REGULAR RESEARCH ARTICLE

Nicotine-Induced Conditional Place Preference Is Affected by Head Injury: Correlation with Dopamine Release in the Nucleus Accumbens Shell

Yuan-Hao Chen, MD, PhD; Tung-Tai Kuo, MA; Eagle Yi-Kung Huang, PhD; Barry J. Hoffer, MD, PhD; Jen-Hsin Kao, PhD; Yu-Ching Chou, PhD; Yung-Hsiao Chiang, MD, PhD; Jonathan Miller, MD

Department of Neurological Surgery, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan, R.O.C (Dr Chen, Kuo, Dr Kao), Graduate Institute of Computer and Communication Engineering, National Taipei University of Technology, Taipei, Taiwan, R.O.C. (Kuo); Department of Pharmacology, National Defense Medical Center, Taipei, Taiwan, R.O.C. (Drs Huang and Kuo); Department of Neurosurgery, Case Western Reserve University School of Medicine, Cleveland, Ohio (Drs Hoffer and Miller), School of Public Health, National Defense Medical Center, Taipei, Taiwan, R.O.C. (Dr Chou); Graduate Program on Neuroregeneration, Taipei Medical University, Taipei, Taiwan, R.O.C. (Dr Chiang).

Correspondence: Yuan-Hao Chen, MD, PhD, 4F, No. 325, 2nd Sec., Cheng-Kung Rd., Neihu Dist., Taipei City, 114, Taiwan, R.O.C. (chenyh178@gmail.com).

Abstract

Background: Traumatic brain injury is known to impact dopamine-mediated reward pathways, but the underlying mechanisms have not been fully established.

Methods: Nicotine-induced conditional place preference was used to study rats exposed to a 6-psi fluid percussion injury with and without prior exposure to nicotine. Preference was quantified as a score defined as (C1 − C2) / (C1 + C2), where C1 is time in the nicotine-paired compartment and C2 is time in the saline-paired compartment. Subsequent fast-scan cyclic voltammetry was used to analyze the impact of nicotine infusion on dopamine release in the shell portion of the nucleus accumbens. To further determine the influence of brain injury on nicotine withdrawal, nicotine infusion was administered to the rats after fluid percussion injury. The effects of fluid percussion injury on conditional place preference after prior exposure to nicotine and abstinence or withdrawal from nicotine were also assessed.

Results: After traumatic brain injury, dopamine release was reduced in the nucleus accumbens shell, and nicotine-induced conditional place preference was significantly impaired. Preference scores of control, sham-injured, and fluid percussion injury groups were 0.1627 ± 0.04204, 0.1515 ± 0.03806, and -0.001300 ± 0.04286, respectively. Nicotine-induced conditional place preference was also seen in animals after nicotine pretreatment, with a conditional place preference score of 0.07805 ± 0.02838. Nicotine preexposure substantially increased tonic dopamine release in sham-injured animals, but it did not change phasic release; nicotine exposure after fluid percussion injury enhanced phasic release, though not to the same levels seen in sham-injured rats. Conditioned preference was related not only to phasic dopamine release (r = 0.8110) but also to the difference between tonic and phasic dopamine levels (r = 0.9521).

Conclusions: Traumatic brain injury suppresses dopamine release from the shell portion of the nucleus accumbens, which in turn significantly alters reward-seeking behavior. These results have important implications for tobacco and drug use after traumatic brain injury.
**Introduction**

Traumatic brain injury (TBI) is often associated with chronic deficits that are not always functionally related to the trauma site (Huang et al., 2014b; Chen et al., 2017a, 2017b). Although widespread processing deficits have been reported, there is evidence that certain physiological networks are uniquely vulnerable to TBI. In particular, alterations in reward processing have been reported, evidenced by increases in tobacco abuse after TBI in both addicts and tobacco-naïve individuals (Ilie et al., 2015a, 2015b). This suggests that mesolimbic-mesocortical dopaminergic reward pathways are affected. We have previously demonstrated that TBI is associated with substantial chronic alterations in nigrostriatal dopamine dynamics, suppressing release and reuptake both ipsilateral and contralateral to the injury (Huang et al., 2014b; Chen et al., 2015). However, the influence of TBI on the mesolimbic-mesocortical dopaminergic pathways is not currently well understood.

Dopamine is a neurotransmitter known to be related to reward (Hoebel, 1985) and is critical for reinforcing effects (Saunders et al., 2013; Vicente et al., 2016) that promote self-administration of drugs of abuse (Di Chiara et al., 2004) as well as compulsions such as eating (Hoebel, 1985; Nestler, 2005). Moreover, dopaminergic neurons in the ventral tegmental area, projecting to the shell of the nucleus accumbens (NAc), play crucial roles in perceptions, which are critical for substance-induced rewards (Benowitz, 2010). Dopaminergic neurons are thought to operate in 2 distinct patterns (Grace, 2000). Tonic firing typically occurs at low frequencies (1–5 Hz) and are related to the basal steady-state concentration of DA in the extracellular compartment. Tonic release can produce a basal input to dopamine receptors, especially to D2 receptors in the mesolimbic system, which includes the NAc (Grace, 1991; Dreyer et al., 2010). In contrast, midbrain dopamine neurons can fire in high-frequency bursts (>20 Hz), referred to as phasic firing, occurring when animals are presented with motivationally salient stimuli that predict drug or rewarding substance availability. This phasic release produces transient increases in dopamine concentration in the NAc that are sufficient to occupy low-affinity dopamine D1 receptors (Phillips et al., 2003; Dreyer et al., 2010). Nicotine elicits dopamine release in the mesolimbic area, the corpus striatum, and the frontal cortex (Brody, 2006), and lesioning the dopaminergic system blocks self-administration of nicotine in rats (Leikola-Pelho and Jackson, 1992). By stimulating nicotinic cholinergic receptors, nicotine augments the release of many neurotransmitters, including dopamine (Wonnacott, 1997; Dajas-Bailador and Wonnacott, 2004). Previous data have shown that prolonging reuptake in the NAc shell can alter the peak level of dopamine induced by burst stimulation (Chen et al., 2017b). Therefore, we hypothesized that TBI-induced suppression of dopamine release in the NAc affects reward processing. In this study, these mechanisms were evaluated, and this hypothesis was tested using animal behavioral models of nicotine-induced conditioned place preference (CPP) and neurochemical analyses.

**Methods**

**Animals**

Adult male Sprague–Dawley rats aged 5 weeks (LASCO Taiwan Co., Ltd.) were used according to procedures that were reviewed and approved by the National Defense Medical Center Animal Care and Use Committee (IACUC protocol no. 16–258). Animals were provided food and water ad libitum and were housed in a 12-h-light/-dark cycle room.

**Nicotine-Induced Conditional Place Preference**

Nicotine hydrogen tartrate was purchased from Sigma-Aldrich, diluted in 0.9% saline, and administered s.c. at a dose of 0.4 mg/kg (Le Foll and Goldberg, 2005). A solution volume of 0.9% saline was equivalent to the volume of nicotine administered.

Conditioned place preference (CPP) tests were used to determine the rewarding effects of nicotine, employing a test apparatus consisting of 3 chambers separated by removable doors, as described elsewhere (Huang et al., 2003) and calculated as a preference score.

Test time for determining place preference was 15 minutes. During a 16-day conditioning period, rats were given saline on odd days and nicotine on even days. Conditioning time was 40 minutes. After saline or nicotine administration, the rat was placed in the corresponding compartment. Place preference was defined as the proportion of this time that a rat remained in the nicotine-paired compartment than in the saline-paired compartment (Huang et al., 2003). If place preference exceeded 60 seconds before the 16-day conditioning period ended, the rat was eliminated from the study. The CPP test was applied in 3 experiments as described below (Figure 1).

**Experiment 1: Nicotine-Induced CPP Test**

To determine whether fluid percussion injury (FPI) affects nicotine-induced CPP and whether preexposure nicotine effects were similar to patients with tobacco use who suffered from brain injury. The animals were separated into 5 groups: (A) CPP group; the rats that only received nicotine-induced CPP training and tests; (B) Sham+CPP; the rats that received a sham operation...
Chen et al. | 951

Experiment 1: Conditioned Place Preference (CPP) Study

The animals were divided into five groups: (A) CPP group, the rats that only received CPP training and tests; (B) Sham+CPP group, the rats that received a sham operation and then CPP tests; (C) FPI+CPP group: the rats received 6 Psi fluid percussion injury (FPI) and then CPP tests; (D) Pre-Nic+CPP group, the rats received 1 week pump implantation releasing nicotine without FPI and then received CPP tests; and (E) Pre-Nic+FPI+CPP group: the rats received nicotine exposure by pump implantation for 1 week and then received FPI followed by CPP training and tests to determine if CPP formation could be altered by nicotine exposure.

Experiment 2: Electrochemistry Study of Dopamine Release

To determine dopamine release changes before and after nicotine-induced CPP in each group, we performed fast scan cyclic voltammetry (FSCV) in the animals before and after nicotine-induced CPP training in each of the above groups. Dopamine releases were collected under single pulse stimulation (10 pulses <25 Hz) to mimic tonic release, whereas dopamine release evoked by burst stimulation (10 pulses <25 Hz) was used to mimic phasic release. Subsequently, tonic and phasic release and differences between tonic and phasic release were calculated.

Experiment 3: The effect of 6 Psi FPI on nicotine withdrawal signs

The rats were divided into three groups: (1) Saline-infusion, rats infused with saline for 7 days; (2) Nic-infusion, rats infused with nicotine (9 mg/kg/d) for 7 days; and (3) Nic-infusion+FPI group, rats infused with nicotine (9 mg/kg/d) for 7 days followed by 6 Psi FPI. CPP training was performed on the rats of all groups.
phasic release (referred as release probability) data were compared between groups to suggest a hypothesis for dopamine release related to reward systems. Moreover, a linear regression analysis of the relationship between the behavioral test data (preference time score) to tonic, phasic, and differences of DA concentration were performed.

**Experiment 3: Abstinence Studies**

To determine the effect of 6 psi FPI on nicotine withdrawal signs in rats, Sprague-Dawley rats were divided into 3 groups: (1) Saline-infusion, rats infused with saline for 7 days; (2) Nic-infusion, rats infused with nicotine (9 mg/kg/d) for 7 days; and (3) Nic-infusion+FPI, rats were infused with nicotine (9 mg/kg/d) for 7 days followed by 6 psi FPI. The animals were placed in the observation chamber at 9:00 AM. After compensation for a new environment for 30 minutes, the frequency of withdrawal signs was counted for 15 minutes by observers (Malin et al., 1992), modified from the standard checklist of opiate abstinence signs (Malin et al., 1988); the nicotine withdrawal signs included: teeth-chattering/chews, writhe/gasps, shakes/tremors, and ptosis. The presence of these signs would be counted as 1 point/min; the point for miscellaneous less frequent signs (yawns, dyspnea, and seminal ejaculation) were summed (Malin et al., 1992). The behavioral observations were performed each day as follows: at baseline, at the last day of nicotine infusion, and at 16 hours after termination of nicotine infusion. These data are shown in Figure 7.

**Surgical Preparation and Fluid Percussion Model**

Procedures were similar to methods in our previous papers (Chen et al., 2015, 2017b). Male Sprague-Dawley rats (6 weeks old) weighing 200 to 250 g were anesthetized with Zoletil (50 mg/kg, i.p.; Vibac). Each rat was placed in a stereotaxic frame, and the scalp and temporal muscle were reflected. A 4.8-mm-diameter craniectomy was then performed over the right parietal cortex, 3.8 mm posterior to the bregma and 2.5 mm lateral to the midline, taking care not to penetrate the dura. A cranial Leur adapter of 2.5 mm in inner diameter was placed on the craniectomy site and tightly mounted to the skull using dental acrylic resin. The cranial Leur adapter was filled with saline and attached to the fluid percussion device (Huang et al., 2014a). A fluid percussion device (model HPD-1700; Dragonfly R&D) was used as described elsewhere to produce TBI in rats (Matsushita et al., 2000). Injury was induced by striking the piston of the device with a weighted metal pendulum released from a predetermined angle. Release from 43° produced a 6-psi (0.48 atm) injury.

**Infusion Pump Implantation and Drug Protocol**

For nicotine or saline infusion, a mini-osmotic pump (ALZET Model 2ML1; DURECT Co.) was implanted in the rat and set to a pumping rate of 10 μL/h. Nicotine was added to 2 mL 0.9% saline to administer 9 mg/kg daily (Cohen and George, 2013). After 7 days, the pump was removed under anesthesia (Cohen and George, 2013).

**Open Field Test**

The locomotor or behavioral activities of rats were quantified using an infrared (IR) cutoff in a novel environment (Stanford, 2007). Four pairs of IR beams spaced 1–15⁄16 in apart were positioned on 2 sides of a 10-in × 18.5-in behavioral chamber (PAS-HC; San Diego Instrument) to measure the frequency of ambulatory activities and total locomotor activity. The former is defined as the number of times a rat crosses 2 or more IR beams within a 15-second detection time interval; the latter is the total number of interruptions detected by a single IR beam.

**Brain Slice Preparation for FSCV**

Brain slices and carbon fibers (7-μm diameter; Goodfellow Corp.) were prepared as described previously (Chen et al., 2008; Good et al., 2013). After the behavioral experiments, a scaffold was used to decapitate the animals. In Experiments 1 and 2, SD rats were decapitated at 48 days or 77 days after birth. In Experiment 3, SD rats were decapitated at 16 hours in the withdrawal period (at 50 days after birth). The animals were decapitated and the brains were removed and placed in a beaker filled with oxygenated (95%O2/5%CO2) ice-cold cutting solution containing (in mM): sucrose 194, NaCl 30, KCl 4.5, MgCl2 1, NaH2PO4 1.2, glucose 10, and NaHCO3 26. A block of brain tissue containing the NAc was glued onto the cutting stage of a vibrating tissue slicer (VT1000, Leica), and the tissue block was immediately submerged in ice-cold, oxygenated cutting solution. Coronal slices 280 μm in thickness containing the NAc were transferred to a holding chamber filled with oxygenated artificial CSF (in mM: NaCl 126, KCl 3, MgCl2 1.5, CaCl2 2.4, NaHPO4 1.2, glucose 11, NaHCO3 26) at 31 °C for 20 to 30 minutes, after which the holding chamber reached room temperature and was maintained there for the rest of the incubation period. The slices were then transferred to a heated chamber (31–33°C) and perfused with normal artificial CSF (2 mL/min) for FSCV recording (Chen et al., 2017b). Carbon fibers were inserted 100 μm into the NAc under stereoscopic magnification. Fast-scan cyclic voltammetry was performed as described elsewhere (Chen et al., 2015, 2017b). For electrochemical detection, the potential of the carbon fiber was driven from -0.4 to 1.0 V then back to -0.4 V using a triangular waveform (400 V/s, 7-ms duration) applied every 100 ms. A 5-second (50-scan) control period was used to obtain a stable background current that was digitally subtracted from that obtained during the peak of the response following electrical stimulation. Peak oxidation currents were converted to dopamine (DA) concentrations using a calibration performed for each electrode with a 1-μM DA standard solution. All signals used in the statistical analyses matched the expected voltammogram profile for DA (Kawagoe et al., 1993).

To assess the capacity of axon terminals to release DA during stimulation, 2 voltammetric signals were obtained at each recording site using a single pulse (tonic) or 10 pulses (phasic) delivered at 25 Hz under various stimulation intensities (1–10 volts). After tonic and phasic dopamine signals were obtained, values were summed and averaged for each intensity across all sites. Differences in peak DA, obtained immediately after each stimulation, were calculated for each intensity and fit to a linear regression model (γ=mx+b; Prism 5.02; GraphPad) where slope m represents the relative change in DA concentration as a function of stimulation intensity (Good et al., 2011; Chen et al., 2017b).

**Statistical Analyses**

Mean dopamine release (input vs output) was analyzed using 2-way ANOVA followed by a Bonferroni posthoc test for multiple comparisons. A 1-way ANOVA and Bonferroni posthoc test were used to analyze nicotine-induced CPP, locomotor activity, DA concentration under maximum stimulation intensity (5 or 10 volts), slope of the linear regression, and
difference in maximum DA concentration. Statistical analyses of abstinence and withdrawal behaviors were performed using paired t tests (baseline vs 16 hours of withdrawal). All data were subjected to repeated-measures ANOVA (pre-test vs post-test), and alpha was set to .05. Posthoc comparisons were performed using an LSD posthoc test. Mixed effects regression analysis for repeated measures was used to evaluate group differences for evoked DA release in the shell of the NAc. All statistical tests were performed using GraphPad Prism 5.02. When $P < .05$ (2-tailed), significance was identified. The details of statistical analysis for all figures were shown in Supplementary data.

Results

Nicotine-Induced CPP Could Be Induced in Animals after Nicotine Preexposure but Was Impaired after FPI

In this first study we focused on behavioral tests for nicotine-induced CPP assessment to determine whether FPI affected nicotine-induced CPP and if preexposure nicotine here was similar to those patients with tobacco use who suffered from brain injury. We first compared nicotine-induced CPP in control, sham, and FPI groups (Figure 2A). FPI affected nicotine-induced CPP formation; CPP could be induced in the control and sham groups but not in the FPI group (Figure 2A). We expressed the CPP results as a preference score of $(C_1 - C_2) / (C_1 + C_2)$, where $C_1$ is time in the nicotine-paired compartment and $C_2$ is time in the saline-paired compartment. Preference scores were $0.1627 ± 0.04204$ ($t = 4.464$, df = 16; pair t test, pre-condition vs post-condition, $P < .001$), $0.1515 ± 0.03806$ ($t = 2.698$, df = 14; pair t test, pre-condition vs post-condition, $P < .05$), and $-0.001300 ± 0.04286$ ($t = 0.5109$, df = 13; pair t test, pre-condition vs post-condition, $P > .05$), respectively. For a behavioral control, locomotion was measured in each group and was not affected by FPI and nicotine pretreatment (Figure 2C–D; 1-way ANOVA $F_{[4,55]} = 0.7741$, $P = .5468$ vs $F_{[4,55]} = 0.3765$, $P = .8245$).

We next analyzed the impact of the history of nicotine pre-exposure on nicotine-induced CPP to further relate our study to smoking history in humans. Nicotine pretreatment for 1 week (Pre-Nic group) was compared with FPI in nicotine pretreatment groups (Pre-Nic+FPI) (Figure 2B). The results indicated that nicotine-induced CPP could be induced in animals after nicotine preexposure. Preference scores were $0.07805 ± 0.02838$ for the Pre-Nic group, significantly different than those before nicotine conditioning ($-0.009641 ± 0.01059$; $t = 2.758$, df = 5; Pair t test, precondition vs postcondition, $P < .05$). In the Pre-Nic+FPI group, preference was $0.02094 ± 0.03550$ compared to $0.06860 ± 0.04207$ ($t = 1.375$, df = 5; Pair t test, precondition vs postcondition, $P > .05$), indicating that CPP was not significantly induced in this group.

Figure 2. Nicotine-induced conditioned place preference (CPP) was used to determine whether fluid percussion injury (FPI) affected the reward behavior and whether preexposure nicotine had an influence. (A) Nicotine-induced CPP could be induced in control ($N = 17$) and sham ($N = 15$) groups, whereas CPP could not be obtained in animals with FPI ($N = 14$). (B) Nicotine preexposure animals (Pre-Nic, $N = 8$) manifested nicotine-induced CPP. Nicotine-induced CPP could be induced in animals with nicotine preexposure, if animals with nicotine preexposure received FPI (Pre-Nic+FPI, $N = 6$), CPP could not be significantly obtained. In each group, white scatter plot: preference before CPP; black scatter plot: preference after CPP. (C) Ambulatory movements of animals and (D) locomotor function were not affected by FPI.
Dopaminergic Transmission in the NAc Shell Was Suppressed after FPI and Was Enhanced in Nicotine Preexposed Animals as Well as after CPP Training

To determine if FPI and nicotine preexposure affected dopaminergic transmission in the NAc shell using FSCV, tonic and phasic dopamine releases from the NAc shell were measured using single-pulse and 10-pulse stimulation, respectively, at 25 Hz. Dopamine signals evoked by various stimulation intensities (1–10 Volts) were studied with input/output (I/O) curves to determine dopamine release patterns. First, to analyze the impact of FPI on release of DA in the NAc shell, we compared the I/O curves in control and FPI groups (Figure 3A–B). Tonic and phasic dopamine release were suppressed in the FPI group, similar to our previous report (Chen et al., 2017b). FPI-only animals also had the lowest phasic DA release (Figure 3A–B, solid red squares). Second, we found that the I/O curves could be elevated (left shift) in both control and FPI groups after CPP training (control+CPP: blue open circle vs FPI+CPP: red open square).

We then analyzed FPI effects on animals with nicotine pre-treatment; the DA release in pre-Nic and Pre-Nic+ FPI groups were compared to determine any FPI effects on DA transmission in animals with nicotine preexposure history (Figure 3C–D). Before CPP training, tonic release in the Pre-Nic group was lower than in the nicotine pretreatment only group (Pre-Nic: green open diamond). After CPP training, tonic release in the Pre-Nic+FPI group (gray open triangle) increased but increments were not as high as in the nicotine pretreatment only group (Pre-Nic: green open diamond). (D) The phasic release signal in nicotine pretreatment only animals (Pre-Nic: green open diamond) showed the highest level after CPP training comparing FPI with nicotine pretreatment groups (Pre-Nic+FPI+CPP: gray open triangle). The variation in I/O curves before and after CPP training in FPI animals with nicotine pretreatment showed the phasic release evoked by higher intensities (>7 V) post-CPP training were higher (Pre-Nic+FPI+CPP group: gray open triangle vs Pre-Nic+FPI: black solid triangle).
highest, and this release enhancement in pre-Nic animals was suppressed by FPI. The order of tonic DA release was pre-
Nic>pre-Nic+FPI>FPI. After CPP training, tonic release was
enhanced in these 2 groups (Figure 3C). Nicotine pretreatment
increased phasic release, but this enhancement was suppressed
after FPI (Figure 3D). Pre-Nic+FPI: black solid triangle vs Pre-Nic:
green solid diamond).

Nicotine-Induced CPP Influence on Dopamine
Release: Nicotine Exposure Tends to Increase
Dopamine Release

To further determine the effect of nicotine-induced CPP on
dopamine release, tonic and phasic releases in brain slice were
quantified under maximal stimulation intensities (10 V) in
before (pre-CPP state, white bar) and after CPP training (post-CPP
state, black bar) animals. For the FPI question, the tonic release
in control and FPI groups was compared in Figure 4A. After CPP
tests, both control and FPI group tonic release signals were
enhanced. Although phasic release before or after CPP training
was markedly suppressed in FPI groups compared with control
groups, it is interesting that significant enhancement in phasic
dopamine release after CPP test could be found in FPI but not in
control group (Figure 4B). The enhancement of tonic (Figure 4C)
and phasic (Figure 4D) release in the Pre-Nic group was reduced
in the Pre-Nic+FPI group. To further determine whether the DA
concentrations before CPP and after CPP training would be a
factor related to CPP formation, we calculated the tonic and
phasic concentration differences before and after CPP training
(peri-CPP difference in tonic level = [Tonic]pre-CPP - [Tonic]post-CPP;
peri-CPP difference in phasic level = [Phasic]pre-CPP - [Phasic]post-CPP
(Figure 4E–F). The peri-CPP difference in tonic release was lowest
in Pre-Nic+FPI. However, the peri-CPP difference in phasic was
different; these differences were lower in control, Pre-Nic and
Pre-Nic+FPI groups, but higher in the FPI only group.

In summary, comparing DA release at stimulation intensities
of 10 V in each group before and after CPP training, we showed
that tonic release was elevated by nicotine pretreatment as
well as with CPP training itself. Phasic release had the same
effect in the FPI groups, suggesting that FPI is associated with
significantly diminished DA release compared to noninjury.
It also suggests that increasing nicotine exposure time tends
to enhance DA release in parallel.

Dopamine Release Capacity Is Reduced after FPI and
Nicotine-Induced CPP

To assess the capacity of axon terminals to release DA during
phasic stimulation, releasing probability at 25 Hz was assessed.
The slopes obtained from the linear regression model of each
group were plotted (Figure 5A). Comparing the control and FPI
groups, the slopes were suppressed with either tonic or phasic
release (Figure 5B). The slope of the FPI group after the CPP test
was still lower than that of the control group (Figure 5B). The
release probability also decreased under pre-CPP conditions
and this became more significant after CPP tests in the nicot-
ine pretreatment group (Figure 5C), which may be the result of the
higher tonic release in this group. In FPI with nicotine pre-
treatment animals (Figure 5, Pre-Nic+FPI), the release probabil-
ity before CPP was lower than the Pre-Nic group. After CPP, the
release probability decreased further.

The release capacity of dopaminergic terminals in each
group, equated to the slope obtained from linear regression,
is shown in Figure 5. For all of the groups, the slope decreased
after nicotine-induced CPP (black bar vs white bar in each
group). After CPP, the largest slope was in the control group
(Control+CPP group) followed by the nicotine pretreatment group
(Pre-Nic+CPP group), and the FPI with nicotine pretreat-
group (Pre-Nic+FPI+CPP group). The lowest slope was in
FPI group (FPI+CPP group) (1-way ANOVA [F7,51 = 43.77, P < .001]).

Preference for the Conditioned Place Is Related Not
only to Phasic Dopamine Release but Also to Release
Probability

As previously shown, nicotine-induced CPP was seen in rats that
were nicotinepretreated and those that were not pretreated, but
it was not significantly found in injured rats (Figure 2A). To fur-
ther determine the relationship between dopamine release and
CPP, after CPP training release probabilities at various stimula-
tion intensities were summed and analyzed using linear regres-
sion. The slope of the linear regression line was highest in the
CPP group followed by the Control+CPP group. It was lowest
in the FPI+CPP group (Figure 5B–C). Linear regression revealed
a relationship in tonic release (Figure 6A), phasic release
(Figure 6B), and release probability (Figure 6C) concentration
differences between pre-CPP and post-CPP states (Figure 6D for
tonic and 6E for phasic) at 10-V stimulation intensity. A posi-
tive correlation was found between phasic release (Figure 6B,
r = 0.8110, P = .1890) and release probability (Figure 6C, r = 0.9521,
P = .0479). Relationships with tonic release were not significant
and even negatively correlated with concentration differences
between pre-CPP and post-CPP preference scores (Figure 6).

Nicotine Withdrawal Is Not Affected by a FPI

To determine whether TBI affects nicotine withdrawal, rats were
exposed to 1 week of nicotine infusion for 7 days after a FPI was
induced. Subsequent withdrawal was studied. Both controls and
injured rats exhibited withdrawal effects when compared with the
saline-infusion group (Figure 7A; 2-way ANOVA [F2,25 = 27.84,
P < .001] saline vs nicotine infusion). Upon nicotine stimula-
tion, DA release was highest among the saline-infusion group
followed by the Nic-infusion group. The Nic-infusion+FPI group
showed much lower values (Figure 7B; 2-way ANOVA [F3,388 = 165.7,
P < .001]). Upon phasic stimulation, the saline-infusion and Nic-
infusion groups showed high values for dopamine release. The
Nic-infusion+FPI group had lower values, but injured animals
without nicotine treatment had the lowest values (Figure 7C;
2-way ANOVA [F2,25 = 157.3, P < .001]). Upon tonic stimulation,
the release capacity of dopaminergic terminals (Figure 7D; 2-way ANOVA [F1,20 = 98.76, P < .001]). The slopes of the
linear regression lines were small for both groups of injured
rats (Figure 7E; 1-way ANOVA [F1,11 = 506.3, P < .001]).

Discussion

In this study, an FPI was found to significantly reduce nicotine-
associated CPP in rats, suggesting that dopamine release in the
shell portion of NAc is affected by this type of injury. This could
play a crucial role in changes in reward or reinforcing behav-
ior. Moreover, preference time seems to correlate with phasic
dopamine release and release probability. In addition, nicotine
withdrawal was not affected by FPI.

As detailed in the Introduction, addictive drugs can prod-
uce physiological changes related to extracellular dopamine
fluctuations that are preferentially augmented in the NAc shell
Figure 4. Nicotine exposure may increase dopamine release. Dopamine concentrations measured with maximal (10 V) stimulation intensities before and after conditioned place preference (CPP) training were analyzed. To determine the fluid percussion injury (FPI) effect, (A) tonic and (B) phasic release values in control and FPI groups were compared. (A) Tonic release was suppressed by FPI. After CPP training, tonic release increased in both control and FPI groups. (B) Either before or after CPP training, phasic release was markedly suppressed in FPI groups compared with control groups. (C) To determine the FPI effect in nicotine preexposed animals, the tonic release data between nicotine pretreated only and FPI with nicotine pretreatment groups were compared. Before CPP training, tonic values in pretreated only animals were higher than in the FPI pretreated animal group. After CPP training, the tonic release increased in both groups, and the tonic release in nicotine pretreatment only animals was again higher than in the nicotine pretreatment with FPI group. (D) For phasic release, analysis between the groups revealed that phasic releases were highest in the nicotine pretreatment only group (Pre-Nic) either before or after CPP training. Phasic release was suppressed in the nicotine pretreatment animals with FPI when compared with nicotine pretreatment only. To further determine whether the DA concentration between before CPP and after CPP training would be a factor related to CPP formation, we calculated the tonic and phasic concentration difference between before and after CPP training (peri-CPP difference in tonic level = [Tonic]_pre-CPP - [Tonic]_post-CPP, peri-CPP different in phasic level = [Phasic]_pre-CPP - [Phasic]_post-CPP). (E) The peri-CPP difference in tonic release was lowest in Pre-Nic+ FPI group. (F) The peri-CPP difference in phasic releases was lower in control, Pre-Nic, and Pre-Nic+ FPI groups, but higher in the FPI only group. **P < .01 and *** < .001, "P < .01 and *** < .001
rather than its core (Di Chiara and Bassareo, 2007). Therefore, we hypothesized that the reward system is affected by TBI through changes in the dopamine system. Impairment of nicotine-induced CPP, reflecting reward activity, was indeed found here in injured rats.

An electrochemical study of brain slices revealed that nicotine infusions suppressed low-frequency, single-pulse-induced dopamine release but enhanced high-frequency stimulation-induced dopamine release (Cragg, 2006). After nicotine infusion for 1 week, desensitization or inactivation of the nicotine acetylcholine receptors (nAChRs) likely occurs. After 1 week, FSCV data suggest that nAChRs were sensitized with higher tonic dopamine releases (Picciotto et al., 2008) (Figure 3A).

Tonic dopamine release I/O curves before and after CPP training were compared (Figure 4), revealing that increases resulted after training. This was also seen in those animals pre-exposed to nicotine. The lowest tonic levels were found before CPP training in the CPP and FPI+CPP groups. The I/O curves for phasic dopamine release in each group suggest that release patterns were similar between the CPP group before and after CPP training and the Pre-Nic+CPP group before and after CPP training. The Pre-Nic+CPP group showed phasic dopamine release both before and after CPP training that was lower than the other groups. The FPI+CPP group showed phasic levels that were higher after CPP training than before training. In fact, the lowest phasic dopamine release levels were in this group before CPP training.

Data from brain slice FSCV assays suggest that nicotine might enhance phasic dopamine release in proportion to pulse or frequency of stimulation (Rice and Cragg, 2004; Cragg, 2006). Because phasic release was low in the FPI+CPP group, incremental increases in DA release after nicotine pretreatment could be seen. Before CPP training, phasic dopamine release in the Pre-Nic+FPI+CPP group was higher than in the FPI+CPP group. After CPP training, phasic dopamine release increased in the latter but remained higher yet in the pretreated rats. There was thus a relationship between phasic dopamine release and nicotine preexposure. In previous studies (Hoebel, 1985; Di Chiara and Bassareo, 2007; Tsai et al., 2009), reward or substance abuse behavior was correlated with phasic dopamine release, and this has now been confirmed and extended by our data.

To assess the release capacity of dopaminergic terminals, the release probability at various stimulation intensities (1–10 V) was analyzed for each group. Multiple linear regression analyses were performed to determine the slopes, which indicated release probabilities. In general, release decreased after CPP training. This phenomenon could be the result of a ceiling effect or the desensitization of nAChRs (Bloem et al., 2014). Among injured groups, the slopes was lower than those found among noninjured groups, whether nicotine pretreated or not. Nicotine-induced CPP was blunted in nicotine pretreated rats. In these groups, higher baseline tonic levels could have reduced the capacity for phasic dopamine release, or again a ceiling effect may be present (Tizabi et al., 2007). Either possibility could lead to a lower releasing probability, thus resulting in reduced nicotine-induced CPP. On the other hand, lower phasic release after FPI could also lead to a lower releasing probability, again producing reduced CPP. Linear regression analysis of the relationship between CPP parameters and release probability suggests that these are strongly correlated.
Upon examination of the possible impact of TBI on the severity of nicotine withdrawal, no significant differences in withdrawal responses were found between controls and rats with induced FPIs, suggesting that TBI has a minimal effect on the negative reinforcement imparted by withdrawal symptoms, which normally induces continued nicotine use to prevent those symptoms.

In sum, the major conclusions and findings in our study are:

1. Nicotine-induced CPP could be induced in control animals and animals with a nicotine preexposure history.
2. Moreover, the change or variation in DA phasic release before and after CPP in each group is found to be one factor that affects the CPP formation.
3. The factors that are associated with CPP formation in animals were correlated by using linear regression as follows:

a) Dopamine release probability (concentration difference between phasic and tonic release): Pearson’s correlation coefficient $r = 0.9521$.

b) Concentration of phasic release: Pearson’s correlation coefficient is $r = 0.8110$.

c) The concentration difference before and after CPP training: Pearson’s correlation coefficient in tonic concentration difference is $r = 0.2828$ and for phasic concentration difference is $r = -0.8201$.

4. Although, the concentration difference between pre- and post-CPP was high in FPI groups, the release probabilities were too low to induce consistent behavior. In humans, reduced capacity for DA release following head injury would lead to a reduction in reward-related DA signaling. Thus, greater amounts of nicotine, or other abused drugs, might be necessary in order to overcome this difference.

Taken together, our findings support the idea that TBI impacts the mesolimbic-mesocortical dopaminergic pathways, thereby reducing nicotine-induced rewards. Therefore, greater stimulation by nicotine might be required to obtain a given level of dopaminergic activity, which in turn might explain increased tobacco abuse among TBI patients. However, in addition to the dopaminergic system, several other neural substrates, including the serotonergic (Wilson et al., 2006; Hein et al., 2007) and noradrenergic systems (Comings and Blum, 2000; Sofuoglu and Sewell, 2009), have been implicated in changes in reward circuitry. Nevertheless, this study demonstrates that TBI has a clear impact on the mesolimbic-mesocortical dopaminergic pathways, reducing dopaminergic release probability in the shell of the NAc, and supporting a connection between reduced rewards.
of nicotine exposure seen in animal behavioral experiments and clinical findings. In conclusion, an FPI was found to markedly reduce nicotine-associated CPP in rats, and this was accompanied by changes in dopamine release in the shell portion of the NAc. This might play a crucial role in changes in reward and reinforcing behavior. Moreover, CPP time scores correlate with phasic dopamine release and release probability. Finally, nicotine withdrawal is not affected by an FPI.

Acknowledgments
This work was supported by the National Science Council of Taiwan (MOST105-2314-B-016-001-MY3), the Tri-Service General Hospital of Taiwan’s Medical Research Project (TSGH-C107-068, TSGH-C107-070, and TSGH-C107-071), and the National Defense Hospital of Taiwan’s Medical Research Project (TSGH-C107-068, Taiwan (MOST105-2314-B-016-001-MY3), the Tri-Service General Medical Center (MAB-107-022). This project was supported in part by philanthropic support from the George R. and Constance P. Lincoln family.

Statement of Interest
None.

References
Benowitz NL (2010) Nicotine addiction. N Engl J Med 362:2295–2303.
Bloom B, Poorthuis RB, Mansvelder HD (2014) Cholinergic modulation of the medial prefrontal cortex: the role of nicotinic receptors in attention and regulation of neuronal activity. Front Neural Circuits 8:17.
Brody AL (2006) Functional brain imaging of tobacco use and dependence. J Psychiatr Res 40:404–418.
Chen YH, Harvey BK, Hoffman AF, Wang Y, Chiang YH, Lupica CR (2008) MPTP-induced deficits in striatal synaptic plasticity are prevented by glial cell line-derived neurotrophic factor expressed via an adeno-associated viral vector. FASEB J 22:261–275.
Chen YH, Huang EY, Kuo TT, Ma HI, Hoffer BJ, Tsai JJ, Chou YC, Chiang YH (2015) Dopamine release impairment in striatum after different levels of cerebral cortical fluid percussion injury. Cell Transplant 24:2113–2128.
Chen YH, et al. (2017a) Impact of traumatic brain injury on dopaminergic transmission. Cell Transplant 26:1156–68.
Chen YH, Huang EY, Kuo TT, Hoffer BJ, Miller J, Chou YC, Chiang YH (2017b) Dopamine release in the nucleus accumbens is altered following traumatic brain injury. Neuroscience 348:180–90.
Cohen A, George O (2013) Animal models of nicotine exposure: relevance to second-hand smoking, electronic cigarette use, and compulsive smoking. Front Psychiatry 4:41.
Comings DE, Blum K (2000) Reward deficiency syndrome: genetic aspects of behavioral disorders. Prog Brain Res 126:325–341.
Cragg SJ (2006) Meaningful silences: how dopamine listens to the ACh pause. Trends Neurosci 29:125–131.
Dajas-Bailador F, Wonnacott S (2004) Nicotinic acetylcholine receptors and the regulation of neuronal signalling. Trends Pharmacol Sci 25:317–324.
Di Chiara G, Bassareo V (2007) Reward system and addiction: what dopamine does and doesn’t do. Curr Opin Pharmacol 7:69–76.
Di Chiara G, Bassareo V, Fenu S, De Luca MA, Spina L, Cadoni C, Acquas E, Carboni E, Valentini V, Lecca D (2004) Dopamine and drug addiction: the nucleus accumbens shell connection. Neuropsychopharmacology 47(Suppl 1):227–241.
Dreyer JK, Herrik KS, Berg RW, Hounsgaard JD (2010) Influence of phasic and tonic dopamine release on receptor activation. J Neurosci 30:14273–14283.
Good CH, Hoffman AF, Hoffer BJ, Chefer VI, Shippenberg TS, Bäckman CM, Larsson NG, Olson L, Gellhaar S, Galtier D, Lupica CR (2011) Impaired nigrostriatal function precedes behavioral deficits in a genetic mitochondrial model of Parkinson’s disease. FASEB J 25:1333–1344.
Good CH, Wang H, Chen YH, Mejias-Aponte CA, Hoffman AF, Lupica CR (2013) Dopamine D4 receptor excitation of lateral habenula neurons via multiple cellular mechanisms. J Neurosci 33:16853–16864.
Grace AA (1991) Phasic vs tonic dopamine release and the modulation of dopamine system responsivity: a hypothesis for the etiology of schizophrenia. Neuroscience 41:1–24.
Grace AA (2000) The tonic/phasic model of dopamine system regulation and its implications for understanding alcohol and psychostimulant craving. Addiction 95:S119–S128.
Hein J, Wrase J, Heinz A (2007) Alcohol-related disorders: etiopathology and therapeutic considerations. Fortschr Neurol Psychiatr 75:10–17.
Hoebel BG (1985) Brain neurotransmitters in food and drug reward. Am J Clin Nutr 42:1133–1150.
Huang EY, Liu TC, Tao PL (2003) Co-administration of dextromethorphan with morphine attenuates morphine rewarding effect and related dopamine releases at the nucleus accumbens. Naunyn Schmiedebergs Arch Pharmacol 368:386–392.
Huang EY, Tsai PF, Kuo TT, Tsai JJ, Chou YC, Ma HI, Chiang YH, Chen YH (2014a) Amantadine ameliorates dopamine-releasing deficits and behavioral deficits in rats after fluid percussion injury. Plos One 9:e86354.
Huang EY, Tsai TH, Kuo TT, Tsai JJ, Tsai PF, Chou YC, Ma HI, Chiang YH, Chen YH (2014b) Remote effects on the striatal dopamine system after fluid percussion injury. Behav Brain Res 267:156–172.
Ilie G, Adlaf EM, Mann RE, Ialomiteanu A, Hamilton H, Rehm J, Asbridge M, Cusimano MD (2015a) Associations between a history of traumatic brain injuries and current cigarette smoking, substance use, and elevated psychological distress in a population sample of Canadian adults. J Neurotrauma 32:1130–1134.
Ilie G, Mann RE, Hamilton H, Adlaf EM, Boak A, Asbridge M, Rehm J, Cusimano MD (2015b) Substance use and related harms among adolescents with and without traumatic brain injury. J Head Trauma Rehabil 30:293–301.
Kawagoe KT, Zimmerman JB, Wightman RM (1993) Principles of voltammetry and microelectrode surface states. J Neurosci Methods 48:225–240.
Le Foll B, Goldberg SR (2005) Nicotine induces conditioned place preferences over a large range of doses in rats. Psychopharmacology (Berl) 178:481–492.
Leikola-Pelho T, Jackson DM (1992) Preferential stimulation of locomotor activity by ventral tegmental microinjections of (-)-nicotine. Pharmacol Toxicol 70:50–52.
Malin DH, Murray JB, Crucian GP, Schweitzer FC, Cook RE, Skolnick MH (1988) Auricular microelectrostimulation: naloxone-reversible attenuation of opiate abstinence syndrome. Biol Psychiatry 24:886–890.
Malin DH, Lake JR, Newlin-Maultsby P, Roberts LK, Lanier JG, Carter VA, Cunningham JS, Wilson OB (1992) Rodent model of nicotine abstinence syndrome. Pharmacol Biochem Behav 43:779–784.
Matsushita Y, Shima K, Nawashiro H, Wada K (2000) Real-time monitoring of glutamate following fluid percussion brain injury with hypoxia in the rat. J Neurotrauma 17:143–153.

Nestler EJ (2005) Is there a common molecular pathway for addiction? Nat Neurosci 8:1445–1449.

Phillips PE, Robinson DL, Stuber GD, Carelli RM, Wightman RM (2003) Real-time measurements of phasic changes in extracellular dopamine concentration in freely moving rats by fast-scan cyclic voltammetry. Methods Mol Med 79:443–464.

Picciotto MR, Addy NA, Mineur YS, Brunzell DH (2008) It is not "either/or": activation and desensitization of nicotinic acetylcholine receptors both contribute to behaviors related to nicotine addiction and mood. Prog Neurobiol 84:329–342.

Rice ME, Cragg SJ (2004) Nicotine amplifies reward-related dopamine signals in striatum. Nat Neurosci 7:583–584.

Saunders BT, Yager LM, Robinson TE (2013) Cue-evoked cocaine “craving”: role of dopamine in the accumbens core. J Neurosci 33:13989–14000.

Sofuoglu M, Sewell RA (2009) Norepinephrine and stimulant addiction. Addict Biol 14:119–129.

Stanford SC (2007) The open field test: reinventing the wheel. J Psychopharmacol 21:134–135.

Tizabi Y, Bai L, Copeland RL Jr, Taylor RE (2007) Combined effects of systemic alcohol and nicotine on dopamine release in the nucleus accumbens shell. Alcohol Alcohol 42:413–416.

Tsai HC, Zhang F, Adamantidis A, Stuber GD, Bonci A, de Lecea L, Deisseroth K (2009) Phasic firing in dopaminergic neurons is sufficient for behavioral conditioning. Science 324:1080–1084.

Vicente AM, Galvão-Ferreira P, Tecuapetla F, Costa RM (2016) Direct and indirect dorsolateral striatum pathways reinforce different action strategies. Curr Biol 26:R267–R269.

Wilson DI, Laidlaw A, Butler E, Hofmann D, Bowman EM (2006) Development of a behavioral task measuring reward “wanting” and “liking” in rats. Physiol Behav 87:154–161.

Wonnacott S (1997) Presynaptic nicotinic ACh receptors. Trends Neurosci 20:92–98.