



Cholinergic modulation of mesolimbic dopamine function and reward

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ARTICLE INFO

Article history:

Received 25 April 2011

Accepted 26 April 2011

Keywords:

Addiction
Acetylcholine
Cocaine
Dopamine
Feeding
Motivation

ABSTRACT

The substantial health risk posed by obesity and compulsive drug use has compelled a serious research effort to identify the neurobiological substrates that underlie the development these pathological conditions. Despite substantial progress, an understanding of the neurochemical systems that mediate the motivational aspects of drug-seeking and craving remains incomplete. Important work from the laboratory of Bart Hoebel has provided key information on neurochemical systems that interact with dopamine (DA) as potentially important components in both the development of addiction and the expression of compulsive behaviors such as binge eating. One such modulatory system appears to be cholinergic pathways that interact with DA systems at all levels of the reward circuit. Cholinergic cells in the pons project to DA-rich cell body regions in the ventral tegmental area (VTA) and substantia nigra (SN) where they modulate the activity of dopaminergic neurons and reward processing. The DA terminal region of the nucleus accumbens (NAc) contains a small but particularly important group of cholinergic interneurons, which have extensive dendritic arbors that make synapses with a vast majority of NAc neurons and afferents. Together with acetylcholine (ACh) input onto DA cell bodies, cholinergic systems could serve a vital role in gating information flow concerning the motivational value of stimuli through the mesolimbic system. In this report we highlight evidence that CNS cholinergic systems play a pivotal role in behaviors that are motivated by both natural and drug rewards. We argue that the search for underlying neurochemical substrates of compulsive behaviors, as well as attempts to identify potential pharmacotherapeutic targets to combat them, must include a consideration of central cholinergic systems.

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1. Introduction

More than three decades of research into the neurobiological substrates of reward have focused attention on the nucleus accumbens (NAc), a ventromedial component of the basal ganglia, as a key structure in the neural systems responsible for translating motivation to action [1–4]. The preponderance of this research effort has centered on dopamine (DA) as the primary neurotransmitter in this regard. Many laboratories have contributed to our understanding of the role of DA in motivated behavior, and certainly the efforts of Dr. Bartley Hoebel in his laboratory at Princeton have been particularly noteworthy. Studies by Hoebel and colleagues have demonstrated increases in the release of DA in the NAc as a function of a variety of behaviors including feeding [5,6], rehydration [7], models of binge eating [8,9] and hypothalamic stimulation [10]. Moreover, his laboratory has been instrumental in demonstrating that many drugs with abuse liability (including cocaine, amphetamine, opiates, nicotine and alcohol) share a common mechanism in their ability to elevate DA levels in the NAc.

Of the many perspectives Hoebel brought to the study of neurochemical modulation of motivated behavior, perhaps the most salient for this review – and one that inspired much of the work presented herein – has been his view (along with that of Dr. Pedro Rada) that acetylcholine constitutes an integral component of the mesolimbic system [11]. Cholinergic pathways interact with key regions in the brain reward circuit, as illustrated in Fig. 1. ACh projections from the nucleus basalis provide input to cortical and subcortical regions of the hippocampus and amygdala. These projections may play a role in learning and memory processes that are implicated in drug-induced dysfunctions like craving and relapse [12,13]. From the DA cell body region of the ventral tegmental area (VTA), DA terminals synapse on medium spiny gamma-aminobutyric acid (GABA)-containing cells and a smaller population of large, aspiny acetylcholine (ACh)-containing interneurons in the NAc [14]. Cholinergic interneurons have large dendritic arbors and an extensive network of axons that contact many cell bodies and terminals within both the core and shell subdivisions of the NAc [15,16]. Thus, in conjunction with DA inputs from VTA, ACh interneurons can modulate the activity of the GABA projection neurons, the primary output neurons of the NAc [17,18].

Cholinergic cells in the laterodorsal tegmental nucleus (LDTg) and the posterior component of the pedunculopontine tegmental nucleus (PPTg) project to the VTA [19,20] where they modulate the activity of

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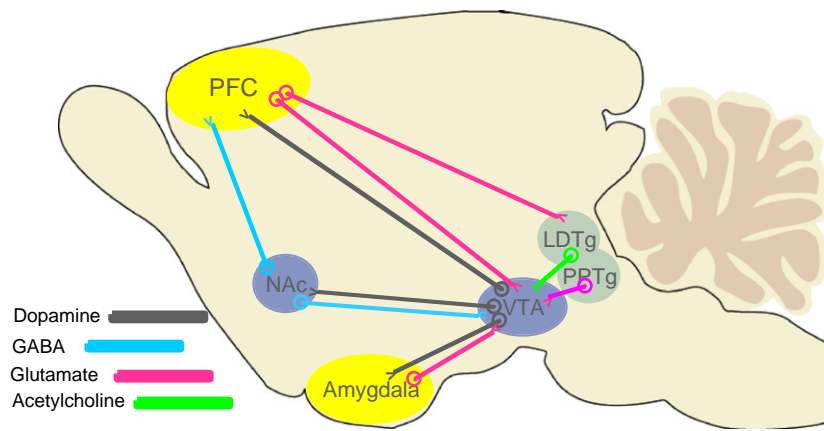


Fig. 1. ACh fibers (green) innervate key components of the mesolimbic system, including projections from nucleus basalis to sub-cortical areas (hippocampus and amygdala). ACh interneurons reside in the NAc and striatum. Another ACh projection system from the lateral dorsal tegmental nucleus (LDTg) projects to the DA cell body region of the ventral tegmental area (VTA). PFC: prefrontal cortex.

DA neurons and reward processing [21–26]. The anterior portion of the PPTg sends cholinergic projections to the substantia nigra [20]. Together with glutamate fibers, the ACh connection from the LDTg to the VTA forms a loop between the midbrain DA cells, the pons and the prefrontal cortex. The ACh input onto DA cell bodies (in the VTA) is therefore a critical link for gating information flow through the mesocorticolimbic and mesostriatal systems.

Several lines of evidence suggest that stimulation of both nicotinic and muscarinic cholinergic receptors can affect mesolimbic DA levels and modify the reinforcing value of self-administered drugs. In this regard, the action of nicotine (the prototypical agonist at nicotinic ACh receptors) on the mesolimbic DA system has been studied extensively (for review see Ref. [27]) as has its interaction with other drugs of abuse such as alcohol [28,29] and cocaine [30,31]. These studies demonstrate that nicotinic activation increases extracellular DA in the NAc, stimulates locomotor activity with repeated exposure, and potentiates the reinforcing value of cocaine.

In comparison to the rather clear-cut situation for nicotinic activation, the effect of muscarinic drugs on stimulus reinforcement is less clear. There is evidence that muscarinic receptor activation can decrease amphetamine-induced hyperactivity [32] and inhibit amphetamine-induced DA release in the NAc [33]. Conversely, muscarinic antagonists enhance the locomotor stimulating effects of amphetamine and cocaine [32,34,35]. Systemic administration of muscarinic agonists and partial agonists has been reported to decrease cocaine self-administration rates in mice [36], whereas co-administration with the muscarinic antagonist, scopolamine decreases cocaine self-administration in rhesus monkeys [37]. In addition, mice lacking the muscarinic M₅ type receptor self-administer less cocaine and show reduced cocaine conditioned place preference compared to their wild-type counterparts [38], suggesting that muscarinic receptors may mediate some component of cocaine reward.

Identifying the precise location of cholinergic effects on psychostimulant reward has been difficult because systemic administration of drugs and genetic deletion models affect receptors throughout the nervous system. However, recent work has focused attention on the NAc as a site of interaction between cholinergic mechanisms and cocaine reinforcement [39]. ACh interneurons in the NAc are known to be responsive to cocaine self-administration [40,41]. Hikida et al. (2001) reported that selective ablation of cholinergic interneurons within the NAc using immunotoxin-mediated cell targeting resulted in increased cocaine-induced locomotor activity and reward value (as measured by conditioned place preference). In contrast, augmentation of ACh levels with acetylcholinesterase inhibitors in lesioned mice had the opposite effect [42,43]. These studies provide strong evidence that cholinergic cells in the NAc play an important role in

modulating the reinforcing value of psychostimulants, but the nature of the cholinergic receptors that are critical for this effect remains unclear.

2. Acetylcholine–dopamine interactions and ingestive behavior

Seminal studies by Hernandez and Hoebel (1988) demonstrated that food reward increased DA in the NAc as measured by microdialysis [5], and Radhakishun and colleagues reported a similar result in the same year [44]. A key question that remained to be addressed, however, was whether DA output in the NAc was part of a general stimulus-induced arousal process or, alternatively, did DA play a role in the transduction of ingestive reward valence. To address these issues, Hoebel and colleagues performed two studies that examined the responses of NAc DA to conditioned taste stimuli. In the first experiment, extracellular dopamine in the NAc of rats was measured before and after they had developed a conditioned aversion to the taste of saccharin. In rats that had not developed an aversion, intra-orally applied saccharin increased DA. In contrast, DA decreased in response to saccharin in rats that had developed an aversion [45]. A complementary study was performed in rats with a conditioned taste preference to a normally aversive tastant, using a procedure eloquently developed by Sclafani and colleagues [46]. DA in the NAc was found to be elevated in response to an appetitively conditioned taste [47]. These studies represented some of the first reports that ingestive-related learning could modify DA output in the NAc.

The fact that mesolimbic DA plays a major role in modulating ingestive related behavior is now well documented. However, the neurochemical history of the corpus striatum is replete with suggestions that DA does not act in isolation but combines its action at least in part with that of the cholinergic system [11,48–51]. In addition to a mesencephalic dopamine input, the caudate and accumbens nuclei contain cholinergic interneurons [52–54], and there are numerous indications that the two systems functionally interact in the striatum [55–59]. Moreover, in the clinical arena, the prescription of dopaminergic antagonists (e.g. for the treatment of psychosis) is usually accompanied by co-administration of anticholinergic agents. Given the history of cholinergic–dopaminergic interactions in the striatum and the similar anatomical connectivity of the subjacent nucleus accumbens, it seemed reasonable to explore the possibility that both DA and ACh may have a combined role in the modulation of motivated behaviors.

Evidence from the Hoebel laboratory has suggested that a particular balance between DA and ACh may indeed exist within the NAc and that changes in this balance may contribute to alterations in ingestive behavior (for a perspective, see Ref. [11]). In rats, presentation of an aversively conditioned taste results in an increase of

DA and ACh work together. there are ACh interneurons in caudate and NAcc

extracellular ACh in the NAc [60], in contrast to the resulting decrease in DA mentioned above. Moreover, it is becoming increasingly apparent that discrete manipulations in the level of ACh activity in NAc can have profound effects on the motivation to eat. For example, hungry rats stop feeding if the DA/ACh balance in the NAc is tilted in favor of excess cholinergic tone (Fig. 2). In this study, rats were implanted with bilateral microdialysis probes in the NAc and allowed to feed ad libitum over the course of their night cycle. Probes implanted in control animals were perfused throughout the night with a standard perfusion medium (Ringer), whereas experimental subjects had the perfusion solution switched to one containing the indirect cholinergic agonist, neostigmine after 3 h. Results showed a dramatic (and nearly complete) cessation of feeding during neostigmine exposure. Lack of eating was apparently not due to incapacitation or immobility, since water intake was unaffected in these animals (data not shown). Conversely, a situation in which ACh is depleted can cause hyperphagia (Fig. 3). For these experiments, rats were trained to lever-press for access to 45 mg food pellets during a 10 min interval each day for several weeks. After a stable baseline of responding was established, one half of the subjects received bilateral NAc injections of ethylcholine aziridinium mustard (AF64A), a compound which has relatively selective toxic effects on ACh neurons, while control animals received vehicle injections. Again, results were profound but in the opposite direction as observed before. In this case, AF64A-treated animals exhibited a 2-fold increase in responding for food. Activity on a food-inactive lever was unaffected (data not shown) indicating that the increase in bar pressing was not simply a consequence of hyperactivity.

3. Cholinergic interactions with mesencephalic dopamine systems and drug reward

Work done in the Hoebel laboratory over a decade ago was some of the first to demonstrate a relevant link **between ACh in the VTA and feeding related behavior** [61]. These findings suggested that an interaction between ACh from pontomesencephalic sources and VTA could also be particularly critical for determining the action of drugs such as cocaine, that increase DA signaling by reuptake blockade. The ability of cocaine to increase extracellular DA is impulse dependent, and the interaction of ACh-mediated excitation of DA neurons, coupled with DA reuptake blockade may effectively increase the impact of cocaine on the mesolimbic system. We have found support for this hypothesis in our finding that repeated exposure to nicotine

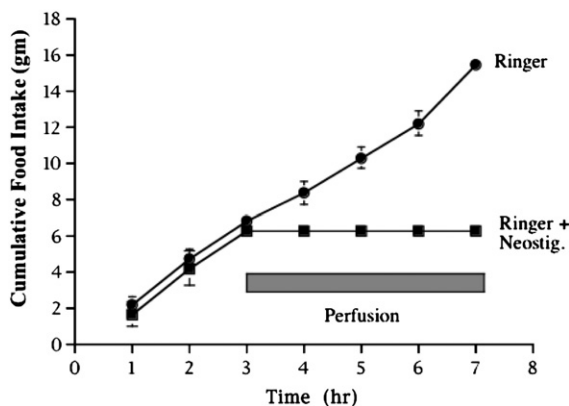


Fig. 2. Bilateral microdialysis probes were implanted into the NAc of rats and probes were perfused with a standard Ringer's solution over the course of 8 h during the dark cycle in non-food deprived rats ($N=10$). Food intake was measured hourly for 3 h, at which point half of the rats had the dialysis perfusion medium switched to a Ringer solution plus the cholinesterase inhibitor, neostigmine ($100 \mu\text{M}$). The remaining rats were maintained on the standard Ringer perfusion medium. Addition of neostigmine to the perfusion Ringer caused a cessation of eating in free-feeding rats.

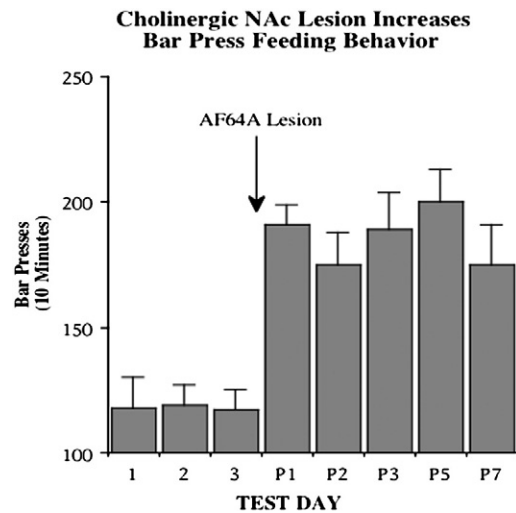


Fig. 3. Lever pressing for 45 mg food pellets was measured for 10 min daily in non-food-deprived rats ($N=8$). Bilateral NAc injection of AF64A, a cholinergic cell toxin, caused a substantial increase in bar pressing for food on the first test day after the lesion and persisted for up to 1 week (P1–P7).

increased break points for cocaine self-administration on a progressive ratio reinforcement schedule [31].

In a separate set of experiments, we measured the response to systemic injections of cocaine of DA released from dendrites in the VTA simultaneously with ACh released from terminals projecting from the pontomesencephalic cholinergic nuclei in rats. Fig. 4 shows that both ACh and DA increased robustly following 20 mg/kg cocaine. The time course of the ACh response was less phasic and the increase lasted longer than the DA response. Importantly, we have found identical responses (both in quality and time course) of DA and ACh to

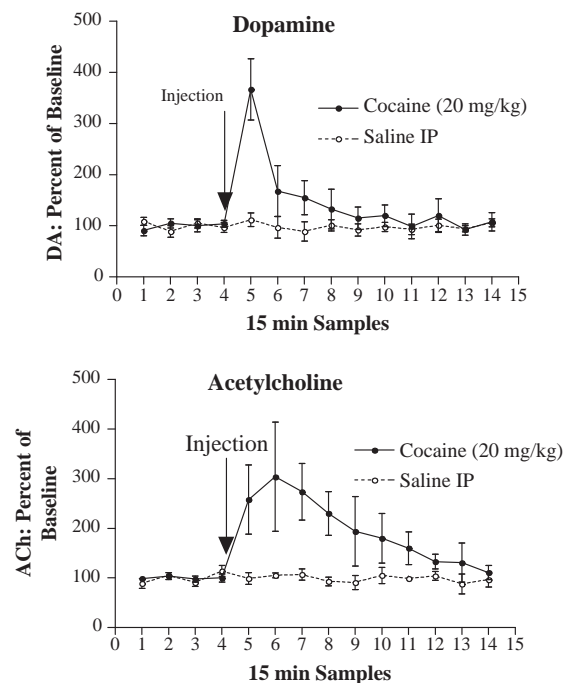


Fig. 4. Systemic injections of cocaine increase ACh and dendritic DA in VTA. Intraperitoneal injections of cocaine caused a robust increase in DA released from dendrites in the VTA (top panel). Measured simultaneously, ACh levels in the VTA also increased dramatically (bottom panel). The time courses of the ACh and DA responses differed; there was a sharp, phasic spike in DA compared to a slower, tonic ACh response.

methamphetamine using microdialysis in the VTA of C57BL/6 mice [62]. We also sought to determine if the increase in VTA DA following cocaine was dependent on cholinergic activity. We found that blockade of nicotinic cholinergic receptors (nAChR) within the VTA (using the nAChR antagonist mecamylamine; MEC) prevented the cocaine-induced increase in somatodendritic DA output in rats (Fig. 5). These results collectively demonstrate that psychostimulants potentially activate the cholinergic projections to the VTA and may be an important finding for understanding the mechanism of ACh/DA interactions in the VTA.

Another proposal we tested was whether *inactivation* of ACh would reduce the behavioral reinforcing effect of cocaine (as measured by decreased cocaine self-administration). Specific pharmacological agents that block or enhance muscarinic receptors in the LDTg change ACh levels in the VTA. We determined that ACh output in the VTA was attenuated by direct, bilateral microinjection of the selective muscarinic type-2 (M2) autoreceptor agonist oxotremorine-sesquifumarate (OxoSQ; Fig. 6). Due to its ability to reduce an excitatory (cholinergic) input onto DA neurons, we hypothesized that OxoSQ would reduce the motivation of rats to self-administer both natural and drug rewards. Animals were therefore tested on progressive ratio (PR) schedules of reinforcement for food pellets and cocaine. We found that OxoSQ microinjection in the LDTg, compared to aCSF, significantly reduced both the number of self-administered pellets and cocaine infusions during the initial half of the session and this reduction was dose-dependent [63]. These data suggest that inactivation of ACh input to the VTA results in lower motivation for both food and cocaine self-administration.

4. Cholinergic antagonist prevents escalation of cocaine self-administration in rats

When rats are allowed increased access time to cocaine for self-administration (from 1-h/day to 6-h/day), they exhibit an escalation in their average daily intake of the drug [64,65]. Rats maintained on 1-h/day access do not show an escalation. We have replicated this finding in our laboratory (see Fig. 7; red circles, solid line [69]). The escalation of drug intake phenomenon is a potential animal model

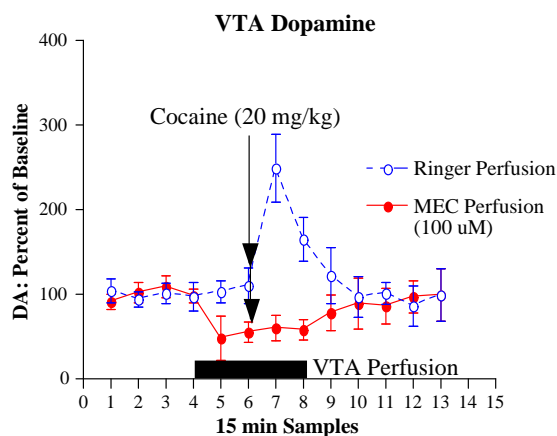


Fig. 5. Perfusion of the non-specific nicotinic receptor antagonist mecamylamine into the VTA blocks DA response to systemic injection of cocaine. The DA response was measured by microdialysis in the VTA of rats. Systemic cocaine (20 mg/kg IP) caused an increase in VTA DA levels under normal perfusion conditions (Ringer; blue line) but cocaine did not increase DA levels when mecamylamine was perfused through the VTA by reverse dialysis (red line). This strongly suggests that the ability of cocaine to increase DA in VTA is dependent on the functional mecamylamine-sensitive nicotinic ACh receptors. Note also that perfusion of VTA with mecamylamine caused a moderate reduction in the amount of DA recovered before the cocaine injection (samples 5 and 6). This suggests that nicotinic cholinergic receptors have an effect on basal DA release from dendrites in the VTA.

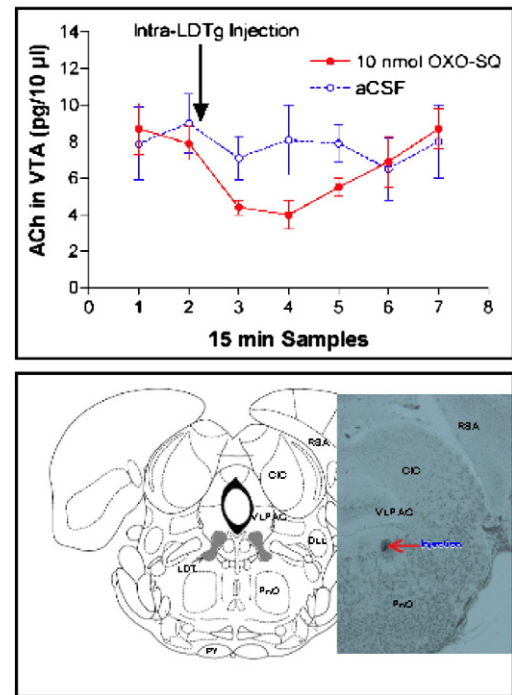


Fig. 6. Intra-LDTg injection of the ACh autoreceptor agonist Oxo-SQ reduced VTA ACh output. Top panel: intra-LDTg injection of oxotremorine-sesquifumarate (Oxo-SQ; an M2 cholinergic autoreceptor agonist) decreased the level of ACh measured in the VTA by microdialysis in rats. Artificial cerebrospinal fluid (aCSF) did not have this effect (N = 4/group). These results show the feasibility of inducing an ACh “deficit” in the VTA using microinjections in the LDTg. The histological slice shows the site of a thionin injection that was infused immediately after the conclusion of the microdialysis. Bottom panel: coronal section of unstained (and unfixed) section from a rat that received an injection of Oxo-SQ into the LDTg (shaded area). Thionin stain was injected immediately after the conclusion of the microdialysis experiment. The red arrow shows the injection site, here compared to a plate taken from the atlas of Paxinos and Watson (Bregma – 8.72).

with strong construct validity for the behavior of some human drug users who, as addiction develops, tend to increase drug intake when cocaine is readily available over extended time periods [66–68].

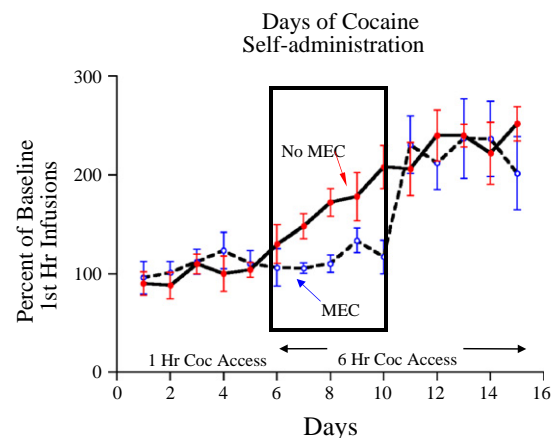


Fig. 7. The graph shows the number of cocaine infusions (normalized to baseline; 0.75 mg/kg/infusion) rats took in the first hour when access was limited to 1-h (Days 1–4) or increased to 6-h per day (Days 5–8). Rats tended to escalate cocaine intake with 6-h access time (upper line) but if mecamylamine (MEC; 20 µg/kg/infusion) was added to the cocaine solution, escalation in cocaine intake did not occur. Note that MEC did not completely eliminate cocaine self-administration but prevented the expression of high-level (i.e. escalated) intake. Adapted from Hansen and Mark (2007) [69].

We examined whether a nicotinic receptor antagonist, mecamylamine (MEC) would affect escalation using the increased cocaine access-time procedure similar to the one described by Ahmed and colleagues. We found that when MEC was added to the cocaine solution that rats self-administered, escalation of intake did not occur (Fig. 7; see also Ref. [69]). Moreover, we found that while MEC blocked the expression of increased cocaine self-administration, it may not have blocked its development because full escalation was observed the day after MEC was removed. These are exciting data because they show, first, that escalation of cocaine intake can be blocked without affecting normal (i.e. non-escalated) intake and, second, that neuroadaptations that underlie the development of increased cocaine-seeking behavior are independent of nicotinic receptors while those that are responsible for expression of higher drug-seeking require nicotinic receptor function.

5. Intra-VTA Injection of mecamylamine reduces high-level cocaine intake

A key goal was to identify whether inactivation of cholinergic input to VTA can reduce expression of escalated cocaine intake. In a recent study, we found that microinjection of the nAChR antagonist mecamylamine (60 µg/side) reduced *escalated* cocaine intake (Fig. 8) but did not reduce cocaine intake below pre-escalation levels. Rats self-administered cocaine for 1 h per day in the pre-escalation phase. The average level of cocaine intake in 1 h access sessions is represented by the dotted line. Rats were then given 6 h per day access and cocaine intake increased. Microinjections of MEC were given between 8 and 12 days after the beginning of the 6 h access sessions. Results are expressed as a percentage of pre-injection baseline, which was the average of the last 2 days of cocaine infusions on the 6 h access schedule. At a concentration of 60 µg/side, intra-VTA injection of mecamylamine significantly reduced escalated intake but did not reduce cocaine self-administration below pre-escalation levels. As a control, we injected the GABA_B agonist baclofen to confirm that intra-VTA injections of a drug known to reduce non-escalated cocaine intake would be effective at reducing cocaine intake below the pre-escalation baseline.

These findings have strong implications for the role of cholinergic systems in controlling drug intake and potentially for the development of treatment strategies. Escalation of self-administration behavior is particularly relevant to cocaine addiction, and the paradigm we (as well as other labs) have used has construct and predictive validity as a model of uncontrolled drug intake in humans. Our data may have important implications for the development of

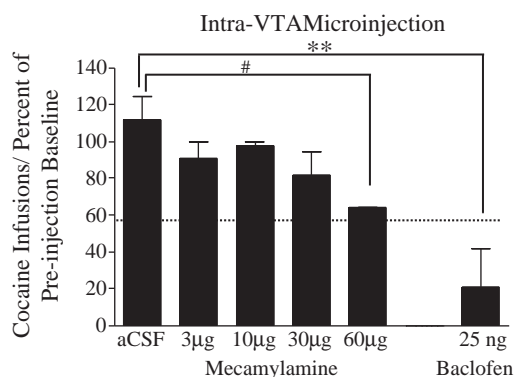


Fig. 8. Intra VTA injection of mecamylamine (MEC; 60 µg/side) reduced cocaine self-administration in rats that had established escalated cocaine intake on a 6 h schedule. Dotted line indicates the average pre-escalation cocaine intake. Note that MEC reduced escalated intake but did not reduce cocaine self-administration below pre-escalation levels. Baclofen was used as a positive control to show that VTA injections could reduce cocaine self-administration below pre-escalation levels, whereas MEC only reduced escalated intake. (N = 5–6 per group; # $P < 0.05$; ** $P < 0.01$).

potential therapeutics. For example, it is possible that new treatments that target nAChRs could interfere with the redevelopment of escalation in addicts when they leave in-patient treatment programs. There is support for this hypothesis since addicts report lower levels of cocaine craving following administration of MEC [70].

Acknowledgments

I (GPM) would like to extend my sincerest gratitude to Dr. Bartley G. Hoebel for his guidance and support throughout the length of this work and all that preceded it. He has been an outstanding role model. The authors would like to thank Tammie Painter for her excellent technical assistance. This work was supported by NIH grants R01 DA14639, T32 DA07262, and P50 DA018165.

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