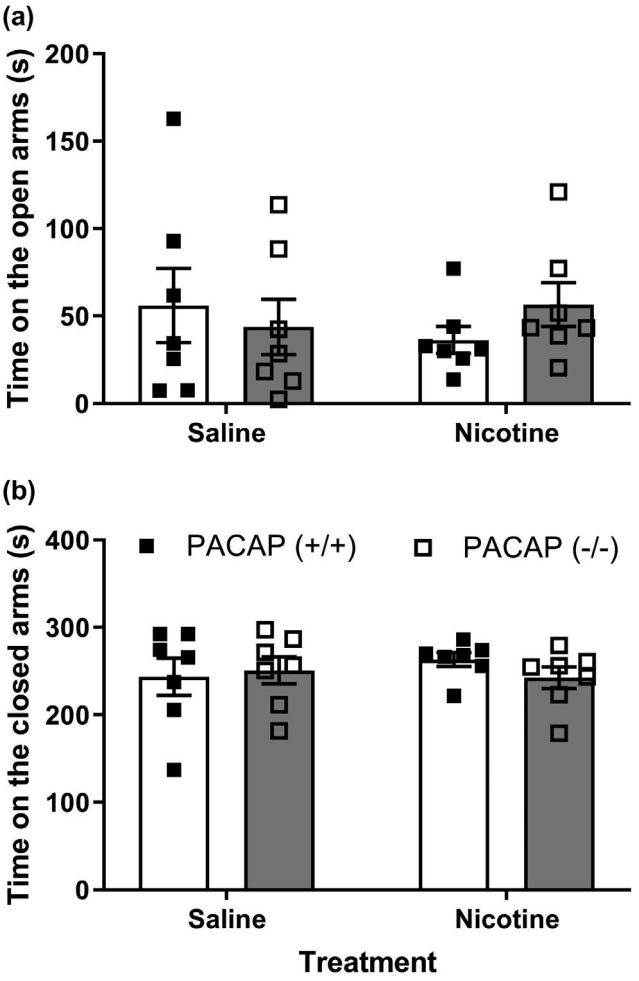


**FIGURE 4** Anxiety-like behaviors in female mice lacking PACAP [PACAP (−/−)] and their wild-type controls [PACAP (+/+)] conditioned with nicotine ($n$ = 7 mice per genotype) or saline ($n$ = 7 mice per genotype). Mice were tested for anxiety-like behaviors 30 min following a challenge dose of mecamylamine (3 mg/kg) in the elevated plus maze (EPM). Data represent the amount of time that mice spent on the open (a) and closed (b) arms of the EPM and analyzed by two-way ANOVA

## 4 | DISCUSSION

The main findings of the present study are that male mice lacking PACAP and their wild-type controls exhibited a comparable CPP response but the affective signs of nicotine withdrawal (i.e., anxiety- and depression-like behaviors) were reduced in male PACAP-deficient mice. In contrast, female mice lacking PACAP showed a blunted CPP response compared to their wild-type controls. However, anxiety- and depression-like behaviors were not different between female mice of the two genotypes. These changes were not observed in saline-treated control mice of the two genotypes. Together, our results suggest that endogenous PACAP is involved in negative affective signs of nicotine withdrawal, and there is a sex-related difference in this regard.

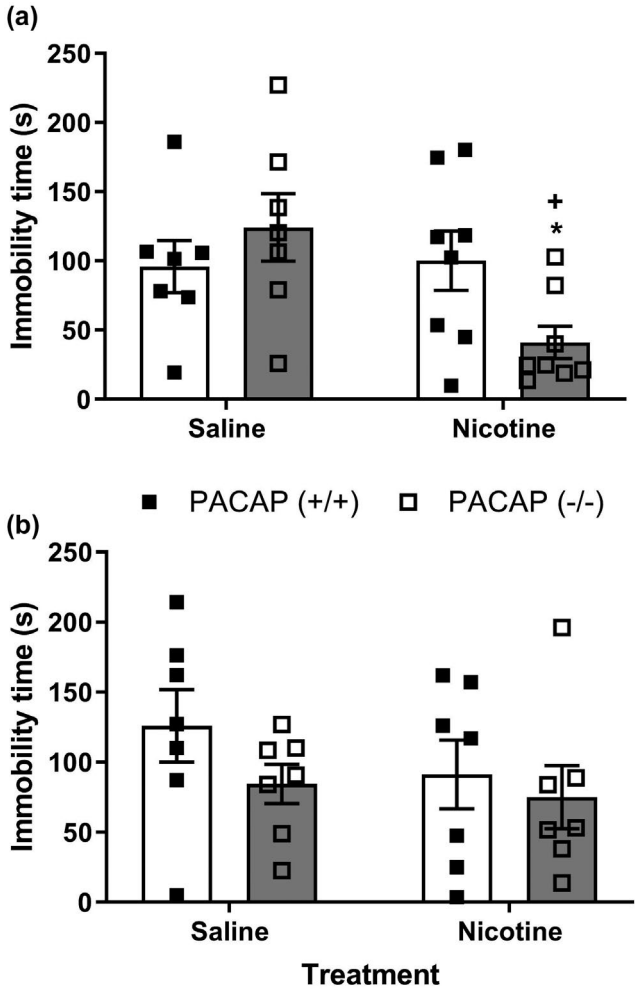


**FIGURE 5** Depression-like behaviors in male (a) and female (b) mice lacking PACAP [PACAP (−/−)] and their wild-type controls [PACAP (+/+)] conditioned with nicotine ($n$ = 7 mice per genotype and sex) or saline ($n$ = 7 mice per genotype and sex). Two hours after the EPM test, mice were forced to swim for 15 min and tested for immobility time in the forced swim test 24 hr later. Data represent the amount of time that mice remained immobile during the last 4 min of the 6-min test period and analyzed by two-way ANOVA. $^{*}p < 0.05$ versus male wild-type mice; $^{+}p < 0.05$ versus saline-pretreated control PACAP-deficient mice

PACAP and its receptors are expressed along the reward circuits and have been implicated in the actions of nicotine (Machaalani, Thawley, Huang, & Chen, 2019; Manavalan et al., 2017; Tseng et al., 2019). We have recently showed that female mice lacking PACAP and their wild-type controls exhibited comparable CPP following conditioning with a lower dose of nicotine (0.25 mg/kg). On the other hand, the aversive effect of a higher dose of nicotine (1 mg/kg) was blunted in female mice lacking PACAP (Tseng et al., 2019). In the present study, we observed a robust CPP response in male and female wild-type mice using the same dose of nicotine (1 mg/kg), which was only blunted in female mice lacking PACAP, suggesting that there may be a sex-related difference in the modulatory action of endogenous PACAP to regulate the rewarding action nicotine.

The aversion in female wild-type mice in our previous study (Tseng et al., 2019) appears in contrast with the CPP response in the present study. However, considering that we used an alternate-day conditioning (saline or nicotine once a day) paradigm in the present study and two conditionings per day (saline and nicotine on the same day) in the previous study, we argue the difference in the outcomes of the two studies may be due to the use of different CPP protocols. Indeed, we did not observe any CPP and even aversion in some wild-type mice following the second set of conditionings, suggesting that tolerance may have developed to the rewarding action of nicotine or the emergence of aversive response might have canceled the CPP response in the wild-type mice. Interestingly, the aversive response observed in some female and male wild-type mice following the second conditioning was absent in their respective mice lacking PACAP, showing that nicotine-induced aversion may have taken more time to develop using the once-daily conditioning paradigm in wild-type mice.

Cessation of nicotine leads to somatic and affective signs of withdrawal. One of the affective signs of nicotine withdrawal is the expression of anxiety in humans (Hughes et al., 1986) and laboratory animals (Bagosi et al., 2016; Damaj, Kao, & Martin, 2003; Elhassan, Bagdas, & Damaj, 2017; Hamouda, Jackson, Bagdas, & Imad Damaj, 2018; Jackson, Papke, & Damaj, 2018; Jackson, McIntosh, Brunzell, Sanjakdar, & Damaj, 2009; Jackson, Muldoon, De Biasi, & Damaj, 2015). Consistent with this notion, we found that male wild-type mice conditioned with nicotine and underwent precipitated withdrawal exhibited a significant increase in anxiety-like behaviors compared to nicotine-pretreated PACAP-deficient mice. Although mice lacking PACAP have been reported to exhibit reduced anxiety-like behaviors (Hashimoto et al., 2001; Hattori et al., 2012; Otto et al., 2001), we did not observe any significant change in the level of anxiety-like behaviors between saline-treated control mice of the two genotypes. Indeed, we found no difference in anxiety-like behaviors between saline-pretreated mice of the two genotypes regardless of sex, showing that the difference in anxiety-like behaviors following nicotine withdrawal was due to nicotine pretreatment and not due to baseline changes in these behaviors between mice lacking PACAP and their wild-type controls. Together, these results suggest that PACAP is involved in anxiety-like behaviors that develop following nicotine withdrawal but there is a sex-related difference in this response, as we found no significant change in anxiety-like behaviors between female mice of the two genotypes undergone nicotine withdrawal.

Depression is another negative affective state that develops following nicotine withdrawal in humans (Hughes et al., 1986) as well as in rodents (Bagosi et al., 2016; Damaj et al., 2003; Elhassan et al., 2017; Hamouda et al., 2018; Jackson et al., 2009, 2015, 2018). Several studies have reported a greater level of depression-like behaviors in mice lacking PACAP compared to their wild-type controls (Ago et al., 2013; Gaszner et al., 2012; Hashimoto et al., 2009, 2010) but this increase may be due to the strain of mouse used to generate the PACAP-deficient mice. For example, naïve PACAP-deficient mice express reduced anxiety-like

behaviors and higher depression-like behaviors ([Hashimoto et al., 2009, 2001]; for review, see also [Lutfy & Shankar, 2019]). Likewise, mice lacking PACAP on a C57BL/6J × 129SvEv mixed background showed decreased anxiety-like behaviors but slight reduction in depression-like behaviors (Hattori et al., 2012). In contrast, mice lacking PACAP on the C57BL/6N background strain exhibited no changes in anxiety-like or depression-like behaviors ([Lehmann, Mustafa, Eiden, Herkenham, & Eiden, 2013], for details see also [Farkas et al., 2017; Kormos et al., 2016]). Our results are consistent with the latter study showing no alterations in anxiety-like and depression-like behaviors in mice lacking PACAP compared to their wild-type controls regardless of the sex of the mice. Nevertheless, we observed a significant decrease in immobility time in male PACAP-deficient mice that underwent nicotine withdrawal compared to their respective wild-type controls. Yet, this difference was not observed in female mice of the two genotypes, suggesting there is a sex-related difference in this response. However, one of the caveats of the current research is that the results are obtained solely in mice lacking PACAP and their wild-type littermates/controls. Therefore, further research using pharmacological tools or other approaches is needed to provide complementary data to that obtained in knockout mice. Additionally, in the present study, we measured affective but not somatic signs of withdrawal and only 4 days after cessation of nicotine because we were interested in affective signs of withdrawal, the main cause of craving and relapse. Also, somatic signs of withdrawal decay and are minimal by this time. Therefore, further studies are needed to assess the role of PACAP in somatic and affective signs of withdrawal immediately after cessation of nicotine treatment.

The hypothalamic–pituitary–adrenal axis mediates the stress response and is implicated in nicotine withdrawal (Grieder et al., 2014; Semba et al., 2004). Considering that PACAP regulates the expression of corticotropic releasing hormone (CRH; Agarwal, Halvorson, & Legradi, 2005; Grinevich, Fournier, & Pelletier, 1997; Stroth & Eiden, 2010; Stroth, Holighaus, Ait-Ali, & Eiden, 2011; Stroth, Liu, Aguilera, & Eiden, 2011), we hypothesize that PACAP by regulating the expression of CRH alters the negative affective states associated with nicotine withdrawal. However, it is unclear why we did not observe any difference in affective signs of withdrawal in female mice. It may be that female do not exhibit robust signs of withdrawal as males, which has been found to be the case in adolescent rats (O'Dell & Torres, 2014). Alternatively, the expression of PACAP is under the influence of sex hormones (Apostolakis, Riherd, & O'Malley, 2005; King, Toufexis, & Hammack, 2017), which may have contributed to this sex-related difference.

Previous studies have implicated several brain regions in the pathophysiology of negative affective states that develop following nicotine withdrawal. Recent evidence implicates habenula in the action of nicotine and in particular in mediation of nicotine aversion and withdrawal (Antolin-Fontes, Ables, Gorlich, & Ibanez-Tallon, 2015; Casarrubea et al., 2015; Eggan & McCallum, 2016; Fowler & Kenny, 2014; Frahm et al., 2011, 2015; Gorlich et al., 2013; Lee, Kang, Chung, & Noh, 2015; Slimak et al., 2014; Zuo et al., 2016). Interestingly, moderate to high levels of PACAP and its receptors have been reported in habenula (Masuo et al., 1991; Vereczki et al., 2006), raising the possibility that PACAP may be involved in these actions of nicotine. Indeed, our earlier report showing that PACAP is involved in the aversive effects of nicotine (Tseng et al., 2019) prompted us to assess the role of endogenous PACAP in nicotine withdrawal. Thus, we propose habenula may be one of the neuroanatomical sites where endogenous PACAP exerts its regulatory action on nicotine withdrawal. However, female mice of the two genotypes did not show any change in negative affective signs of nicotine withdrawal, requiring further investigations to define the underlying mechanism of this sex-related difference and identify the neuronal circuits involved in this process. The bed nucleus of stria terminalis (BNST) may be another potential brain area which may be involved in this process. BNST not only shows characteristic sexual dimorphism (Hines, Davis, Coquelin, Goy, & Gorski, 1985) but also receives dense PACAP-ergic innervation (Hammack et al., 2010). Notably, nicotine self-administration affects neuronal activity in the BNST in responses to stress (Yu & Sharp, 2012). However, further studies are needed to tease out the underlying mechanism of this sexual dimorphic regulatory action of PACAP. For example, we need to examine if gonadectomy reverses this dichotomy or administration of sex hormones to the opposite gender would reverse the phenotype.

In summary, the rewarding effect of nicotine was altered only in female PACAP-deficient mice. In contrast, the negative affective states that develop following nicotine withdrawal were only affected in male mice lacking PACAP, suggesting that endogenous PACAP is involved in nicotine reward and dependence but there is a sex-related difference in this regard. The current result suggests that PACAP-ergic system may be a novel target to develop medications to treat negative affective states associated with nicotine withdrawal at least in men. However, the result of this study needs to be interpreted with caution because voluntary nicotine intake and withdrawal in humans is not the same as withdrawal precipitated by mecamylamine in mice treated with nicotine by an experimenter.

## DECLARATION OF TRANSPARENCY

The authors, reviewers and editors affirm that in accordance to the policies set by the *Journal of Neuroscience Research*, this manuscript presents an accurate and transparent account of the study being reported and that all critical details describing the methods and results are present.

### ACKNOWLEDGMENTS

The authors thank Ms. Kaitlin Rickerl and Ms. Neda Ibrahim for their assistance with the analysis of locomotor activity data. The present study was supported by a Tobacco-Related Disease Research Program (TRDRP) grant (24RT-0023H) and by a Western University of Health Sciences intramural grant to KL.

## CONFLICT OF INTEREST

The authors declare no potential sources of conflict of interest.

## AUTHOR CONTRIBUTIONS

All authors take responsibility for the integrity of the data and the accuracy of the data analysis. *Conceptualization*, K.L.; *Methodology*, K.L.; *Investigation*, S.N., P.M., and A.H.; *Validation*, S.N., A.H., and P.M.; *Formal Analysis*, S.M.A. and K.L.; *Writing - Original Draft*, S.N., S.M.A. and K.L.; *Writing - Review & Editing*, S.M.A. and K.L.; *Visualization*, S.M.A. and K.L.; *Supervision*, K.L; *Project Management*, K.L.: *Funding Acquisition*, K.L.

## DATA AVAILABILITY STATEMENT

The data that support the findings of the present study will be made available upon request from the corresponding author.

## ORCID

*Kabirullah Lutfy* https://orcid.org/0000-0003-0350-3693

## REFERENCES

Agarwal, A., Halvorson, L. M., & Legradi, G. (2005). Pituitary adenylate cyclase-activating polypeptide (PACAP) mimics neuroendocrine and behavioral manifestations of stress: Evidence for PKA-mediated expression of the corticotropin-releasing hormone (CRH) gene. *Brain Research. Molecular Brain Research*, *138*(1), 45–57. https://doi.org/10.1016/j.molbrainres.2005.03.016

Ago, Y., Hiramatsu, N., Ishihama, T., Hazama, K., Hayata-Takano, A., Shibasaki, Y., … Matsuda, T. (2013). The selective metabotropic glutamate 2/3 receptor agonist MGS0028 reverses psychomotor abnormalities and recognition memory deficits in mice lacking the pituitary adenylate cyclase-activating polypeptide. *Behavioural Pharmacology*, *24*(1), 74–77. https://doi.org/10.1097/FBP.0b013e32835cf3e5

Antolin-Fontes, B., Ables, J. L., Gorlich, A., & Ibanez-Tallon, I. (2015). The habenulo-interpeduncular pathway in nicotine aversion and withdrawal. *Neuropharmacology*, *96*(Pt B), 213–222. https://doi.org/10.1016/j.neuropharm.2014.11.019

Apostolakis, E. M., Riherd, D. N., & O'Malley, B. W. (2005). PAC1 receptors mediate pituitary adenylate cyclase-activating polypeptide- and progesterone-facilitated receptivity in female rats. *Molecular Endocrinology*, *19*(11), 2798–2811. https://doi.org/10.1210/me.2004-0387

Bagosi, Z., Palotai, M., Simon, B., Bokor, P., Buzás, A., Balangó, B., … Szabó, G. (2016). Selective CRF2 receptor agonists ameliorate the anxiety- and depression-like state developed during chronic nicotine treatment and consequent acute withdrawal in mice. *Brain Research*, *1652*, 21–29. https://doi.org/10.1016/j.brainres.2016.09.044

Bardo, M. T., & Bevins, R. A. (2000). Conditioned place preference: What does it add to our preclinical understanding of drug reward? *Psychopharmacology (Berl)*, *153*(1), 31–43. https://doi.org/10.1007/s002130000569

Can, A., Blackwell, R. A., Piantadosi, S. C., Dao, D. T., O'Donnell, K. C., & Gould, T. D. (2011). Antidepressant-like responses to lithium in genetically diverse mouse strains. *Genes, Brain, and Behavior*, *10*(4), 434–443. https://doi.org/10.1111/j.1601-183X.2011.00682.x

Can, A., Grahame, N. J., & Gould, T. D. (2012). Affect-related behaviors in mice selectively bred for high and low voluntary alcohol consumption. *Behavior Genetics*, *42*(2), 313–322. https://doi.org/10.1007/s10519-011-9505-y

Carmody, T. P., Brischetto, C. S., Matarazzo, J. D., O'Donnell, R. P., & Connor, W. E. (1985). Co-occurrent use of cigarettes, alcohol, and coffee in healthy, community-living men and women. *Health Psychology*, *4*(4), 323–335. https://doi.org/10.1037/0278-6133.4.4.323

Casarrubea, M., Davies, C., Faulisi, F., Pierucci, M., Colangeli, R., Partridge, L., … Di Giovanni, G. (2015). Acute nicotine induces anxiety and disrupts temporal pattern organization of rat exploratory behavior in hole-board: A potential role for the lateral habenula. *Frontiers in Cellular Neuroscience*, *9*, 197. https://doi.org/10.3389/fncel.2015.00197

Colwell, C. S., Michel, S., Itri, J., Rodriguez, W., Tam, J., Lelièvre, V., … Waschek, J. A. (2004). Selective deficits in the circadian light response in mice lacking PACAP. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology*, *287*(5), R1194–R1201. https://doi.org/10.1152/ajpregu.00268.2004

Commons, K. G., Cholanians, A. B., Babb, J. A., & Ehlinger, D. G. (2017). The rodent forced swim test measures stress-coping strategy, not depression-like behavior. *ACS Chemical Neuroscience*, *8*(5), 955–960. https://doi.org/10.1021/acschemneuro.7b00042

Damaj, M. I., Kao, W., & Martin, B. R. (2003). Characterization of spontaneous and precipitated nicotine withdrawal in the mouse. *Journal of Pharmacology and Experimental Therapeutics*, *307*(2), 526–534. https://doi.org/10.1124/jpet.103.054908

David, D. J., Renard, C. E., Jolliet, P., Hascoet, M., & Bourin, M. (2003). Antidepressant-like effects in various mice strains in the forced swimming test. *Psychopharmacology (Berl)*, *166*(4), 373–382. https://doi.org/10.1007/s00213-002-1335-4

DiFranza, J. R., & Guerrera, M. P. (1990). Alcoholism and smoking. *Journal of Studies on Alcohol*, *51*(2), 130–135. https://doi.org/10.15288/jsa.1990.51.130

Doherty, K., Kinnunen, T., Militello, F. S., & Garvey, A. J. (1995). Urges to smoke during the first month of abstinence: Relationship to relapse and predictors. *Psychopharmacology (Berl)*, *119*(2), 171–178. https://doi.org/10.1007/BF02246158

Eggan, B. L., & McCallum, S. E. (2016). 18-Methoxycoronaridine acts in the medial habenula to attenuate behavioral and neurochemical sensitization to nicotine. *Behavioral Brain Research*, *307*, 186–193. https://doi.org/10.1016/j.bbr.2016.04.008

Elhassan, S., Bagdas, D., & Damaj, M. I. (2017). Effects of nicotine metabolites on nicotine withdrawal behaviors in mice. *Nicotine & Tobacco Research*, *19*(6), 763–766. https://doi.org/10.1093/ntr/ntx045

Farkas, J., Kovács, L. Á., Gáspár, L., Nafz, A., Gaszner, T., Ujvári, B., … Gaszner, B. (2017). Construct and face validity of a new model for the three-hit theory of depression using PACAP mutant mice on CD1 background. *Neuroscience*, *354*, 11–29. https://doi.org/10.1016/j.neuroscience.2017.04.019

Fiore, M. C., Schroeder, S. A., & Baker, T. B. (2014). Smoke, the chief killer—Strategies for targeting combustible tobacco use. *New England Journal of Medicine*, *370*(4), 297–299. https://doi.org/10.1056/NEJMp1314942

Fowler, C. D., & Kenny, P. J. (2014). Nicotine aversion: Neurobiological mechanisms and relevance to tobacco dependence vulnerability. *Neuropharmacology*, *76 Pt B*, 533–544. https://doi.org/10.1016/j.neuropharm.2013.09.008

Frahm, S., Antolin-Fontes, B., Gorlich, A., Zander, J. F., Ahnert-Hilger, G., & Ibanez-Tallon, I. (2015). An essential role of acetylcholine-glutamate synergy at habenular synapses in nicotine dependence. *Elife*, *4*, e11396. https://doi.org/10.7554/eLife.11396

Frahm, S., Slimak, M. A., Ferrarese, L., Santos-Torres, J., Antolin-Fontes, B., Auer, S., … Ibanez-Tallon, I. (2011). Aversion to nicotine is regulated by the balanced activity of beta4 and alpha5 nicotinic receptor subunits in the medial habenula. *Neuron*, *70*(3), 522–535. https://doi.org/10.1016/j.neuron.2011.04.013

Gaszner, B., Kormos, V., Kozicz, T., Hashimoto, H., Reglodi, D., & Helyes, Z. (2012). The behavioral phenotype of pituitary adenylate-cyclase activating polypeptide-deficient mice in anxiety and depression tests is accompanied by blunted c-Fos expression in the bed nucleus of

the stria terminalis, central projecting Edinger-Westphal nucleus, ventral lateral septum, and dorsal raphe nucleus. *Neuroscience*, *202*, 283–299. https://doi.org/10.1016/j.neuroscience.2011.11.046

George, O., Ghozland, S., Azar, M. R., Cottone, P., Zorrilla, E. P., Parsons, L. H., … Koob, G. F. (2007). CRF-CRF1 system activation mediates withdrawal-induced increases in nicotine self-administration in nicotine-dependent rats. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(43), 17198–17203. https://doi.org/10.1073/pnas.0707585104

Ghatei, M. A., Takahashi, K., Suzuki, Y., Gardiner, J., Jones, P. M., & Bloom, S. R. (1993). Distribution, molecular characterization of pituitary adenylate cyclase-activating polypeptide and its precursor encoding messenger RNA in human and rat tissues. *Journal of Endocrinology*, *136*(1), 159–166. https://doi.org/10.1677/joe.0.1360159

Gorlich, A., Antolin-Fontes, B., Ables, J. L., Frahm, S., Slimak, M. A., Dougherty, J. D., & Ibanez-Tallon, I. (2013). Reexposure to nicotine during withdrawal increases the pacemaking activity of cholinergic habenular neurons. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(42), 17077–17082. https://doi.org/10.1073/pnas.1313103110

Grieder, T. E., Herman, M. A., Contet, C., Tan, L. A., Vargas-Perez, H., Cohen, A., … George, O. (2014). VTA CRF neurons mediate the aversive effects of nicotine withdrawal and promote intake escalation. *Nature Neuroscience*, *17*(12), 1751–1758. https://doi.org/10.1038/nn.3872

Grinevich, V., Fournier, A., & Pelletier, G. (1997). Effects of pituitary adenylate cyclase-activating polypeptide (PACAP) on corticotropin-releasing hormone (CRH) gene expression in the rat hypothalamic paraventricular nucleus. *Brain Research*, *773*(1–2), 190–196. https://doi.org/10.1016/S0006-8993(97)01011-1

Hammack, S. E., Roman, C. W., Lezak, K. R., Kocho-Shellenberg, M., Grimmig, B., Falls, W. A., … May, V. (2010). Roles for pituitary adenylate cyclase-activating peptide (PACAP) expression and signaling in the bed nucleus of the stria terminalis (BNST) in mediating the behavioral consequences of chronic stress. *Journal of Molecular Neuroscience*, *42*(3), 327–340. https://doi.org/10.1007/s12031-010-9364-7

Hamouda, A. K., Jackson, A., Bagdas, D., & Imad Damaj, M. (2018). Reversal of nicotine withdrawal signs through positive allosteric modulation of alpha4beta2 nicotinic acetylcholine receptors in male mice. *Nicotine & Tobacco Research*, *20*(7), 903–907. https://doi.org/10.1093/ntr/ntx183

Hashimoto, H., Hashimoto, R., Shintani, N., Tanaka, K., Yamamoto, A., Hatanaka, M., … Baba, A. (2009). Depression-like behavior in the forced swimming test in PACAP-deficient mice: Amelioration by the atypical antipsychotic risperidone. *Journal of Neurochemistry*, *110*(2), 595–602. https://doi.org/10.1111/j.1471-4159.2009.06168.x

Hashimoto, H., Shintani, N., Tanaka, K., Mori, W., Hirose, M., Matsuda, T., … Baba, A. (2001). Altered psychomotor behaviors in mice lacking pituitary adenylate cyclase-activating polypeptide (PACAP). *Proceedings of the National Academy of Sciences of the United States of America*, *98*(23), 13355–13360. https://doi.org/10.1073/pnas.231094498

Hashimoto, R., Hashimoto, H., Shintani, N., Ohi, K., Hori, H., Saitoh, O., … Kunugi, H. (2010). Possible association between the pituitary adenylate cyclase-activating polypeptide (PACAP) gene and major depressive disorder. *Neuroscience Letters*, *468*(3), 300–302. https://doi.org/10.1016/j.neulet.2009.11.019

Hattori, S., Takao, K., Tanda, K., Toyama, K., Shintani, N., Baba, A., … Miyakawa, T. (2012). Comprehensive behavioral analysis of pituitary adenylate cyclase-activating polypeptide (PACAP) knockout mice. *Frontiers in Behavioural Neurosciences*, *6*, 58. https://doi.org/10.3389/fnbeh.2012.00058

Hines, M., Davis, F. C., Coquelin, A., Goy, R. W., & Gorski, R. A. (1985). Sexually dimorphic regions in the medial preoptic area and the bed nucleus of the stria terminalis of the guinea pig brain: A description and an investigation of their relationship to gonadal steroids in adulthood. *Journal of Neuroscience*, *5*(1), 40–47. https://doi.org/10.1523/JNEUROSCI.05-01-00040.1985

Hughes, J. R., & Hatsukami, D. (1986). Signs and symptoms of tobacco withdrawal. *Archives of General Psychiatry*, *43*(3), 289–294. https://doi.org/10.1001/archpsyc.1986.01800030107013

Hughes, J. R., Hatsukami, D. K., Mitchell, J. E., & Dahlgren, L. A. (1986). Prevalence of smoking among psychiatric outpatients. *American Journal of Psychiatry*, *143*(8), 993–997. https://doi.org/10.1176/ajp.143.8.993

Hunt, W. A., Barnett, L. W., & Branch, L. G. (1971). Relapse rates in addiction programs. *Journal of Clinical Psychology*, *27*(4), 455–456. https://doi.org/10.1002/1097-4679(197110)27:4<455:AID-JCLP2270270412>3.0.CO;2-R

Isola, R., Vogelsberg, V., Wemlinger, T. A., Neff, N. H., & Hadjiconstantinou, M. (1999). Nicotine abstinence in the mouse. *Brain Research*, *850*(1–2), 189–196. https://doi.org/10.1016/S0006-8993(99)02131-9

Jackson, A., Papke, R. L., & Damaj, M. I. (2018). Pharmacological modulation of the alpha7 nicotinic acetylcholine receptor in a mouse model of mecamylamine-precipitated nicotine withdrawal. *Psychopharmacology (Berl)*, *235*(7), 1897–1905. https://doi.org/10.1007/s00213-018-4879-7

Jackson, K. J., McIntosh, J. M., Brunzell, D. H., Sanjakdar, S. S., & Damaj, M. I. (2009). The role of alpha6-containing nicotinic acetylcholine receptors in nicotine reward and withdrawal. *Journal of Pharmacology and Experimental Therapeutics*, *331*(2), 547–554. https://doi.org/10.1124/jpet.109.155457

Jackson, K. J., Muldoon, P. P., De Biasi, M., & Damaj, M. I. (2015). New mechanisms and perspectives in nicotine withdrawal. *Neuropharmacology*, *96*(Pt B), 223–234. https://doi.org/10.1016/j.neuropharm.2014.11.009

Kenny, P. J., & Markou, A. (2001). Neurobiology of the nicotine withdrawal syndrome. *Pharmacology, Biochemistry and Behavior*, *70*(4), 531–549. https://doi.org/10.1016/S0091-3057(01)00651-7

King, S. B., Toufexis, D. J., & Hammack, S. E. (2017). Pituitary adenylate cyclase activating polypeptide (PACAP), stress, and sex hormones. *Stress*, *20*(5), 465–475. https://doi.org/10.1080/10253890.2017.1336535

Kormos, V., Gáspár, L., Kovács, L. Á., Farkas, J., Gaszner, T., Csernus, V., … Gaszner, B. (2016). Reduced response to chronic mild stress in PACAP mutant mice is associated with blunted FosB expression in limbic forebrain and brainstem centers. *Neuroscience*, *330*, 335–358. https://doi.org/10.1016/j.neuroscience.2016.06.004

Lee, H., Kang, M. S., Chung, J. M., & Noh, J. (2015). Repeated nicotine exposure in adolescent rats: Reduction of medial habenular activity and augmentation of nicotine preference. *Physiology & Behavior*, *138*, 345–350. https://doi.org/10.1016/j.physbeh.2014.11.034

Lehmann, M. L., Mustafa, T., Eiden, A. M., Herkenham, M., & Eiden, L. E. (2013). PACAP-deficient mice show attenuated corticosterone secretion and fail to develop depressive behavior during chronic social defeat stress. *Psychoneuroendocrinology*, *38*(5), 702–715. https://doi.org/10.1016/j.psyneuen.2012.09.006

Lister, R. G. (1990). Ethologically-based animal models of anxiety disorders. *Pharmacology & Therapeutics*, *46*(3), 321–340. https://doi.org/10.1016/0163-7258(90)90021-S

Lutfy, K., & Shankar, G. (2019). Emerging evidence for the role of pituitary adenylate cyclase-activating peptide in neuropsychiatric disorders. *Progress in Molecular Biology and Translational Science*, *167*, 143–157. https://doi.org/10.1016/bs.pmbts.2019.06.009

Machaalani, R., Thawley, M., Huang, J., & Chen, H. (2019). Effects of prenatal cigarette smoke exposure on BDNF, PACAP, microglia and gliosis expression in the young male mouse brainstem. *Neurotoxicology*, *74*, 40–46. https://doi.org/10.1016/j.neuro.2019.05.009

Mackowick, K. M., Heishman, S. J., Wehring, H. J., Liu, F., McMahon, R. P., & Kelly, D. L. (2012). Illicit drug use in heavy smokers with and

without schizophrenia. *Schizophrenia Research*, *139*(1–3), 194–200. https://doi.org/10.1016/j.schres.2012.04.012

Mackowick, K. M., Lynch, M. J., Weinberger, A. H., & George, T. P. (2012). Treatment of tobacco dependence in people with mental health and addictive disorders. *Current Psychiatry Reports*, *14*(5), 478–485. https://doi.org/10.1007/s11920-012-0299-2

Manavalan, S., Getachew, B., Manaye, K. F., Khundmiri, S. J., Csoka, A. B., McKinley, R., … Tizabi, Y. (2017). PACAP protects against ethanol and nicotine toxicity in SH-SY5Y cells: Implications for drinking-smoking co-morbidity. *Neurotoxicity Research*, *32*(1), 8–13. https://doi.org/10.1007/s12640-017-9727-8

Masuo, Y., Ohtaki, T., Masuda, Y., Nagai, Y., Suno, M., Tsuda, M., & Fujino, M. (1991). Autoradiographic distribution of pituitary adenylate cyclase activating polypeptide (PACAP) binding sites in the rat brain. *Neuroscience Letters*, *126*(2), 103–106. https://doi.org/10.1016/0304-3940(91)90529-3

Miller, N. S., & Gold, M. S. (1998). Comorbid cigarette and alcohol addiction: Epidemiology and treatment. *Journal of Addictive Diseases*, *17*(1), 55–66. https://doi.org/10.1300/J069v17n01_06

Miyata, A., Arimura, A., Dahl, R. R., Minamino, N., Uehara, A., Jiang, L., … Coy, D. H. (1989). Isolation of a novel 38 residue-hypothalamic polypeptide which stimulates adenylate cyclase in pituitary cells. *Biochemical and Biophysical Research Communications*, *164*(1), 567–574. https://doi.org/10.1016/0006-291X(89)91757-9

O'Dell, L. E., & Torres, O. V. (2014). A mechanistic hypothesis of the factors that enhance vulnerability to nicotine use in females. *Neuropharmacology*, *76 Pt B*, 566–580. https://doi.org/10.1016/j.neuropharm.2013.04.055

Oslin, D., Liberto, J. G., O'Brien, J., Krois, S., & Norbeck, J. (1997). Naltrexone as an adjunctive treatment for older patients with alcohol dependence. *American Journal of Geriatric Psychiatry*, *5*(4), 324–332. https://doi.org/10.1097/00019442-199700540-00007

Otto, C., Martin, M., Wolfer, D. P., Lipp, H. P., Maldonado, R., & Schutz, G. (2001). Altered emotional behavior in PACAP-type-I-receptor-deficient mice. *Brain Research. Molecular Brain Research*, *92*(1–2), 78–84. https://doi.org/10.1016/s0169-328x(01)00153-x

Petit-Demouliere, B., Chenu, F., & Bourin, M. (2005). Forced swimming test in mice: A review of antidepressant activity. *Psychopharmacology (Berl)*, *177*(3), 245–255. https://doi.org/10.1007/s00213-004-2048-7

Picciotto, M. R., Brunzell, D. H., & Caldarone, B. J. (2002). Effect of nicotine and nicotinic receptors on anxiety and depression. *NeuroReport*, *13*(9), 1097–1106. https://doi.org/10.1097/00001756-200207020-00006

Picciotto, M. R., & Zoli, M. (2002). Nicotinic receptors in aging and dementia. *Journal of Neurobiology*, *53*(4), 641–655. https://doi.org/10.1002/neu.10102

Porsolt, R. D. (1979). Animal model of depression. *Biomedicine*, *30*(3), 139–140.

Porsolt, R. D., Bertin, A., & Jalfre, M. (1977). Behavioral despair in mice: A primary screening test for antidepressants. *Archives Internationales de Pharmacodynamie et de Therapie*, *229*(2), 327–336.

Reglodi, D., Kiss, P., Horvath, G., Lubics, A., Laszlo, E., Tamas, A., … Szakaly, P. (2012). Effects of pituitary adenylate cyclase activating polypeptide in the urinary system, with special emphasis on its protective effects in the kidney. *Neuropeptides*, *46*(2), 61–70. https://doi.org/10.1016/j.npep.2011.05.001

Reglodi, D., Kiss, P., Szabadfi, K., Atlasz, T., Gabriel, R., Horvath, G., … Tamas, A. (2012). PACAP is an endogenous protective factor-insights from PACAP-deficient mice. *Journal of Molecular Neuroscience*, *48*(3), 482–492. https://doi.org/10.1007/s12031-012-9762-0

Reglodi, D., Tamas, A., Koppan, M., Szogyi, D., & Welke, L. (2012). Role of PACAP in female fertility and reproduction at gonadal level—Recent advances. *Frontiers in Endocrinology*, *3*, 155. https://doi.org/10.3389/fendo.2012.00155

Rimm, E. B., Chan, J., Stampfer, M. J., Colditz, G. A., & Willett, W. C. (1995). Prospective study of cigarette smoking, alcohol use, and the risk of diabetes in men. *BMJ*, *310*(6979), 555–559.

Semba, J., Wakuta, M., Maeda, J., & Suhara, T. (2004). Nicotine withdrawal induces subsensitivity of hypothalamic-pituitary-adrenal axis to stress in rats: Implications for precipitation of depression during smoking cessation. *Psychoneuroendocrinology*, *29*(2), 215–226. https://doi.org/10.1016/S0306-4530(03)00024-6

Slimak, M. A., Ables, J. L., Frahm, S., Antolin-Fontes, B., Santos-Torres, J., Moretti, M., … Ibanez-Tallon, I. (2014). Habenular expression of rare missense variants of the beta4 nicotinic receptor subunit alters nicotine consumption. *Frontiers in Human Neuroscience*, *8*, 12. https://doi.org/10.3389/fnhum.2014.00012

Stroth, N., & Eiden, L. E. (2010). Stress hormone synthesis in mouse hypothalamus and adrenal gland triggered by restraint is dependent on pituitary adenylate cyclase-activating polypeptide signaling. *Neuroscience*, *165*(4), 1025–1030. https://doi.org/10.1016/j.neuroscience.2009.11.023

Stroth, N., Holighaus, Y., Ait-Ali, D., & Eiden, L. E. (2011). PACAP: A master regulator of neuroendocrine stress circuits and the cellular stress response. *Annals of the New York Academy of Sciences*, *1220*, 49–59. https://doi.org/10.1111/j.1749-6632.2011.05904.x

Stroth, N., Liu, Y., Aguilera, G., & Eiden, L. E. (2011). Pituitary adenylate cyclase-activating polypeptide controls stimulus-transcription coupling in the hypothalamic-pituitary-adrenal axis to mediate sustained hormone secretion during stress. *Journal of Neuroendocrinology*, *23*(10), 944–955. https://doi.org/10.1111/j.1365-2826.2011.02202.x

Tseng, A., Singh, P., Marquez, P., Hamid, A., & Lutfy, K. (2019). The role of endogenous pituitary adenylyl cyclase activating polypeptide (PACAP) in nicotine self-administration, reward and aversion. *Pharmacology, Biochemistry and Behavior*, *181*, 46–52. https://doi.org/10.1016/j.pbb.2019.04.007

Vaudry, D., Falluel-Morel, A., Bourgault, S., Basille, M., Burel, D., Wurtz, O., … Vaudry, H. (2009). Pituitary adenylate cyclase-activating polypeptide and its receptors: 20 years after the discovery. *Pharmacological Reviews*, *61*(3), 283–357. https://doi.org/10.1124/pr.109.001370

Vereczki, V., Koves, K., Csaki, A., Grosz, K., Hoffman, G. E., & Fiskum, G. (2006). Distribution of hypothalamic, hippocampal and other limbic peptidergic neuronal cell bodies giving rise to retinopetal fibers: Anterograde and retrograde tracing and neuropeptide immunohistochemical studies. *Neuroscience*, *140*(3), 1089–1100. https://doi.org/10.1016/j.neuroscience.2006.02.081

Yu, G., & Sharp, B. M. (2012). Nicotine modulates multiple regions in the limbic stress network regulating activation of hypophysiotrophic neurons in hypothalamic paraventricular nucleus. *Journal of Neurochemistry*, *122*(3), 628–640. https://doi.org/10.1111/j.1471-4159.2012.07785.x

Zuo, W., Xiao, C., Gao, M., Hopf, F. W., Krnjević, K., McIntosh, J. M., … Ye, J.-H. (2016). Nicotine regulates activity of lateral habenula neurons via presynaptic and postsynaptic mechanisms. *Scientific Reports*, *6*, 32937. https://doi.org/10.1038/srep32937

## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

**FIGURE S1** Dose-response effect of nicotine in inducing conditioned place preference (CPP) in male C57BL/6J mice. Mice were tested for baseline place preference on day 1 (D1, conditioned with saline

or nicotine (0.25 or 1 mg/kg, s.c.) for 15 min for six days and then tested for CPP on day 8 (D8). Data are mean (±*SEM*) of the amount of time that mice spent in each chamber on D1 and D8. ***$p < 0.001$ vs. VPCh on that day

**FIGURE S2** Distance traveled the first (days 2 and 3, D2/3) and last (days 6 and 7, D6/7) two conditioning days in female mice lacking PACAP (-/-) and their wild-type controls (+/+) conditioned with saline in both conditioning chambers (upper panel) or conditioned with nicotine in one chamber (DPCh) and saline in the other conditioning chamber (VPCh) (lower panel). Data are mean (±*SEM*) of distance traveled by mice of time in the VPCh and DPCh chambers on D2/3 and D6/7. *$p < 0.05$, ***$p < 0.001$ decreased distance traveled by the mice in the DPCh vs. their respective VPCh

Supplemental Data

Transparent Peer Review Report

Transparent Science Questionnaire for Authors

**How to cite this article:** NegaS, Marquez P, Hamid A, Ahmad SM, Lutfy K. The role of pituitary adenylyl cyclase activating polypeptide in affective signs of nicotine withdrawal. *J Neurosci Res*. 2020;98:1549–1560. https://doi.org/10.1002/jnr.24649