Life Sciences 234 (2019) 116784

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Adolescent nicotine challenge promotes the future vulnerability to opioid addiction: Involvement of lateral paragigantocellularis neurons

Mona Torabi[a], Hossein Azizi[a,*], S. Mohammad Ahmadi-Soleimani[b], Ameneh Rezayof[c]

[a] Department of Physiology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
[b] Neuroscience Research Center, Torbat Heydariyeh University of Medical Sciences, Torbat Heydariyeh, Iran
[c] Department of Animal Biology, School of Biology, College of Science, University of Tehran, Tehran, Iran

ARTICLE INFO

Keywords:
Adolescence
Nicotine
Morphine tolerance
Morphine withdrawal
Single unit recording
LPGi

ABSTRACT

Tobacco smoking is recognized as a life-threatening risk factor worldwide. Initiation of smoking primarily occurs during adolescence which is a critical developmental phase characterized by specific neurobehavioral alterations. The effect of adolescent nicotine exposure on vulnerability to opioid addiction has not been previously addressed. Furthermore, lateral paragigantocellularis (LPGi) is a key modulator of opiate effects. In this study we investigated the effect of adolescent nicotine treatment on development of morphine tolerance and dependence as well as LPGi neuronal responses to morphine during adulthood.

Male Wistar rats received subcutaneous injections of either nicotine or saline during adolescence and then development of morphine tolerance and dependence was assessed during adulthood by tail-flick and withdrawal tests, respectively. In vivo single-unit recording was performed to examine the LPGi neuronal activities.

Results indicated that adolescent nicotine exposure significantly facilitates the development of tolerance to analgesic effect of morphine and increases the expression of morphine withdrawal signs in adulthood. Also, it was observed that following adolescent nicotine treatment, the extent of morphine-induced excitation is attenuated in LPGi neurons of adult rats. Moreover, the onset of morphine-induced inhibition was increased in these animals. Neither the baseline, nor the regularity of firing was affected in our observations.

It could be concluded that nicotine challenge during adolescence may enhance the future vulnerability to opioid addiction through induction of persistent neuroadaptations in LPGi neurons.

1. Introduction

Tobacco smoking is considered as a leading cause of morbidity and mortality worldwide [1] Each year, around 6 million smokers die at the result of tobacco-related diseases. Epidemiological reports indicate that the initiation of smoking primarily occurs during adolescence and that the majority of adult smokers have had the experience of tobacco exposure before the age 18 [2].

Adolescence is a critical biological period during which persistent alterations occurs in CNS development and function. These mainly include progressive maturation of neural circuits and enhanced plasticity of brain regions required for executive performance, reward processing and expression of motivated behaviors [3]. Indeed, these changes are associated with the advent of specific behavioral manifestations including novelty-seeking and risk-taking behaviors [4,5]. Therefore, adolescents are not only at a higher risk for the early use of cigarettes, but also, they are probably differentially vulnerable to the undesirable effects of psychoactive chemicals in tobacco such as nicotine.

Studies on animal models have demonstrated that adolescents are more sensitive to the rewarding properties of nicotine and express less aversion to nicotine than adults [6–10]. It has been revealed that nicotine administration during adolescence sensitizes rats to the rewarding effects of nicotine in adulthood [11,12]. According to the gateway hypothesis, exposure to nicotine-containing products in adolescence is linked to the use of other drugs of abuse later in life. Those who have initiated smoking prior to age 15, are estimated to be 80 times more susceptible to experience cocaine abuse in adulthood [13]. Also, studies in rodents have shown that early-adolescent nicotine exposure can induce preference to amphetamine and morphine in adulthood [14]. Adolescent nicotine treatment has also been reported to dose-dependently increase opioid consumption in adult male rats [15].

Nicotine effects are mainly mediated by the activity of nicotinic acetylcholine receptors (nAChRs) which play critical roles in a variety of neurophysiological processes such as cognition, reward and

* Corresponding author.
E-mail address: azizih@modares.ac.ir (H. Azizi).

https://doi.org/10.1016/j.lfs.2019.116784
Received 8 July 2019; Received in revised form 11 August 2019; Accepted 20 August 2019
Available online 21 August 2019
0024-3205/

analgesia [16]. Activation of nAChRs promotes the release of different neurotransmitters, such as dopamine, noradrenaline, acetylcholine, glutamate, serotonin and GABA [17]. Furthermore, several studies have provided evidence indicating that nicotine administration can induce the release of endogenous opioid peptides [18–20], which known to be involved in analgesic and addictive effects of nicotine [21]. This idea is based on the results of previous studies in which administration of opioid receptor antagonist naloxone precipitated nicotine abstinence syndrome [22] and prevented nicotine-induced antinociception in animal models [23,24].

Lateral paragigantocellularis (LPGi) nucleus, located in the rostral ventrolateral medulla, is a critical modulator of opiate-related effects including tolerance and dependence [25,26]. It has been shown that electrical stimulation of LPGi region can elicit the expression of morphine withdrawal-like behaviors [27]. At the level of brain neural circuitries mediating drug-triggered behaviors, the modulatory role of LPGi nucleus is indispensable [28]. When the LPGi nucleus, as the main source of excitatory afferents to locus coeruleus (LC), is lesioned [29] or isolated from the circuit [30], the expected withdrawal-induced hyperactivity of LC neurons, which is temporally associated with opioid withdrawal signs, is dramatically attenuated.

Considering the existing literature in support of possible interactions between endogenous opioid system and nicotinic receptors, the present study was designed. In this work, we first aimed to assess the long-term effects of adolescent nicotine exposure on development of morphine tolerance and dependence at behavioral level. Then, we further investigated whether this adolescent nicotine exposure can persistently affect the LPGi neuronal response to acute morphine injection in adulthood.

2. Materials and methods

2.1. Animals

All experiments were performed on naïve male Wistar rats obtained from Razi institute (Tehran, Iran) at postnatal day (PND) 22. Subjects were kept four per cage in a colony room under constant temperature with ad libitum access to water and food (standard rodent pellet chow). The cage floors were covered with woodchip bedding and animals were checked daily to ensure their health and convenience. The circadian rhythm was controlled under a 12:12 h regular light/dark cycle (lights on at 7:00 a.m.). All experimental interventions were approved and done according to the protocols set by the committee of animal care and use in Tarbiat Modares University, Tehran, Iran. One week prior to the beginning of experiments, animals were given daily opportunities (1 h sessions per day) to get acclimated with the lab environment and instruments.

A total number of 99 rats were used in 7 experimental groups according to the following order: 20 rats for morphine withdrawal test (Groups 1 and 2: Saline-Morphine and Nicotine-Morphine groups, $n = 10$ for each group), 34 rats used for tail flick test (Groups 3, 4 and 5: Saline-Morphine, Nicotine-Morphine and Saline-Saline groups, $n = 13$, 13 and 8 respectively). Finally, 45 rats were used for electrophysiological recording (Groups 6 and 7: Saline- and Nicotine-treated, $n = 23$ and 22 respectively). It should be noted that distinct behavioral and electrophysiological assessments were performed on different groups of rats by the same experimenter during the light cycle between 09:00 and 15:00.

2.2. Study timeline

As illustrated in Fig. 1, animals received nicotine exposure during their adolescence period (PND 28–42) which was followed by a drug-free interval (PND 43–64). All experimentations (withdrawal test, tail flick test and electrophysiological recording) were performed between the PND 65–75 corresponding adulthood developmental stage in rats.

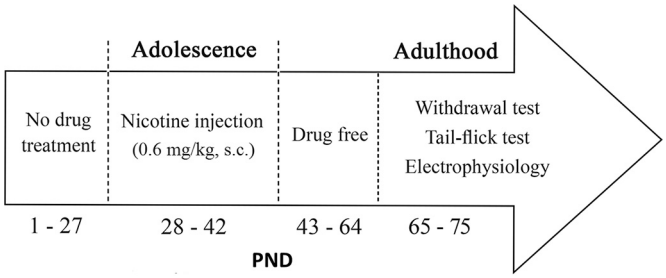

Fig. 1. Schematic timeline indicating the sequence of experimental protocols. As shown, animals experienced adolescent nicotine exposure between PND 28–42 which was followed by a drug-free period (between PND 43–64). All Behavioral and electrophysiological assessments were performed on different groups of rats within the adulthood developmental stage (i.e. PND 65–75).

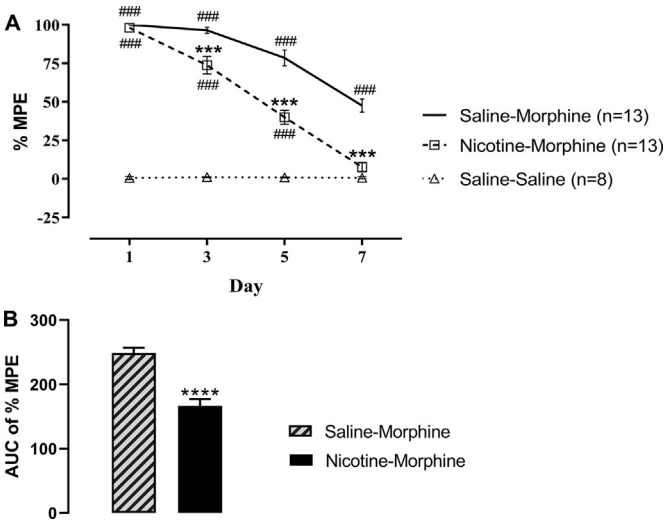

Fig. 2. The effect of adolescent nicotine exposure on development of morphine analgesic tolerance. A) The analgesic effect of morphine (3.5 mg/kg; s.c.) is illustrated as % MPE on days 1, 3, 5, and 7 in different experimental groups. Two-way ANOVA, ***$P < 0.001$ vs. Saline-Morphine, ### $P < 0.001$ vs. Saline-Saline B) The area under the curve (AUC) calculated for the obtained % MPEs during the experimental period. Unpaired two-tailed Student's *t*-test, **** $P < 0.0001$ vs. Saline-Morphine (control) group. Data are presented as mean ± standard error of the mean (SEM).

2.3. Drugs

The following drugs were used in this study: 1) Morphine sulfate powder (obtained from Temad Co., Tehran, Iran) was dissolved in physiological saline (sterile sodium chloride 0.9%); 2) Nicotine (as a base (−) nicotine hydrogen tartrate salt; obtained from Sigma-Aldrich, USA) was dissolved in saline and the pH was adjusted to 7.0 (± 0.2) with NaOH and 3) Naloxone hydrochloride powder (Sigma-Aldrich, USA) was also dissolved in saline similar to morphine and nicotine. The mentioned solutions were all freshly prepared before each experiment. In order to prepare the required dose of nicotine (0.6 mg/kg), the following calculations were made: Step 1) A total amount of 1.5 mg nicotine was dissolved in 5 ml saline which equals 0.3 mg/ml. Step 2: In order to obtain our selected dose of nicotine (i.e. 0.6 mg/kg), we considered 2 ml from the solution prepared in step 1 (i.e. 2 × 0.3 mg/ml). Thus, we got our final solution (2 ml containing 0.6 mg nicotine for 1 kg weight). Finally, we calculated the required dose proportionate to the animal's weight.

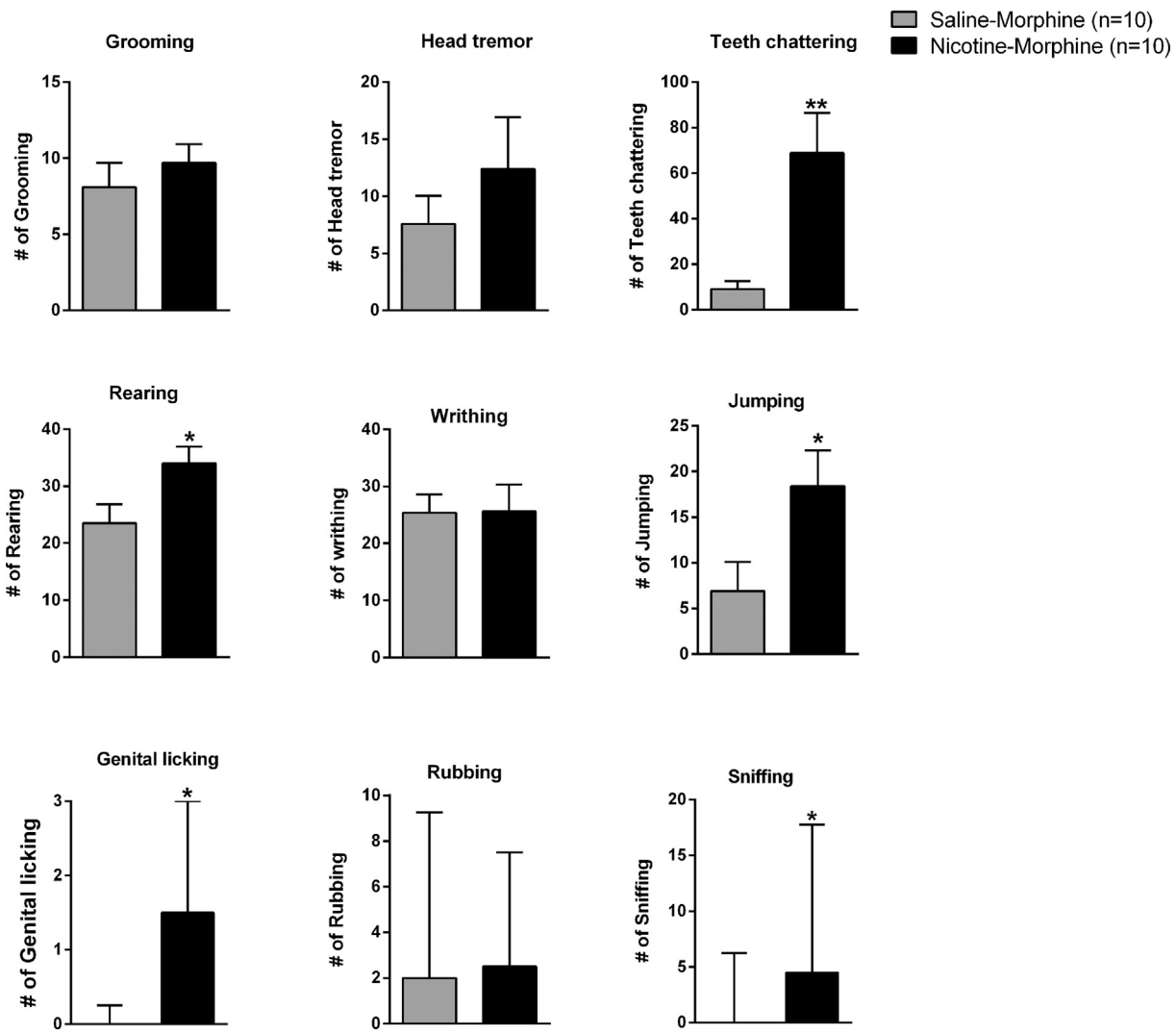

Fig. 3. The effect of adolescent nicotine exposure on severity of naloxone-induced morphine withdrawal signs. Rats received nicotine (0.6 mg/kg/day, s.c.) during adolescence for 7 consecutive days and then undergone chronic morphine exposure (10 mg/kg, b.i.d., s.c.) for 9 days during adulthood. Naloxone administration (2 mg/kg, i.p.) was done on day 10 (i.e. PND 74). Unpaired two-tailed Student's t-test was used for analysis of normally distributed data including grooming, head tremor, teeth chattering, rearing, writhing, and jumping. For these signs data are shown as mean ± standard error of the mean (SEM). Mann–Whitney *U* test was used to analyze non-parametric data including sniffing, genital licking and rubbing. For these signs data are shown as median with interquartile range. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. saline/morphine group.

2.4. Experimental procedures

2.4.1. Adolescent nicotine exposure

In order to assess the persistent effects of nicotine, a rat model of adolescent nicotine exposure was designed based on a previous study [31]. For this purpose, rats received subcutaneous (s.c.) injections of nicotine (0.6 mg/kg) or saline (as vehicle) at the volume of 2 ml/kg once a day (at 10:00 a.m.) between the PND 28–42 and as aforementioned, during the next 22 days, animals experienced a clear drug-free period (Fig. 1).

2.4.2. Monitoring the development of morphine tolerance by tail flick test

Development of tolerance to analgesic effects of morphine was assessed during adulthood in rats undergone adolescent nicotine/saline exposure. For induction of analgesic tolerance in adult rats, animals received 3.5 mg/kg/day morphine injections (s.c.) for 7 consecutive days (PND 65–71) and analgesic tests were done every other day (i.e. PNDs 65, 67, 69 and 71). A tail flick analgesia meter (IITC life science, USA) was used to measure the antinociceptive responses 10 min before and 30 min after each morphine injection. In this apparatus, a concave mirror, surrounding a 150 W electrical bulb, reflects the light beam on the dorsal part of the animal's tail and the animal flicks its tail upon feeling the heat-induced pain. In our study, the intensity of light was adjusted to yield a baseline tail flick latency of 2–4 s. in all experiments. Also, in order to minimize the possibility of tissue damage, a cut-off time of 10 s. was considered in all experiments [32,33]. For each test, tail flick latency was calculated as the mean of three sequential recordings. Morphine-induced analgesic effect was calculated as the percentage of maximum possible effect (%MPE) through the following formula: $\%MPE = [(T1 - T0) / (T2 - T0)] \times 100$; where T0 is the latency prior to treatment, T1 is the latency after treatment and T2 is the pre-determined cutoff time. All experiments were performed in the same lab with constant cues and environmental conditions.

2.4.3. Monitoring the intensity of opioid dependence by morphine withdrawal test

Morphine withdrawal test is a standard and well established behavioral method commonly used by the researchers for quantification of drug dependence intensity in animal models [26,34,35]. Mechanistically, chronic administration of opioid agonists induces neuronal

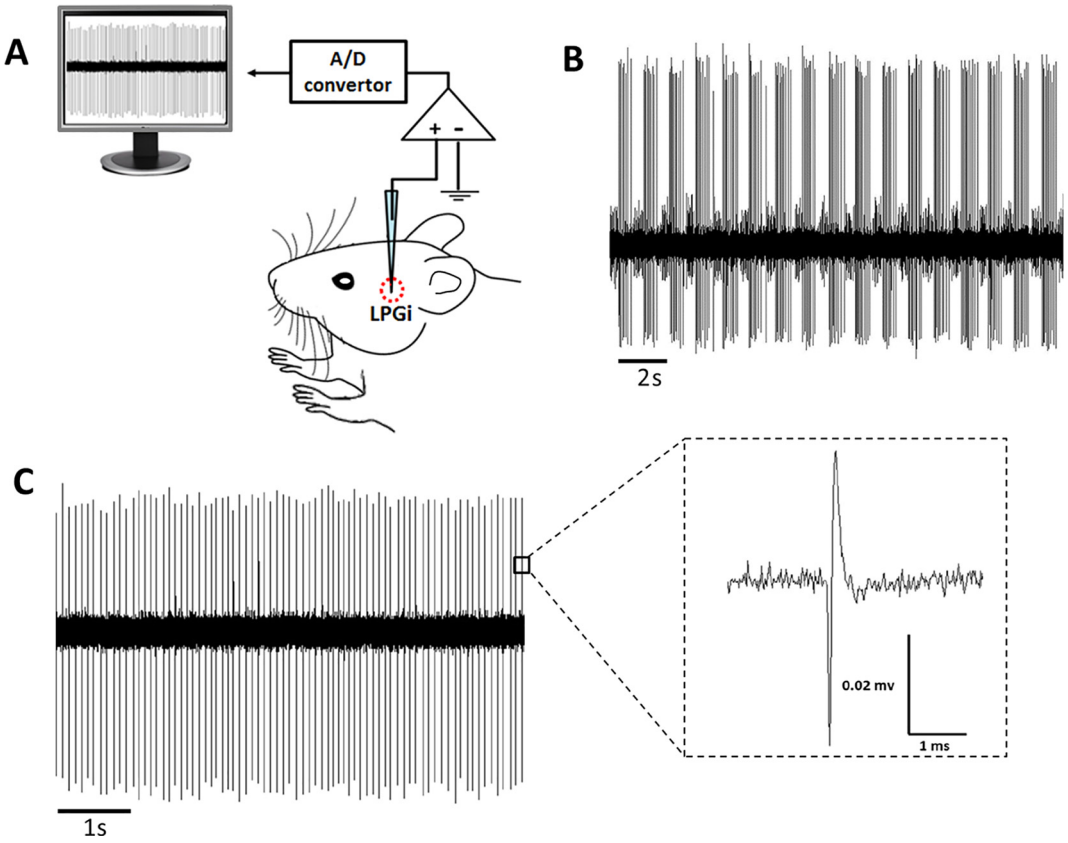

Fig. 4. In vivo single unit recording and electrophysiological identification of LPGi unit discharges. A) A block diagram displaying the experimental set up used for in vivo single unit recording in anesthetized rat. B) A sample signal indicating the burst-like extracellular activity of neurons within the RVLM region. C) Left side: A sample trace of neuronal activity recorded from the LPGi region (spikes emerged after disappearance of the mentioned burst-like pattern). Right side: represents the typical waveform of the LPGi extracellular spikes.

adaptations in certain brain circuits. However, when opioid ligands are either spontaneously or pharmacologically detached from their receptors, the mentioned neuronal pathways are super-activated resulting in simultaneous behavioral manifestations known as "opioid withdrawal signs" [28,36]. These signs mainly include chewing (mastication), defecation (discharge of feces), genital licking, grooming (manipulation of the head or body), head tremor (shaking of the head), teeth chattering (teeth grinding), sniffing (brief audible inhalations), jumping, writhing (twisting of the abdomen), rearing (standing on hind limbs to explore) etc.

In order to investigate the long-term effect of adolescent nicotine treatment on the intensity of morphine dependence, nicotine- or saline-treated rats were rendered dependent on morphine during adulthood using a procedure described previously [37,38]. Briefly, morphine was injected (10 mg/kg, s.c., b.i.d. at 08:00 and 17:00) for 9 consecutive days. Then, on day 10, the last dose of morphine was injected and withdrawal syndrome was pharmacologically induced 2 h later by intraperitoneal (i.p.) injection of naloxone (2 mg/kg). In other words, the entire dependence protocol was conducted between PNDs 65–75. Afterwards, each rat was transferred to a transparent cylindrical test chamber for behavioral assessment (50 cm in height, 30 cm in diameter and the bottom covered with woodchip). The expression of withdrawal signs was visually observed and recorded for 25 min after naloxone injection by the same experimenter who was blinded to the treatments. Furthermore, in each test day, rats from both experimental groups were assessed.

2.4.4. Surgical procedure and electrophysiological recording

For extracellular single unit recording, adult rats were anesthetized by acute i.p. injection of urethane (1.2 g/kg) which was repeated when necessary at a lower maintenance dose (0.15 g/kg) [39]. In order to facilitate breathing and prevent apnea under anesthesia, rats were first tracheostomized and then fixed in a rodent stereotaxic frame (Narishige, Japan). Next, the scalp was longitudinally cut and underlying tissues were dissected to expose the skull surface. Afterwards, a small hole was carefully drilled right above the stereotaxic coordinates of LPGi region (12 mm posterior to bregma and 1.5 mm lateral to the midline) [40]. Dura was cautiously removed using the curved tip of a 30-gauge stainless steel needle under a dissecting microscope. The spontaneous unit activity of LPGi neurons were recorded by borosilicate glass micropipettes (OD: 1.2 mm, ID: 0.94 mm, Sutter instruments, CA, USA) filled with a combination of 0.5 M sodium acetate solution and 2% pontamine sky blue dye with the tip resistance of 3–10 MΩ. The recording electrode was smoothly inserted through the hole into the region of interest i.e. LPGi nucleus, 10.2–10.8 mm below the skull surface [25] using a hydraulic microelectrode driver (MO-10 OIL hydraulic micromanipulator, Narishige, Japan). Extracellular signals were amplified (×1 k) by an AC differential amplifier (DAM 80, WPI, USA). Sampling rate and band pass filter were adjusted at 20 kHz and 0.3–3 kHz, respectively. The amplified analog signals were monitored on an oscilloscope (Hitachi, Japan) while being transported to an audio-analyzer (Fredrick Haer, USA), digitized by an analog-to-digital board (PowerLab 4/30, ADInstruments Pty Ltd., Australia) and saved on a computer. For off-line analysis of the obtained spikes, Lab Chart 7 software, equipped with the spike histogram module, was used (ADInstruments, Australia).

During the insertion of microelectrode towards the LPGi, a sudden burst-like train of spontaneous discharge appeared. This typical pattern (Fig. 4B), which occurs in phase with the animal's respiratory movements, originates form the activity of respiratory neurons within the

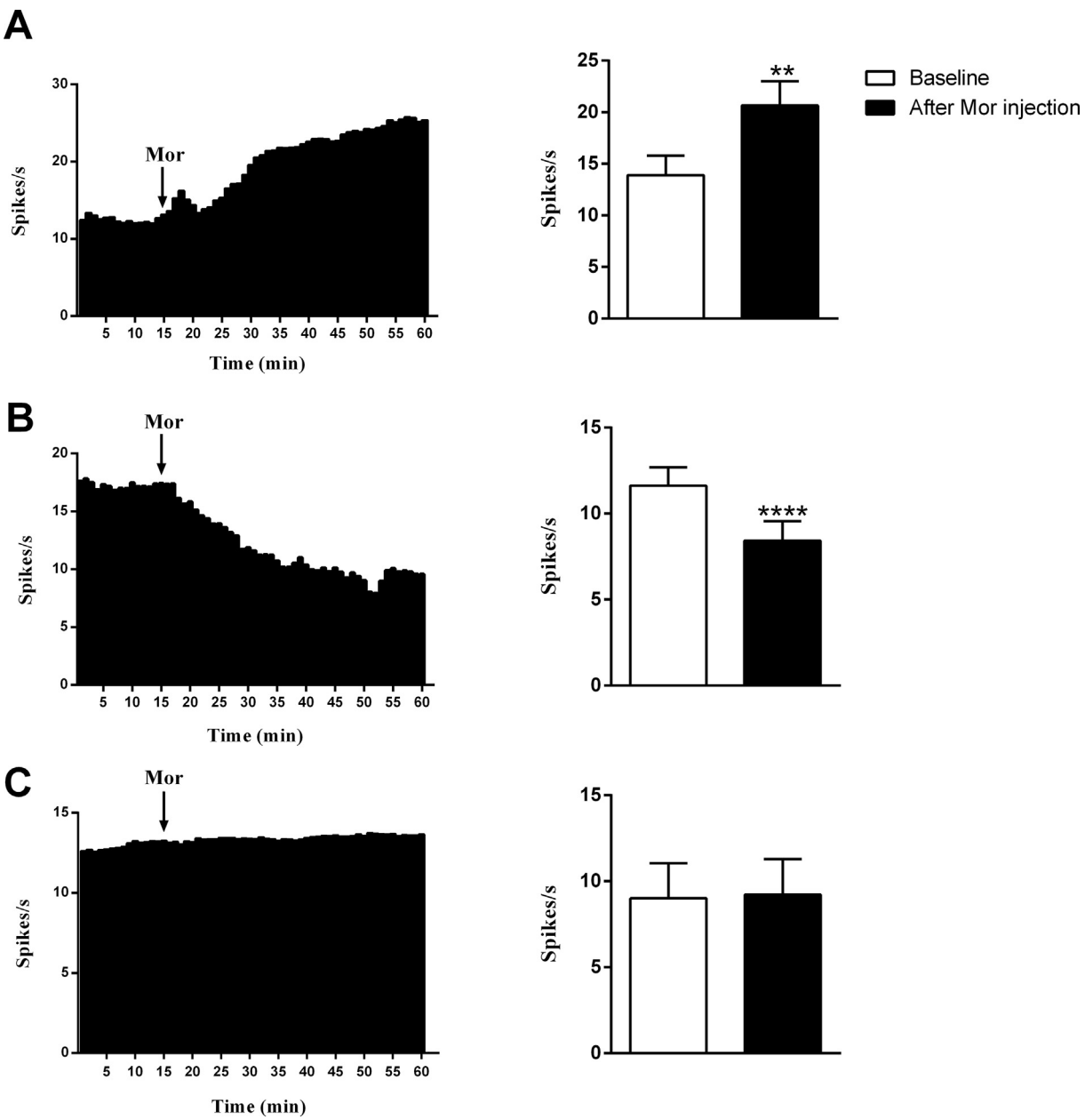

Fig. 5. Heterogeneity of LPGi neuronal responses to morphine injection in saline-treated rats. Sample histograms (A, B and C) and respective bar graphs indicate heterogeneous responses of LPGi neurons to acute morphine (3.5 mg/kg, i.p.) injection (i.e. excitatory, inhibitory and no response, respectively) in rats received saline during adolescence. Paired Student's t-test, $^{**}P < 0.01$, $^{****}P < 0.0001$, compared to the baseline. Data are presented as means ± standard error of the mean (SEM).

rostral ventrolateral medulla (RVLM) [41] and serves as an electrophysiological landmark to ensure the accuracy of recording site [42]. The neuronal activities emerged following the disappearance of last respiratory signals were considered as the LPGi spikes.

In each experiment (both saline- and nicotine-treated groups), the baseline activity of LPGi neurons was recorded for 15 min and then morphine sulfate was administered (3.5 mg/kg, i.p.). In our study, the responses of LPGi neurons to acute morphine injection was calculated by mean ± 2 × standard deviation (SD) and classified as "excitatory response" and "inhibitory response" representing increased and decreased firing rate compared to the baseline level, respectively. Furthermore, the coefficient of variation (CV) was calculated for interspike intervals (ISIs) from the following formula: SD/mean of ISIs × 100. This index was used to reach a quantitative estimate of alterations in the spike regularity among different experimental groups.

2.5. Statistical analysis

Statistical analysis was performed by GraphPad Prism software (version 6.01 for Windows, USA). In order to check the normality of data distribution, Kolmogorov Smirnov or the Shapiro–Wilk tests were used. Data from morphine tolerance test were compared among different experimental groups by two-way ANOVA with repeated measures followed by Bonferroni's post hoc comparisons. Area under the curve (AUC) data was analyzed by unpaired Student's *t*-test. For the assessment of morphine dependence, results were presented as the number of withdrawal signs occurred during each recording session. Normally distributed data were analyzed by unpaired Student's *t*-test and for non-parametric findings; Mann–Whitney *U* test was used. The effect of acute morphine administration on the spontaneous discharge rate of LPGi neurons was analyzed by Student's *t*-test. In cases of normal

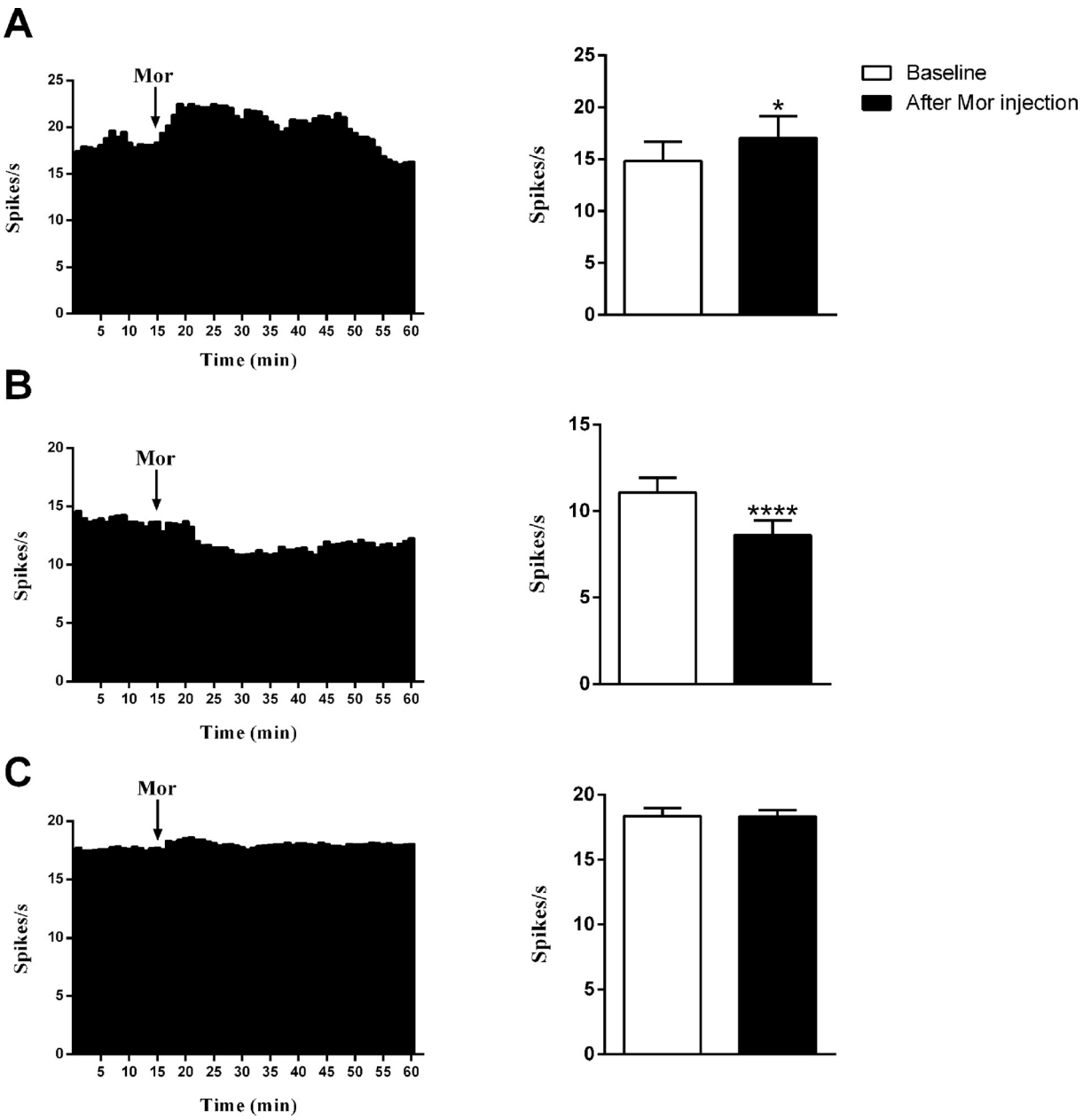

Fig. 6. Heterogeneity of LPGi neuronal responses to morphine injection in nicotine-treated rats. Sample histograms (A, B and C) and respective bar graphs indicate heterogeneous responses of LPGi neurons to acute morphine (3.5 mg/kg, i.p.) injection (i.e. excitatory, inhibitory and no response, respectively) in rats received nicotine during adolescence. Paired Student's t-test, *$P < 0.05$, ****$P < 0.0001$, compared to the baseline. Data are presented as means ± standard error of the mean (SEM).

distribution, data are presented as mean ± standard error of the mean (SEM), otherwise, results are shown as median with interquartile range. In all experiments, the $p < 0.05$ was considered statistically significant.

3. Results

3.1. Adolescent nicotine administration facilitates the development of morphine analgesic tolerance in adulthood

As indicated in Fig. 2A, analgesic tolerance (defined as the gradual reduction of drug effect) was developed following chronic morphine treatment (3.5 mg/kg, s.c. for 7 consecutive days) in both "nicotine-morphine" and "saline-morphine" groups. However, tolerance process was accelerated in rats pre-treated with nicotine during adolescence (nicotine-treated group) compared to the control counterparts. Statistically, two-way ANOVA revealed significant difference in %MPE of the two experimental groups ($P < 0.001$, 73.8 ± 5.7 vs. 96.4 ± 1.9, 39.9 ± 4.5 vs. 78.5 ± 5.1 and 7.6 ± 3 vs. 47.5 ± 4.2, for days 3, 5 and 7, respectively).

In other words, %MPE on day 7, is still significantly different in saline-morphine group vs. saline-saline group ($P < 0.001$, 47.5 ± 4.2 vs. 0.6 ± 0.6, respectively), while in nicotine-morphine group, %MPE is reduced to the control level on the same day (not significantly different, 7.6 ± 3 vs. 0.6 ± 0.6). Furthermore, as shown in Fig. 2B, area under curve (AUC) values for the entire experimental period were significantly decreased in nicotine-morphine group compared to control rats ($P < 0.001$, 166.7 ± 10.57 vs. 248.8 ± 8.4, respectively).

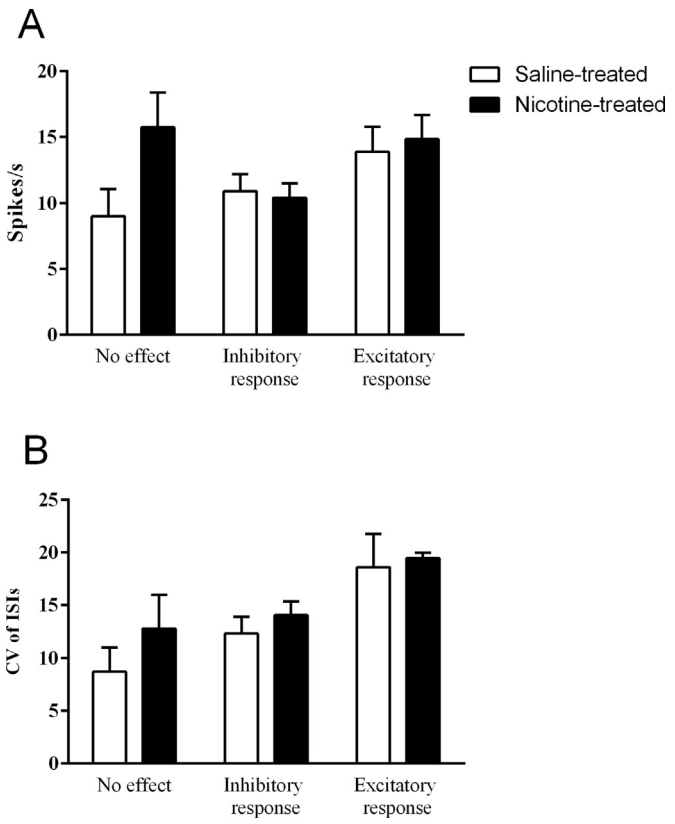

Fig. 7. The effect of adolescent nicotine administration on frequency of baseline activity and regularity of firing rate in LPGi neurons during adulthood. As shown, for the three response categories (no effect, excitatory and inhibitory), neither the baseline firing rate (A) nor the CV of ISIs (B) were significantly altered following morphine injection during adulthood in nicotine-treated group compared to the saline-treated subjects. CV: coefficient of variation, ISI: interspike interval. Unpaired Student's t-test, data are averaged and presented as means ± standard error of the mean (SEM).

3.2. Adolescent nicotine administration potentiates naloxone-induced morphine withdrawal signs in adulthood

Somatic signs of morphine withdrawal were visually assessed in morphine-dependent rats following naloxone injection (2 mg/kg, i.p.). Results indicated that adolescent nicotine (vs. saline) exposure potentiates the intensity of morphine dependence in adulthood. This was revealed by the observation of more frequent behavioral manifestations (5 out of 9 withdrawal signs) in nicotine-morphine vs. saline-morphine group (Fig. 3). The results of unpaired two-tailed *t*-test (for parametric data) were as follows: teeth chattering (68.9 ± 17.6 vs. 9.1 ± 3.5, $p < 0.01$), rearing (34 ± 2.9 vs. 23.5 ± 3.3, $p < 0.05$) and jumping (18.4 ± 3.9 vs. 6.9 ± 3.2, $p < 0.05$). In addition, the results of Mann–Whitney *U* test (for non-parametric data) were as follows: genital licking (1.5 (0.0–3.0) vs. 0.0 (0.0–0.25), $p < 0.05$) and sniffing (4.5 (1.0–17.75) vs. 0.0 (0.0–6.25), $p < 0.05$).

3.3. Heterogeneity in LPGi neuronal responses to acute morphine injection

The effect of acute morphine injection on spontaneous firing rate of LPGi neurons was examined in adult rats that have undergone nicotine or saline (as vehicle) exposure during adolescence (i.e. nicotine/saline-treated groups, respectively). In both groups, morphine injection (3.5 mg/kg, i.p.) was done after 15 min of baseline recording. As shown in Figs. 5 and 6, acute morphine induced heterogeneous (hereafter called "excitatory" and "inhibitory") responses in LPGi neurons. In addition, the firing rates of some neurons were not affected by morphine administration ("no effect"). Totally, 45 LPGi neurons were recorded in 45 rats (23 and 22 neurons in saline-and nicotine-treated groups, respectively). In saline-treated group, morphine induced excitation in 4 neurons (baseline: 13.9 ± 1.9 spikes/s vs. 20.6 ± 2.3 spikes/s after morphine administration) and inhibition in 15 neurons (baseline: 11.6 ± 1.1 spikes/s vs. 8.4 ± 1.1 spikes/s after morphine administration). Furthermore, the baseline activity of 4 neurons was not significantly altered following acute morphine administration (9 ± 2 spikes/s vs. 9.2 ± 2.1 spikes/s). In nicotine-treated rats, morphine induced excitation in 3 neurons (14.8 ± 1.8 spikes/s vs. 17 ± 2.1 spikes/s) and inhibited the activity of 16 neurons (11.1 ± 0.8 spikes/s vs. 8.6 ± 0.8 spikes/s). Moreover, the firing rate of 3 neurons was not affected by morphine injection (18.3 ± 0.6 spikes/s vs. 18.3 ± 0.5 spikes/s). It is worthwhile mention that distribution of neuronal response types was not significantly different (data not shown) in nicotine-vs. saline-treated animals (for excitatory responses: 13.63% vs. 17.39%, for inhibitory responses: 72.72% vs. 65.21% and for no effect cases 13.63% vs. 17.39%).

3.4. The effect of adolescent nicotine exposure on baseline spontaneous activity and regularity of firing rate in LPGi neurons

As indicated in Fig. 7A, the baseline spontaneous activity of LPGi neurons in all three response categories was not affected by adolescent nicotine exposure compared to the corresponding responses in control (saline-treated) animals (for "no effect" observations: 15.7 ± 2.6 vs. 9 ± 2, for "inhibitory" responses: 10.41 ± 1.06 vs. 10.89 ± 1.28 and for "excitatory" responses: 14.8 ± 1.8 vs. 13.9 ± 1.9 in nicotine- and saline treated rats, respectively). In addition, in order to assess whether adolescent nicotine exposure could induce long-lasting effects on firing regularity of unit discharge in LPGi neurons, coefficient of variation (CV) for interspike intervals (ISIs) was calculated for each LPGi neuronal class (excitatory, inhibitory and no effect) in both experimental groups. Results indicated that no alteration occurs in CV of ISIs in any of response types following adolescent nicotine administration compared to the corresponding response type in saline-treated group (Fig. 7B), (for excitatory responses: %19.5 ± 0.5 vs. %18.6 ± 3.1, for inhibitory responses: %14 ± 1.3 vs. %12.3 ± 1.5 and for no effect cases: %12.8 ± 3.2 vs. %8.7 ± 2.3).

3.5. The extent and timing of LPGi neuronal responses to acute morphine injection in adult rats undergone adolescent nicotine or saline treatment

In saline-treated animals, the extent of morphine-induced excitation was 50.19 ± 5.96% compared to the baseline level. This enhancement was significantly attenuated in rats received nicotine during adolescence (Fig. 8A), (50.2 ± 6% vs. 14.6 ± 0.5% in saline- and nicotine-treated rats respectively). However, adolescent nicotine exposure did not significantly alter the inhibitory effect (Fig. 8B), (29.7 ± 4.9% vs. 22.9 ± 3.7% in saline and nicotine- treated rats, respectively). Furthermore, for all recorded neuronal activities, the time taken to observe both the onset (mean ± 2SD, Fig. 8C and D) and the time to peak of morphine inhibitory/excitatory effect were calculated. As shown, the onset of morphine inhibitory effect was increased in animals pre-treated with nicotine in adolescence compared to saline-treated animals (11.7 ± 1 s. vs. 7.1 ± 1.3 s. in nicotine- and saline-treated rats, respectively), but no significant difference was observed in the same index for morphine excitatory effect (1 s. vs. 1.2 ± 0.2 s. in nicotine- and saline-treated groups respectively). Adolescent nicotine exposure did not alter the time taken to observe the maximum effect of morphine (data not shown) compared to the corresponding neuronal category in saline-treated group (for inhibitory responses: 39.1 ± 2.2 s. vs. 39.1 ± 1.7 s. and for excitatory responses: 14.7 ± 9.2 s. vs. 26.2 ± 10.3 s.).

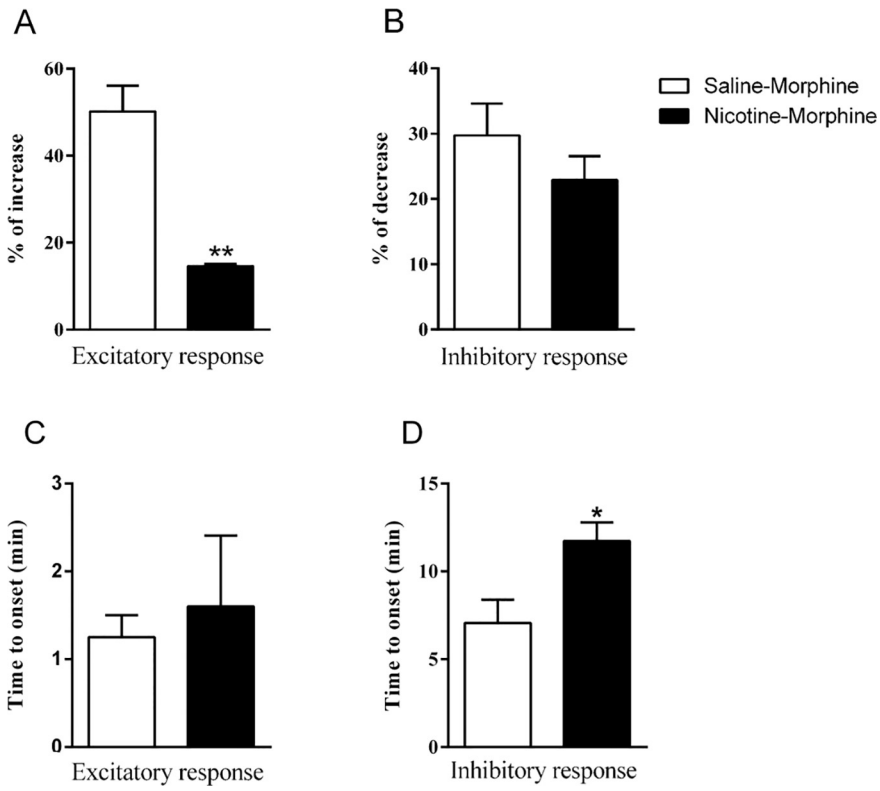

Fig. 8. The maximum extent and the onset of morphine effect in adult rats received nicotine or saline during adolescence. A) and B) indicate the percentage of increase and decrease in firing rate of LPGi neurons, respectively following morphine injection during adulthood in nicotine-treated rats compared to the control (saline-treated) animals. C) and D) indicate time taken to observe the onset (mean ± 2SD) of morphine excitatory and inhibitory effects, respectively. Unpaired Student's t-test, *P < 0.05 and **P < 0.01, data are expressed as mean ± standard error of the mean (SEM).

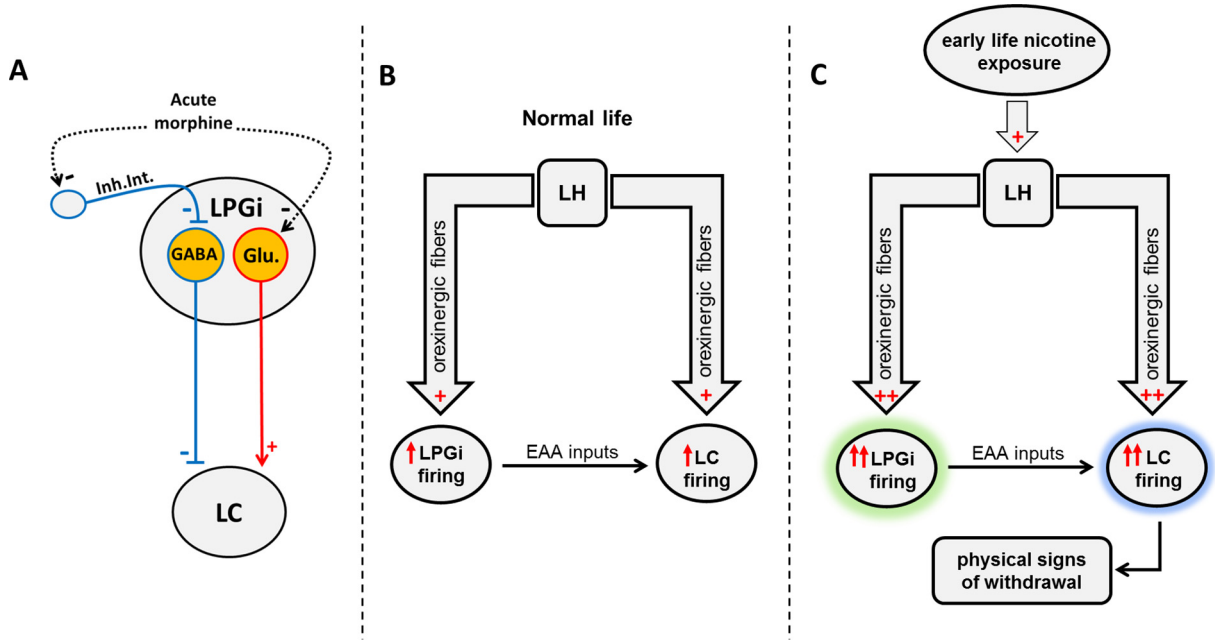

Fig. 9. Schematic representation of hypothetical mechanisms underlying persistent effects of adolescent nicotine exposure on neuroadaptation occurring within the paragiganto-coerulear pathway.

A) Paragiganto-coerulear (LPGi to LC) circuit [28] contains two main category of GABAergic [48,57,58] and glutamatergic [28,43] neurons. Acute morphine administration inhibits the firing rate of LC through direct (inhibition of glutamatergic transmission) or indirect (disinhibition of GABAergic transmission) mechanisms. Adolescent nicotine exposure may reduce the sensitivity of interneurons to morphine within the indirect path which results in attenuated inhibition of GABAergic transmission to LC. On the other hand, adolescent morphine exposure may reduce the sensitivity of glutamatergic neurons to morphine which leads to the LC excitation as well. B) Indicates orexinergic fibers originating from LH and their projections to LPGi and LC as well as the existing excitatory transmission between the two nuclei. C) Indicates how prolonged nicotine pre-treatment can potentiate orexin-mediated excitation of LPGi to LC pathway and the subsequent expression of withdrawal signs. Abbreviations: Glu., Glutamatergic, Inh.Int., Inhibitory interneuron, LH., lateral hypothalamus, EAA., excitatory amino acid.

4. Discussion

The results of our behavioral experiments revealed that adolescent nicotine exposure facilitates the development of morphine-analgesic tolerance and also potentiates the intensity of morphine dependence which was characterized by expression of more naloxone-induced withdrawal signs. Interestingly, similar findings had been obtained in our previous study in which animals underwent adolescent morphine (rather than nicotine) exposure [34]. This similarity in long lasting behavioral effects motivated us to further investigate whether adolescent nicotine or morphine exposure can affect the function of brain neuronal circuits through similar cellular mechanisms. To this end, two independent series of electrophysiological experiments were conducted and the effect of adolescent morphine/nicotine exposure on later (adulthood) responses of LPGi neurons to morphine was examined in rats. In case of morphine, our results were obtained and published earlier [25]. In brief, adolescent morphine treatment increased the baseline firing rate of LPGi neurons and potentiated the morphine-induced inhibitory effect in adulthood, however, distribution of response types (excitatory and inhibitory), regularity of ISIs and the onset of morphine effect were not affected. In the present study, the same heterogeneous response categories to acute morphine injection were observed in our recordings. This heterogeneity in response could be attributed to the extensive neurochemical diversity of LPGi neurons as reported in previous studies [43–46].

Moreover, in nicotine-treated rats (received nicotine during adolescence); neither the baseline activity of LPGi neurons, nor the regularity of ISIs was altered in adulthood (Fig. 7). However, the interesting point is that adolescent nicotine exposure suppressed the morphine-induced excitatory effect. Taken together, it seems that adolescent exposure with either nicotine or morphine results in neuronal adaptations with the same alignment (i.e. decreased excitation and increased inhibition, respectively) representing reduced level of excitability in specific, but not necessarily the same, LPGi neurons. In addition, the latency of inhibitory responses to morphine was significantly increased in nicotine-treated animals compared to the control group. In other words, adolescent nicotine exposure delayed the onset of inhibitory responses to morphine administration. This may in turn indicate reduced sensitivity of opioid receptors in some LPGi neurons at the result of nicotine pre-treatment during adolescence.

Another similar finding between the two mentioned studies was that in both cases, distributions of response types (% of cells displaying excitatory or inhibitory response to morphine) did not change and instead the maximum effect of morphine was altered. This also strengthens the idea that the long-lasting effects of nicotine and morphine on LPGi neurons might have been mainly occurred at receptor level (e.g. alteration of receptor sensitivity).

Now it is time to discuss how persistent neuroadaptations of LPGi neurons induced by adolescent nicotine treatment might have potentiated the intensity of morphine withdrawal signs as observed in our study. Here, it is very important to highlight again the role of LPGi to LC circuit in modulation of opiate dependence which has been mentioned earlier and comprehensively reviewed elsewhere [28]. In nutshell, direct excitatory afferents of LPGi to LC are required for the hyperactivity of LC neurons as a critical prerequisite for the expression of withdrawal signs [28]. Previous studies have shown that the rat LPGi acts as a relay station for mediating the nicotine-induced effects in LC nucleus. More precisely, nicotine has been shown to excite the LC neurons and this effect is abolished by pharmacological blockade of LPGi to LC excitatory neurotransmission [47]. Thus one possibility is that those neurons which are normally excited by acute morphine injection in LPGi (probably through disinhibition), are the inhibitory interneurons projecting to LC and causing it to turn off. Consistently, the existence of such inhibitory projections has been previously reported [48,49].

Thus, attenuation of these morphine excitatory effects in LPGi (similar to what we observed in this study following nicotine-pretreatment) may result in hyperactivity of LC and the subsequent expression of withdrawal signs. This effect has also been observed in literature, where nicotine administration has been found to suppress the activity of GABAergic neurons in LPGi nucleus [50]. This hypothesis is further supported by our other finding i.e. facilitation of tolerance to morphine analgesic effect in adult rats undergone adolescent nicotine exposure. In other words, development of morphine tolerance in LPGi neurons, which is already reported in literature [51], is intensified at behavioral level due to reduction of LPGi neuronal responsiveness to morphine challenge in adulthood that is induced by early nicotine pretreatment. In this regard, occurrence of nicotine-morphine cross tolerance has previously been suggested [52]. In addition, it is believed that chronic nicotine intake can act as a gateway for the induction of dependence to other drugs of abuse such as opioids [53]. Furthermore, there are numerous evidence suggesting mutual regulatory mechanisms between nicotine receptors and endogenous opioid system [18–21].

There is also another mechanistic approach for the interpretation of our results. Endogenous orexin neuropeptides, originating from the lateral hypothalamus neurons, are well established as critical modulators of opioid-induced neuroadaptations in specific brain structures such as LPGi and LC [26,28,33,54]. Normally, orexinergic projections of LH excite both LPGi and LC neurons (schematically shown in Fig. 9B), i.e. LC receives direct (LH-mediated) and indirect (LH-LPGi-mediated) excitatory inputs [28]. During opioid withdrawal, activity of these orexinergic projections dramatically increases resulting in hyperactivity of LC and the subsequent expression of withdrawal signs [28]. Interestingly, there is an evidence indicating that prolonged nicotine exposure increases the expression of orexin and its receptors in LH [55]. In addition, immunohistochemical experiments support the idea that nicotine-induced activation of LC neurons is mediated by orexinergic inputs [56]. Thus, our second hypothesis is that prolonged administration of nicotine may potentiate orexin-mediated excitatory transmission within the paragiganto-coerulear pathway (Fig. 9C).

5. Conclusion

Collectively, we believe that prolonged exposure with nicotine during the critical period of adolescence, in which most brain networks are still developing, can persistently alter the future susceptibility to opioid effects and this may in turn be mediated via the long-term changes occurring in the responsiveness of LPGi neurons to opioid ligands which alters the excitatory transmission to the coerulear region in the brain stem. Indeed, future studies are required to mechanistically investigate the suggested hypotheses.

Acknowledgement

This study is supported by the Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.

Author contributions

HA and AR conceived and designed the experiments. MT performed the experiments. MT, HA, SMA and AR analyzed the data. MT, SMA and HA wrote the manuscript. The authors discussed and approved the final manuscript.

Declaration of competing interest

The authors declare no conflict of interest.

References

[1] American Cancer Society, Cancer Facts and Figures 2010, American Cancer Society, Atlanta, 2010.
[2] U.S. Department of Health and Human Services, Preventing Tobacco Use Among

Youth and Young Adults: a Report of the Surgeon General, Department of Health and Human Services, Atlanta, GA, 2012.
[3] M. Yuan, S.J. Cross, S.E. Loughlin, F.M. Leslie, Nicotine and the adolescent brain, J. Physiol. 593 (2015) 3397–3412, https://doi.org/10.1113/JP270492.
[4] L.P. Spear, Adolescent neurodevelopment, J. Adolesc. Health 52 (2013) S7–13, https://doi.org/10.1016/j.jadohealth.2012.05.006.
[5] L.P. Spear, The adolescent brain and age-related behavioral manifestations, Neurosci. Biobehav. Rev. 24 (2000) 417–463.
[6] J.D. Belluzzi, A.G. Lee, H.S. Oliff, F.M. Leslie, Age-dependent effects of nicotine on locomotor activity and conditioned place preference in rats, Psychopharmacology 174 (2004) 389–395, https://doi.org/10.1007/s00213-003-1758-6.
[7] C.E. Wilmouth, L.P. Spear, Adolescent and adult rats' aversion to flavors previously paired with nicotine, Ann. N. Y. Acad. Sci. 1021 (2004) 462–464, https://doi.org/10.1196/annals.1308.065.
[8] M.J. Shram, D. Funk, Z. Li, A.D. Lê, Periadolescent and adult rats respond differently in tests measuring the rewarding and aversive effects of nicotine, Psychopharmacology 186 (2006) 201–208, https://doi.org/10.1007/s00213-006-0373-8.
[9] J.M. Brielmaier, C.G. McDonald, R.F. Smith, Immediate and long-term behavioral effects of a single nicotine injection in adolescent and adult rats, Neurotoxicol. Teratol. 29 (2007) 74–80, https://doi.org/10.1016/j.ntt.2006.09.023.
[10] O.V. Torres, H.A. Tejeda, L.A. Natividad, L.E. O'Dell, Enhanced vulnerability to the rewarding effects of nicotine during the adolescent period of development, Pharmacol. Biochem. Behav. 90 (2008) 658–663, https://doi.org/10.1016/j.pbb.2008.05.009.
[11] W. Adriani, V. Deroche-Gamonet, M. Le Moal, G. Laviola, P.V. Piazza, Preexposure during or following adolescence differently affects nicotine-rewarding properties in adult rats, Psychopharmacology 184 (2006) 382–390, https://doi.org/10.1007/s00213-005-0125-1.
[12] D. Kota, S.E. Robinson, M. Imad Damaj, Enhanced nicotine reward in adulthood after exposure to nicotine during early adolescence in mice, Biochem. Pharmacol. 78 (2009) 873–879, https://doi.org/10.1016/j.bcp.2009.06.099.
[13] S. Lai, H. Lai, J.B. Page, C.B. McCoy, The association between cigarette smoking and drug abuse in the United States, J. Addict. Dis. 19 (2000) 11–24, https://doi.org/10.1300/J069v19n04_02.
[14] M. Alajaji, M.F. Lazenka, D. Kota, L.E. Wise, R.M. Younis, F.I. Carroll, A. Levine, D.E. Selley, L.J. Sim-Selley, M.I. Damaj, Early adolescent nicotine exposure affects later-life cocaine reward in mice, Neuropharmacology 105 (2016) 308–317, https://doi.org/10.1016/j.neuropharm.2016.01.032.
[15] L.C. Klein, Effects of adolescent nicotine exposure on opioid consumption and neuroendocrine responses in adult male and female rats, Exp. Clin. Psychopharmacol. 9 (2001) 251–261.
[16] N.L. Benowitz, Pharmacology of nicotine: addiction, smoking-induced disease, and therapeutics, Annu. Rev. Pharmacol. Toxicol. 49 (2009) 57–71, https://doi.org/10.1146/annurev.pharmtox.48.113006.094742.
[17] A.A. Jensen, B. Frølund, T. Liljefors, P. Krogsgaard-Larsen, Neuronal nicotinic acetylcholine receptors: structural revelations, target identifications, and therapeutic inspirations, J. Med. Chem. 48 (2005) 4705–4745, https://doi.org/10.1021/jm040219e.
[18] S. Kishioka, N. Kiguchi, Y. Kobayashi, F. Saika, Nicotine effects and the endogenous opioid system, J. Pharmacol. Sci. 125 (2014) 117–124.
[19] A.A. Houdi, K. Pierzchala, L. Marson, M. Palkovits, G.R. Van Loon, Nicotine-induced alteration in Tyr-Gly-Gly and met-enkephalin in discrete brain nuclei reflects altered enkephalin neuron activity, Peptides 12 (1991) 161–166.
[20] R. Isola, H. Zhang, G.A. Tejwani, N.H. Neff, M. Hadjiconstantinou, Acute nicotine changes dynorphin and prodynorphin mRNA in the striatum, Psychopharmacology 201 (2009) 507–516, https://doi.org/10.1007/s00213-008-1315-4.
[21] L. Galeote, B.L. Kieffer, R. Maldonado, F. Berrendero, Mu-opioid receptors are involved in the tolerance to nicotine antinociception, J. Neurochem. 97 (2006) 416–423, https://doi.org/10.1111/j.1471-4159.2006.03751.x.
[22] X. Liu, C. Jernigan, Activation of the opioid μ1, but not δ or κ, receptors is required for nicotine reinforcement in a rat model of drug self-administration, Prog. Neuro-Psychopharmacol. Biol. Psychiatry 35 (2011) 146–153, https://doi.org/10.1016/j.pnpbp.2010.10.007.
[23] M.R. Zarrindast, M. Pazouki, S. Nassiri-Rad, Involvement of cholinergic and opioid receptor mechanisms in nicotine-induced antinociception, Pharmacol. Toxicol. 81 (1997) 209–213.
[24] H.L. Tripathi, B.R. Martin, M.D. Aceto, Nicotine-induced antinociception in rats and mice: correlation with nicotine brain levels, J. Pharmacol. Exp. Ther. 221 (1982) 91–96.
[25] H. Salmanzadeh, H. Azizi, S.M. Ahmadi Soleimani, N. Pachenari, S. Semnanian, Chronic adolescent morphine exposure alters the responses of lateral paragigantocellular neurons to acute morphine administration in adulthood, Brain Res. Bull. 137 (2018) 178–186, https://doi.org/10.1016/j.brainresbull.2017.12.007.
[26] S.M. Ahmadi-Soleimani, M. Ghaemi-Jandabi, H. Azizi, S. Semnanian, Orexin type 1 receptor antagonism in lateral Paragigantocellularis nucleus attenuates naloxone precipitated morphine withdrawal symptoms in rats, Neurosci. Lett. 558 (2014) 62–66, https://doi.org/10.1016/j.neulet.2013.10.064.
[27] N. Liu, R.W. Rockhold, I.K. Ho, Electrical stimulation of nucleus paragigantocellularis induces opioid withdrawal-like behaviors in the rat, Pharmacol. Biochem. Behav. 62 (1999) 263–271, https://doi.org/10.1016/s0091-3057(98)00164-6.
[28] S.M. Ahmadi-Soleimani, H. Azizi, H.S. Gompf, S. Semnanian, Role of orexin type-1 receptors in paragiganto-coerulear modulation of opioid withdrawal and tolerance: a site specific focus, Neuropharmacology 126 (2017) 25–37, https://doi.org/10.1016/j.neuropharm.2017.08.024.
[29] K. Rasmussen, G.K. Aghajanian, Withdrawal-induced activation of locus coeruleus neurons in opiate-dependent rats: attenuation by lesions of the nucleus paragigantocellularis, Brain Res. 505 (1989) 346–350, https://doi.org/10.1016/0006-8993(89)91466-2.
[30] A. Kaeidi, H. Azizi, M. Javan, S.M. Ahmadi Soleimani, Y. Fathollahi, S. Semnanian, Direct Facilitatory role of Paragigantocellularis neurons in opiate withdrawal-induced hyperactivity of rat locus Coeruleus neurons: an in vitro study, PLoS One 10 (2015) e0134873, , https://doi.org/10.1371/journal.pone.0134873.
[31] R.L. Pomfrey, T.A. Bostwick, B.B. Wetzell, A.L. Riley, Adolescent nicotine exposure fails to impact cocaine reward, aversion and self-administration in adult male rats, Pharmacol. Biochem. Behav. 137 (2015) 30–37, https://doi.org/10.1016/j.pbb.2015.08.004.
[32] Y. Ranjbar-Slamloo, H. Azizi, Y. Fathollahi, S. Semnanian, Orexin receptor type-1 antagonist SB-334867 inhibits the development of morphine analgesic tolerance in rats, Peptides 35 (2012) 56–59, https://doi.org/10.1016/j.peptides.2012.02.023.
[33] S. Mohammad Ahmadi Soleimani, H. Azizi, J. Mirnajafi-Zadeh, S. Semnanian, Orexin type 1 receptor antagonism in rat locus coeruleus prevents the analgesic effect of intra-LC met-enkephalin microinjection, Pharmacol. Biochem. Behav. 136 (2015) 102–106, https://doi.org/10.1016/j.pbb.2015.07.010.
[34] H. Salmanzadeh, H. Azizi, S. Semnanian, Adolescent chronic escalating morphine administration induces long lasting changes in tolerance and dependence to morphine in rats, Physiol. Behav. 174 (2017) 191–196, https://doi.org/10.1016/j.physbeh.2017.03.014.
[35] H. Azizi, J. Mirnajafi-Zadeh, K. Rohampour, S. Semnanian, Antagonism of orexin type 1 receptors in the locus coeruleus attenuates signs of naloxone-precipitated morphine withdrawal in rats, Neurosci. Lett. 482 (2010) 255–259, https://doi.org/10.1016/j.neulet.2010.07.050.
[36] E.J. Nestler, Historical review: molecular and cellular mechanisms of opiate and cocaine addiction, Trends Pharmacol. Sci. 25 (2004) 210–218, https://doi.org/10.1016/j.tips.2004.02.005.
[37] G.H. Fan, L.Z. Wang, H.C. Qiu, L. Ma, G. Pei, Inhibition of calcium/calmodulin-dependent protein kinase II in rat hippocampus attenuates morphine tolerance and dependence, Mol. Pharmacol. 56 (1999) 39–45, https://doi.org/10.1124/mol.56.1.39.
[38] M. Ghaemi-Jandabi, H. Azizi, S.M. Ahmadi-Soleimani, S. Semnanian, Intracoerulear microinjection of orexin-a induces morphine withdrawal-like signs in rats, Brain Res. Bull. 130 (2017) 107–111, https://doi.org/10.1016/j.brainresbull.2017.01.010.
[39] M. Ghaemi-Jandabi, H. Azizi, S. Semnanian, Blockade of orexin type 1 receptors inhibits the development of morphine tolerance in lateral paragigantocellularis nucleus: an electrophysiological approach, Brain Res. 1578 (2014) 14–22, https://doi.org/10.1016/j.brainres.2014.06.033.
[40] G. Paxinos, C. Watson, The Rat Brain in Stereotaxic Coordinates: Hard Cover Edition, Elsevier, 2006.
[41] R. Kanjhan, J. Lipski, B. Kruszewska, W. Rong, A comparative study of pre-sympathetic and Bötzinger neurons in the rostral ventrolateral medulla (RVLM) of the rat, Brain Res. 699 (1995) 19–32, https://doi.org/10.1016/0006-8993(95)00814-7.
[42] C. Sirieix, D. Gervasoni, P.-H. Luppi, L. Léger, Role of the lateral paragigantocellular nucleus in the network of paradoxical (REM) sleep: an electrophysiological and anatomical study in the rat, PLoS One 7 (2012) e28724, , https://doi.org/10.1371/journal.pone.0028724.
[43] M. Ennis, G. Aston-Jones, Activation of locus coeruleus from nucleus paragigantocellularis: a new excitatory amino acid pathway in brain, J. Neurosci. 8 (1988) 3644–3657.
[44] G. Aston-Jones, B. Astier, M. Ennis, Inhibition of noradrenergic locus coeruleus neurons by C1 adrenergic cells in the rostral ventral medulla, Neuroscience 48 (1992) 371–381, https://doi.org/10.1016/0306-4522(92)90497-p.
[45] P.G. Guyenet, B.S. Young, Projections of nucleus paragigantocellularis lateralis to locus coeruleus and other structures in rat, Brain Res. 406 (1987) 171–184, https://doi.org/10.1016/0006-8993(87)90781-5.
[46] G. Drolet, E.J. Van Bockstaele, G. Aston-Jones, Robust enkephalin innervation of the locus coeruleus from the rostral medulla, J. Neurosci. 12 (1992) 3162–3174.
[47] Z. Chen, G. Engberg, The rat nucleus paragigantocellularis as a relay station to mediate peripherally induced central effects of nicotine, Neurosci. Lett. 101 (1989) 67–71, https://doi.org/10.1016/0304-3940(89)90442-4.
[48] O. Dehkordi, R.M. Millis, G.C. Dennis, E. Jazini, C. Williams, D. Hussain, A. Jayam-Trouth, Expression of alpha-7 and alpha-4 nicotinic acetylcholine receptors by GABAergic neurons of rostral ventral medulla and caudal pons, Brain Res. 1185 (2007) 95–102, https://doi.org/10.1016/j.brainres.2007.09.027.
[49] L. Verret, L. Léger, P. Fort, P.-H. Luppi, Cholinergic and noncholinergic brainstem neurons expressing Fos after paradoxical (REM) sleep deprivation and recovery, Eur. J. Neurosci. 21 (2005) 2488–2504, https://doi.org/10.1111/j.1460-9568.2005.04060.x.
[50] O. Dergacheva, X. Wang, M.R. Lovett-Barr, H. Jameson, D. Mendelowitz, The lateral paragigantocellular nucleus modulates parasympathetic cardiac neurons: a mechanism for rapid eye movement sleep-dependent changes in heart rate, J. Neurophysiol. 104 (2010) 685–694, https://doi.org/10.1152/jn.00228.2010.
[51] A. Haghparast, S. Semnanian, Y. Fathollahi, Morphine tolerance and dependence in the nucleus paragigantocellularis: single unit recording study in vivo, Brain Res. 814 (1998) 71–77, https://doi.org/10.1016/s0006-8993(98)01029-4.
[52] M.R. Zarrindast, N. Faraji, P. Rostami, H. Sahraei, H. Ghoshouni, Cross-tolerance between morphine- and nicotine-induced conditioned place preference in mice, Pharmacol. Biochem. Behav. 74 (2003) 363–369, https://doi.org/10.1016/s0091-3057(02)01002-x.
[53] S. Lai, H. Lai, J.B. Page, C.B. McCoy, The association between cigarette smoking and drug abuse in the United States, J. Addict. Dis. 19 (2000) 11–24, https://doi.org/

10.1300/J069v19n04_02.
[54] S. Mohammad Ahmadi Soleimani, H. Azizi, N. Pachenari, J. Mirnajafi-Zadeh, S. Semnanian, Enhancement of μ-opioid receptor desensitization by orexin-a in rat locus coeruleus neurons, Neuropeptides 63 (2017) 28–36, https://doi.org/10.1016/j.npep.2017.03.004.
[55] J.K. Kane, S.L. Parker, S.G. Matta, Y. Fu, B.M. Sharp, M.D. Li, Nicotine up-regulates expression of orexin and its receptors in rat brain, Endocrinology 141 (2000) 3623–3629, https://doi.org/10.1210/endo.141.10.7707.
[56] O. Dehkordi, J.E. Rose, M.I. Dávila-García, R.M. Millis, S.A. Mirzaei, K.F. Manaye, A. Jayam-Trouth, Neuroanatomical relationships between orexin/Hypocretin-containing neurons/nerve fibers and nicotine-induced c-Fos-activated cells of the reward-addiction Neurocircuitry, J. Alcohol. Drug Depend. 5 (2017), https://doi.org/10.4172/2329-6488.1000273.
[57] E. Sapin, D. Lapray, A. Bérod, R. Goutagny, L. Léger, P. Ravassard, O. Clément, L. Hanriot, P. Fort, P.-H. Luppi, Localization of the brainstem GABAergic neurons controlling paradoxical (REM) sleep, PLoS One 4 (2009) e4272, , https://doi.org/10.1371/journal.pone.0004272.
[58] L. Verret, P. Fort, D. Gervasoni, L. Léger, P.-H. Luppi, Localization of the neurons active during paradoxical (REM) sleep and projecting to the locus coeruleus noradrenergic neurons in the rat, J. Comp. Neurol. 495 (2006) 573–586, https://doi.org/10.1002/cne.20891.