Published in final edited form as:

Rev Neurosci. 2014 ; 25(2): 207–221. doi:10.1515/revneuro-2013-0067.

Critical role of peripheral sensory systems in mediating the neural effects of nicotine following its acute and repeated exposure

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Abstract

It is well established that the reinforcing properties of nicotine depend upon its action on nicotinic acetylcholine receptors expressed by brain neurons. However, when administered systemically, nicotine first phasically activates nicotinic receptors located on the afferents of sensory nerves at the sites of drug administration before reaching the brain and directly interacting with central neurons. While this peripheral action of nicotine has been known for years, it is usually neglected in any consideration of the drug's reinforcing properties and experience-dependent changes of its behavioral and physiological effects. The goal of this work is to review our recent behavioral, electrophysiological, and physiological data, suggesting the critical importance of peripheral actions of nicotine in mediating its neural effects following acute drug exposure and their involvement in alterations of NIC effects consistently occurring following repeated drug exposure. Since nicotine by acting peripherally produces a rapid sensory signal to the CNS that is followed by slower, more prolonged direct drug actions in the brain, these two pharmacological actions interact in the CNS during repeated drug use with the development of Pavlovian conditioned association. This within-drug conditioning mechanism could explain experience-dependent changes in the physiological, behavioral, and human psycho-emotional effects of nicotine, which in drug-experienced individuals always represent a combination of pharmacological and learning variables.

Keywords

learning; metabolic brain activation; neuronal activation; peripheral action of nicotine; sensory signal; pharmacological or within-drug conditioning

...the external environment is not just the passive recipient of our unchecked efferent violence but also the constant source and provider ... of all the innumerable stimuli which impinge upon the living organism and supply it, via afferent inputs, not only with specific information but indeed with the basic energies upon which efferent activity lastly depends.

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(K. H. Ginzl, 1975)

Introduction

It is well established that nicotine (NIC)'s action on centrally located nicotinic acetylcholine receptors is essential in mediating its reinforcing properties (Picciotto and Corrigall, 2002). However, before reaching the brain and interacting with these receptors, NIC transiently activates nicotinic receptors on the afferents of sensory nerves at the sites of its entry (i.e., lung alveoli, nasal and oral cavities) and within the circulatory system (Anand, 1996; Jonsson et al., 2002; Ginzl, 1975; Liu and Simon, 1996; Steen and Reeh, 1993; Walker et al., 1996). Although it is known that this peripheral action of NIC is essential in mediating its primary sensory and cardiovascular effects (Anand, 1996; Comroe, 1960; Ginzl, 1975), it is usually neglected in considerations of the drug's reinforcing properties and experience-dependent changes in its behavioral, and physiological effects (see, however, Bevins et al., 2012; Engberg and Hajos, 1994). The goal of this work is to review our recent behavioral, electrophysiological and physiological findings (Tang and Kiyatkin, 2010; Lenoir and Kiyatkin, 2011; Lenoir et al., 2013), suggesting the critical importance of the peripheral actions of NIC in mediating its neural effects following acute and chronic exposure. Based on these data, we substantiate the hypothesis that the peripheral actions of NIC, by creating a rapid neural signal and interacting with subsequent direct drug actions in the brain, play an important role in the development of NIC-selective neural sensitization—a basic phenomenon underlying experience-dependent changes in behavioral, physiological, and psycho-emotional effects of this drug occurring during its repeated exposure.

Techniques used for evaluating neural effects of NIC following acute and repeated exposure

For direct evaluation of NIC-induced changes in neural activity and their underlying mechanisms we used *electroencephalography* (EEG) with subsequent high-speed analysis of its power and frequency characteristics. To assess structural specificity of the neural response, electrical activity was simultaneously recorded from the cortex and the ventral tegmental area (VTA), a critical structure of the motivational-reinforcement circuit (Wise, 2004). The great advantage of EEG is its dynamic nature, allowing a high-speed (second scale) analysis resolution that is critical for evaluating the latency and time-course of rapid neural responses. In addition to EEG, we also recorded *neck electromyographic* (EMG) activity, a centrally mediated physiological parameter that reflects tonic and phasic changes in motor output. In contrast to locomotion that is insensitive or of little value during certain behavioral responses (i.e., fear-related freezing, intense stereotypy), EMG activity is highly sensitive to even very weak sensory stimuli such as an audio signal or intravenous (iv) saline injection (Kiyatkin and Smirnov, 2010). In contrast to single-unit recording, which has a number of significant limitations when used in freely moving rats (i.e., cell heterogeneity, silent/active cells, limited recording time, necessity of large data samples), EEG and EMG signals may be recorded with high temporal resolution for extended periods following repeated daily sessions in the same animals, providing an integral measure of basal activity state and event-related neural and motor responses. While the total power of the EEG signal,

a universal measure of electrical synchronization/desynchronization (Buzsaki, 2008; Steriade and Carley, 2005), was the primary parameter for evaluating the dynamics of neural response, additional information on alterations in neural activity was obtained by EEG spectral analysis, which can reveal dynamic, time-dependent alterations in specific activity bands. While non-specific with respect to individual neurons, frequency analysis of the EEG signal provides valuable information on activity changes of large neuronal populations.

For assessing the physiological effects of NIC and their changes following repeated drug exposure, we used *thermorecordings* in freely moving rats. Similar to the electrophysiological studies, these recordings were conducted in chronic experiment, thus allowing an examination of the temperature effects of NIC and related drugs during multiple sessions. In these studies, temperatures were monitored from three locations: the brain (nucleus accumbens or NAcc), temporal muscle, and facial skin. While brain temperature is an important homeostatic parameter, it fluctuates in rats within a relatively large range (3–4°C), normally at low levels during slow-wave sleep and increasing in response to various arousing stimuli, during various behaviors, and after exposure to different drugs (see for review Kiyatkin, 2010, 2013). Since brain temperature depends upon metabolism-related intra-brain heat production and heat dissipation by cerebral blood flow, simultaneous temperature recording from the temporal muscle makes it possible to reveal a component of brain temperature response related to metabolic neural activity. While drug-induced changes in metabolic neural activity could be also evaluated by using PET and fMRI and these approaches were used to study nicotine in humans (Garavan et al., 2000; Stampeton et al., 2003; Zuo et al., 2011), these techniques are based on indirect measures (glucose metabolism or fluctuations in local cerebral blood flow) and not applicable in behaving animals. In contrast, brain temperature could be recorded in freely moving rats with high temporal resolution during multiple sessions, thus making possible to examine experience-dependent changes in drug responses. Skin temperature provides a sensitive index of peripheral vasoconstriction, the known effect of any arousing stimuli and NIC (Altschule, 1951; Baker et al., 1976; Comroe, 1960; Ginzel, 1975).

Our electrophysiological and temperature recordings were combined with the monitoring of locomotion as an index of behavioral activation. It is well known that NIC affects locomotion and its repeated intermittent administration results in progressive enhancement or sensitization of drug-induced locomotor responses (Benwell and Balfour, 1992; Clark and Kumar, 1983; Domino et al., 2001; Mao and McGehee, 2010; Vezina et al., 2007). Thus, this simple measure allowed us to correlate changes in physiological parameters with animal behavior elicited by acute and repeated NIC exposure. Moreover, this parameter was important to clarify the role of peripheral drug actions by using peripherally acting nicotinic agonists and antagonists (see below).

Pharmacological tools for evaluating the contribution of peripheral vs. central actions of nicotine

In our studies, we employ iv NIC ([–]nicotine hydrogen tartrate) injections at a low, behaviorally relevant dose (10–30 µg/kg) that is optimal for development and maintenance of operant self-administration behavior in rats (Cox et al., 1984; Donny et al., 1995). This

NIC formulation easily crosses the blood-brain barrier (BBB), thus acting on both peripherally and centrally located nicotinic receptors. Although humans use NIC preferentially by smoking, providing rapid drug entry to the brain (Rose et al., 2010; Berridge et al., 2010), iv injection compared to other means of drug delivery is a valid route of drug administration in rats to study the basic mechanisms underlying its neural and physiological effects.

To examine the contribution of peripheral actions of NIC to its neural effects, we used two pharmacological tools, hexamethonium bromide (HEXA) and NIC pyrrolidine methiodide (NIC_{PM}), highly charged molecules that are unable to cross the BBB (Barlow and Dobson, 1955; Aceto et al., 1983; Oldendorf et al., 1993; Wasserman, 1972; Ginzel, 1973, 1975; Woods et al., 2008). By using HEXA pretreatment, we were able to examine how the blockade of peripheral actions of NIC affects its acute neural and behavioral effects and their changes following repeated NIC administration. In contrast, NIC_{PM} activates only the peripheral pool of nicotinic receptors, thus allowing the examination of their impact in mediating neural and behavioral effects of NIC. Although we recently confirmed, by using ultra-sensitive mass-spectrometry, that NIC_{PM} cannot be detected in brain tissue after iv administration at 0.1 mg/kg dose (Lenoir et al., 2013), direct data on its affinity with respect to different subtypes of nicotinic and other receptors remains unknown. However, a basic similarity in receptor affinity of both molecules could be suggested because intraventricular administration of both drugs induces similar antinociceptive effects and these effects are attenuated by mecamylamine, a non-selective antagonist of nicotinic acetylcholine receptors (Aceto et al., 1983). Finally, NIC_{PM} could serve another important purpose when used in NIC-experienced individuals. If peripheral actions of NIC are critical for the development of neural sensitization, activation of peripheral nicotinic receptors in these NIC-experienced individuals should induce a conditioned neural or physiological response, which is different from the initial effect of this drug before NIC treatment.

Acute effects of nicotine

When recorded in freely moving rats extensively habituated to the recording environment, the electrical activity in both the cortex and VTA typically shows high-magnitude, low frequency fluctuations (synchronized activity), which are transiently interrupted by episodes of low-magnitude, high-frequency activity that occur either spontaneously or in response to sensory stimuli. While the synchronized activity is a typical electrophysiological feature of slow-wave sleep, the so-called desynchronized activity is an index of neural activation. As shown in Fig. 1, iv NIC injection at a low self-administering dose results in very rapid and almost synchronous change in cortical and VTA EEG signals and, a little later, the appearance of strong EMG activation. Importantly, the NIC-induced EEG desynchronization does not differ qualitatively from a transient activation response that occurs after iv saline injection, a weak visceral sensory stimulus, but it is much stronger and longer quantitatively. NIC-induced EEG desynchronization was drastically delayed during urethane-induced anesthesia, suggesting the importance of natural physiological conditions (freely moving animal) to observe undisturbed drug-induced neural effects.

During quantitative analyses of EEG activities (Fig. 2, left panel), we found that EEG total power was rapidly decreased by NIC, reaching significance in the middle of a 15-s injection. Importantly, this rapid effect was very similar in both the cortex and VTA and for two low doses (10 and 30 $\mu\text{g/kg}$), while its duration was clearly shorter for a smaller dose (a and b). The EMG signal showed similar dynamics but the changes were quantitatively larger (c). The total power of this signal began to increase during the NIC injection, peaked immediately after its end, and slowly decreased thereafter. The electrophysiological effects of NIC clearly differed in its magnitude and duration from the effects of saline, which also induced a rapid decrease in EEG total power and a tiny EMG response.

Rapid, powerful effects were found when the EEG signal was divided into individual frequency bands (Fig. 2, right panel). In this case, alpha activity decreased (f) and high-frequency beta and gamma activities increased (g and h), representing the classic EEG activation triad typical of any arousing stimuli. These changes were equally rapid for both low NIC doses and similar in both structures, with slightly stronger effects for 30 $\mu\text{g/kg}$. NIC administration also decreased low-frequency delta activity; these effects were identical in both structures and for both drug doses. In contrast, cortical theta activity rapidly and transiently increased only after NIC at 30 $\mu\text{g/kg}$ but this type of activity decreased in the VTA for 4–6 min post-injection. Although saline injection also induced similar effects and while they were equally rapid, they were much weaker and more transient (<1 min).

NIC injected at a low dose (30 $\mu\text{g/kg}$) also induced relatively weak locomotor activation, small down-up-down changes in NAcc and muscle temperatures, but strongly decreased skin temperature, suggesting peripheral vasoconstriction (Fig. 3, a–c). These effects continued for about 20 min post-injection. Skin temperature decrease occurred with the shortest latency, within or immediately after the injection. After the NIC injection, temperature in the NAcc increased more rapidly than in the muscle, suggesting a transient metabolic neural activation, evident within 8–10 min post-injection (b). In contrast, a decrease in skin-muscle differential, the most accurate measure of peripheral vasoconstriction, was stronger and much more prolonged, being evident within 20–30 post injection.

The role of peripheral actions of NIC in mediating its acute neural effects

To evaluate the role of peripheral actions of NIC in mediating its central effects, two pharmacological strategies were used. First, we used an antagonist strategy and examined how electrophysiological and behavioral effects of NIC are affected by HEXA pre-treatment. Second, we used an agonist strategy to examine to what extent NIC_{PM} , a peripherally acting NIC analog, mimics the effects of NIC.

As shown in Fig. 4, HEXA pre-treatment (5 mg/kg, iv, 8 min preceding NIC injection) significantly attenuated NIC-induced EEG desynchronization in both the cortex and VTA (A–B). While NIC after HEXA pretreatment continued to decrease rapidly EEG total powers in both brain structures, all effects were significantly weaker than those before drug pre-treatment and close to those induced by saline injections. HEXA pretreatment also drastically attenuated NIC-induced EMG activation, but this effect was clearly larger than

that induced by saline (C). Similar to NIC, NIC_{PM} induced rapid EEG desynchronization and EMG activation but its effects were drastically shorter than those of NIC (Fig. 4 D–E). The effects of NIC_{PM} in drug-naïve rats resembled those of saline but they were significantly stronger and more prolonged for each parameter.

NIC_{PM} also resembled NIC in its effects on NAcc, muscle, and skin temperatures (Fig. 3). In both cases, locomotor activity as well as NAcc and muscle temperatures slightly increased after iv administration of both drugs in equal doses (30 µg/kg). Although NIC_{PM} also decreased skin temperature, this effect was clearly weaker than that of NIC.

To directly test how blockade of peripheral nicotinic receptors affects locomotor effects of NIC, locomotor activity was monitored in four groups of rats that received different treatments (Saline-Saline, HEXA-Saline, Saline-NIC, HEXA-NIC). In contrast to all our studies in which drugs were delivered under stress- and cue-free conditions, in this experiment drugs were injected iv but manually via an injection port on the animal's head. With this type of administration, both saline control and NIC injections resulted in similar levels of locomotor activation (Fig. 5). In contrast, iv injection of HEXA (5 mg/kg) induced much weaker locomotor activation than that of saline, suggesting its attenuating effect on the saline-induced motor response. This SAL-HEXA difference was evident for ~10 min post-injection, corresponding to the duration of HEXA effect with iv administration. The same inhibiting effect was seen in another HEXA group that received NIC injection, and locomotor effects of NIC were strongly attenuated by HEXA pretreatment. This effect was evident for both immediate and long-term effects of NIC. A total locomotor response in this group was significantly weaker than in Saline-Saline and HEXA-Saline groups (Fig. 5B). Hence, NIC's action on peripherally located nicotinic receptors is a significant factor in drug-induced locomotor activation.

Experience-dependent changes in neural, physiological and behavioral effects of NIC

Similar to other drugs of abuse, the locomotor effects of NIC are enhanced or sensitized following repeated intermittent drug injections (Benwell and Balfour, 1992; Clark and Kumar, 1983; Domino et al., 2001; Mao and McGehee, 2010; Vezina et al., 2007). Consistent with these data, we found that rats that received 6 iv NIC injections during two treatment days and tested after one drug-free day showed a significantly larger locomotor response to NIC (Fig. 6A). While the overall magnitude of the sensitized response increased two-fold, locomotion occurred with shorter latencies and peaked at earlier times after drug administration. We also found that repeated NIC experience dramatically affects the temperature effects of NIC, with a significant, robust enhancement of brain and muscle temperature elevations and a stronger decrease in skin temperatures (Fig. 6A–B). In addition, an increase in the NAcc-muscle differential, an index reflecting metabolic brain activation (Kiyatkin, 2010) becomes larger and a decrease in skin-muscle differential becomes more pronounced, reflecting stronger vasoconstrictive effect of NIC. Therefore, not only locomotor but also physiological effects of NIC are enhanced or sensitized after previous drug experience.

In contrast to the physiological effects, EEG desynchronization and EMG activation remained equally strong in NIC-experienced animals but became more prolonged (Fig. 7A–C). A weak enhancement was found only for the immediate effects of NIC in the cortex (G–H). In contrast to the stability of the electrophysiological effects of NIC, both the EEG and EMG effects induced by NIC_{PM} became weaker or habituated after previous experience with this drug (Fig. 7D–F). This effect was evident in each structure for both the immediate and long-term effects of the drug (G–H). Therefore, the acute neural effects of NIC remain relatively stable following repeated experience, but the effects of selective activation of peripheral nicotinic receptors rapidly habituate following repeated use of NIC_{PM}.

The role of peripheral actions of NIC in the development of NIC-induced neural sensitization

If the peripheral actions of NIC are important for the development of NIC-induced neural sensitization, the effects of selective activation of peripheral nicotinic receptors by NIC_{PM} should be changed in animals after NIC experience. Similar to the appearance of a conditioned response to sensory stimulus repeatedly paired with natural reinforcers such as food, NIC_{PM} used in NIC-experienced subjects could induce a conditioned response.

As shown in Fig. 8A, NIC_{PM} used in NIC-experienced rats induced much stronger physiological effects than those seen with this substance in drug-naïve rats. In this case, it induced strong increases in NAcc and muscle temperatures and strong decreases in skin temperatures, which were significantly larger than those induced by this drug in drug-naïve conditions. Since the effects of NIC_{PM} underwent habituation following its repeated exposure, even larger differences were found between the effects of NIC_{PM} in rats repeatedly exposed to either NIC or NIC_{PM}. This strong change in physiological effects contrasted to NIC_{PM}-induced locomotion, which did not change significantly after NIC experience (Fig. 8B). Dramatic changes in temperature effects of this drug are especially evident with rapid time-course resolution analysis (Fig. 8C). In this case, both the increases in NAcc temperature and decreases in skin temperature induced by NIC_{PM} in NIC-experienced rats were similar to those induced by NIC itself. They also were quite different from the initial effects of this drug. Therefore, a clear conditioned activation was evident in this case at the physiological level, but it was absent at the level of locomotion.

Rapid development of conditioned neural activation was also evident in our electrophysiological data. After a relatively short NIC experience (two days, 6 injections), NIC_{PM} induced a strong and prolonged EEG desynchronization and powerfully increased EMG activity (Fig. 9A), greatly exceeding the effects of this drug in both drug-naïve and experienced (with repeated NIC_{PM} injections) conditions (Fig. 9B).

To examine the role of peripheral actions of NIC in the development of drug-induced locomotor sensitization, we also used a pharmacological strategy. In this case, we compared the NIC-induced locomotor activation (30 µg/kg) in two groups of rats that received identical NIC pretreatment (6 iv injections at 30 µg/kg dose during two days), with each NIC injection preceded (–5 min) by the injection of either saline or HEXA (5 mg/kg). In addition to these two active drug treatment groups, NIC-induced locomotion was assessed in rats of

two control groups that received Saline-Saline and HEXA-Saline pretreatment. Figure 10 shows the final result of this experiment, namely the mean locomotor response induced by NIC in rats with different pretreatment histories (Saline-Saline, HEXA-Saline, Saline-NIC, HEXA-NIC).

This experiment produced two primary findings. First, it confirmed that repeated NIC exposure enhances the acute locomotor effects of NIC. Second, in rats that received NIC after HEXA pretreatment, the locomotor effects of NIC were drastically reduced, suggesting a full blockade of the development of locomotor sensitization. Unexpectedly, NIC-induced locomotion in rats after HEXA-NIC pretreatment (blue) was significantly weaker than that found in rats that had never received NIC (black). While this finding was unexpected, it could be related to procedural differences in this experiment. In contrast to our electrophysiological and thermorecording studies, in which drugs and saline were delivered via catheter extensions, thus excluding any stressful influences associated with iv injections, all injections in our behavioral experiment were performed manually into the injection ports on rat's head. Under these conditions, saline induced locomotor activation (see Fig. 5), which became weaker with repeated injections. Therefore, the locomotor response to the first NIC injection (but after 6 previous saline injections) in the control Saline-Saline group could have been enhanced due to a habituation of the motor effects induced by the procedure of drug administration. While this explanation seems valid, the definite reasons for this difference remain unclear and are under investigation now.

General discussion

NIC after iv injection is rapidly distributed by the circulating blood within the body, affecting multiple nicotinic acetylcholine receptors widely expressed in the peripheral and central nervous system. In this case, NIC local concentrations fall rapidly from very large values at the site of administration to much lower levels in distant body locations, including the brain (Berridge et al., 2010; Rose et al., 2010). Although NIC's action on centrally located nicotinic receptors is essential in mediating its reinforcing properties and its ability to induce NIC dependence (Picciotto and Corrigan, 2002), the studies reviewed here suggest that the rapid, transient action of NIC on the afferents of sensory nerves at the administration sites plays an important role in mediating acute neural effects of this drug and their consistent changes following drug experience.

Peripheral and central contributions to nicotine's neural effects

NIC_{PM}, a highly charged NIC analog, which does not enter the brain after systemic administration, is an important tool to dissociate the peripheral actions of NIC from its central ones. When used in drug-naïve conditions, NIC_{PM} mimicked the electrophysiological effects of weak natural somato-sensory stimuli, inducing transient EEG desynchronization and brief EMG activation. Consistent with the known habituation of neural effects induced by simple somato-sensory stimuli (Sandler and Tsitolovsky, 2008; Hendry et al., 1999; Stancak, 2006), the electrophysiological effects of NIC_{PM} significantly weakened (i.e. habituated) following repeated drug exposure. Decreases in neural activation induced by NIC_{PM} paralleled EMG changes, suggesting that habituation also extends to

peripheral motor output. The arousing effects of NIC_{PM} virtually fully disappeared so they became similar to those of iv saline) after minimal experience.

The electrophysiological effects of regular NIC, which acts both in the brain and periphery were much stronger than those of NIC_{PM}, suggesting central drug actions as a major contributor. Moreover, the effects of NIC did not habituate following repeated drug use. In both drug-naïve and drug-experienced conditions, NIC induced equally strong EEG desynchronization and EMG activation, which became more prolonged in rats previously exposed to NIC. The NIC-induced EMG activation also remained equally strong in both groups, suggesting the lack of experience-dependent habituation in drug-induced motor output. This finding suggests the differences between EMG, which reflects tonic and phasic changes in muscular activity, and locomotion, which clearly increases following repeated NIC exposure.

Conditioned neural activation induced by NIC_{PM} after NIC experience

While the neural and motor effects of NIC_{PM} rapidly habituated to the levels produced by saline, this drug induced powerful EEG desynchronization after NIC experience. This effect could be viewed as a manifestation of conditioned neural activation triggered by a selective activation of the peripheral pool of nicotinic receptors. This effect was equally strong in the cortex and VTA, which both showed robust increases in high frequency β and γ frequencies, which greatly exceeded those induced by this drug in both drug-naïve and experienced conditions and were quite similar to those induced by NIC itself. This change was coupled with strong EMG activation, suggesting that conditioning also occurs at the level of centrally regulated motor output. Robust conditioned activation was also found at the levels of physiological parameters. In contrast to very weak activating effects of NIC_{PM} in drug-naïve conditions, the same drug in the same dose induced strong increases in brain and muscle temperatures and powerful and prolonged peripheral vasoconstriction—the novel effects not seen both before NIC pretreatment and after repeated pretreatment with NIC_{PM}. By these parameters, the effects of NIC_{PM} after previous NIC exposure were stronger than the initial effects of NIC itself and similar to the effects induced by NIC after previous exposure. However, powerful potentiation of electrophysiological and physiological effects of NIC_{PM} was not accompanied by changes in locomotion, which remained the same regardless of pretreatment.

Conclusions and Functional implications

Although associative learning is viewed as an important contributor to drug-induced behavioral sensitization (Di Chiara, 2000; Kalivas and Stewart, 1991; Robinson and Berridge, 1993), it is usually considered as the result of interaction between environmental stimuli that precede drug intake and the drug *per se*. However, this stimulus-drug interaction could be more complex in the case of NIC, which by itself has two different, time-shifted actions that are mediated via the drug's interaction with peripherally and centrally located nicotinic receptors. The data presented in this review suggest the critical role of peripheral actions of NIC in mediating its rapid neural and physiological effects. In addition to the pharmacological evidence provided by using peripherally acting nicotinic agonists and antagonists, very short, second-scale latencies of NIC-induced neural activation are

inconsistent with possible direct actions of NIC in the brain. A certain time is always necessary for the drug to reach brain vessels from the site of administration, cross the blood-brain barrier, and diffuse passively to the appropriate receptor sites. In contrast, NIC rapidly but transiently activates nicotinic receptors abundantly expressed on afferents of sensory nerves at the sites of its entry (e.g., lung alveoli, nasal and oral cavities) and within the circulatory system (Anand, 1996; Jonsson et al., 2002; Juan, 1982; Ginzel, 1975; Liu and Simon, 1996; Steen and Reeh, 1993; Walker et al., 1996) and induces an excitatory neural signal that rapidly reaches the brain via visceral somato-sensory pathways. While this study clarifies the nature of this first, rapid and transient, action of NIC, it is still unclear at the mechanistic level how the neural effects triggered by this peripheral action are affected by direct actions of NIC on brain neurons. This direct central action appears with much longer latencies and is maintained for a longer time after systemic drug administration at human-relevant doses, and this action is critical for fixation in memory of traces associated with the initial, sensory effects of NIC, possibly playing a key role in nicotine reinforcement (Huston et al., 1977; Huston and Oitzl, 1989).

While it is well established that this direct action of NIC on brain neurons is essential for the development of NIC-induced neural sensitization, the present studies provide clear evidence that peripheral actions of NIC are also critically involved in this process. Co-existence in NIC of two initially independent pharmacological actions that are mediated via peripheral and central nicotinic acetylcholine receptor, allows their interaction within the same neural substrates based on principles of Pavlovian conditioning. Due to this interaction, the initial sensory effects of NIC acquire new properties of interoceptive cues, thus affecting the changes in behavioral and physiological effects of this drug after repeated exposure. Therefore, the development of conditioned association could occur not only for external (exteroceptive) stimulus and the reinforcer (drug), but also to the drug itself (pharmacological or within-drug conditioning), which shares the dual properties of a sensory stimulus and reinforcer. Therefore, the effects of the drug in experienced individuals are always different from those seen in drug-naïve individuals and they always represent “conditioned drug effects”, reflecting a joint contribution of pharmacological and learning variables (Stewart, 1992).

The ability of NIC to serve as its own sensory signal could explain its high addictive potential in humans and the rapid transformation of experimental smoking into a highly compulsive habit. Similar to the known shift of neural activation from a primary reinforcer to its sensory predictors (Schultz, 1998), the initially unpleasant or aversive sensory stimulation associated with NIC consumption (smoking *per se*) becomes in drug-experienced individuals the source of powerful neural activation and the most desired aspect of smoking. Consistent with this idea, smoking becomes much less rewarding and satisfying when the peripheral actions of NIC on the sensory afferents of the upper respiratory tract are blocked by local anesthetics (Rose et al., 1985). On the other hand, interoceptive actions of NIC provide only a part of sensory experience of smoking, explaining why habitual smokers often perceive similar immediate subjective and autonomic responses during smoking of NIC-free cigarettes (Butschky et al., 1995; Westman et al., 1996). Obviously, these responses are triggered by multiple non-NIC sensory stimuli associated with smoking,

which in smokers become powerful conditioned stimuli. This basic mechanism of pharmacological conditioning could explain the persistent nature of NIC addiction in humans and limited success in its pharmacological correction. On the other hand, it suggests that attempts to reduce NIC addiction could be more effective if they also target the immediate conditioned effects of the drug rather than focusing exclusively on decreasing its unconditioned rewarding effects in the brain.

Acknowledgments

This study was supported by the Intramural Research Program of NIDA-IRP. The Author greatly appreciates the editorial assistance of Drs. Ken Wakabayashi and Mary Pfeiffer.

References

- Aceto MD, Awaya H, Martin BR, May EL. Antinociceptive action of nicotine and its methiodide derivatives in mice and rats. *Br J Pharmacol.* 1983; 79:869–876. [PubMed: 6140048]
- Altschule MD. Emotion and the circulation. *Circulation.* 1951; 3:444–454. [PubMed: 14812675]
- Anand A. Role of aortic chemoreceptors in the hypertensive response to cigarette smoke. *Respir Physiol.* 1996; 106:231–238. [PubMed: 9017841]
- Baker MA, Cronin MJ, Mountjoy DG. Variability of skin temperature in the waking monkey. *Am J Physiol.* 1976; 230:449–55. [PubMed: 816208]
- Barlow RB, Dobson NA. Nicotine monomethiodide. *J Pharm Pharmacol.* 1955; 7:27–34. [PubMed: 1322247]
- Benwell ME, Balfour DJ. The effects of acute and repeated nicotine treatment on nucleus accumbens dopamine and locomotor activity. *Br J Pharmacol.* 1992; 105:849–56. [PubMed: 1504716]
- Berridge MS, Apana SM, Nagano KK, Berridge CE, Leisure GP, Boswell MV. Smoking produces rapid rise of [¹¹C]nicotine in human brain. *Psychopharmacology (Berl).* 2010; 209:383–394. [PubMed: 20232056]
- Bevins RA, Barrett ST, Polewan RJ, Pittenger ST, Swalve N, Charntikov S. Disentangling the nature of the nicotine stimulus. *Behav Processes.* 2012; 90:28–33. [PubMed: 22119845]
- Buzsaki, G. *Rhythms of the Brain.* Oxford University Press; Oxford: 2006.
- Butschky MF, Bailey D, Henningfield JE, Pickworth WB. Smoking without nicotine delivery decreases withdrawal in 12-hour abstinent smokers. *Pharmacol Biochem Behav.* 1995; 50:91–96. [PubMed: 7700960]
- Clark PB, Kumar R. The effects of nicotine on locomotor activity in non-tolerant and tolerant rats. *Br J Pharmacol.* 1983; 78:329–337. [PubMed: 6131718]
- Comroe JH. The pharmacological actions of nicotine. *Ann NY Acad Sci.* 1960; 27:48–51. [PubMed: 13694943]
- Cox BM, Goldstein A, Nelson WT. Nicotine self-administration in rats. *Br J Pharmacol.* 1984; 83:49–55. [PubMed: 6487896]
- Di Chiara G. Role of dopamine in the behavioral actions of nicotine related to addiction. *Eur J Pharmacol.* 2000; 393:295–314. [PubMed: 10771025]
- Domino EF. Nicotine induced behavioral locomotor sensitization. *Prog Neuropsychopharmacol Biol Psych.* 2001; 25:59–71.
- Donny EC, Caggiula AR, Knopf S, Brown C. Nicotine self-administration in rats. *Psychopharmacology.* 1995; 122:390–394. [PubMed: 8657839]
- Engberg G, Hajos M. Nicotine-induced activation of locus coeruleus neurons-an analysis of peripheral versus central induction. *Naunyn-Schmiedeberg's Arch Pharmacol.* 1994; 349:443–446. [PubMed: 8065457]
- Garavan H, Pankiewicz J, Bloom A, Cho JK, Sperry L, Ross TJ, Salmeron BJ, Risinger R, Kelley D, Stein EA. Cue-induced craving: neuroanatomical specificity for drug users and drug stimuli. *Am J Psychiatry.* 2000; 157:1789–98. [PubMed: 11058476]

- Ginzel KH. Muscle relaxation by drugs which stimulate sensory nerve endings. II. The effects of nicotinic agents. *Neuropharmacology*. 1973; 13:149–64. [PubMed: 4787642]
- Ginzel KH. The importance of sensory nerve endings as sites of drug action. *Naunyn-Schmiedeberg's Arch Pharmacol*. 1975; 288:29–56. [PubMed: 1099463]
- Hendry, SC.; Hsiao, SS.; Brown, MC. Fundamentals of Sensory Systems. In: Zigmond, MJ.; Bloom, FE.; Landis, SC.; Roberts, JL.; Squire, LR., editors. *Fundamental Neuroscience*. San Diego: Academic Press; 1999. p. 657-670.
- Huston JP, Mondadory C. Memory and reinforcement: a model. *Proceedings of CIANS, Prague*, 1975. *Activ Nerv Super*. 1977; 19:17–9.
- Huston JP, Oitzl MS. The relationships between reinforcement and memory: Parallels in the rewarding and mnemonic effects of the neuropeptide substance P. *Neurosci Biobehav Rev*. 1989; 13:171–80. [PubMed: 2478940]
- Jonsson M, Kim C, Yamamoto Y, Runold M, Lindahl SG, Eriksson LI. Atracurium and vecuronium block nicotine-induced carotid body chemoreceptor responses. *Acta Anaesthesiol Scand*. 2002; 46:488–494. [PubMed: 12027840]
- Kalivas PW, Stewart J. Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. *Brain Res Rev*. 1991; 16:223–44. [PubMed: 1665095]
- Kiyatkin EA. Brain temperature homeostasis: physiological fluctuations and pathological shifts. *Front Biosci*. 2010; 15:73–92.
- Kiyatkin EA. The hidden side of drug action: brain temperature changes induced by neuroactive drugs. *Psychopharmacology*. 2013; 225:765–780. [PubMed: 23274506]
- Kiyatkin EA, Smirnov MS. Rapid EEG desynchronization and EMG activation induced by intravenous cocaine in freely moving rats: a peripheral, nondopamine neural triggering. *Am J Physiol*. 2010; 298:R285–R300.
- Lenoir M, Kiyatkin EA. Critical role of peripheral actions of intravenous nicotine in mediating its central effects. *Neuropsychopharmacology*. 2011; 36:2125–2138. [PubMed: 21654739]
- Lenoir M, Tang JS, Woods AS, Kiyatkin EA. Rapid sensitization of physiological, neuronal, and locomotor effects of nicotine: Critical role of peripheral drug actions. *J Neurosci*. 2013; 33:9937–9949. [PubMed: 23761889]
- Liu L, Simon SA. Capsaicin and nicotine both activate a subset of rat trigeminal ganglion neurons. *Am J Physiol*. 1996; 270:C1807–C1814. [PubMed: 8764165]
- Mao D, McGehee DS. Nicotine and behavioral sensitization. *J Mol Neurosci*. 2010; 40:154–163. [PubMed: 19669944]
- Oldendorf WH, Stoller BE, Harris FL. Blood-brain barrier penetration abolished by N-methyl quaternization of nicotine. *Proc Natl Acad Sci U S A*. 1993; 90:307–11. [PubMed: 8419935]
- Picciotto MR, Corrigan WA. Neuronal systems underlying behaviors related to nicotine addiction: neural circuits and molecular genetics. *J Neurosci*. 2002; 22:3338–3341. [PubMed: 11978809]
- Robinson TE, Berridge KC. The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res Rev*. 1993; 18:247–91. [PubMed: 8401595]
- Rose JE, Tashkin DP, Ertle A, Zinser MC, Lafer R. Sensory blockade of smoking satisfaction. *Pharmacol Biochem Behav*. 1985; 23:289–293. [PubMed: 4059314]
- Rose JE, Mukhin AG, Lokitz SJ, Turkington TG, Herskovic J, Behm FM, Garg S, Garg PK. Kinetics of brain nicotine accumulation in dependent and nondependent smokers assessed with PET and cigarettes containing 11C-nicotine. *Proc Natl Acad Sci*. 2010; 107:5190–5195. [PubMed: 20212132]
- Sandler, U.; Tsitolovsky, L. *Neural Cell Behavior and Fuzzy Logic*. Springer; 2008.
- Schultz W. Predictive reward signal of dopamine neurons. *J Neurophysiol*. 1998; 80:1–27. [PubMed: 9658025]
- Stancak A. Cortical oscillatory changes occurring during somatosensory and thermal stimulation. *Prog Brain Res*. 2006; 159:237–252. [PubMed: 17071235]
- Stapleton JM, Gilson SF, Wong DF, Villemagne VL, Dannais RF, Grayson RF, Henningfield JE, London ED. Intravenous nicotine reduces glucose metabolism: a preliminary study. *Neuropsychopharmacology*. 2003; 28:765–72. [PubMed: 12655323]

- Steen KH, Reeh PW. Actions of cholinergic agonists and antagonists on sensory nerve endings in rat skin, in vitro. *J Neurophysiol.* 1993; 70:397–405. [PubMed: 8103089]
- Steriade, M.; McCarley, RW. *Brain Control of Wakefulness and Sleep.* Kluwer Academic/Plenum Publishers; New York: 2005.
- Stewart J. Neurobiology of conditioning to drugs of abuse. *Ann NY Acad Sci.* 1992; 654:335–346. [PubMed: 1321575]
- Tang JS, Kiyatkin EA. Fluctuations in central and peripheral temperatures induced by intravenous nicotine: Central and peripheral contributions. *Brain Res.* 2010; 1383:141–53. [PubMed: 21295014]
- Vezina P, McGehee DS, Green WN. Exposure to nicotine and sensitization of nicotine-induced behaviors. *Prog Neuropsychopharmacol Biol Psychiatry.* 2007; 31:1625–1638. [PubMed: 17936462]
- Walker JC, Kendal-Reed M, Keiger CJ, Bencherif M, Silver WL. Olfactory and trigeminal responses to nicotine. *Drug Dev Res.* 1996; 38:160–168.
- Wassermann O. Studies on the pharmacokinetics of bis-quaternary ammonium compounds. 3 Autoradiographic studies on the substituent-dependent distribution pattern of 3H-hexamethonium derivatives in mice Naunyn Schmiedeberg's. *Arch Pharmacol.* 1972; 275:251–61.
- Westman EC, Behm FM, Rose JE. Dissociating the nicotine and airway sensory effects of smoking. *Pharmacol Biochem Behav.* 1996; 53:309–315. [PubMed: 8808137]
- Wise RA. Dopamine, learning and motivation. *Nat Rev Neurosci.* 2004; 5:483–494. [PubMed: 15152198]
- Woods AS, Moyer SC, Jackson SN. Amazing stability of phosphate-quaternary amine interactions. *J Proteome Res.* 2008; 7:3423–7. [PubMed: 18578519]
- Zuo Y, Lu H, Vaupel DB, Zhang Y, Chefer SI, Rea WR, Moore AV, Yang Y, Stein EA. Acute nicotine-induced tachyphylaxis is differentially manifest in the limbic system. *Neuropsychopharmacology.* 2011; 36:2498–512. [PubMed: 21796109]

Figure 1.

Original examples of simultaneous recordings of cortical and VTA EEG and neck EMG, showing rapid neural responses to iv injections of nicotine (30 μg/kg) and saline in freely moving rats. The last graph shows dramatic slowing of electrophysiological effects of nicotine in rats anesthetized with urethane. The onset of injections is shown by arrows and its duration (15 s) is shown by horizontal lines above and below electrophysiological records.

Figure 2. EEG desynchronization and EMG activation induced by iv nicotine (10 and 30 $\mu\text{g/kg}$) in drug-naïve freely moving rats. Left panel show mean ($\pm$ SEM) changes in cortical (a) and VTA EEG (b) and EMG total powers (c) and two right panels (d–h) show mean ($\pm$ SEM) changes in individual EEG frequency bands (delta, theta, alpha, beta, gamma) separately for the cortex and VTA. Filled symbols show values significantly different from baseline. Original data were presented in Lenoir and Kiyatkin, 2011, where all details of the protocol and statistical analyses could be found.

Figure 3.

Mean ($\pm$ SEM) temperatures in the NAcc, temporal muscle and skin induced by nicotine (30 μ g/kg) and nicotine_{PM}, its peripherally acting analog (30 μ g/kg), in drug-naïve freely moving rats (drug doses are expressed as free-base nicotine). Top graphs show temperature changes relative to pre-injection baseline; middle graphs show NAcc-Muscle and Skin-Muscle temperature differentials; and bottom graphs show locomotor activity. Filled symbols indicate values significantly different from baseline. Original data were presented in Tang and Kiyatkin, 2011.

Figure 4.

Pretreatment with hexamethonium (HEXA), a blocker of peripheral nicotinic receptors, significantly attenuates nicotine-induced EEG desynchronization and EMG activation (A–C), while nicotine_{PM}, peripherally acting nicotine analog, induces equally rapid but much shorter electrophysiological effects than regular nicotine (D–F). Each graph shows data obtained with three types of injections (A–C: nicotine [30 µg/kg], HEXA [5 mg/kg] + nicotine [30 µg/kg], and saline; D–F: nicotine [30 µg/kg], nicotine_{PM} [30 µg/kg], and saline). Graphs show mean (±SEM) changes in total power of cortical EEG signal (A and D), VTA EEG signal (B and E) and neck EMG signal (C and F). Original data were presented in Lenoir and Kiyatkin, 2011.

Figure 5.

Pre-treatment with hexamethonium (HEXA), a blocker of peripheral nicotinic receptors, significantly attenuates locomotor activation induced by nicotine (30 μ g/kg). A shows the time-course of locomotor activity and B shows mean changes in locomotion (for 20 min) in four groups of rats that received two consecutive injections (Saline-Saline, HEXA-Saline, Saline-nicotine, HEXA-nicotine). Horizontal line with asterisks in A indicates time interval, where differences between HEXA-NIC and Saline-NIC were significant ($p < 0.05$). Asterisks in B indicate a significant difference in locomotion between these groups ($p < 0.01$). Original data were presented in Lenoir et al., 2013.

Figure 6.

Temperature and locomotor effects of nicotine show dramatic enhancement (sensitization) after relatively short drug experience. A shows changes in NAcc, muscle, and skin temperatures (top), NAcc-Muscle and Skin-Muscle temperature differentials (middle) and locomotion (bottom) induced by nicotine (30 μ g/kg) in drug-naive conditions and after previous nicotine exposure (6 injections during two days). B shows mean ($\pm$ SEM) values of individual temperature parameters in animals of different experimental groups. Horizontal lines of respective colors in (A) show durations of time intervals, when the change in each parameter was significantly different from baseline ($p < 0.05$). Asterisks in (B) denote statistical difference vs. saline and small circle denotes difference between two nicotine groups (at least $p < 0.05$). Original data were presented in Lenoir et al., 2013.

Figure 7.

Electrophysiological effects of iv nicotine (30 µg/kg) remain highly stable after drug experience, with a tendency to prolongation, but the same effects of nicotine_{PM} (30 µg/kg) habituate following repeated drug exposure. Two left columns show changes in total power of cortical EEG (A and D), VTA EEG (B and E) and neck EMG (C and F) induced by nicotine (30 µg/kg) in drug-naive and experienced rats. Right column show between-group differences, shown separately for rapid (5–60 s) and slow (60–600 s) components of electrophysiological responses. Asterisks show statistical significance between groups *, $p < 0.05$; ***, $p < 0.001$). Original data were presented in Lenoir et al., 2013.

Figure 8.

Nicotine_{PM}, a peripherally acting nicotinic agonist, induces robust temperature effects but not affects locomotion after previous nicotine experience. A–C show changes in NAcc, muscle and skin temperature (top), NAcc-Muscle and Skin-Muscle temperature differentials (middle) and locomotion induced by nicotine_{PM} (30 µg/kg) in rats after nicotine experience (6 injections during 2 days). B shows between-group differences [circles show differences vs. nicotine_{PM} (naive) group; asterisks show differences between nicotine_{PM} (nicotine_{PM}) and nicotine_{PM} (nicotine) groups, and hash symbol show differences between nicotine_{PM} (nicotine) and nicotine (nicotine) groups; all $p < 0.05$]; and C shows initial temperature effects analyzed at high temporal resolution. Original data were presented in Lenoir et al., 2013.

Figure 9.

Nicotine_{PM}, a peripherally acting nicotinic agonist, induces robust and prolonged EEG desynchronization and EMG activation after previous nicotine experience. Left column show changes in total power of cortical EEG (A), VTA EEG (B) and neck EMG (C) induced by nicotine_{PM} (30 µg/kg) in rats that received previous treatment with either nicotine or nicotine_{PM}. Right panel show between-group differences in these parameters separately for rapid (5–60 s) and slow (60–600 s) components of electrophysiological responses. Green lines in A–C show time intervals with significant between-group differences. Asterisks in

D–F show statistical significance between groups ***; $p < 0.001$). Original data were presented in Lenoir et al., 2013.

Figure 10.

Co-administration of nicotine with hexamethonium (HEXA) blocks the development of locomotor sensitization that occurs following repeated nicotine exposure. A shows the time-course of locomotor activity and B shows mean values of locomotion (for 20 min post-injection) induced by nicotine (30 μ g/kg) in rats exposed to different pre-treatments (Saline-Saline, HEXA-Saline, Saline-nicotine, HEXA-nicotine). Rats that were previously exposed to nicotine showed significantly larger or sensitized locomotor responses to this drug, but when nicotine injections were conducted after HEXA pre-treatment, locomotor response to nicotine was significantly smaller. Asterisk in A shows time interval, where differences between HEXA-NIC and Saline-NIC were significant ($p < 0.05$). Asterisks in B show significance of between-group differences (*, $p < 0.05$; ***, $p < 0.001$).