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Age-contingent influence over accumbal neurotransmission and the locomotor stimulatory response to acute and repeated administration of nicotine in Wistar rats

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ABSTRACT

Nicotine addiction is one of the leading contributors to the global burden of disease, and early onset smokers report a more severe addiction with lower chance of cessation than those with a late onset. Preclinical research supports an age-dependent component to the rewarding and reinforcing properties of nicotine, and the aim of this study was to define behavioral adaptations and changes in accumbal neurotransmission that arise over 15 days of intermittent nicotine treatment (0.36 mg/kg/day) in rats of three different ages (5 weeks, 10 weeks, 36 weeks old). Repeated treatment increased the locomotor stimulatory response to nicotine in all age groups, but significantly faster in the two younger groups. In addition, nicotine decreased rearing activity in a way that sustained even after repeated administration in aged rats but not in the younger age groups. Electrophysiological field potential recordings revealed a decline in input/output function in the nucleus accumbens (NAc) of animals intermittently treated with nicotine starting at 5 weeks of age, but not in older animals. In drug naïve rats, acute administration of nicotine modulated both accumbal dopamine output and excitatory transmission in a partially age-dependent manner. Fifteen days of intermittent nicotine treatment did not alter the acute effect displayed by nicotine on dopamine levels or evoked field potentials. The data presented here show that both acute and repeated nicotine administration modulates accumbal neurotransmission and behavior in an age-contingent manner and that these age-dependent differences could reflect important neurobiological underpinnings associated with the increased vulnerability for nicotine-addiction in adolescents.

1. Introduction

Smoking is the single most preventable cause of death, and one of the leading risk factors for the global burden of disease (Lim et al., 2012). The risk for developing nicotine addiction is partially believed to be associated with genetic, social and psychosocial factors, but there also appears to be an age-dependent component to the rewarding and reinforcing properties of nicotine (Breslau and Peterson, 1996; Placzek et al., 2009). Adolescents report more positive effects after their first smoking experience and early onset smokers develop a more severe addiction with lower chance of cessation than those who start later in life (Chen and Millar, 1998; Kendler et al., 2013).

Nicotine produces its reinforcing effects by activating nicotinic acetylcholine receptors (nAChRs) in the ventral tegmental area (VTA), leading to increased dopamine output in the nucleus accumbens (NAc) (Di Chiara and Imperato, 1988; Imperato et al., 1986; Nisell et al., 1994; Rowell et al., 1987). Nicotine-induced effects on accumbal neurotransmission has recurrently been linked to both the acute rewarding and reinforcing properties of nicotine, as well as to the behavioral adaptations that arise as a result of continual administration (Imperato et al., 1986; Museo and Wise, 1990; Pontieri et al., 1996). Following a repeated and intermittent administration protocol, nicotine produces drug-seeking behavior and sensitization to the locomotor stimulatory properties of the drug (Benwell and Balfour, 1992; Clarke and Kumar, 1983; Corrigall

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et al., 1992; Di Chiara, 2000; Ericson et al., 2010, 2000b). Humans have also been suggested to exhibit a sensitized response to nicotine and to conditioned cues following repeated nicotine exposure, but the role of this phenomenon in drug abuse is unclear (Boileau et al., 2006; Parker and Gilbert, 2008; Robinson and Berridge, 1993).

Age-dependent properties of nicotine are supported by preclinical studies on rodents, demonstrating that neurobiological responses may vary across age (Christensen et al., 2014). Despite having faster nicotine metabolism, adolescent rats are more sensitive to the rewarding and reinforcing properties of nicotine, and less sensitive to the aversive effects (Doremus-Fitzwater et al., 2010; Shram et al., 2006; Torres et al., 2008; Vieira-Brock et al., 2013). Younger rodents also show stronger conditioned place preference to nicotine, and increased nicotine self-administration (Belluzzi et al., 2004; Levin et al., 2007; Shram et al., 2006; Torres et al., 2008). In addition, cross-sensitization to other drugs of abuse, such as amphetamine, cocaine and ethanol (Blomqvist et al., 1996; Collins and Izenwasser, 2004; Desai and Terry, 2003; Ericson et al., 2000a; Itzhak and Martin, 1999; Johnson et al., 1995; Soderpalm et al., 2000), is more pronounced in adolescent rats as compared to adult animals (Collins and Izenwasser, 2004; Mojica et al., 2014; Santos et al., 2009). Age-related differences in dopaminergic excitability and the nicotine sensitivity within the reciprocal VTA-NAc circuit have been suggested to contribute to the increased risk of nicotine addiction in adolescent animals and humans (Placzek et al., 2009). Thus the aim of this study was to further assess the influence of age on behavioral adaptations and accumbal neurotransmission after acute and intermittent administration of nicotine in Wistar rats for up to 15 days.

2. Methods

2.1. Drugs and solutions

Nicotine tartrate was dissolved in 0.9% NaCl, adjusted to pH 7.2–7.4 with $NaHCO_3$ and administered at 1.0 mg/kg s.c. (0.36 mg/kg nicotine, free base). Modified artificial cerebrospinal fluid (aCSF) contained (in mM); 194 sucrose, 30 NaCl, 4.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 26 $NaHCO_3$, 1.2 NaH_2PO_4 and 10 D-glucose, while regular aCSF consisted of 124 NaCl, 4.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 26 $NaHCO_3$, 1.2 NaH_2PO_4 and 10 D-glucose. Ringer solution contained; (in mM) 140 NaCl, 1.2 $CaCl_2$, 3.0 KCl, 1.0 $MgCl_2$. All drugs were purchased from Sigma-Aldrich, Stockholm, Sweden.

2.2. Animals

Male Wistar rats (Taconic, Ejby, Denmark), 4, 9 or 35 weeks at the time of arrival, were group housed (5, 3 or 2 per cage, respectively) at constant room temperature (21 °C) and humidity (60%). The animals were kept under regular light–dark conditions (lights on at 6:00 am and off at 6:00 pm) with *ad libitum* access to food and tap water. The animals were allowed to adapt to the novel environment for one week before any experiments were initiated. The experimental protocols were approved by the Ethics Committee of Animal Experiments, Gothenburg, Sweden.

2.3. Nicotine treatment

The animals were randomly assigned to receive either nicotine or saline over a period of three weeks. Subcutaneous injections of nicotine (0.36 mg/kg) were given five times a week (15 injections in total). This protocol has repeatedly been shown to induce robust behavioral sensitization to nicotine, which sustains even 7 months after the last drug-injection (Morud et al., 2015b). The experiments were conducted over a three-month period in an integrated manner, using three different batches of animals. A fourth batch of 9 weeks old animals was used to define acute withdrawal effects on input/output function and nicotine-induced dopamine-elevations in the NAc.

2.4. Locomotor activity

The horizontal and vertical movements were recorded once weekly at baseline and following an s.c. injection of either saline or nicotine as described previously (Ericson et al., 2000b; Morud et al., 2015b). In brief, each animal was placed in a 70 × 70 cm locomotion test box (Kungsbacka mät-och reglerteknink AB, Kungsbacka, Sweden), lit by a weak light bulb. Rays of infrared light form grids in two layers, which detect movement when the animal breaks one of the beams of light in either layer. The subjects were allowed to acclimatize to the testing environment for 30 min before the injection was administered and locomotion was recorded for an additional 30 min after the injection. The activity was recorded in five min bins as locomotion (beam breaks in a consecutive order), rearing (vertical beam breaks) and center time (relative number of beam breaks performed in the center of the activity box as compared to the peripheral regions and corners).

2.5. Electrophysiology

To avoid short-term effects on neurotransmission caused by withdrawal, brain slices were prepared ten days after the final nicotine-injection as previously described (Clarke and Adermark, 2010). However, a subset of recordings was also performed on brain slices sectioned 3 days after the final drug-administration to define treatment-effects on evoked PS amplitudes during the first week of withdrawal. The animal was deeply anesthetized with isoflurane before decapitation; the brain was removed and submerged in ice-cold modified aCSF, bubbled with 95% O_2/5% CO_2. Coronal slices (400 μM thick) were prepared and equilibrated for 15 min at 30 °C, and then for a minimum of one hour in aCSF at room temperature. The hemispheres were separated and placed in the recording chambers under a continuous flow of aCSF pre-heated to 30 °C. A stimulating electrode (monopolar tungsten electrode, World Precision Instruments, FL, USA, type TM33B) was placed in the NAc (Fig. 4A) and population spikes (PS) were activated at a frequency of 0.05 Hz. The amplitude of the PS consistently reflects the efficacy of excitatory synaptic input (Misgeld et al., 1979), and treatment effects on striatal neurotransmission was estimated by stepwise increasing afferent stimulation strength (24 μA-96 μA). Stimulus intensity was set to induce a PS amplitude half of the maximal response and the release probability was assessed by administering paired pulse stimulations, with a 50 ms interpulse interval. Paired pulse ratio (PPR) was assessed by dividing the amplitude from PS2, evoked at half max stimulation strength, with the amplitude of PS1. In a subset of experiments changes in PS amplitude induced by nicotine (1 μM) was evaluated in acutely isolated brain slices from nicotine-naïve rats at three different ages (4 weeks, 10 weeks, 35 weeks), and in rats treated with nicotine or vehicle for 3 weeks starting at 10 weeks of age. In these recordings, a stable baseline was recorded for 10 min before slices were perfused with nicotine for an additional 30 min.

2.6. In vivo microdialysis

In vivo microdialysis was performed in the NAc of awake and freely moving rats as previously described (Morud et al., 2015a). In brief, 4, 10 and 35 weeks old Wistar rats, or rats treated for 3 weeks with either vehicle or nicotine starting from 9 weeks of age, were anesthetized with isoflurane and placed in a stereotaxic apparatus (Kopf Instruments, CA, USA). A central incision was made to expose the skull and holes were drilled for anchoring screws and for implantation of an I-shaped custom-made dialysis probe into the NAc. The coordinates for 4 and 35 weeks old rats were calibrated from adult coordinates based on the relative distance between the two skull sutures lambda and bregma (4 weeks: AP +1.4 mm, ML −1.2 mm relative to bregma and DV −6.8 mm relative to brain surface. 10 weeks, and drug-treated rats: AP: +1.65 mm, ML: −1.4 mm relative to bregma, DV: −7.8 mm relative to brain surface. 35 weeks: AP +2.0 mm, ML −1.6 mm relative to bregma, DV −7.8 relative to brain surface) (Paxinos and Watson, 2007). The probe and the anchoring screws were fixated to the scull using Harvard cement (DAB Dental AB, Gothenburg, Sweden). Animals were allowed two days of recovery. On the day of experiments probes were connected to a microperfusion pump (U-864 Syringe Pump, AgnTho's, Lidingö, Sweden), and perfused with Ringer solution (2 μl/min) for two hours to obtain a balanced fluid exchange. Samples were collected every 20 min and after obtaining a stable baseline for at least 40 min animals were injected with nicotine (0.36 mg/kg s.c.). Dopamine was analyzed using a HPLC system with electrochemical detection as previously described (Morud et al., 2015a). Animals were sacrificed immediately after the experiment and brains were removed for confirmation of probe placement. Only animals with accurate probe placement were included in the data analysis (Fig. 5C).

2.7. Data analysis

All data was analyzed using Microsoft Excel and GraphPad Prism 6. Data from the locomotion measurements and field potential recordings were analyzed using Mann-Whitney and two-way ANOVA with Bonferroni post-hoc test. Changes in PPR were analyzed using unpaired t-test. Gaussian distribution was tested with D'Agostino and Pearson omnibus normality test. All data is presented as mean ± SEM, and the level of significance was set to $p < 0.05$.

3. Results

3.1. Locomotor activity

Measuring locomotion following drug or vehicle administration assessed behavioral sensitization to the stimulatory properties of nicotine or vehicle injections. All rats received a total of 15 injections over a 3-week period. Over the three weeks of treatment a two-way ANOVA revealed a significant effect by both age and treatment (treatment: $F = 99_{(3, 249)}$, $p < 0.001$; age: $F = 19_{(5, 83)}$,