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Fluctuations in central and peripheral temperatures induced by intravenous nicotine: central and peripheral contributions

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Abstract

Nicotine (NIC) is a highly addictive substance that interacts with different subtypes of nicotinic acetylcholine receptors widely distributed in the central and peripheral nervous systems. While the direct action of NIC on central neurons appears to be essential for its reinforcing properties, the role of peripheral actions of this drug remains a matter of controversy. In this study, we examined changes in locomotor activity and temperature fluctuations in the brain (nucleus accumbens and ventral tegmental area), temporal muscle, and skin induced by intravenous (iv) NIC at low human-relevant doses (10 and 30 $\mu\text{g/kg}$) in freely moving rats. These effects were compared to those induced by social interaction, an arousing procedure that induces behavioral activation and temperature responses via pure neural mechanism procedure, and iv injections of a peripherally acting NIC analogue, NIC pyrrolidine methiodide (NIC-PM) used at equimolar doses. We found that NIC at 30 $\mu\text{g/kg}$ induces a modest locomotor activation, rapid and strong decrease in skin temperature, and weak increases in brain and muscle temperature. While these effects were qualitatively similar to those induced by social interaction, they were much weaker and showed a tendency to increase with repeated drug administrations. In contrast, NIC-PM did not affect locomotion and induced much weaker than NIC increases in brain and muscle temperatures and decreases in skin temperature; these effects showed a tendency to be weaker with repeated drug administrations. Our data indicate that NIC's actions in the brain are essential to induce locomotor activation and brain and body hyperthermic responses. However, rapid peripheral action of NIC on sensory afferents could be an important factor in triggering its central effects, contributing to neural and physiological activation following repeated drug use.

Keywords

nicotinic receptors; vasoconstriction; brain hyperthermia; peripheral sensory nerves; nicotine pyrrolidine methiodide; conditioning

1. Introduction

Nicotine (NIC) is a highly addictive substance that interacts with different subtypes of nicotinic receptors widely distributed in the central and peripheral nervous systems (see Changeux, 2010 for review). Despite numerous targets, the direct action of NIC on brain

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neurons is usually viewed as the primary mechanism underlying physiological, behavioral, and psychoactive effects of this drug. However, changes in the activity of central neurons could also result from interaction of NIC with afferent terminals of sensory nerves and the ascending signal carried rapidly to the CNS from the periphery via neural pathways. Sensory nerves densely innervate all primary locations of NIC administration (i.e., arteries, veins, abdominal cavity, lung alveoli) and abundantly express nicotinic receptors. Nicotinic receptors are present on afferents of vagal bronchopulmonary nerves (Gu et al., 2008), nasal trigeminal nerves (Alimohammadi and Silver, 2000; Flores et al., 1996), skin sensory nerves (Steen and Reeh, 1993), perivascular sensory nerves (Juan, 1982) and numerous sensory fibers innervating the heart and major blood vessels (Ginzel, 1975; Monti-Bloch and Eyzaguirre, 1980). The direct stimulation by NIC of afferents of these nerves results in activation of primary sensory neurons in both trigeminal, nodose and dorsal root ganglia (Genzen et al., 2001; Liu and Simon, 1996; Xu et al., 2007, 2010) as well as rapid changes in impulse activity of central monoamine neurons (Engberg and Hajos, 1994; Erhardt et al., 2002).

Although NIC rapidly reaches the brain after smoking or intravenous (iv) administration (Berridge et al., 2010; Muzic et al., 1998; Rose et al., 2010) and easily crosses the blood-brain barrier, thus diffusing to and interacting with central neurons, this direct action should be preceded by the drug's interaction with multiple nicotinic receptors on visceral sensory nerves. While this action stands apart from the reinforcing properties of NIC, which are mediated via its interaction with brain substrates (see Balfour, 2009; Corrigan, 1991; Di Chiara, 2000 for review), it is critical in mediating sensory (Alimohammadi and Silver, 2000; Walker et al., 1996) and cardio-vascular effects of NIC (Barlow and Dobson, 1955; Ginzel, 1975; Larson and Haig, 1943). All major cardio-vascular effects of iv NIC at low doses (i.e., bradycardia, fluctuations in blood pressure, peripheral vasoconstriction) occur within second-scale latencies, depend upon the site of drug administration, and are attenuated by peripherally acting NIC antagonists. Despite the peripheral triggering, these effects are centrally mediated; they are organized and executed by the CNS in response to the receptor stimulation via descending sympathetic and parasympathetic pathways.

This study had two goals. First, we examined the effects of intravenous (iv) NIC at low, human-relevant doses on locomotor activity and temperature fluctuations in the brain, temporal muscle, and skin and compared these effects with those induced by social interaction, an arousing procedure that induces behavioral activation and temperature responses via pure neural mechanism (Kiyatkin and Wise, 2001). In contrast to the traditional pharmacological studies that employ large-dose drug administrations, NIC was used at two low doses (10 and 30 $\mu\text{g/kg}$), within the optimal range maintaining self-administration in rats (Cox et al., 1984; Donny et al., 1995) and comparable to the doses received by humans during smoking (0.7-2.1 mg/70 kg). Second, to evaluate the relative contribution of the central and peripheral actions of NIC in its centrally mediated effects, we compared the effects of NIC with those induced by its peripherally acting analogue, NIC pyrrolidine methiodide (NIC-PM), used at equimolar doses (10 and 30 $\mu\text{g/kg}$). Although the affinity of NIC-PM to different subtypes of nicotinic receptors remains unknown (see Discussion), this quaternary derivative of NIC cannot cross the blood-brain barrier, has comparable to NIC effects with intraventricular administration (Aceto et al., 1983) and was used previously to mimic the peripheral effects of NIC (Zuo et al., 2009).

To represent brain temperature, we chose the nucleus accumbens (NAcc) and ventral tegmental area of midbrain (VTA), two deep brain structures tightly involved in behavioral regulation and mediation of the reinforcing properties of addictive drugs (Wise and Bozarth, 1987). Although alterations in brain temperature are determined primarily by metabolic neural activity, they are also affected by arterial blood inflow, which could either retain

metabolic heat in brain tissue or accelerate its removal to the body and then to external environment. To evaluate the contribution of arterial blood inflow from the periphery to brain temperature fluctuations, we also recorded temperatures from the temporal muscle. This muscle is non-motor (non-thermogenic), and its temperature is determined primarily by arterial blood inflow. Since the brain and temporal muscle receive arterial blood supply from the same carotid arteries (being equally affected by temperature influences from the body core), relative temperature changes in the brain site vs. the muscle (i.e., brain-muscle differentials) could serve as a useful tool to reveal the contribution of metabolic brain activity to overall brain temperature changes. We also recorded temperatures from facial skin. In contrast to the muscle, skin temperature depends primarily on the vessel state, thus providing a reliable measure of vasoconstriction or vasodilatation. Therefore, thermorecording in freely moving rats could be a useful tool to characterize both the drug-induced neural activation and its peripheral manifestations as change in body temperature and vasoconstriction (see Kiyatkin, 2010 for review). All thermorecording measurements were supplemented by monitoring locomotor activity, thus allowing examination of the relationships between physiological and behavioral effects of our experimental manipulations.

2. Results

2.1. Effects of NIC

Figure 1 shows changes in temperature and locomotion induced by iv NIC at 10 and 30 $\mu\text{g}/\text{kg}$ dose in freely moving rats. Data were obtained in 8 drug-naïve rats; 25 injections were analyzed in each group.

As can be seen, NIC at 10 $\mu\text{g}/\text{kg}$ dose did not significantly affect locomotor activity as well as brain and muscle temperatures but induced a rapid and relatively prolonged decrease in skin temperature, suggesting peripheral vasoconstriction (A1, B1 and D1). In contrast, NIC at 30 $\mu\text{g}/\text{kg}$ dose increased locomotor activity, increased brain and muscle temperatures, and decreased skin temperature (A2, B2 and D2). The latter effect was equally rapid, but stronger and more prolonged than that of a lower dose. While skin temperature decrease has the shortest onset latency (the effect was significant at the first minute post-injection), brain and muscle temperatures increased much more slowly, with the first significant values at 6 and 8 min post-injection for both brain sites and temporal muscle, respectively. The vasoconstrictive effect (evaluated by subtraction of muscle temperature from skin temperature) of NIC was stronger and more prolonged, taking about 40 min for recovery at both doses. Brain-muscle temperature differentials were virtually unchanged by NIC at a low dose, but significantly increased for both brain sites for about 10 min post-injection.

Since data shown in Fig. 1 is an average of three injections for each drug dose, we also evaluated differences in temperature responses (for the NAcc and Skin) separately for each of three injections of a session (Fig. 2). Following NIC administration at low dose, NAcc temperatures showed a weak decrease following the first ever injection but increases following the second and third injections of a session. In contrast, skin temperature decreases were about the same for each of three injections. NIC at 30 $\mu\text{g}/\text{kg}$ dose also decreased NAcc temperatures following the first injection but clear increases ($\sim 0.4^\circ\text{C}$) were seen following the second and third injections. Rapid drop of skin temperatures was evident with each injection and it was more prolonged with the initial injection.

2.2. Responses elicited by social interaction and saline injections

Fig. 3 (left panel) shows changes induced by presentation of another male conspecific to the recorded male rats (social interaction). Data were obtained in 10 rats (10 tests). Injections of

saline were conducted in 9 rats both before and after drug injections; a total of 30 tests were analyzed.

As can be seen, social interaction resulted in powerful locomotor activation (D), strong and prolonged increases in brain and muscle temperature, and rapid, transient decrease in skin temperature (A and B). All these changes were much stronger and more prolonged than those induced by NIC. While NAcc and VTA temperatures peaked at about 1.3°C above the baseline, skin temperature decrease averaged of ~1.2°C below resting baseline. Temperature increase in both brain sites was stronger and more rapid than in temporal muscle, resulting in a significant increase in brain-muscle differential for about 20 min (C). While less phasic, this change was stronger than that for NIC at 30 µg/kg. Similarly, vasoconstrictive effect (evaluated by subtraction of muscle temperature from skin temperature) of social interaction was stronger than that for NIC (see C in Fig. 1).

While saline injection did not result in any changes in brain and muscle temperatures as well as locomotion, there was a transient decrease in skin temperature (Fig. 3). This effect was rapid, relatively weak (~0.25°C), and evident within ~10 min post-injection.

Table summarizes mean temperature changes induced by our procedures during the first 10 min post-injection or post-stimulation. Compared to saline, NIC at the lowest dose had a significantly stronger effect on skin temperature, but did not affect NAcc temperature. NIC at a larger dose significantly affected both parameters. Maximal changes occurred during social interaction; both brain temperature increases and skin temperature decreases were significantly stronger than those induced by all tested drugs.

2.3. Effects of NIC-PM

Figure 4 shows changes in temperature and locomotion induced by iv NIC-PM at 10 and 30 µg/kg dose. Data were obtained in drug-naïve 6 rats; 16 and 20 injections were analyzed for each dose, respectively.

As can be seen, NIC-PM at both doses had no effect on locomotion (D) and induced much weaker temperature changes in each recording location (A and B) than regular NIC. At both doses, NIC-PM induced a transient decrease in skin temperature, which was weaker and shorter than that for NIC. This effect was stronger and longer lasting for the low drug dose (~20 vs. ~14 min). Similarly, NIC-PM-induced brain and muscle temperature increases were also stronger with the lower dose.

Since data shown in Fig. 4 is an average of three injections for each drug dose, we also evaluated differences in temperature responses (for the NAcc and Skin) separately for each of three injections of a session (Fig. 5). As can be seen, the effects were weak for each drug dose and each injection, but changes in NAcc temperature were relatively stronger for NIC-PM at 10 µg/kg dose. In contrast to NIC, NAcc temperature increases induced by NIC-PM at 10 µg/kg were stronger for the first and second drug injections and weaker for the last injection of a session. While NIC at 30 µg/kg induced weaker changes following each injection, an increase was seen only with the first injection of a session.

As shown in the Table, the effects of NIC-PM on brain and skin temperatures were significantly weaker than those of NIC for each dose. While the vasoconstrictive effect of NIC-PM was stronger than that of saline, NAcc temperature increase was stronger than that of saline only for 10 µg/kg dose

2.4. High-resolution evaluation of temperature responses. Differences between the NAcc and VTA

To evaluate between-structure differences in temperature dynamics, NAcc and VTA temperatures were analyzed with high temporal resolution (10-s bins) for the first 5 min post-injection or post-stimulation (Fig. 6). As can be seen, NAcc showed a more rapid temperature increase than the VTA in each condition. The between-structure difference was maximal during social interaction (B1), when the increases in absolute temperatures were maximal. The difference was weaker for NIC 30 $\mu\text{g/kg}$ (B3) and NIC-PM 10 $\mu\text{g/kg}$ (B4), when their increases were much weaker. Finally, no differences were revealed for NIC 10 $\mu\text{g/kg}$ (B2), which did not affect brain temperatures. Interestingly, between-structural differences appeared very rapidly (within 10-20 s), reached maximum at ~90 s, and later disappeared, with both structures having similar temperature values.

To evaluate the strength of peripheral vasoconstrictive effects of our procedures, we compared mean changes in skin temperatures at high temporal resolution (10-s bin) for the first 5 min post-injection or post-stimulation (Fig. 7). As shown in this figure, both social interaction and all drugs tested induced very rapid decrease in facial skin temperature, which was maximal for social interaction, weaker but also strong for NIC at both doses, even weaker for NIC-PM, and minimal for saline injection. In each case, the effect occurred with 20-30 or 30-40 s onset latencies, quickly progressed to its nadir at 3-5 min, and slowly returned toward baseline. Interestingly, both for NIC and NIC-PM skin temperature showed a similar decrease immediately after the injection, but this decrease was most prolonged for NIC 30 $\mu\text{g/kg}$ (~240 s), shorter for NIC 10 $\mu\text{g/kg}$ (~130 s), and minimal for NIC-PM 10 $\mu\text{g/kg}$ (~60 s). The rate of skin temperature decrease was clearly stronger for social interaction.

3. Discussion

This study revealed that NIC at the 30 $\mu\text{g/kg}$ dose, one that is optimal for maintaining iv self-administration in rats (Cox et al., 1984; Donny et al., 1995), induces locomotor activation and significant temperature responses in each recording location. While the temperature increases in the NAcc and VTA were moderate (0.3-0.4°C), they were more rapid and stronger than those in temporal muscle, suggesting metabolic neural activation as a primary factor responsible for brain temperature elevation. NIC at 10 $\mu\text{g/kg}$ was clearly at the threshold dose to affect brain and body temperatures. However, NIC at both doses effectively decreased facial skin temperatures, suggesting peripheral vasoconstriction, a known centrally mediated effect of NIC that is triggered via activation of peripheral nicotinic receptors (Ginzel, 1975). This effect appeared within surprisingly short onset latencies (20-30 s after the start of 20-s injection), rapidly reached its nadir (3-5 min), and slowly decreased within 20-30 min post-injection; the effect was clearly dose-dependent. Therefore, NIC used in self-administering dose induces both a *peripheral vasoconstriction* and *metabolic brain activation*, resulting in moderate increases in brain and body temperatures.

While quantitatively weaker, the effects of NIC shared several important similarities with the effects of social interaction, an arousing procedure that is triggered via activation of sensory afferents (visual and olfactory stimuli) and subsequent neural activation. In addition to motor hyperactivity, social interaction resulted in strong and prolonged temperature increases in both brain sites and the muscle as well as rapid and strong decreases in skin temperature, suggesting peripheral vasoconstriction. Similar to NIC, temperature increases in both brain sites were quicker and stronger than in temporal muscle, pointing at metabolic neural activation as a factor triggering brain temperature increase. Therefore, it is possible to suggest that NIC may induce neural activation by engaging a similar mechanism - *stimulation of peripheral nicotinic receptors* located on afferents of sensory nerves within

the cardio-vascular system. The direct stimulation by NIC of afferents of these nerves results in activation of primary sensory neurons, with rapid transmission of the signal to the CNS.

The hyperthermic and movement-stimulatory effects of NIC at low iv doses are in obvious contrast to the hypothermic effects of NIC reported in cats, rats and monkeys with relatively large drug doses (0.1-1.0 mg/kg) (Rezvani and Lewin, 2004; Rushkin et al., 2007; Vinkers et al., 2009). This hypothermic effect was associated with decreased locomotion and it was mimicked by intra-ventricular drug administrations (10-100 μ g), which also induced vasodilatation, tachipnoe, and panting (Baird and Lang, 1973; Hall, 1972, 1982). These effects were attenuated by centrally acting NIC receptor blockers (mecamylamine), which alone increased body temperature and induced vasoconstriction, piloerection, and intense shivering. Since intra-ventricular NIC administration by-passes interactions with any peripheral sensory afferents, this hypothermic effect coupled with vasodilatation, tachipnoe, and panting could reflect a true central pharmacological action of NIC. Therefore, it appears that physiological effects of NIC drastically differ upon the dose and the site of drug administration. Interestingly, despite a significant temperature increasing effect of iv NIC on brain and muscle temperatures, we also observed a weak temperature decrease following the first injection of a session (see Fig. 2C). No increase or a weak decrease was also typical of the first NIC injection at the lowest dose.

While the increases in brain and muscle temperatures induced by NIC were relatively slow and weak, skin temperature dropped very rapidly and strongly, obviously reflecting peripheral vasoconstriction. It is well established that this physiological effect of NIC is mediated via stimulation of vascular chemoreceptors, increased afferent input to the CNS, and subsequent activation of descending sympathetic vasomotor mechanisms (Ginzel, 1975). While peripheral vasoconstriction promotes heat accumulation in the organism, it alone cannot explain temperature increases in brain sites, which exceeded in their speed and amplitude those in temporal muscle. This effect, however, appears to contribute to accelerating temperature increases in the brain sites seen within the several minutes post-injection.

NIC-PM, a quaternary methiodide analogue of NIC that cannot cross the blood-brain barrier (Aceto et al., 1983; Gillis and Levis, 1956), was used in this study to verify the contribution of peripheral actions of NIC in mediating its locomotor and temperature effects. Although data on the affinity of this compound to different types of NIC receptors are still lacking, we assumed that NIC-PM mimics the actions of NIC in the periphery but will lack its action in the brain. As found in this study, the effects of NIC-PM used at equimolar doses dramatically differed from those of NIC. First, in contrast to weak locomotor activating effects of NIC, this drug at the tested doses did not affect spontaneous locomotion. Second, this drug had much weaker than NIC effects on skin temperature, which were only slightly stronger than those induced by saline. Third, NIC-PM also had weaker effects on brain and body temperatures, which were evident only for the low drug dose tested during the initial session. Despite a three-fold increase in dose, the effects of NIC-PM at 30 μ g/kg tested during the next daily session were clearly weaker than those for 10 μ g/kg in all parameters (see Fig. 4). Finally, the effects of NIC-PM showed a clear tendency to weaken following repeated injections within the session (see Fig. 5). This could explain an unusual decrease in effects of this drug with dose increase. This “habituation” or “desensitization” of brain temperature responses for NIC-PM was in contrast to an obvious “sensitization” seen with regular NIC. While NIC at 30 μ g/kg slightly decreased NAcc temperature during the first injection, it clearly increased it following the second and third injections of the session (see Fig. 2C). These changes in NIC's ability to increase brain temperature were associated with progressive weakening of vasoconstrictive effect with repeated drug injections (D). Decrease in skin temperature was stronger and more prolonged after the first NIC injection

of a session, and weakened after the second and third injections. These opposite changes in skin and brain temperatures are inconsistent with the idea that vasoconstriction *per se* (i.e., a factor for heat accumulation in the body) is a significant contributor to drug-induced brain temperature increases. Therefore, it appears that NIC's actions in the brain are essential for inducing locomotor activation and hyperthermic responses in the brain and body. The direct actions of NIC on brain cells also appears to be important to induce strong vasoconstriction, which is only partially dependent upon stimulation of peripheral nicotinic receptors.

However, this conclusion could be valid if NIC-PM mimics regular NIC in its affinity to specific subtypes nicotinic receptors that are responsible for the effects seen in this study. While NIC is a universal agonist, which interacts with all subgroups of nicotinic acetylcholine receptors, selectivity and affinity of NIC-PM to different subtypes of nicotinic receptors is still unknown. It is known, however, that NIC and NIC-PM are equipotent in their pressor effects in cats (Barlow and Dobson, 1955), dogs (Larson and Haig, 1943), and monkeys (Zuo et al., 2009) as well as show comparable antinociceptive effects with central administration in mice and rats (Aceto et al., 1983). NIC methiodide also has about a half of NIC's relative potency assessed in neuro-muscular preparation (Barlow et al., 1969). However, data with other methiodide derivatives indicate that changes in molecular structure and charge could affect drug's receptor affinity and selectivity. Despite often use as a peripheral analogue of cocaine, its methiodide derivative, for example, is 184-, 31- and 123-fold less effective than regular cocaine in inhibition of dopamine, norepinephrine and serotonin uptake and 40-fold weaker in inhibition of Na⁺ channels (Hill et al., 2009). This also could be true for NIC-PM, which as a charged molecule could have altered affinity to different subtypes of nicotinic cholinoreceptors and interact with other receptor types. The effects of centrally administered NIC-PM on tail-flick responses were blocked only partially by both mecamylamine and hexamethonium, while mecamylamine fully blocked the effects of regular NIC (Aceto et al., 1983). Finally, it has been recently shown that quaternizing the N group of a methyl substituent of NIC decreased its potency (1/EC₅₀) more than 100-fold at $\alpha 4\beta 2$ and $\alpha 7$ receptors, a centrally located subtypes of nicotinic acetylcholine receptors (Xing et al., 2010). Also, the maximum response generated by this quaternary analog is less than 10% of the maximum response measured with acetylcholine on these human receptors (W. Kem, personal communication).

While our present data suggest that NIC's actions on brain neural substrates are essential for its behavioral and physiological effects, interaction of this drug with peripheral nicotinic receptors on sensory nerves could play an important role in triggering these effects and maintaining brain activation following repeated NIC administration. As showed with intra-ventricular administration, NIC decreases body temperature and locomotion by acting selectively in the brain (see above). This effect was also seen following systemic drug administrations in drug-naïve animals (Rezvani and Levin, 2004; Vinkers et al., 2009). Weak brain and muscle temperature decrease was also typical for the very first NIC administration (10 μ g/kg) in our study (see Fig. 2C). On the other hand, NIC undeniably interacts with peripheral NIC receptors on sensory nerves in the lungs (in case of smoking), nasal cavity (in case of snorting), and within the entire cardiovascular system before reaching the brain (in case of iv and eventually all other types of NIC self-administration). This interaction occurs at the peripheral gates of NIC administration, when drug levels are transiently very large and rapidly decay when the drug is travelling within the cardiovascular system. Therefore, the initial, rapid effects of NIC could derive from its action on peripheral NIC receptors, activation of visceral sensory afferents and rapid ascending signal to the brain immediately after drug injection. Despite peripheral triggering, these effects are in fact centrally mediated and involve descending sympathetic and parasympathetic pathways. After NIC finally reaches brain receptive substrates (but at much lower concentrations than in blood vessels; see Rose et al., 1999; 2-3 ng/ml or 12-19 nM for

cigarette smoking in humans), two actions (direct and indirect) interact at the same neural substrates, thus jointly contributing to the behavioral and physiological effects of NIC and allowing their interaction following repeated drug use. In the case of NIC-PM, the second, true central action is absent, and the initial excitatory effects (which are stronger than those for NIC, compare Fig. 2 and 5) rapidly habituate following repeated drug injections. This pattern of change is similar to that seen with other sensory stimuli (light or sound), when the initial weak temperature increases seen with the first stimulus presentations rapidly habituate following repeated tests (Kiyatkin et al., 2002). In the case of regular NIC, the effects on peripheral neural substrates are followed by slower and more prolonged actions in the brain. Similar to simple sensory stimuli, which after repeated pairing with primary reinforcers, acquire properties of conditioned stimuli and induce new, conditioned responses (Pavlov, 1929), the effects of NIC (which shares properties of both a sensory stimulus and reinforcer) following repeated administration are also changed due to within-drug conditioning. This interaction appears to be important for NIC learning and NIC sensitization, explaining why the initial sensory experience of tobacco smoking eventually becomes a persistent habit.

4. Materials and Methods

4.1. Subjects

Fourteen Long-Evans male rats (Taconic, Germantown, NY), weighing 420–480 g and housed under a 12 h light cycle (lights on at 7:00 AM), with *ad libitum* food and water, were used. Protocols were performed in compliance with the Guide for the Care and Use of Laboratory Animals (NIH, Publications 865-23) and were approved by the Animal Care and Use Committee, NIDA-IRP.

4.2. Surgery

All animals were implanted with four thermocouple electrodes as previously described (Kiyatkin and Brown, 2005). Animals were anesthetized intraperitoneally (ip) with 3.3 ml/kg of Equithesin (sodium pentobarbital, 32.5 mg/kg and chloral hydrate, 145 mg/kg) and mounted in a stereotaxic apparatus. Five holes were drilled through the skull: three for securing screws and two for thermocouple insertions over the NAcc shell (1.2 mm anterior to bregma, 0.9 mm lateral to bregma) and VTA (6.0 mm posterior to bregma, 2.0 mm lateral to bregma) using the coordinates of Paxinos and Watson (1998). The *dura mater* was retracted and the thermocouple probes were slowly lowered to the desired target depths (for NAcc: 7.4 mm and for VTA: 8.4 mm with 10° angle, measured from the skull surface). A third thermocouple probe was implanted subcutaneously along the nasal ridge with the tip approximately 15 mm anterior to bregma. A fourth thermocouple probe was implanted in the deep temporal muscle (*musculus temporalis*). The probes were secured with dental cement to the three stainless steel screws threaded into the skull. During the same surgery session, animals were also implanted with a jugular iv catheter. For jugular catheter implantation, a 10 mm incision was made in the neck to expose the jugular vein. A catheter was then inserted into, and was secured to, the vein. The catheter ran subcutaneously to the head mount and secured with dental cement. Rats were allowed three days recovery and two more days of habituation (6 h sessions) to the testing environment before the start of testing.

4.3. Experimental Protocol

All tests occurred inside a Plexiglas chamber (32×32×32 cm) equipped with four infrared motion detectors (Med Associates, Burlington, VT, USA), placed inside a sound attenuation chamber. Rats were brought to the testing chamber at ~09:30 AM and attached via a flexible cord and electrical commutator to thermal recording hardware (Thermes 16, Physitemp, Clifton, NJ, USA). A catheter extension was also attached to the internal catheter, thereby allowing remote, unsignalled iv injections. Since rats are exceptionally sensitive to

extraneous influences, special care was taken to exclude all possible influences immediately before and during drug injections. Temperatures were recorded with a time resolution of 10 s and movement was recorded as the number of infrared beam breaks per 1 min. Room temperature was maintained at 23-24°C and controlled by another thermocouple located in the recording chamber.

Each of the fourteen rats underwent at least five daily recording sessions. The first recording session was always habituation session, when the rats were housed in the testing chamber for ~6-8 hours, with continuous recording of temperatures and spontaneous locomotion. During the second session, after habituation to the testing chamber (~2 hours), rats received three iv injections of saline (with ~2-h inter-injection intervals) and they were exposed once to social interaction. During the third recording session, rats received three injections of either NIC (8 rats) or NIC-PM (6 rats) at 10 µg/kg (0.2 ml over 20 s). During the fourth recording session, the rats received three more iv injections of saline. During the fifth recording session, rats received three injections of either NIC or NIC-PM at 30 µg/kg (0.2 ml over 20 s). Two-hour intervals were maintained between each consecutive drug injection.

Both drugs were used in equimolar doses calculated as salt base. Nicotine hydrogen tartrate (Sigma) and nicotine pyrrolidine methiodide (synthesized by Dr. Yi Zhang (National Institute of Mental Health, NIH) # 5/01/2008 and kindly provided to us by Dr. B. Vaupel; relative purity is estimated as at least 95%) were freshly dissolved in saline to deliver drugs in 10 µg/kg (or 62 nM/kg) and 30 µg/kg (or 186 nM/kg) doses. Concentrations of delivered drugs were: 20 µg/ml and 60 µg/ml for low and high doses, respectively. Solutions of both NIC and NIC-PM were slightly acidic (~4.5 and 5.0, respectively).

The social interaction was executed by placing an unfamiliar, conspecific male rat matched for age and weight in the testing chamber for three minute. Since our motion detection equipment cannot distinguish between two animals, locomotor activity during the social interaction *per se* (3 min) represents the activity of two rats.

4.4. Histology and Data Analysis

When recording was completed, all rats were anesthetized, decapitated, and had their brains removed for sectioning and confirmation of probe placement. Brains were cut on a cryostat into 45 µm slices and placed on glass slides. All probes were located within the medial portion of ventral striatum (NAcc shell) and in the VTA, as described in Paxinos and Watson (1998).

Temperature and movement data were analyzed with 1-min time bins and were presented as both absolute and relative changes with respect to the moment of stimulus presentation or drug administration. Both a one-way ANOVA with repeated measures and post-hoc Fisher tests were used for statistical evaluation of temperature and movement responses induced by an arousing stimulus and drugs tested. Student's t-test was used for comparisons of between-site and between-condition differences in temperature and locomotion. For high-resolution temperature analyses, we used 10-s values. Between-treatment differences were evaluated based on statistical comparisons of basal brain temperatures, absolute and relative temperature changes, and mean values of locomotor responses.

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Figure 1.

Mean ($\pm$ sem) changes in temperature and locomotion induced by iv administration of NIC at 10 μ g/kg (left panel) and 30 μ g/kg (right panel) doses in awake, freely moving rats.

A=absolute temperatures in each recording location, $^{\circ}$ C; B=relative temperature changes (value for the last pre-injection minute=0), $^{\circ}$ C; C=brain-muscle and skin-muscle temperature differentials, $^{\circ}$ C; D=locomotion, counts/min. Each graph contains data obtained in eight rats with three tests conducted at the same recording session (n=25 for each drug dose). Filled symbols indicate values significantly different vs. baseline ($p < 0.05$, Fisher post-hoc test that followed one-way ANOVA used to assess the effect of time on the parameters). Vertical hatched lines in each graph show the moments of drug injections.

Figure 2.

Mean relative changes in NAcc (A and C) and skin (B and D) temperatures following the first, second and third NIC injections of a session. With nicotine at 10 $\mu\text{g/kg}$, NAcc temperatures did not change following each drug injection, while with NIC at 30 $\mu\text{g/kg}$ a weak decrease following the initial injection had been changed into increases with the second and third injections. Skin temperature rapidly decreased following each NIC injection, but the decrease was strongest and most prolonged following the initial injection of a session and became weaker and more transient with subsequent injections. For picture clarity standard errors are shown only for the first NIC injections at each dose.

Figure 3.

Mean ($\pm$ sem) changes in temperature and locomotion induced by iv saline administration (right) and presentation of another same-sex animal (social interaction, left) to awake, freely moving rats. A=absolute temperatures in each recording location, °C; B=relative temperature changes (value for the last pre-injection minute=0), °C; C=brain-muscle and skin-muscle temperature differentials, °C; D=locomotion, counts/min. Each graph contains data obtained in 10 rats; social interaction test was conducted once in each rat and saline injections were done at least three times in each rat. Filled symbols indicate values significantly different vs. baseline ($p < 0.05$, Fisher post-hoc test that followed one-way ANOVA used to assess the effect of time on the parameters). Hatched vertical lines in each graph (=0 min) show the start of social interaction (second rat is placed in the cage) or saline injection.

Figure 4.

Mean ($\pm$ sem) changes in temperature and locomotion induced by iv administration of NIC-PM at 10 μ g/kg (left panel) and 30 μ g/kg (right panel) doses in awake, freely moving rats. A=absolute temperatures in each recording location, $^{\circ}$ C; B=relative temperature changes (value for the last pre-injection minute=0), $^{\circ}$ C; C=brain-muscle and skin-muscle temperature differentials, $^{\circ}$ C; D=locomotion, counts/min. Each graph contains data obtained in six rats with three tests conducted at the same recording session (n=16 and 20 for 10 and 30 μ g/kg, respectively). Filled symbols indicate values significantly different vs. baseline ($p<0.05$, Fisher post-hoc test that followed one-way ANOVA used to assess the effect of time on the parameters). Hatched vertical lines in each graph show the moments of drug injections.

Figure 5.

Mean relative changes in NAcc (A and C) and skin (B and D) temperatures following the first, second and third NIC-PM injections of a session. With a 10 µg/kg dose, NAcc temperatures weakly increased with each administration; the change was maximal during the first and second injections of the session. NIC-PM at 30 µg/kg induced much weaker responses that were only slightly stronger than those induced by saline.

Figure 6.

High-speed analysis of temperature changes (10-s bins for 5 min) induced in the NAcc and VTA by social interaction (1), nicotine at 10 (2) and 30 µg/kg (3) and nicotine-PM at 10 µg/kg (4). Top graphs (A1-4) show relative temperature changes in two brain structures and lower graphs (B1-4) show NAcc-VTA temperature differences. As can be seen, temperature increases in the NAcc were consistently more rapid and stronger than those in the VTA. The difference appeared at ~20-30 s, peaked at ~80 s, and slowly disappeared at about 3 min post-stimulus or post-injection.

Figure 7.

High-speed analysis of skin temperature decreases (10-s bins for 5 min) induced by social interaction and iv injections of saline, NIC, and NIC-PM. As can be seen, temperature decrease became evident at 10-20 s post-stimulus and accelerated at ~60-80 s. The effect was maximal with social interaction, weaker with NIC, and minimal with NIC-PM. Interestingly, the rate of decrease with both NIC derivatives was similar during the first 40-50 s from the injection start, but then the curves became separate, with much stronger effects for NIC than for NIC-PM.

Table

Rapid changes in NAcc and Skin temperatures induced by NIC, NIC-MT, saline, and social interaction in freely moving rats

	NAcc	Skin
Saline	0.046±0.007	-0.073±0.026
Nicotine 10	0.007±0.006	-0.409±0.039***
Nicotine 30	0.129±0.024*	-0.646±0.056***
Nicotine PM 10	0.102±0.019*	-0.184±0.016**
Nicotine PM 30	0.027±0.007	-0.185±0.024**
Social Interaction	0.802±0.146***	-0.818±0.089***

Each value represents mean (±sem) change in temperature (in °C) for 10 min post-stimulus or post-injection. Asterisks show values significantly different from saline

*
p<0.05

**
p<0.01

<0.001, Student's t-test