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Effects of mecamylamine on flash-evoked potentials, body temperature, and behavior in Long-Evans rats

Bruce E. Hetzler *, Elizabeth J. Beckman

Department of Psychology, Lawrence University, 711 E. Boldt Way, Appleton, WI 54911 USA

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ABSTRACT

This experiment examined the effects of mecamylamine, a nicotinic acetylcholine receptor antagonist, on flash-evoked potentials (FEPs) recorded from the visual cortex (VC) and superior colliculus (SC) of chronically implanted male Long-Evans rats, and on body temperature and open field behavior. FEPs were recorded at 20 and 35 min following intraperitoneal injections of saline, and of 0.3, 3.0, and 10.0 mg/kg mecamylamine on separate days. The 0.3 mg/kg dose did not produce significant effects. The amplitude of VC components N_{30}, P_{48}, and P_{87} increased, N_{150} and P_{231} decreased, and P_{23}, N_{40}, N_{58}, and N_{68} were unchanged following administration of the 10.0 mg/kg dose. In the SC, component P_{28} was unaffected, P_{39} was reduced, and N_{49} was augmented by the 10.0 mg/kg dose. All component peak latencies were increased by the 3.0 and 10.0 mg/kg doses. Significant hypothermia was also produced by the 3.0 and 10.0 mg/kg doses, suggesting that this was the basis for the increased latencies. The 10.0 mg/kg dose produced a significant decrease in movement during the recording sessions. In subsequent open field observations, both line crossings and rearings were reduced by the 3.0 and 10.0 mg/kg doses. The results suggest that endogenous acetylcholine acting on nicotinic acetylcholine receptors plays at most a modest role in producing FEPs recorded from the VC and SC.

1. Introduction

The flash-evoked potential (FEP) is a complex electrical response that occurs immediately following the presentation of a brief flash of light. In laboratory animals, it can be easily recorded from both cortical and subcortical sites. The individual components of FEPs are representations of neural pathways that are activated during the photic stimulation (Fox and Rosenfeld, 1972). In this regard, visual stimulation increases acetylcholine (ACh) efflux in the visual cortex (VC) (Collier and Mitchell, 1966; Fournier et al., 2004; Laplante et al., 2005), and this released ACh presumably contributes to the production or elaboration of one or more of the components of the FEP recorded from the VC. Since ACh release in the cerebral cortex is involved in neuronal plasticity, attention and learning (Levin and Simon, 1998; Verdier and Dykes, 2001; Sarter et al., 2005; Dotigny et al., 2008), knowledge of the cholinergic specificity of components of the FEP could prove useful in the assessment of cholinergically mediated cognitive processes.

Another visual center involved in attention that receives a major cholinergic input is the superior colliculus (SC), a structure involved in attention and orientation (Goldberg and Robinson, 1978; Binns, 1999). However, while the VC receives its cholinergic input from the nucleus basalis in the basal forebrain (Carey and Rieck, 1987), the main cholinergic input to the superficial visual layers of the SC originates in the contralateral parabigeminal nucleus (Sefton and Martin, 1984; Binns, 1999). The components of the SC FEP show a polarity reversal in the stratum griseum superficiale layer of the SC (Dyer and Annau, 1977), and ACh receptors have been identified throughout the superficial layers of the SC (Clarke et al., 1984). There should therefore be a cholinergic involvement in the production of FEPs recorded from the SC as well as the VC.

ACh has effects on two broad classes of ACh receptors: muscarinic and nicotinic (mAChR and nAChR). The focus of the current study is nAChRs, since activation of nAChRs selectively enhances thalamocortical synapses (Gil et al., 1997), most likely via the nAChRs that have been identified in layers III and IV of the primary VC (Clarke et al., 1984), and nAChRs are also prominent in the SC (Clarke et al., 1984). There are a variety of subtypes of nAChRs, composed of distinct combinations of at least 12 neuronal subunits (∝2–∝10 and ß2–ß4; Levin and Simon, 1998; Vizi and Lendvai, 1999; Metherate, 2004), with ∝4ß2 and the homomeric ∝7 receptors being the most common high-affinity nicotinic receptors in the brain (Origlia et al., 2008).

The activity of nAChRs can be altered by administration of agonists and antagonists. While nicotine can be used as an exogenous agonist at nAChRs, mecamylamine is an antagonist of nAChRs, exhibiting both competitive and noncompetitive properties (Stolerman et al., 1997). It

* Corresponding author. Tel.: +1 920 832 6704; fax: +1 920 832 6962.
E-mail address: hetzlerb@lawrence.edu (B.E. Hetzler).

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is readily absorbed from the gastro-intestinal tract, and easily crosses the blood-brain barrier (Young et al., 2001). Although mecamylamine has been used in the past as a ganglionic blocker to treat hypertension, it is now viewed as having many potential clinical applications (Young et al., 2001). For example, mecamylamine is being studied in efforts to curb various drug dependencies, including nicotine (tobacco), alcohol, and cocaine (Levin et al., 2000).

In regard to FEPs, the extent to which mecamylamine alters individual components can be viewed as an indication of the involvement of endogenous ACh acting on nAChRs to produce or regulate the sensory responses (Metherate, 2004). However, despite the large number of studies which have examined the effects of nicotine on FEPs recorded from human and animal subjects (see Hetzler and Theinpeng, 2004 for summary), there has been no systematic evaluation of the effects of mecamylamine on FEPs.

One recent study examined the influence of cholinergic drugs on VC visual evoked potentials (VEPs) produced by sinusoidal gratings in isoflurane anesthetized rats (Kang and Vaucher, 2009). Intracortical injection of mecamylamine did not alter the amplitude of either the early negative wave or the later positive wave, but did prevent a long-term enhancement of the VEPs produced by either intracortical infusion of the ACh analog carbachol or electrical stimulation of the horizontal limb of the diagonal band of Broca. In other work by Bale et al. (2005), VC VEPs produced by a vertical wave grating were recorded from awake but restrained rats. The dependent variables were the spectral amplitude at the stimulus rate (F1) and twice the stimulus rate (F2). Mecamylamine (6 mg/kg, ip) significantly reduced F2 but not F1 amplitude. A dose of 3 mg/kg had no effect. Using urethane-anesthetized rats, Lewandowski (1996) stimulated the dorsal lateral geniculate nucleus (dlgn) and recorded VEPs from the VC. Mecamylamine (4 mg/kg, iv) did not alter either the VEP or reticular facilitation of that VEP. Knott et al. (1999) examined the effects of mecamylamine (20 mg) on P300 amplitude recorded from human subjects during visual and auditory continuous performance tasks. Mecamylamine significantly attenuated P300 amplitude in the visual condition. In regard to the SC, we are unaware of any studies that have examined the effects of mecamylamine on FEPs recorded from that structure.

The present study sought to determine the effects of mecamylamine on visual system electrophysiology by examining the effects of a range of mecamylamine dosages on FEPs recorded from both the VC and SC of Long-Evans rats. In addition to recording FEPs, body temperature was also measured, since drugs affecting the ACh system are capable of altering body temperature (Clark and Clark, 1980), and such changes in body temperature may then result in secondary changes in evoked potential parameters (Hetzler and Dyer, 1984; Hetzler et al., 1988). Finally, since mecamylamine can alter behavior in rats (e.g., Andrews et al., 1994; Rezvani et al., 2002), we monitored gross body movement during and after the evoked-potential recording sessions.

2. Materials and methods

2.1. Animals

Twenty-seven adult, male Long-Evans hooded rats (Harlan, Indianapolis, IN), about 4 months old and weighing 345–422 g at the time of surgery, were used in the study. The rats were housed individually in standard cages in a room with a light/dark cycle (light from 07:00 to 19:00 h) and climate control (temperature about 22 °C and humidity about 50%). Purina Lab Chow (St. Louis, MO) and tap water were provided *ad libitum*. All procedures were approved by the Lawrence University Animal Care and Use Committee.

2.2. Electrode implantation surgery

At least 1 week before adaptation, recording electrodes in the VC and the SC were implanted while the animals were under the effect of pentobarbital anesthesia. The rats first received an intraperitoneal (ip) injection of atropine sulfate (0.06 mg) to minimize respiratory distress during anesthesia. They were then anesthetized with an ip injection of 50 mg of sodium pentobarbital/kg body weight. The VC electrode (0–80×1/8 in. stainless steel screw) was placed 6 mm posterior to bregma and 3 mm lateral to the right of the midline. Similar screw electrodes placed over the ipsilateral and the contralateral frontal cortex provided for a recording reference and grounding, respectively. SC recordings were made from a twisted pair of nichrome wires (each 250 μm in diameter), insulated to the tip, with a vertical intertip distance of 1 mm. With the skull surface of the animal located in a horizontal plane (König and Klippel, 1963), the bipolar SC electrode was implanted 6.5 mm posterior to bregma and 1.5 mm lateral to the left of the midline and then lowered 4.7 mm below the surface of the skull. All electrodes were led to a 5-hole plastic cap (Wire Pro #223-1605), and the whole assembly was secured to the skull with additional screws and dental acrylic. After surgery, animals were handled briefly on a daily basis before testing.

At the conclusion of the experiment, placements of the SC electrodes were histologically verified (Hetzler et al., 1981). Results for the SC are reported for those 23 animals in which the lower member of the electrode pair penetrated the superficial layer of the SC (Dyer and Annau, 1977). VC recordings from seven animals were unusable because of problems with the cap or amplifiers.

2.3. Chemicals

Mecamylamine hydrochloride (Sigma Product No. M9020, St. Louis, MO) was dissolved in 0.9% saline to obtain the different concentrations, which were injected ip in a volume of 2 ml/kg. Doses of 0.3, 3.0, and 10.0 mg mecamylamine/kg body weight were used. The goal was to utilize a range of dosages commonly found in rat studies (Moran, 1993; Rezvani et al., 2002; Miller and Segert, 2005).

2.4. Evoked potential procedure

Evoked potentials were amplified with Tektronix 122 preamplifiers (Tektronix, Beaverton, OR) with high and low filter settings of 1.0 kHz and 0.8 Hz for both the VC and the SC. Amplified waveforms were averaged (2000-Hz sample rate, 400 ms epoch, 100 epochs averaged per FEP) by an IBM PS/ValuePoint computer connected to a Modular Instruments M100 Mainframe containing the following modules: M202 Fast A/D, M210 Memory, and M214 Data Acquisition Timer. Fifty milliseconds of the epoch occurred before the application of the evoking stimulus. Evoking stimuli were presented with an interstimulus interval of 2 s. Data collection was controlled with an S-215 Signal Averager program (Modular Instruments, West Chester, PA).

Recordings were obtained while animals were located inside a shielded recording chamber with dim background illumination of about 3 lx. The testing box, which was located inside the shielded chamber, measured 10×10×10 in. and was constructed of white Plexiglas on three sides, the top, and the bottom, whereas the front panel was clear. Shielded Microdot cables (Microdot Connectors, South Pasadena, CA), which are designed to reduce artifacts associated with cable movements (Fox and Rosenfeld, 1972), were attached to the top of the chamber with a mercury swivel, allowing freedom of movement. Flash stimuli were presented by a Grass Model PS22C photostimulator (Grass Instrument Division, Astro-Med, West Warwick, RI) with an intensity setting of 8 (measured as 133 lx-s using a Model DR-1600 Photometer with a Model D-1500-2B Multiprobe; Gamma Scientific, San Diego, CA). The flash lamp of the photostimulator was placed in a small sound-attenuating chamber to eliminate the auditory click present with each flash. The flash lamp

was positioned outside the shielded recording chamber and was visible to the rat through a clear Plexiglas window.

The animals were given 2 days of familiarization to the testing procedures, followed by 2–3 days of rest before actual data collection. On each testing day, the animal received one drop of 1% ophthalmic atropine in each eye to maintain constant pupil dilation (Gelatt, 1981). Ten minutes after the eye drops were given, the animal was injected ip with physiological saline (0.9% sodium chloride, 2 ml/kg), 0.3, 3.0, or 10.0 mg mecamylamine hydrochloride per kg of body weight (2 ml/kg). Fifteen minutes later, the animal was placed in the testing chamber. Evoked potentials were collected 5 and 15 min after the animal was in the chamber (i.e., 20 and 35 min after the injection). The sequence of four injections was counterbalanced across animals, and the animals were given 2 days of rest between each of the four testing days. Thus, all of the rats received all of the treatments in this study.

Evoked potentials were collected simultaneously from the VC and the SC at 20 and 35 min following injection. These time intervals were selected based on prior studies investigating the behavioral effects of mecamylamine on rats (e.g., Clarke and Fibiger, 1990; Moran, 1993; Levin and Lippiello, 1999; Newman et al., 2002). In addition, gross body movement was measured during FEP collection by two intersecting photocell sensors (S23-01; Coulbourn Instruments, Allentown, PA) that were positioned diagonally in the cage corners 3.5 cm above the floor of the cage. Electronic counters tallied the number of beam interruptions.

2.5. Body temperature

Immediately after FEP testing, the animal was placed in a restraining tube, and a rectal thermistor probe (YSI No. 402; Yellow Springs Instruments, Yellow Springs, OH) was inserted 10 cm into the rectum. A rectal temperature reading (YSI 49TA Tele-Thermometer; Yellow Springs Instruments) was then taken. Ambient temperature (21 °C) was controlled by a wall thermostat, and was recorded at the conclusion of each test.

2.6. Open field behavior

Following body temperature measurement, each animal was observed for 4 min in a 0.92 m-square open field, with walls 46 cm high. The field was constructed of standard plywood, and was painted gray. The Plexiglas floor of the apparatus was divided into 36 equal squares, 15 cm on each side. Overhead fluorescent lights provided illumination of approximately 635 lx. On each testing day, the animal was placed in the same middle square of the open field, and allowed to explore for four minutes. Line crossings (movement of all four limbs of the rat across a line) and rearings (rising onto two legs) were tallied, and general qualitative observations were made of each animal's behavior.

2.7. FEP component identification

Fig. 1 presents group average FEP waveforms for the VC, while Fig. 3 presents group average FEP waveforms for the SC. Components studied in the present experiment are identified in the 20 min saline traces. Following the component identification procedure described in Hetzler & Theinpeng (2004), in the present study FEP components were designated by their polarity and by their latency from the onset of the light flash. Baseline-to-peak amplitudes and peak latencies were obtained for nine VC components (P_{23}, N_{30}, N_{40}, P_{48}, N_{58}, N_{68}, P_{87}, N_{150}, and P_{231}; see Fig. 1). Components P_{28}, P_{39}, and N_{49} were similarly analyzed in the SC waveforms (see Fig. 3). The baseline-to-peak amplitudes consisted of the difference between the mean voltage of 50 ms of prestimulus activity and the peak voltage. Peak latencies were measured from the onset of the evoking stimulus.

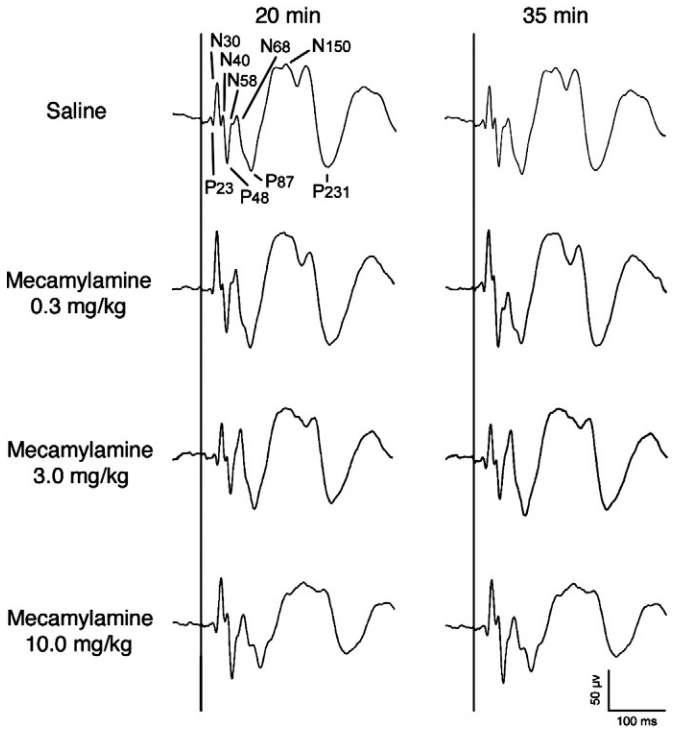

Fig. 1. Group average visual cortex (VC) flash-evoked potential (FEP) waveforms ($n = 20$) for each dose of mecamylamine and each time interval. Vertical lines represent onset of the evoking stimulus. Individual FEP components are named by polarity and latency from the onset of the evoking stimulus. The effects of mecamylamine varied with the component. In general, the amplitudes of components N_{30}, P_{48}, and P_{87} were increased by mecamylamine, while the amplitudes of components N_{150} and P_{231} were decreased by mecamylamine. Amplitudes of components P_{23}, N_{40}, N_{58}, and N_{68} were unchanged.

2.8. Data analysis

VC, SC, and photocell data were subjected to two factor (i.e., mecamylamine dose and time) repeated measures analyses of variance (ANOVA). When a significant main effect, or drug × time interaction, was found, individual means were compared with the Dunnett test. The saline treatment and 20-min recording interval data served as the basis for comparisons in the Dunnett tests for the VC, SC, and photocell data. That is, the 35-min data were compared to the 20-min data, while each dosage of mecamylamine was compared to the saline treatment. Body temperature, open field line crossings, and open field rearings data were subjected to a repeated measures analysis of variance in which drug treatment was the repeated factor. A significant main effect was followed by the Dunnett test, in which each mecamylamine dose was compared to saline. In all analyses, statistical significance was assumed when $P < .05$ for two-tailed comparisons. Changes in evoked potential amplitudes and latencies resulting from time-related factors are not discussed in the Results. Likewise, significant drug × time interactions are described only in relation to the main effects of the drug.

3. Results

3.1. Visual cortex: amplitude

Group mean evoked potentials for each dose of mecamylamine at both testing time intervals are displayed in Fig. 1, while baseline-to-peak amplitude data are presented for each component in Fig. 2. Only the 10 mg/kg dose of mecamylamine produced significant effects on component amplitudes, but not all components were significantly affected. Relative to saline, the 10 mg/kg dose of mecamylamine increased the amplitude of early components N_{30} [$F(3,57) = 4.340$,

$p=0.008$] and P_{48} [$F(3,57)=2.830$, $p=0.046$], but increased the amplitude of component P_{87} at only the 20 min recording interval [drug×time interaction: $F(3,57)=4.098$, $p=0.011$]. In contrast, both late components N_{150} [$F(3,57)=3.470$, $p=0.022$] and P_{231} [$F(3,57)=7.730$, $p<0.001$] were significantly depressed in amplitude by the 10 mg/kg dose of mecamylamine.

3.2. Visual cortex: latency

ANOVA test results for the main effects of mecamylamine on VC component latencies are shown below in Table 1. Significant drug×time interactions were also present for components P_{23}, N_{40}, P_{48}, N_{58}, and P_{231}. Dunnett test comparisons revealed that both the 3 and 10 mg/kg doses significantly increased the latency of all components at both the 20 and 35 min recording intervals, with the exception of component P_{231}, which was not increased at the 20 min interval by the 3 mg/kg dose of mecamylamine. The greatest increase in latency for all components was observed following administration of the 10 mg/kg dose. For example, at 35 min the latency of P_{23} was increased by 2.7 ms by the 3 mg/kg dose of mecamylamine in comparison to the saline latency, and by 3.1 ms by the 10 mg/kg dose. For N_{30} latency, the increases were 2.75 and 3.4 ms for the 3 and 10 mg/kg doses of mecamylamine, respectively (data not shown).

Table 1
ANOVA test results for VC FEP latencies.

P_{23}: $F(3,57)=38.516$, $p<0.001$
N_{30}: $F(3,57)=48.526$, $p<0.001$
N_{40}: $F(3,57)=64.911$, $p<0.001$
P_{48}: $F(3,57)=53.385$, $p<0.001$
N_{58}: $F(3,57)=24.426$, $p<0.001$
N_{68}: $F(3,57)=14.430$, $p<0.001$
P_{87}: $F(3,57)=37.255$, $p<0.001$
N_{150}: $F(3,57)=20.456$, $p<0.001$
P_{231}: $F(3,57)=19.521$, $p<0.001$

3.3. Superior colliculus: amplitude

Group mean SC evoked potentials are displayed in Fig. 3. Baseline-to-peak amplitude data for each component are presented in Fig. 4. Mecamylamine had differential effects on the 3 components. While the amplitude of P_{28} was not significantly altered by any dose of mecamylamine, P_{39} was significantly reduced in amplitude by the 10 mg/kg dose [$F(3,66)=3.005$, $p=0.037$]. In contrast, N_{49} was significantly increased at the 35 min recording interval by both the 3 and 10 mg/kg doses [drug×time interaction: $F(3,66)=4.253$, $p=0.008$].

Table 2
ANOVA test results for SC FEP latencies.

P_{28}: $F(3,66)=17.483$, $p<0.001$
P_{39}: $F(3,66)=73.138$, $p<0.001$
N_{49}: $F(3,66)=60.679$, $p<0.001$

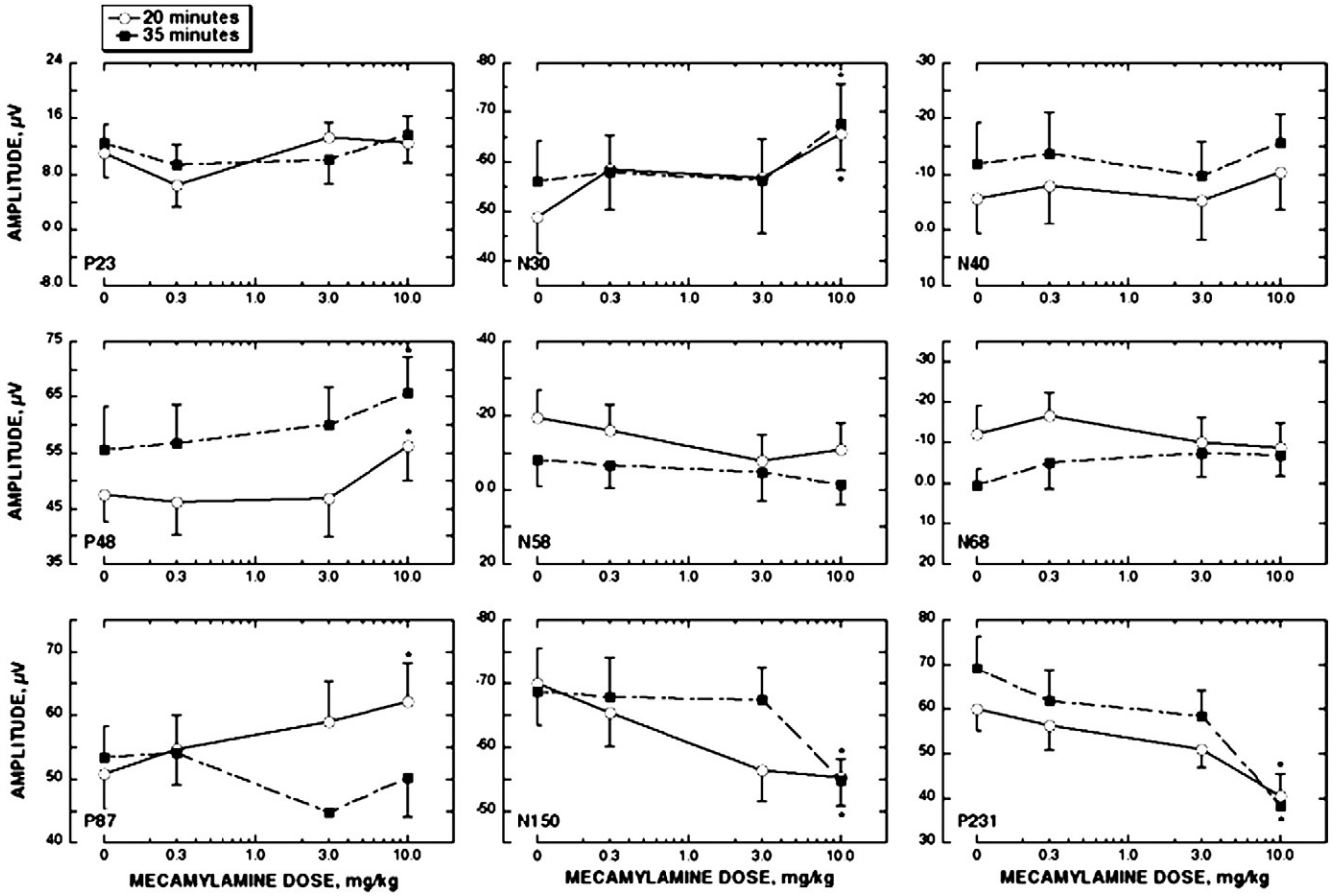

Fig. 2. Peak amplitudes of VC FEP components ($n=20$) as a function of mecamylamine dosage and time interval. Each panel illustrates the results for one FEP component, named by polarity and latency from the onset of the evoking stimulus. Vertical bars above or below the mean values represent ±1 S.E.M, respectively. *$P<.05$ when compared to the saline value by a two factor repeated measures ANOVA followed by Dunnett test comparisons.

3.4. Superior colliculus: latency

ANOVA test results for the main effects of mecamylamine on SC component latencies are shown below in Table 2. The latencies of all 3 components were significantly increased by both the 3 and 10 mg/kg doses of mecamylamine. A significant drug × time interaction for component P_{39} [$F(3,66) = 4.583$, $p = 0.006$] reflected a significant latency increase at the 35 min recording interval following administration of the 0.3 mg/kg dose as well. The greatest increase in latency again resulted from administration of the 10 mg/kg dose of mecamylamine. For example, at 35 min, the latency of P_{28} was increased by 2.5 ms by the 3 mg/kg dose of mecamylamine, and by 3.4 ms by the 10 mg/kg dose (data not shown).

3.5. Body movement

Fig. 5 (upper left panel) presents the mean number of photocell beam interruptions recorded during 3.5 min of evoked potential recording for each recording session. Although the main drug effect was not significant, there was a significant drug × time interaction [$F(3,78) = 3.841$, $p = 0.013$]. The 10.0 mg/kg dose of mecamylamine resulted in significantly fewer photocell beam interruptions at the 20 min recording interval than were observed following saline administration. The mean number of line crossings during the 4-min observation period following evoked potential testing is shown in the lower left panel of Fig. 5. Both the 3 and 10 mg/kg doses of mecamylamine produced a significant reduction in the number of line crossings [$F(3,78) = 7.894$, $p < 0.001$]. Likewise, both the 3 and 10 mg/kg doses of mecamylamine produced a significant reduction in the number of rearings in comparison to the number observed following saline administration [$F(3,78) = 17.117$, $p < 0.001$; lower right panel, Fig. 5].

Behavioral observations in the open field were also made during this 4-min time. Behavior after saline administration included ambulation around the perimeter, turning in circles, rearing (particularly in corners of the maze), sniffing, grooming, and gazing. Subjects tended not to enter the middle of the maze, and generally spent the latter portion (roughly 2 min) of the 4-min trial in a corner, facing the wall. When treated with 0.3 mg/kg mecamylamine, the behavior was very similar to that observed following saline administration, although the rats tended to enter the center of the maze more often than those in any other treatment group. Finally, after receiving the 0.3 mg/kg dose, subjects seemed to spend more time grooming during the final two minutes spent in the maze than the subjects injected with any other treatment. When the subjects received the 3.0 mg/kg dose of mecamylamine, they appeared more sluggish in the open field, and they did not enter the middle of the maze. Similar to both the saline and 0.3 mg/kg mecamylamine treatment groups, the 3.0 mg/kg-treated rats generally spent the final two minutes of each trial in a corner of the maze, facing the corner. After administration of the 10.0 mg/kg dose of mecamylamine, there was a drastic decline in the speed of ambulation, and walking often appeared wobbly. They tended not to explore the entire maze, but more often one or two sides of it, and did not enter the middle of the maze. When given this high dose, the rats typically terminated ambulatory behavior earlier in the trial than when treated with other doses, facing a corner and gazing, exhibiting a shivering, almost hiccup-like behavior.

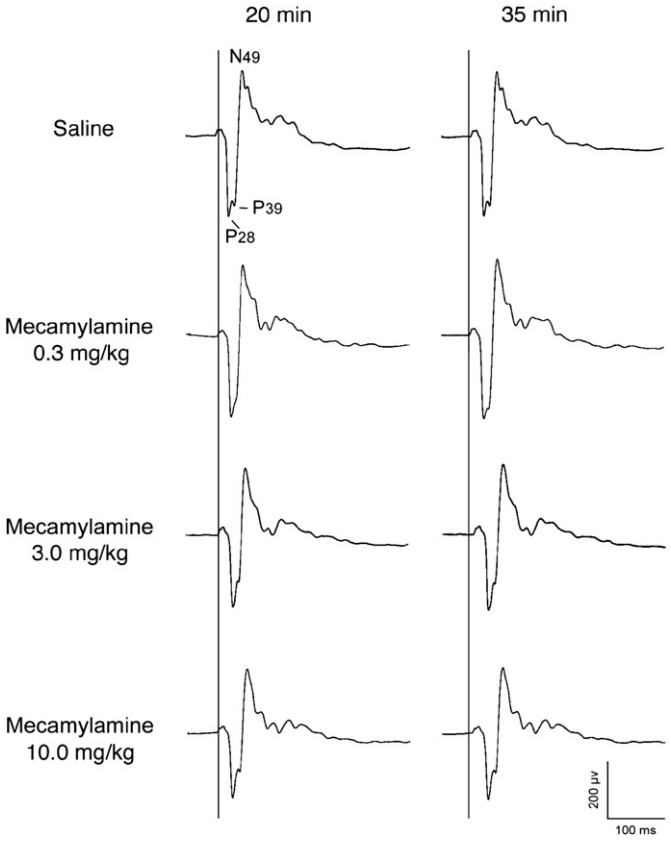

Fig. 3. Group average superior colliculus (SC) FEP waveforms ($n = 23$) for each dose of mecamylamine at both time intervals. Vertical lines represent onset of the evoking stimulus. Individual FEP components are named by polarity and latency from the onset of the evoking stimulus. The effects of mecamylamine varied with the component and time interval. While the amplitude of component P_{28} was unchanged, component P_{39} was depressed by the 10.0 mg/kg dose of mecamylamine, and component N_{49} was increased by both the 3.0 and 10.0 doses of mecamylamine at only the 35 min recording interval.

3.6. Body temperature

Fig. 5 (upper right hand corner) presents body temperature data. Both the 3.0 and the 10.0 mg/kg doses of mecamylamine significantly decreased body temperature compared to saline [$F(3,78) = 153.193$, $p < 0.001$]. The 3.0 mg/kg dose lowered body temperature by about 1.1 °C, while body temperature was lowered by about 1.9 °C by the 10 mg/kg dose of mecamylamine.

4. Discussion

Basal forebrain cholinergic neurons comprise a cortical input system which modulates information processing in all cortical areas, including the VC, and ACh release in the cerebral cortex is involved in neuronal plasticity, attention and learning (Levin and Simon, 1998; Verdier and Dykes, 2001; Sarter et al., 2005; Dotigny et al., 2008). The impact of ACh on cognitive processes such as attention could be exerted on the processing of sensory information as early as the primary cortical sensory areas (Gilbert et al., 2000), and visual stimulation increases ACh efflux in the VC (Collier and Mitchell, 1966; Fournier et al., 2004; Laplante et al., 2005). In this regard, high densities of [^{3}H]nicotine binding have been found in layers III and IV in the VC (Clarke et al., 1984), and it is the activation of nAChRs acting at presynaptic sites on glutamatergic neurons that enhances transmission at thalamocortical synapses (Sarter et al., 2005; Origlia et al., 2008).

Artificial activation of nAChRs via systemic administration of nicotine clearly alters sensory evoked potentials, indicating effects on sensory processing (e.g., Hetzler and Theinpeng, 2004; Metherate, 2004). On the other hand, the role of endogenous ACh on nAChRs in the VC can be assessed by preventing the release of ACh through destruction of the cell bodies of origin of the basal forebrain pathway, or the administration of an nAChR antagonist, such as mecamylamine (Metherate, 2004).

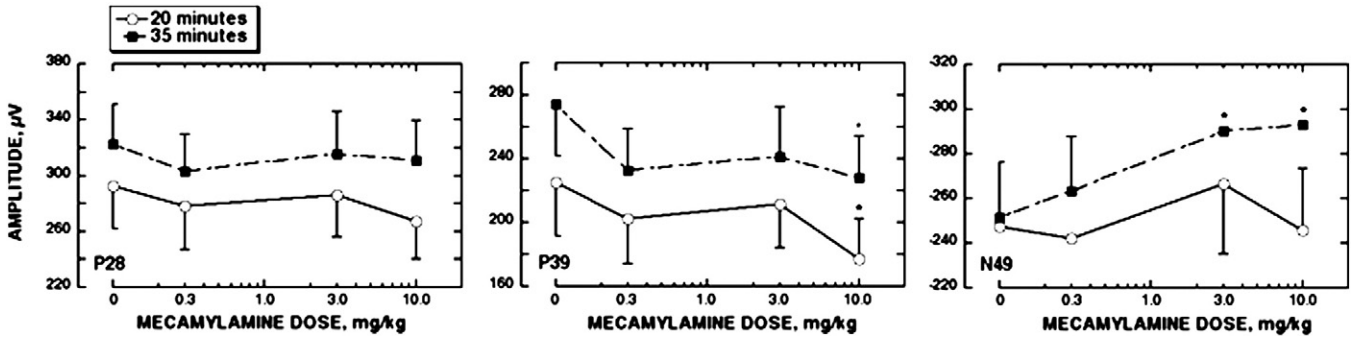

Fig. 4. Peak amplitudes of SC FEP components ($n = 23$) as a function of mecamylamine dosage and time interval. Each panel illustrates the results for one FEP component, named by polarity and latency from the onset of the evoking stimulus. Vertical bars above or below the mean values represent ±1 S.E.M, respectively. $^{*}P < .05$ when compared to the saline value by a two factor repeated measures ANOVA followed by Dunnett test comparisons at individual time intervals.

However, it remains to be demonstrated that destruction of the basal forebrain cholinergic pathway to the VC has any clear effect on cortical FEPs recorded from rats. Bringmann (1995) recorded VC FEPs from male Long-Evans rats after unilateral ibotenic acid lesion of the nucleus basalis magnocellularis or the pedunculopontine tegmental nucleus. The nucleus basalis lesion did not alter N_{31} peak latency or the amplitude of any component examined (up to a latency of 200 ms). Although the pedunculopontine tegmental lesion shortened the latency of N_{31} in the lesioned hemisphere, it also did not alter peak amplitudes (up to 200 ms).

Using the other approach in the present study, mecamylamine produced a series of dose-dependent changes in the VC FEP. While the lower doses of 0.3 and 3.0 mg/kg were ineffective in altering the amplitudes of FEP VC components, the high dose of 10.0 mg/kg significantly altered the amplitude of several components: N_{30}, P_{48}, P_{87}, N_{150}, and P_{231}. The first component of the VC FEP, P_{23}, is typically viewed as the correlate of the presynaptic geniculate volley (Siegel and Sisson, 1993), and is often unaffected by pharmacological manipulations (e.g., Hetzler and Burkard, 1999), as was the case in the present study.

Since component N_{30} represents a geniculocortical synaptic process produced by glutamate release in layer IV of the VC (Siegel and Sisson, 1993; Meeren et al., 1998), and activation of nAChRs modulate thalamocortical synapses (Sarter et al., 2005), it might be expected that mecamylamine and nicotine would have opposite effects on this component, and that is indeed the case. N_{30} was increased in amplitude by the 10.0 mg/kg dose of mecamylamine, while past research using nicotine (Hetzler and Theinpeng, 2004) has demonstrated a nicotine-induced reduction in the amplitude of this component.

P_{48} represents a nongeniculate, non-glutamate-mediated inhibitory postsynaptic potential on the pyramidal cells of cortical layers V and VI (Siegel and Sisson, 1993; Meeren et al., 1998). Interestingly, both the high doses of mecamylamine and nicotine enhance the amplitude of this component (Hetzler and Theinpeng, 2004; Hetzler and Martin, 2006). The amplitude of this component can also be increased by the muscarinic blocking agents atropine and scopolamine (Schwartzbaum and Kreinick, 1975; Hetzler and Smith, 1984). It would therefore appear that the amplitude of this component is sensitive to any modification of cholinergic brain systems.

N_{58} and N_{68}, like component N_{40}, were unaffected by the nAChR antagonist mecamylamine, but in past research were enhanced (at least transiently) by the nAChR agonist nicotine (Hetzler and Theinpeng, 2004). Siegel and Sisson (1993) state that components N_{58} and N_{68} reflect glutamate-mediated intracortical or subcortical (but not direct geniculate) input to the VC, while Bringmann (1994) speculates that this broad negative portion of the waveform is cholinergically generated, since physostigmine enhanced its amplitude, a finding that we noted in prior research (Hetzler and Smith, 1984). In that early work, we also found that atropine decreased the amplitude of this portion of the waveform, demonstrating a muscarinic involvement. While the amplitude of this portion of the waveform can therefore be manipulated by cholinergic drugs, the lack of an effect of mecamylamine indicates that release of ACh does not normally play a role in its production.

Component P_{87} was briefly enhanced by mecamylamine and also in earlier work by nicotine (Hetzler and Theinpeng, 2004). This is the last of the so-called secondary components (in addition to P_{48}, N_{58} and N_{68}), which are thought to result from connections between the SC, brain stem, and diffuse thalamic projections (see Creel et al., 1974).

Finally, the amplitudes of both of the late components N_{150} and P_{231} were depressed by the 10.0 mg/kg dose of mecamylamine. In our earlier study, nicotine also depressed the late positive wave, but had no significant effect on the late negative wave (Hetzler and Theinpeng, 2004). N_{150} reflects secondary (or rebound) activation of cortical pyramidal cells (Meeren et al., 1998), and is generally viewed as the first component of the flash-evoked after-discharge that is produced by a thalamic recurrent inhibitory system involving both the dorsal lateral geniculate nucleus (dLGN) and the thalamic reticular nucleus (Rhodes and Fleming, 1970; Creel et al., 1974; Bigler, 1977). Since nAChRs are located on the somatodendritic area of dLGN relay neurons, on GABAergic terminals which make contact with the relay neurons, and on cells in the thalamic reticular nucleus (Lena and Changeux, 1997), there are numerous places where blockade of nAChRs could have an impact. Most likely, the effects on these components are not cortical, however, since mecamylamine enhanced the primary component N_{30} while depressing these later components.

Components of the SC FEP show a polarity reversal in the stratum griseum superficiale (Dyer and Annau, 1977). The main cholinergic input to the superficial visual layers of the SC originates in the contralateral parabigeminal nucleus, which, in turn, receives visual input from the ipsilateral SC (Sefton and Martin, 1984; Binns, 1999). Components P_{28} and P_{39} of the SC FEP are part of the early positive complex. Mecamylamine had no effect on the amplitude of P_{28}, but the 10 mg/kg dose significantly depressed the amplitude of P_{39}. In contrast, the later negative wave N_{49} was augmented at the 35 min interval by both the 3 and 10 mg/kg doses of mecamylamine. Interestingly, these effects with the nAChR antagonist mecamylamine are almost exactly opposite of what was reported in our earlier study with the nAChR agonist nicotine (Hetzler and Theinpeng, 2004). In that study, P_{27} was reduced in amplitude by both the .7 and 1.0 mg/kg doses of nicotine, P_{37} was unaffected, and N_{48} was reduced in amplitude by several nicotine doses. The results of our two studies thereby appear to demonstrate a compelling role for nAChRs in the production of SC FEP components, and are in line with the finding that visual responses of neurons located in the stratum griseum superficiale of rats are inhibited by iontophoretic administration of lobeline (a nicotinic agonist) and augmented by hexamethonium (a nicotinic antagonist) (Binns, 1999).

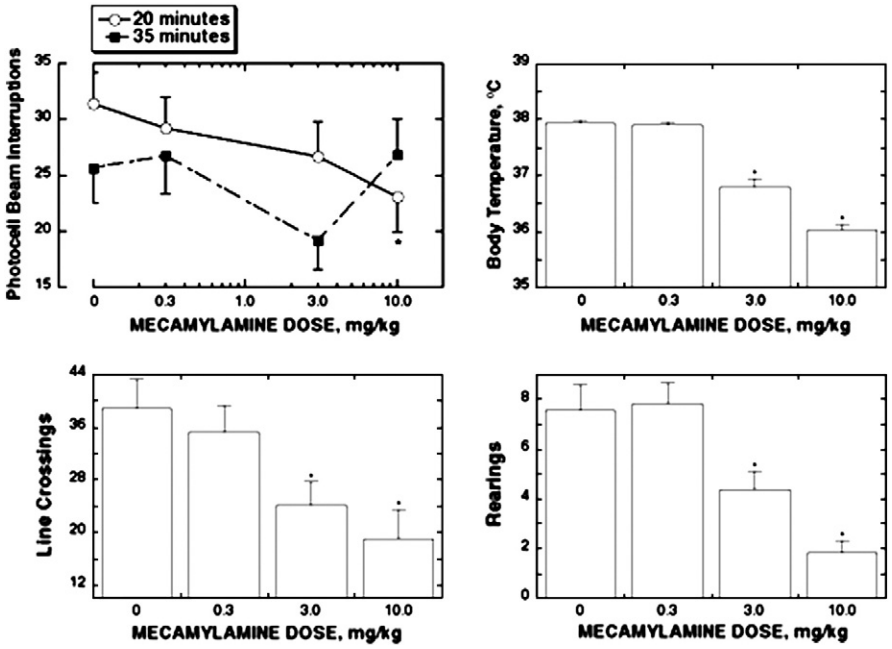

Fig. 5. This figure illustrates the effects of mecamylamine on body movement and body temperature. Upper left panel: body movement ($n=27$) as measured by photocell beam interruptions during the 3.5-min evoked potential test sessions. Data are presented as a function of mecamylamine dosage and time interval. Vertical bars above or below the mean values represent ± 1 S.E.M, respectively. $^{*}P<.05$ when compared to the saline value by a two factor repeated measures ANOVA followed by Dunnett test comparisons. Lower left panel: mean line crossings ($n=27$) during a 4-min open field test session that followed evoked potential collection. Data are presented as a function of mecamylamine dosage. Vertical bars above the mean values represent $+1$ S.E.M. $^{*}P<.05$ when compared to the saline value by a repeated measures ANOVA followed by Dunnett test comparisons. Lower right panel: mean rearings ($n=27$) during a 4-min open field test session that followed evoked potential collection. $^{*}P<.05$ when compared to the saline value by a repeated measures ANOVA followed by Dunnett test comparisons. Upper right panel: body temperature was measured with a rectal probe immediately after FEP testing. $^{*}P<.05$ when compared to the saline value by a repeated measures ANOVA followed by Dunnett test comparisons.

However, this conclusion must be tempered since the selectivity of mecamylamine for nAChRs might be compromised following administration of doses higher than 1.0 mg/kg, since higher doses can block NMDA glutamate receptors (O'Dell and Christensen, 1988; Clarke et al., 1994; Matta et al., 2007). One of the most potent noncompetitive NMDA receptor blockers is MK-801 (Dizocilpine; Foster, 1991), and in past research we examined the effects of MK-801 (0.1, 0.3, and 1.0 mg/kg) on FEPs, body temperature, and behavior in rats (Hetzler and Burkard, 1999). MK-801 reduced most FEP component latencies and increased body temperature at most doses tested. MK-801 also increased line crossings in an open field. These effects of MK-801 are generally opposite with those reported following mecamylamine administration in the present study, indicating that blockage of NMDA glutamate receptors is most likely not involved in the mecamylamine-induced changes in these dependent variables. For most VC FEP component amplitudes, the impact of MK-801 was also generally dissimilar to that of mecamylamine: P1 (P_{23} in the present study) was unchanged, N1 (N_{30}) was augmented, K1 (N_{40}) was augmented, P2 (P_{48}) was split into two portions by the intrusion of K2 with P2A (P_{48}) decreasing in amplitude and P2B (P_{58}) increasing, N2 (N_{68}) became much more positive, P3 (P_{87}) was substantially augmented over the entire recording session, and N3 (N_{150}) was augmented. Thus, the only two components affected in similar ways by mecamylamine and MK-801 were P1 (P_{23}), which was unchanged by either drug, and N1 (N_{30}), which was enhanced by both drugs. In the SC, component P1 (P_{28} in the present study) was not altered by MK-801, while P3 (P_{39}) was reduced in amplitude by the 0.3 and 1.0 mg/kg doses. N4A (N_{49}) was reduced by only the 1.0 mg/kg dose. These effects on the early positive SC FEP component amplitudes bear a strong resemblance to those reported in the present study with the highest dose of mecamylamine. It therefore may be that changes in the amplitude of the primary VC and SC component amplitudes induced by the 10.0 mg/kg dose of mecamylamine might actually result from blockade of NMDA glutamate receptors, since mecamylamine blocks NMDA receptors at the MK-801 site (Fu et al., 2008).

All FEP peak latencies in the VC and SC were increased by both the 3.0 and 10.0 mg/kg doses of mecamylamine. Those few past studies which have examined the effects of mecamylamine on VEP latencies have not reported changes in peak latencies (Knott et al., 1999; Kang and Vaucher, 2009). The two other studies did not measure latencies (Lewandowski, 1996; Bale et al., 2005).

The increased latencies observed in the present study most likely resulted from mecamylamine-induced hypothermia, which we observed following both the 3.0 and 10.0 mg/kg doses. Several past studies on the effects of mecamylamine on body temperature have reported hypothermia in rats (Leduc, 1961; Tsoucaris-Kupper and Schmitt, 1972) and mice (Martin et al., 1989). The relationship between reduced body temperature and increased FEP peak latency (but not peak amplitude) is clear (Hetzler et al., 1988), and would be expected to influence the results of any mecamylamine study involving freely moving rats. Based on our earlier work (Hetzler et al., 1988), for VC component P_{23} the estimated latency increase from a 1.0 °C decrease in body temperature is about 1.4–1.7 ms, whereas for component N_{30} the expected increase in latency is about 1.6–2.2 ms. These estimates can account for nearly all of the observed mecamylamine-induced increases in latency observed for VC components P_{23} and N_{30}. Finally, we note in this regard that the Kang and Vaucher (2009) rat study did not find a change in the peak latency with rectal temperature maintained at 37 °C.

In our study, the 10.0 mg/kg dose of mecamylamine resulted in a significant decrease in photocell beam interruptions at the 20 min interval during the recording sessions. Photo beam interruptions detected any movement that broke the beam, such as general ambulation. However, the equipment was not able to discriminate between various types of movements. Following electrophysiological testing, both the 3.0 and 10.0 mg/kg doses significantly reduced both the mean number of line crossings and rearings in an open field.

Past research has indicated a dose-dependent effect of mecamylamine on motor behaviors in rats. Thus, while a dose of 1.5 mg/kg had

no effect on cage crosses or repeated moves (Stolerman et al., 1997), mecamylamine produced a dose related increase in response time in a delayed matching to position procedure (Andrews et al., 1994), a dose related decrease in percent hit in an operant visual signal detection task (Rezvani et al., 2002), a dose related decrease in total responses in a two-lever nicotine oral self-administration model (Glick et al., 1996), and a dose related deficit in T-maze alternation and T-maze discrimination (Moran, 1993). On the other hand, low doses of mecamylamine (0–5.0 mg/kg) did not have any cataleptic effect in rats (Levin and Lippiello (1999)), and doses of 0.3–3.0 mg/kg did not alter basal activity in an automated open field (Miller and Segert, 2005). In mice, mecamylamine doses of 10.0 and 30.0 mg/kg produced significant reductions in open field activity (Chiappetta and Jarvik, 1969).

In conclusion, a dose of mecamylamine that is selective for nAChRs (0.3 mg/kg) did not produce any significant effects on FEP amplitudes or latencies, body temperature, or behavior in rats. While behavioral, body temperature, and FEP latency effects were observed following administration of the 3.0 mg/kg dose, significant FEP amplitude changes occurred almost entirely following only the 10.0 mg/kg dose of mecamylamine. This is a relatively high dose which may not be selective for nAChRs. This fact, in addition to comparisons with our past studies on the effects of nicotine (Hetzler and Theinpeng, 2004) and MK-801 (Hetzler and Burkard, 1999), as well as the lesion study by Bringmann (1995), suggests that endogenous ACh acting on nAChRs plays at most a modest role in producing these FEPs recorded from the VC and SC.

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