Current Topics in Behavioral Neurosciences 24

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The Neuropharmacology of Nicotine Dependence

Springer

Current Topics in Behavioral Neurosciences

Volume 24

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The Neuropharmacology of Nicotine Dependence

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ISSN 1866-3370 ISSN 1866-3389 (electronic)
Current Topics in Behavioral Neurosciences
ISBN 978-3-319-13481-9 ISBN 978-3-319-13482-6 (eBook)
DOI 10.1007/978-3-319-13482-6

Library of Congress Control Number: 2014956492

Springer Cham Heidelberg New York Dordrecht London

Printed on acid-free paper

Springer International Publishing AG Switzerland is part of Springer Science+Business Media (www.springer.com)

Preface

When one of us (DJKB) first started studying the psychopharmacology of nicotine some 40 years ago the numbers of researchers interested in the topic was small and could probably be accommodated around a large dinner table. Our understanding of the potential hazards of smoking was at a fairly early stage as was our understanding of the neural mechanisms that mediated the behavioral responses to nicotine. At that time smoking was considered to be a habit, not an addiction, and was still widely accepted. Readers who are not old enough to remember those times may be familiar with the television series, *Mad Men*. That series gives you an impression of how acceptable smoking was. Even into the 1980s, the fact that neurones within the brain expressed nicotinic receptors was still debated among some researchers. We have come a long way since that time, and now it is not unusual to have 1,000 delegates or more at conferences on nicotine and tobacco, and sessions dedicated to nicotine are not uncommon at many neuroscience conferences. Moreover, public health policy is now driven by a sound evidence base relating both to the toxicity of primary and second-hand (also known as environmental) tobacco smoke and the plethora of neuroscience studies that have established nicotine as one of the most widely studied recreational drugs. The primary purpose of the chapters in this book and its companion volume is to explore the extent to which the wide range of approaches adopted to investigate the behavioral responses to nicotine and the molecular and neural mechanisms that mediate these effects have opened our eyes to the properties of this unique and fascinating drug.

It goes without saying that one of the principal factors that drives the study of nicotine psychopharmacology is its established role in the addiction to tobacco. It is appropriate, therefore, that this second volume is dedicated specifically on this issue. The chapters in this volume not only describe the ways in which research at a basic level, largely using animal models, have revealed the complex mechanisms that seem to underpin the role of nicotine in tobacco smoking, but also the ways in which the results of these studies translate to our understanding of the dependence on tobacco experienced by most habitual smokers. A number of the chapters show how modern imaging technologies have allowed us to relate directly findings in animal models to the effects of nicotine and tobacco smoke in the human brain.

We have sought to take a logical approach to the issue by first addressing the neurobiological and psychological mechanisms that contribute to the rewarding, perhaps better called the reinforcing, properties of nicotine. We then turn to the mechanisms that underpin the effects of nicotine withdrawal and relapse, chapters that will have a particular resonance with smokers. The final chapter returns to the issue of the role of underlying psychiatric illnesses in tobacco dependence. It focuses on the ways in which animal studies have contributed to our understanding of the reasons that this group seems to be especially vulnerable to tobacco dependence and resistant to treatment.

We hope that the volumes *The Neurobiology and Genetics of Nicotine and Tobacco* and *The Neuropharmacology of Nicotine Dependence* will provide readers with a contemporary overview of the current research on nicotine psychopharmacology and its role in tobacco dependence from leaders in this field of research and that they will prove valuable to those who are developing their own research programs in this important topic.

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Imaging Tobacco Smoking with PET and SPECT

Kelly P. Cosgrove, Irina Esterlis, Christine Sandiego, Ryan Petrulli and Evan D. Morris

Abstract Receptor imaging, including positron emission computed tomography (PET) and single photon emission computed tomography (SPECT), provides a way to measure chemicals of interest, such as receptors, and neurotransmitter fluctuations, in the living human brain. Imaging the neurochemical mechanisms involved in the maintenance and recovery from tobacco smoking has provided insights into critical smoking related brain adaptations. Nicotine, the primary addictive chemical in tobacco smoke, enters the brain, activates beta2-nicotinic acetylcholine receptors (β_2*-nAChRs) and, like most drugs of abuse, elicits dopamine (DA) release in the ventral striatum. Both β_2*-n*A*ChRs and DA signaling are critical neurosubstrates underlying tobacco smoking behaviors and dependence and have been studied extensively with PET and SPECT brain imaging. We review the imaging literature on these topics and describe how brain imaging has helped inform the treatment of tobacco smoking.

Keywords Brain imaging · Smoking · Nicotine · Nicotinic acetylcholine receptors · Dopamine · PET · SPECT

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D.J.K. Balfour and M.R. Munafò (eds.), *The Neuropharmacology of Nicotine Dependence*, Current Topics in Behavioral Neurosciences 24,
DOI 10.1007/978-3-319-13482-6_1

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1 Introduction

Positron emission computed tomography (PET) and single photon emission computed tomography (SPECT) are unique among imaging techniques in the ability to measure specific molecules in the brain. They have revolutionized our ability to measure chemicals in the brains of living people. Brain chemicals, including receptors present in low concentrations (nM–pM range), are measured using "trace" doses of highly specific radioactive drugs (called radiotracers) and imaged with a PET or SPECT camera. There are differences in the physics and chemistry used in PET versus SPECT, but the outcome—a measure of receptor availability—is the same (Fig. 1). The primary addictive chemical in tobacco smoke, nicotine, activates β_2*-containing nicotinic acetylcholine receptors (β_2*-nAChRs). Although there are other combinations of subunits that assemble to form nAChRs (see chapter entitled Structure of Neuronal Nictinic Receptors; volume 23), the β_2*-nAChRs are pivotal (e.g., they are critical for the reinforcing effects of nicotine), and so this site has been a primary point of interest for radiotracer imaging in the smoking field. In addition, when nicotine activates β_2*-nAChRs located on mesolimbic dopamine (DA) neurons in the ventral tegmental area, this results in neuronal firing and dopamine release in the nucleus accumbens (Imperato et al. 1986). There are several SPECT and PET radiotracers that label β_2*-nAChRs and are used to measure occupancy and changes in receptor availability, and several radiotracers that label dopamine D2/3 receptors and allow for measurement of fluctuations in synaptic dopamine. Both β_2*-nAChRs and DA signaling are critical neurosubstrates underlying tobacco smoking behaviors and dependence. In this chapter, we will review the imaging literature that has provided insights into the molecular mechanisms of tobacco smoking with a focus on studies examining β_2*-nAChR availability and DA neurotransmission.

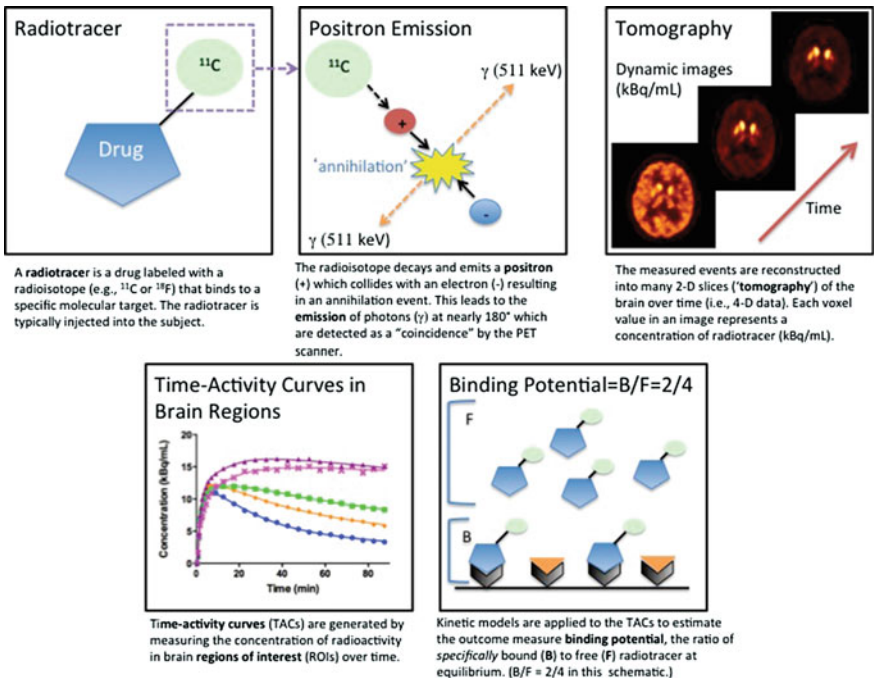

Fig. 1 Description of the use of PET brain imaging to determine the outcome measure of receptor availability

2 Imaging β2*-Nicotinic Acetylcholine Receptors

2.1 Preclinical Studies

The nAChRs that contain the β_2*-subunit are critical for mediating the effects of nicotine in the brain including the reinforcing effects (Picciotto et al. 1998), dopamine release (Epping-Jordan et al. 1999; Koranda et al. 2013), sensitivity to nicotine (Cosgrove et al. 2010; Marubio et al. 1999; Tritto et al. 2004), and the incentive aspects of motivation (Brunzell et al. 2010) (see chapter entitled The Role of Mesoaccumbens Dopamine in Nicotine Dependence; this volume). In addition, there is a wealth of literature showing that nicotine and tobacco smoking robustly upregulate (i.e., increase numbers of) β_2*-nAChRs throughout the brain (Abreu-Villaca et al. 2003; Benwell et al. 1988; Breese et al. 1997; Kassiou et al. 2001; Marks et al. 1992). Preclinical studies administering nicotine at various doses and routes of administration to rats, mice, and monkeys—as well as postmortem human studies—have all indicated that nicotine and tobacco smoke result in significantly more β_2*-nAChRs throughout the brain compared to saline (animals) or to not smoking (humans). We now know that nicotine itself is responsible for this upregulation. Nicotine acts in the cell to help the receptor subunits assemble and

then acts to chaperone the receptors to the cell membrane (Srinivasan et al. 2010). Our goal was to measure this upregulation in living human tobacco smokers. However, first we needed to work out the proper experimental timing.

Nicotine and the radiotracers used in these studies both bind to the same receptor in the brain—the nAChR containing the β_2*-subunit. When nicotine is present in the brain, it may block the receptor and prevent the radiotracer from binding, which would confound our ability to measure β_2*-nAChR availability. Our preclinical experiment consisted of two monkeys drinking nicotine (diluted in water and sweetened with Tang to make it more appetizing) for 6 weeks. After 6 weeks, the monkeys were taken off nicotine. One monkey was scanned at 1 day into nicotine withdrawal, and the other was scanned at 2 days into nicotine withdrawal. Surprisingly, the data showed a *decrease* in radiotracer binding which was not consistent with the literature. To probe further, the monkeys consumed nicotine for two additional weeks and then were scanned at 7 days of withdrawal. At that point, there was a robust increase in radiotracer binding suggestive of an upregulation of β_2*-nAChRs that was consistent with the preclinical literature. Taken together, these data suggested that nicotine remains in the brain during early withdrawal and may take up to 7 days to clear. Levels of cotinine (the major metabolite of nicotine) in the monkeys were measured over the 7 days of withdrawal. Cotinine progressively declined over the week, not completely clearing or reaching nonsmoker levels until 7 days of abstinence. The cotinine data nicely mirrored the brain data. Once cotinine had cleared, it was possible to measure nicotine-induced upregulation of β_2*-nAChRs in the brain. In the human studies discussed below, low cotinine levels are typically used as an indicator of abstinence and that nicotine has cleared so that smokers can be imaged with β_2*-nAChR radiotracers.

2.2 *Imaging the Upregulation of β_2*-nAChRs in Tobacco Smokers*

Based on the preclinical monkey study, our group imaged β_2*-nAChRs in human tobacco smokers at 7–9 days of smoking abstinence (early phase withdrawal). In this and similar studies, the subjects were required to quit smoking and not use any medications or nicotine replacement strategies such as the nicotine patch, because all forms of nicotine would bind the β_2*-nAChR and block the radiotracer from binding. In order to help the subjects quit smoking, we used contingency management techniques (Staley et al. 2006). In the first paper, we demonstrated that tobacco smokers at 7–9 days of abstinence have significantly higher β_2*-nAChR availability in the cortex, striatum, and cerebellum compared to a group of age- and sex-matched nonsmokers (Fig. 2). This work in our laboratory (Staley et al. 2006) and others (Mamede et al. 2007; Mukhin et al. 2008) confirmed that it is possible to measure the upregulation phenomenon in human smokers in vivo.

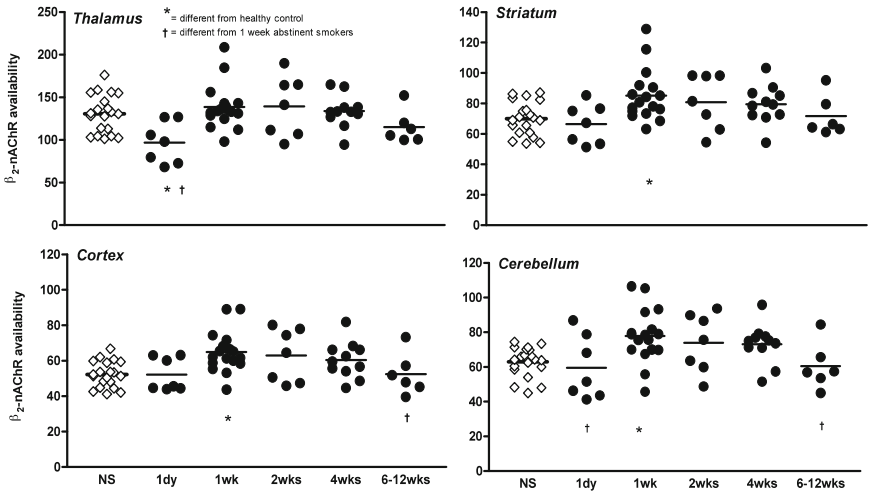

Fig. 2 β_2*-nAChR availability (V_T/f_P) is shown in individual nonsmokers (*open diamonds*) and tobacco smokers (*filled circles*) at 1 day, 1, 2, 4, and 6–12 weeks of abstinence in the thalamus, striatum (average of caudate and putamen), cortex (average of cortical regions including parietal, frontal, anterior cingulate, temporoinsular, and occipital cortex), and cerebellum. The line in each scatter plot represents the mean value of those subjects. *Asterisk* indicates significant difference from control nonsmokers after Bonferroni's correction using two-sample *t*-tests. *Dagger* indicates significant difference from 1 week abstinent smokers after Bonferroni's correction using planned post-hoc between-group comparisons subsequent to the analysis of repeated measures mixed-effects regression models including the overall effect of abstinent smoker group

2.3 Imaging the Normalization of β_2*-nAChRs in Tobacco Smokers

There is also evidence from preclinical (Collins et al. 1990; Pietila et al. 1998) and postmortem human (Breese et al. 1997) studies that the β_2*-nAChRs do not stay upregulated and eventually return to control levels. The postmortem study (Breese et al. 1997) indicated that smokers who had quit smoking at least two months prior to their death had β_2*-nAChRs levels similar to controls. However, smokers in the study had quit anywhere from 2 months to 30 years prior to their death, so the study did not shed light on the acute time course of receptor changes (e.g., during acute withdrawal in the first few months of abstinence, when relapse rates are high). In our next study, we imaged β_2*-nAChR changes over the first few months of abstinence in tobacco smokers (Cosgrove et al. 2009). As shown in Fig. 2, at one day of abstinence, nicotine is still present in the brain blocking the radioligand from binding to the receptor and there is no difference in β_2*-nAChR availability compared to the group of nonsmokers. At one week of abstinence, there is higher β_2*-nAChR availability in smokers compared to nonsmokers consistent with the previous study (Staley et al. 2006). Then even at 2 and 4 weeks of abstinence, receptor availability remains high and does not return to nonsmoker control levels

until 6–12 weeks of abstinence. This study (Cosgrove et al. 2009) and others (Brody et al. 2013b; Mamede et al. 2007) demonstrate that upregulation of β_2*-nAChRs is initially persistent, but that β_2*-nAChRs normalize over approximately 6–12 weeks of abstinence from cigarettes and all other nicotine-containing products. These brain changes parallel the clinical course of smoking cessation in which craving, relapse, and withdrawal symptoms slowly dissipate over the first few months of abstinence even though relapse may occur months or years after the last cigarette.

Relationships between β_2*-nAChR availability and clinical correlates have been reported in these studies. The type of cigarette smoked modulates the degree of upregulation. Cigarettes containing menthol, which are used by up to 1/3 of smokers, lead to higher β_2*-nAChR availability than non-menthol-containing cigarettes (Brody et al. 2013a). Additionally, a primary advantage of neuroreceptor imaging studies (vs. postmortem studies) is that we can record behavior and examine correlations between behaviors of interest and brain chemistry. At one week of abstinence, β_2*-nAChR availability in the sensorimotor cortex was negatively correlated with the urge to smoke to relieve withdrawal symptoms (Staley et al. 2006). At four weeks of abstinence subjects with higher β_2*-nAChR availability in the cerebellum reported both a greater desire to smoke and a greater urge to smoke to relieve withdrawal (Cosgrove et al. 2009). This suggests that magnitude of upregulation may play a role in craving over the course of abstinence and that managing the time course of the normalization may help individuals who are more likely to relapse in response to high levels of craving. For example, it is possible that nicotine replacement strategies may be effective because they continue to activate β_2*-nAChRs. This leads to continued upregulation and the individual can "wean" the receptors off of nicotine as the dose of nicotine is decreased over time.

2.4 Sex Differences in β_2-nAChR Availability*

There is a large literature demonstrating sex differences in tobacco smoking behaviors. In general, men tend to smoke for the nicotine reinforcement, or nicotine per se in the cigarette, whereas women tend to smoke more for the sensory cues associated with smoking, as well as affect and stress regulation (Perkins 2009; Perkins et al. 1999; Perkins and Scott 2008). There are also two preclinical studies showing that male rats and mice exposed to nicotine exhibited greater nAChR upregulation than female rats and mice exposed to nicotine (Koylu et al. 1997; Mochizuki et al. 1998). We wanted to determine if there were sex differences in β_2*-nAChR availability between men and women smokers compared to nonsmokers. Consistent with the preclinical literature, male smokers had significantly higher β_2*-nAChR availability compared to male nonsmokers (9–17 %), but women smokers had similar β_2*-nAChR availability compared to women nonsmokers (1–3 %) (Cosgrove et al. 2012). This was a striking finding given in all the studies demonstrating that nicotine and tobacco smoking upregulate β_2*-nAChRs

throughout the brain. Considering known behavioral sex differences in tobacco smoking, these findings make sense and provide a biological mechanism that may underlie some of the behaviors. Specifically, men smoke for the nicotine in cigarettes, they are more responsive to nicotine replacement therapy as a cessation strategy, and men's brains are responsive to nicotine, exhibiting upregulation of β_2*-nAChRs. Women smoke for affect regulation and for reasons other than the nicotine, they do not respond as well to nicotine replacement strategies, and their brains do not respond to nicotine by increasing β_2*-nAChRs. The bottom line is that novel treatment strategies targeting other receptor systems need to be evaluated to more effectively help women quit smoking. All the current strategies act at the β_2*-nAChR, and, of course, all nicotine replacement strategies act at that site. For example, varenicline (Chantix) is a partial agonist at the β_2*-nAChR, and even bupropion (Zyban) is a nicotinic antagonist.

2.5 Nicotine Occupancy of β_2-nAChRs*

In addition to receptor changes, imaging studies have informed our knowledge about what happens in the brain after someone smokes a cigarette. For example, after one puff of a cigarette, approximately 50 % of all β_2*-nAChRs in the brain are occupied by nicotine. After smoking one or two cigarettes, the receptors are saturated, so up to 100 % of β_2*-nAchRs are occupied by nicotine (Brody et al. 2006a; Esterlis et al. 2013). We know that nicotine doesn't *clear* the brain immediately, and in fact dependent smokers have a slower process of nicotine accumulation going *into* the brain from a cigarette than do nondependent smokers (Rose et al. 2010). Indeed, in one study, habitual smokers did not show evidence of puff-associated spikes in nicotine, but rather a gradual accumulation of nicotine during smoking (Rose et al. 2010). Both of these ideas—rapid accumulation and puff-associated spikes of nicotine—had been proposed to explain the maintenance of tobacco dependence. So with a slow kinetic profile and with most receptors in a smoker occupied by nicotine throughout the day, why do people keep smoking? This brings up some important points about tobacco smoking. People smoke for many different reasons, and nicotine reinforcement is only one component. The reinforcement or pleasure derived from nicotine, like many other drugs of abuse, is necessary in driving the initial phases of drug-seeking behavior. However, as the addiction progresses, many people may continue to smoke in order to avoid withdrawal symptoms and due to the many conditioned cues that have become ingrained, which are a part of the compulsive, repetitive nature of tobacco smoking. Additionally, there are over 4,000 chemical compounds that are produced when a cigarette burns; all of these compounds are in tobacco smoke and are inhaled. Thus, while nicotine is the primary addictive component of tobacco smoke, there are additional compounds such as MAO-A and MAO-B inhibitors that likely play a role.

Other imaging studies have demonstrated that even smoking a denicotinized cigarette, which supposedly has very low nicotine content, leads to occupancy of