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The effects of cotinine on information processing in nonsmokers

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The Effects of Cotinine on Information Processing in Nonsmokers

Karen E. Herzig

Abstract

Cotinine is the major metabolite of nicotine, present in the bodies of smokers in larger amounts and for a longer time period than nicotine. Studies have suggested beneficial effects of nicotine on cognition, but the cognitive effects of cotinine have not been reported. The slowing of mental performance observed during tobacco withdrawal peaks in the first one or two days after smoking cessation, after nicotine has been eliminated, while cotinine is still present. Pilot study findings showed that 20 mg oral cotinine tended to slow reaction time (RT) in nonsmokers. Given the suggestion of slowing effects by cotinine, it is possible that cotinine contributes to tobacco withdrawal symptoms.

Here 16 nonsmokers were tested on three doses of cotinine: 30, 60, and 90 mg, and placebo, in a within-subjects, pre-post design. Half of the subjects were women. Subjects were tested on a choice RT task, in which RT, amplitudes and latencies of N100 and P300 event-related brain potential (ERP) components, and errors were recorded. A verbal recall task with short and long lists was also used. Heart rate, blood pressure, mood, subjective sensations, and saliva cotinine levels were also measured.

Cotinine significantly impaired verbal recall on the long list. On the short list cotinine effects were bidirectional; 30 mg improved recall, 60 mg showed little change on average, and 90 mg nonsignificantly impaired recall. Cotinine displayed a nonsignificant but consistent dose-related slowing of all information processing measures. These slowing effects were consistent in direction with those of the pilot study. Accuracy and ERP amplitudes were unaffected. Cotinine thus appears to have some weak, generalized slowing effect on information processing and specific effects on memory, which may reflect impairment of long term memory consolidation and differential effects, depending on dose, on short term memory processes. These effects were not mediated by any subjective, physiological, or mood changes. No gender effects were observed. The acute effects of cotinine seen here imply the possibility of more significant cognitive effects when regular smokers, who have chronically high cotinine levels in their bodies, stop smoking.

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Chapter 1
Introduction

Over 50 million people in the United States continue to smoke cigarettes, despite known health risks associated with their use (U.S. Department of Health and Human Services, 1988). Smokers often report that smoking helps them think and concentrate, which suggests that this effect may contribute to tobacco addiction (Russell et al., 1974). Difficulty concentrating is a major symptom of tobacco withdrawal (American Psychiatric Association, 1987; Hughes et al., 1990; Jaffe, 1990), and the negative effects of smoking cessation on mental performance are often cited as one important reason for relapse to smoking (U.S. Department of Health and Human Services, 1988). An understanding of the mechanisms by which smoking deprivation may affect cognitive function could lead to more effective smoking cessation treatments, yet effects of nicotine and tobacco withdrawal on cognition are still not clearly or specifically established.

Using a variety of cognitive tasks, Snyder et al. (1989) found significantly increased reaction times (RTs) and decreased accuracy in smokers deprived of nicotine for ten days. Other investigators reported that amplitudes of the auditory P300 and N100 components of the event-related potential (ERP) were reduced during a 10-day nicotine deprivation period (Herning et al., 1986). Authors reporting deprivation effects have generally observed impairment to occur or peak at between 12-48 hours (Schneider et al., 1984; Snyder et al., 1989; Snyder & Henningfield, 1989). Assessments of tobacco deprivation may not be measuring the effects solely of nicotine, however. Cotinine is the major metabolite of nicotine (Gorrod & Jeiner, 1975). It is present in the bodies of smokers in larger amounts and for a longer time period than nicotine. The elimination half-life of nicotine averages two to three hours, while the half-life of cotinine averages 15-19 hours (Benowitz et al., 1982; Benowitz et al., 1983). Regular smokers therefore maintain high levels of cotinine in their bodies over 24 hours of each day, and after nicotine deprivation begins cotinine remains present for several days. It is therefore possible that withdrawal effects occurring within the first few days of smoking cessation (when they are strongest) may be mediated, at least in part, by

cotinine. To my knowledge, however, no one has yet reported any testing of cotinine on human information processing.

Under the direction of Drs. Callaway, Halliday, and Benowitz, I recently conducted exploratory testing of the effects of 20 milligrams (mg) oral cotinine on 13 male nonsmokers. In that pilot study results indicated a tendency for cotinine to slow RT relative to placebo. Following completion of the pilot study we learned that Keenan et al. (1994) had tested subjects going through smoking cessation on a larger dose of cotinine and had observed significant increases in some symptoms of withdrawal effects on mood. Cotinine produced significantly greater self-reported anxiety and restlessness than placebo and significantly less self-reported pleasantness and sedation. The Keenan et al. study was the first directly to associate cotinine with an increase in tobacco withdrawal symptoms. That finding, in conjunction with our pilot study results, prompted the initiation of this investigation, the purpose of which is to test the direct effects of larger and multiple doses of cotinine on information processing in nonsmokers. The use of nonsmokers will avoid any confounding effects of nicotine.

Chapters 2 and 3 describe the methods and results, respectively, of the pilot study. Chapter 4 describes methods used in the present study, and Chapter 5 presents the results. Chapter 6 offers a discussion of these results and suggestions for future research.

The rest of this chapter provides an overview of the literature on the effects of nicotine and tobacco withdrawal on human cognition. It also introduces some salient points about the pharmacokinetics of cotinine and known physiological and behavioral effects of cotinine in animals. A description of the rationale for using RT and ERP measures in testing drug effects follows. The final section summarizes how this study will address some of the issues in the literature.

Effects of Nicotine on Human Cognition

--Methodological Concerns

Despite reports by smokers that smoking helps them think and concentrate, it has proved difficult to demonstrate unequivocally that nicotine affects specific cognitive processes. Methodological problems challenge straightforward

interpretations of many empirical results. Two principal problems are that most investigators have failed to use nonsmokers as controls and have not measured blood nicotine levels in the subjects being tested. A major question is whether the effects of smoking or nicotine when tested only in deprived smokers indicate direct effects or alleviation of deprivation effects. The use of nonsmokers as controls avoids the ambiguity of interpretation and argues for direct effects of nicotine. Nonsmokers experience side effects such as dizziness and nausea from smoking and other routes of nicotine administration, however, and they may require different doses of nicotine than smokers do (see Le Houezec et al., 1994). The use of nondeprived smokers as an alternative introduces the possible problem of acute tolerance to nicotine inhibiting the demonstration of its direct effects (West & Hack, 1991). Minimally-deprived smokers or non-addicted "chippers" are possible alternatives, but the best measure of a baseline would include nonsmokers as well. Accordingly, the many studies in which only deprived smokers are used are inadequate demonstrations of the direct effects of smoking or nicotine.

Without obtaining nicotine blood levels, one is not justified in attributing any observed effects to nicotine. The delivered dose of nicotine cannot be predicted from the nicotine content of a cigarette, because intake varies by inhalation intensity, duration, and number of puffs. Even when using nicotine tablets or gum, rate of chewing, amount swallowed, and factors affecting buccal absorption prevent clear determinations of the administered dose (Le Houezec & Benowitz, 1991). Although some researchers use expired CO and machine-smoked measures of nicotine levels of specific cigarette brands to infer amounts of nicotine inhaled, very few studies have incorporated direct blood nicotine measures.

Routes of administration other than smoking provide stronger evidence that effects are due to nicotine and not tar or other chemical or behavioral aspects or physiological sensations of smoking. Nicotine from tablets or gum is absorbed more slowly, however, and nicotine levels do not peak as sharply as with cigarettes (Benowitz et al., 1988); Le Houezec & Benowitz, 1991). Intravenous infusions with periodic "shots" of nicotine mimic the pharmacokinetic effects of smoking (Ashton et al., 1978), but have limited practical use. Nicotine nasal spray (West & Jarvis, 1986) and subcutaneous nicotine injections are promising routes, but reports of their use are scarce as yet, and some evidence suggests that subcutaneous

injections may create a confounding anxiety in subjects (Le Houezec et al., 1994).

Other methodological concerns include arguments that overnight deprivation, as is commonly used, is extreme and unrealistic. For example, effects observed with such subjects can be interpreted as reflecting effects of only the first cigarette of the day, but not those of most cigarettes in a smoker's day (Church, 1989; Hindmarch et al., 1990). Amounts of smoking (often more than one cigarette) in laboratory experiments may also be unrealistically high, since some evidence exists that smokers smoke cigarettes more intensely in the laboratory than in natural settings (Knott, 1989). There are also individual differences in effects of nicotine for heavy versus light smokers (Le Houezec & Benowitz, 1991; Peeke & Peeke, 1984) and for low versus high CO-absorbing smokers (Knott, 1985; Michel et al., 1987; Nil & Battig, 1989). These differences make comparisons across studies difficult and may conceal real effects.

--Information Processing Findings

The majority of existing evidence of nicotine effects on cognition is flawed by one or another of the problems just described. Nonetheless, researchers have tested smoking or nicotine on a variety of tasks, and evidence exists for cognitive effects. In sustained attention tasks, for example, there are consistent findings that smoking and nicotine tablets prevent the normal increase in errors with time-on-task in deprived smokers, although results showing this benefit in nonsmokers are inconsistent (Wesnes & Warburton, 1978; Wesnes et al., 1983). Using the "letter cancellation task," a measure of sustained attention, dose-related improvements over baseline have been observed in deprived smokers who smoked and were administered three doses of nicotine gum (Parrott & Craig, 1992). Another measure of sustained attention is the "Rapid Visual Information Processing" (RVIP) task, in which the subject responds as quickly as possible to the occurrence of three consecutive even or odd digits during a rapid presentation of one stimulus after another on a computer screen. Wesnes & Revell (1984) and Wesnes & Warburton (1984b) found that with deprived smokers nicotine tablets and high nicotine versus low nicotine cigarettes counteracted deleterious effects of scopolamine on hit rate and RT, without affecting response bias. And high versus low or no nicotine cigarettes produced improvement over baseline in deprived

smokers (Wesnes & Warburton, 1983; Wesnes & Warburton, 1984a). Nicotine gum had the same effect (Parrott & Craig, 1992; Parrott & Winder, 1989). Thus, nicotine appears to enhance sustained attention, at least in deprived smokers.

Direct problem solving effects, if they exist, can also be interpreted as an enhancement of attention. Nicotine speeds the adoption of an effective initial strategy in the Luchins Water Jug task, but slows the subject's later adaptation to a new strategy. This has been interpreted as a facilitation and maintenance of narrow, focused attention (Wesnes & Warburton, 1985). Dunne et al. (1986) demonstrated a lack of effect with nicotine gum in nonsmokers, however, on verbal and numerical problem-solving tasks, although this may be because high nicotine levels are hard to achieve in nonsmokers (see Le Houezec et al., 1994).

A number of studies also show nicotine improving performance by reducing the negative effects of other factors, such as fatigue (Wesnes & Revell, 1984, reported no performance improvement when long rest period preceded testing; Wesnes & Warburton, 1983, reported improvements on task only at latest of multiple posttests), noise (Herning & Pickworth, 1985), or scopolamine (Wesnes & Warburton, 1984a). Such findings support the idea of an attentional effect, whether as a facilitation of sustained attention or improving the efficiency of a filter to lessen effects of distractors. That smoking has been shown to speed habituation may be evidence for a stimulus filter effect (Church, 1989).

Wesnes and Warburton (1983; 1984a; 1984b) theorize that nicotine activates the central cholinergic pathway, which alters arousal and changes stimulus selection processing efficiency. Findings on vigilance and sustained attention tasks in which scopolamine impairs performance and nicotine reverses the impairment support this notion. Improvement by nicotine of cognitive performance on recognition tasks in Alzheimer's Disease patients, known to have a cholinergic deficit, also support a cholinergic basis for nicotine effects (Newhouse et al., 1988). Nicotine also affects other neurotransmitters, however, including norepinephrine and dopamine, which have reciprocally antagonistic effects with cholinergic muscarinic activity (Corrigal, 1991; Stolerman, 1991). There is at least one study in which nicotine failed to reverse scopolamine effects (Rusted & Eaton-Williams, 1991).

To the extent that nicotine activates the cholinergic system, according to Callaway et al. (1992), it should constrain or narrow information processing

operations, consistent with findings that nicotine filters out distracting stimuli such as incidental context features (Andersson & Hockey, 1977) or noise (Hasenfratz et al., 1989; Michel et al., 1987). Cholinergic effects would also be consistent with selective effects on stimulus, rather than response-related processing (Naylor et al., 1993). A few researchers have used ERP measures and RT tasks to investigate selective effects of drugs on component mental processes, and some results have suggested stimulus processing effects of nicotine. For example, nicotine decreased P300 latency, used as a measure of stimulus evaluation (Edwards et al., 1985; Herning & Pickworth, 1985; Le Houezec et al., 1994; Michel & Battig, 1989), and N100 amplitude, which reflects early stimulus processing (Knott, 1985). At least two studies have failed to find an effect of nicotine on the Stroop task, a measure of response processing (Parrott & Craig, 1992; Wesnes & Revell, 1984), although at least one study has demonstrated an effect (Wesnes & Warburton, 1978).

Le Houezec et al. (1994) recently tested the effects of nicotine on nonsmokers. They addressed some of the methodological issues previously discussed, by using nonsmokers, measuring blood levels of nicotine at regular intervals pre and post drug administration, and by using subcutaneous injections rather than gum or tablets. They used a choice RT task in which two levels of stimulus complexity and two levels of response complexity were manipulated, and they measured N100 and P300 amplitudes and latencies. Nicotine speeded mean RT compared with a saline-injection control and a no-injection control group, but the effect was only significant when compared to the no-injection control group. Nicotine speeded P300 latency in the hardest stimulus-by-response task condition, which suggests an effect on stimulus processing. Surprisingly, however, nicotine slowed P300 latency in the other task conditions--so an interpretation of increased stimulus evaluation processing efficiency may be too simple. Nicotine also altered the speed-accuracy tradeoff function normally seen when subjects trade accuracy for speed and vice versa. Nicotine increased the number of RTs at the fast end of the RT distribution, without decreasing accuracy, as would happen with placebo if subjects were given instructions to increase speed. The authors suggested that an effect on attention might account for this result.

It appears that the bulk of evidence supports a general attentional mechanism for nicotine effects on information processing, possibly involving some

selective effect on stimulus, as opposed to response-related processing. An arousal mechanism has also been proposed to explain some nicotine effects. Study results with both humans and animals suggest that nicotine typically produces a general central nervous system activation, as observed in the electroencephalogram (EEG), at least when the pre-nicotine arousal state is low (Knott, 1989). In addition, the contingent negative variation (CNV), which is associated with activation, is increased in amplitude at lower doses of nicotine and decreased at higher doses (Ashton et al., 1978). EEG and ERP evidence of activation and biphasic effects, together with the notion of the U-shaped relation between arousal and performance and findings that performance on simple tasks is improved while performance on complex tasks is hampered by higher arousal (Levine et al., 1975), have contributed to a general arousal theory. The theory holds that smokers are able to use nicotine to control their level of general arousal, to perform optimally in a variety of situations (Frearson et al., 1988). Given existing findings, a general mechanism seems plausible. It has been argued, however, that arousal is not a unitary construct and does not of itself explain the cognitive effects of nicotine, although it likely plays some role in a complete explanation (Church, 1989; Knott, 1989).

--Learning and Memory

Learning and memory experiments, employing mostly verbal recall and recognition tasks, show inconsistent results and generally fail to demonstrate specific benefits of nicotine, at least with pre-trial administration of the drug. Andersson (1975) found that in deprived smokers smoking impaired immediate memory and improved delayed recall, interpreting this as a possible enhancement of memory consolidation. Andersson and Hockey (1977) found that smoking impaired incidental, but not intentional learning in deprived smokers, which they interpreted as a narrowing of attention by nicotine. Peeke and Peeke (1984), however, in a series of experiments, found that pre-trial (but not post-trial) smoking improved delayed recall and immediate recall for high nicotine cigarettes in deprived smokers. They found no incidental learning or recognition task effects. They interpreted their results as indicating a general, attentional effect, rather than any specific learning or memory benefit, since pre-trial smoking could affect

attention and motivational factors as well as memory-related ones, while post-trial smoking would only affect consolidation. Also, the finding of an incidental learning or recognition task effect would have implied an effect on memory processes in the absence of attentional requirements. Warburton et al. (1986) also concluded that a general effect of nicotine is more likely than a specific encoding effect. In that study, deprived smokers exhibited state-dependent memory effects with smoking and nicotine tablets. There was some evidence of nicotine facilitating recognition, but no effect on categorization or organization of to-be-remembered material. In Houston et al., (1978) the authors found no evidence for a specific effect of smoking on category clustering or any general recall improvement.

In a study by Rusted and Eaton-Williams (1991), testing deprived smokers, scopolamine impaired immediate recall and nicotine improved it, although nicotine did not reverse effects of scopolamine. Nicotine improved recall more for a longer list than a shorter one, but reportedly did not show differential effects on easy versus hard encoding conditions. The authors suggested that nicotine effects are not related to encoding efficiency, but to sustained attention (manipulated by word list length), and that one reason for inconsistent results in the literature may be differences in attentional demands of the various learning tasks, including the range of different list lengths on verbal recall tasks.

In a rare study of nonsmokers (Dunne et al., 1986) found impairment on recognition and recall tasks with nicotine gum relative to placebo. West and Hack (1991) tested regular and occasional smokers, with and without deprivation, and found that smoking speeded memory search time on the Sternberg task for both groups, across abstinence conditions. This is one of the few learning and memory-related findings suggesting a specific cognitive improvement with smoking.

Recently, Colrain et al. (1992) reported that post-trial nicotine administration improved paired-associate list learning. Warburton et al. (1992) argued that facilitation by nicotine of immediate recall may reflect an attentional effect, but post-trial nicotine effects on delayed recall demonstrate an improvement of memory consolidation. Their subjects smoked after presentation of word list items for a few seconds, during which time they were instructed to rehearse the words. Extended rehearsal was prevented by a distractor task that immediately followed.

When subjects smoked they had better recall than when they smoked a placebo cigarette. The authors interpreted the effect as an enhancement of consolidation, which would improve memory storage. Rusted and Warburton (1992) extended this finding to a paradigm in which a list was learned without instructions to rehearse, with and without a subsequent distractor condition. Post-trial nicotine significantly improved delayed recall compared with placebo, but only when no distractor task followed. Again, this was interpreted as a facilitation of consolidation. Peeke and Peeke (1984) failed to find an effect of post-trial smoking on delayed recall, however.

For the most part, one could argue that learning and memory studies provide more support for a general, attentional cognitive enhancement, if any. This seems true for the other types of cognitive studies as well, and evidence is much stronger for effects in deprived smokers than in nonsmokers. There may also be a selective effect on stimulus, as opposed to response processes, and possibly, an effect on consolidating memory storage. Thus far, however, smokers' claims of improved thinking and concentration after smoking have not been unequivocally corroborated by tests of nicotine's direct effects.

Effects of Tobacco Withdrawal on Cognition

Although difficulty concentrating is a commonly recognized symptom of tobacco withdrawal, and subjects' self-report ratings of difficulty concentrating significantly increase following smoking cessation (Hughes et al., 1991), direct tests of tobacco deprivation effects on cognitive tasks are few and have yielded inconsistent results. Elgerot (1978) demonstrated better performance on verbal and nonverbal reasoning tasks, including an arithmetic task, after 15 hours of deprivation compared to a smoking condition in a within-subjects design. Kleinman et al. (1973) found that 24-hour deprived smokers recalled more words on an easy paired-associates list than nondeprived smokers, but they recalled fewer words on a hard list in the same task.

Some consistency of impairment has been demonstrated with vigilance tasks. Heimstra et al. (1967) found deprived smokers to be worse than nondeprived smokers or nonsmokers on measures of vigilance, RT, and tracking

error in a simulated driving task after six hours of deprivation. Hatsukami et al. (1989) found impaired performance on RT, accuracy, and increased variability of RT in a vigilance task. Hughes et al. (1989) found no significant changes in RT or accuracy, but found increased variability in RT for deprived smokers compared to nondeprived smokers or nonsmokers. Deprivation did not cause a fatigue effect (change in performance from beginning to end of task).

Snyder and Henningfield (1989) tested smokers on a battery of tasks, and after twelve hours of deprivation administered either placebo gum or 2 mg or 4 mg nicotine gum (within-subjects) and retested performance. The tasks comprising the "Performance Assessment Battery" (PAB) included a two-letter search task, a six-letter search task, a logical reasoning task, a digit recall task (immediate memory), and a rapid arithmetic test. All tasks were timed, and RT and accuracy were dependent measures. Placebo gum significantly increased RT on all tasks above baseline, while the 2 mg and 4 mg nicotine gum produced dose-related decreases in RT to the same or better performance than baseline. This finding served as direct evidence for slowed performance following deprivation of nicotine specifically, rather than some other aspect of smoking, since the slowing was reversed by nicotine gum. There were no significant effects on accuracy and no effects on self-reported mood ratings.

In a separate study Snyder et al. (1989) also administered the PAB to smokers at one, four, eight, and 24 hours and at days two through eleven after smoking cessation. The subjects then resumed smoking and were tested at one, four, eight, and 24 hours later. Again, RT was significantly slowed on all tasks at 24 hours (and usually at 48 hours) following cessation of smoking. Some detriments persisted for the entire deprivation period, although generally they showed some recovery by the tenth day. Reversal to baseline occurred within 24 hours of resumed smoking. Accuracy decreased significantly only in the two memory tasks (digit recall, addition/subtraction), which involved reliance on immediate working memory. Unfortunately, the PAB did not include any verbal memory tasks. Again, mood ratings were not affected, demonstrating the independence of cognitive effects.

Hatsukami et al. (1989) found that significant impairment did not occur until 24 hours had elapsed, a finding that is consistent with Snyder et al. (1989), in

which peak impairment occurred at 24-48 hours post smoking cessation, and with a number of other studies (Hatsukami et al., 1989).

A preponderance of the available data therefore appears to suggest that a day after nicotine deprivation begins some significant impairment on cognitive tasks exists, although the range and characterization of that impairment has not yet been defined. It appears to affect speed and consistency of performance speed on rapidly-timed tasks, but effects on accuracy are uncertain, and reports of verbal memory and other task effects are few and inconsistent.

Cotinine

Cotinine is the major metabolite of nicotine (Gorrod & Jenner, 1975). Blood cotinine levels are associated with number of cigarettes smoked per day and the nicotine content of those cigarettes (Pomerleau et al., 1983). Pre-abstinence blood cotinine levels are significantly correlated with the severity of tobacco withdrawal symptoms and difficulty maintaining abstinence from smoking (Pomerleau et al., 1983; Zeidenberg et al., 1977). Cotinine blood levels average 250-300 ng/ml in cigarette smokers, although they can range as high as 900 ng/ml, and average levels exceed nicotine concentrations by tenfold. While the terminal half-life of nicotine averages two to three hours, with considerable variability among individuals (Benowitz et al., 1982), the half-life of cotinine averages 16-19 hours. Although nicotine levels fluctuate throughout the day, cotinine levels remain relatively constant over a 24-hour period in a regular smoker. The time for blood concentrations of cotinine to decline to a nonsmoking level ranges from two days to one week, with an average of about four days (Benowitz et al., 1983). Nicotine, on the other hand, would be eliminated in 10-15 hours.

Between the time nicotine is eliminated following smoking cessation and several days to a week later, then, cotinine is still present in the abstinent smoker's body. What, if any, effects does it exert during that time? Cotinine has been assumed to be fairly inert, with weak, if any, effects. There is a growing body of evidence, however, indicating that cotinine is more active than has been supposed.

Cotinine is widely distributed in the mouse, including the brain (Bowman et al., 1963). Animal data indicates that cotinine has a hypotensive effect (Dominiak

et al., 1985). It also produces EEG arousal (Yamamoto & Dominico, 1965). Cotinine has been demonstrated to have physiological effects in animals, effects that are dissociated from those of nicotine. It is distributed differently than nicotine (Bowman et al., 1963), it relaxes smooth muscle (Kim et al., 1968), and it significantly reduces central serotonin turnover to a greater degree than nicotine and blocks reuptake and retention of serotonin under conditions when nicotine does not (Fuxe et al., 1979). Andersson et al. (1993) demonstrated not only that cotinine and nicotine affect aldosterone and prolactin levels in the rat differently, but that cotinine produces opposite effects that counteract those of nicotine. The antagonistic influences of nicotine and cotinine on serum prolactin do not affect leutinizing hormone and therefore suggest activity in the brain rather than exclusively peripheral activity. The authors further suggest that cotinine may play some role in nicotine withdrawal effects.

Cotinine also has behavioral effects on animals that are distinct from those of nicotine. Risner et al. (1985) demonstrated that cotinine produced dose-dependent decreases in rates of fixed-interval and fixed-ratio responding in beagle dogs, while nicotine produced a different pattern of increases and decreases. Cotinine also produced bidirectional effects, depending on dose, in the response rates of squirrel monkeys, exhibiting a pattern of effects different from those of nicotine. Goldberg et al. (1989) also showed differential effects of cotinine and nicotine on rats' response rates. Cotinine effects, though less potent than those of nicotine, were not blocked by mecamylamine (a nicotinic cholinergic antagonist), though mecamylamine did block nicotine effects. Brioni and Arneric (1993) tested rats on an avoidance task and found that, while treatment with nicotine improved learning, treatment with cotinine tended to impair it.

Data on the behavioral effects of cotinine in humans is sparse. Benowitz et al. (1983) infused abstinent smokers with cotinine and noted reductions in self-reported desire to smoke, irritability, anxiety, and tension, with no concomitant changes in blood pressure, heart rate, or skin temperature (effects that are sensitive to low concentrations of nicotine). The authors noted, however, that these subjective changes were similar in magnitude to changes previously seen from pre to post treatment with placebo. They concluded that cotinine exerts little, if any, behavioral or physiological effects in smokers. Keenan et al. (1994) tested

deprived smokers and found significant differences in subjective self-report ratings of withdrawal symptoms between intravenous administration of cotinine and placebo. Cotinine significantly increased ratings of restlessness, anxiety, tension, insomnia, sedation, and reduced ratings of pleasantness. Minimal effects on heart rate and blood pressure were observed.

The Keenan et al. study was the first to support the idea of behavioral effects of cotinine in humans, and further, to provide evidence of a role for cotinine in tobacco withdrawal symptoms. Those investigators did not examine cognitive effects, however. Given the physiological and behavioral evidence of antagonistic or differing effects of nicotine and cotinine in animals, the elimination time course of the two substances in humans, the findings of Keenan et al., and our pilot study results demonstrating a tendency for cotinine to slow RT, it appears that cotinine could contribute to the cognitive decrements seen in nicotine withdrawal.

Testing Drug Effects Using RT tasks and Event-Related Potentials

Drug investigations focusing on performance have paid less attention to underlying neurocognitive processes mediating performance. Instead of determining merely that a drug improves or impairs, speeds or slows performance, chronometric methods exist by which to determine which components of information processing are most affected. Serial models have provided one framework for studying the operation of discrete information processing components. Serial models assume that performance on a task such as a choice RT task can be accounted for by the operations of separate hypothetical processes, which transform information so that an appropriate output can be generated. Different models propose different stages; one example includes the following stages in serial order: pre-processing, feature extraction, identification, response choice, response programming, and motor adjustment (Sanders, 1980).

The Additive Factors Method (AFM) described by Sternberg (1969) provides a research methodology for identifying the activity of variables on specific stages. One assumes that the time taken to make a response is the sum of times taken to complete each of the operations involved in generating that response.

Using the AFM, one can isolate cognitive processes by manipulating variables that change RT and then examining their relative effects on RT. If two variables influence different processing stages, their effects on RT will be additive. If two variables influence a common processing stage, however, their joint effects on RT will produce an interaction. Thus, the interaction between a drug and some task variable localizes the action of the drug to a particular (hypothetical) processing stage.

Physiological data can also be used to provide convergent evidence of drug effects on a particular processing stage. Event-related potentials (ERPs) have traditionally been used in this context. ERPs are small patterns of positive and negative voltage peaks in the ongoing electroencephalogram (EEG). Recorded at the scalp, they are time-locked to specific sensory or cognitive events, and can be seen by computer averaging the EEG over a number of trials (Donchin et al., 1986).

The latency of particular ERP components demarcate where in the flow of information particular effects occur. The most commonly studied example is the P300, a positive component occurring between 300 and 600 ms post-stimulus, which is sensitive to a variety of cognitive variables. The P300 consistently appears as a discriminative response to a specific stimulus in a series, which the subject has identified as either surprising or task-relevant. Accordingly, researchers have assumed that P300 peak latency is preceded by an evaluation of the stimulus (Donchin, 1979; Kutas et al., 1977). The term "stimulus evaluation" has been described as including encoding, recognition, and classification of stimuli. There is evidence that earlier components may be better measures of some aspects of stimulus evaluation, such as pattern recognition and stimulus discrimination (Ritter et al., 1982; 1883). The process preceding P300 peak latency apparently includes some kind of categorization, since Kutas et al. (1977) observed that P300 latency increased as complexity of stimulus categorization increased. It has been asserted not that P300 latency is a manifestation of a stimulus evaluation process, but that it is contingent on completion of such a process (Donchin, 1979; McCarthy & Donchin, 1981).

The most important definitional aspect of stimulus evaluation, and the basis of its usefulness, is its alleged independence from response-related processes.

Evidence for the existence of these two separable sets of processes was originally published by Kutas et al. (1977), who demonstrated a dissociation of P300 latency from RT measures. The correlation between P300 latency and RT was higher with instructions emphasizing accuracy on a discrimination task, and lower with instructions emphasizing speed. P300 latency was sensitive to changes in complexity of the stimulus categorization, but not to response instructions, whereas RT was sensitive to the instructions. In addition, on error trials in the speed condition, RT preceded P300. The authors suggested that this dissociation supported the use of P300 latency as a new chronometric measure, indexing a subset of processes separable from overall RT.

McCarthy and Donchin (1981) provided a direct test of the hypothesis that P300 latency reflects stimulus evaluation independently from response selection and execution. Because RT had been additively affected by stimulus discrimination and stimulus-response (S-R) compatibility variables, pursuant to the AFM those variables could be said to influence two separate stages. McCarthy and Donchin reasoned that manipulations of stimulus discriminability should influence stimulus evaluation time, while manipulations of S-R compatibility should influence response processing time. P300 latency was affected by stimulus discriminability and not by S-R compatibility, while RT was affected additively by both variables, supporting their hypothesis. This finding was replicated and extended by Magliero et al. (1984). While McCarthy and Donchin had noise versus no-noise stimulus discrimination conditions, Magliero et al. manipulated four levels of noise in the stimulus display. They found that P300 latency and RT increased monotonically with increases in noise. The S-R compatibility manipulation, as for McCarthy and Donchin, involved instructions "same" versus "opposite" prior to each trial, such that the subject was to respond to the target word "right" with the right hand and "left" with the left hand in the same condition, and the opposite with the opposite condition. While RT was affected by S-R compatibility, the effect on P300 latency was very small. The authors stated that relative, not absolute, insensitivity of P300 to S-R compatibility supports the use of P300 as an index of stimulus evaluation.

Although not as well-studied as P300, N100, a negative peak occurring at approximately 200 ms post-stimulus, depending on the task, has been characterized

as reflecting the latency of early visual processes, such as feature extraction, pattern recognition, or engagement of spatial attention (Hillyard et al., 1973; Luck et al., 1990). It has also been interpreted as indicating a generalized drug-response component that reflects increases in arousal or alertness (Naylor et al., 1993).

Callaway and his colleagues have tested the effects of a number of psychoactive drugs in a choice RT task paradigm in which RT, errors, and N100 and P300 latencies are recorded, to infer the processing stages affected. They have determined specific information processing effects for a variety of drugs, including d-amphetamine, yohimbine, clonidine, pimozide, and scopolamine. Their paradigm has been used, for example, to infer that cholinergic drugs affect primarily stimulus-related processes (affecting P300 and/or N100 latencies), while aminergic drugs affect primarily response-related processing (affecting RT with relatively little effect on P300 latency). In some cases, drugs have affected N100 latency, without affecting P300, further specifying an information processing locus of stimulus processes prior to full evaluation of the stimulus (Naylor et al., 1993). Recently they tested the effects of subcutaneous nicotine in nonsmokers, employing their standard paradigm, and found speeding effects on RT and P300 latency in the difficult task condition (Le Houezec et al., 1994), which is consistent with other findings of cholinergic effects on stimulus-related processes (Callaway et al., 1985; Brandeis et al., 1992).

Summary

Given the pharmacokinetics of nicotine and cotinine and the evidence for their distinct and sometimes opposing physiological and behavioral effects in animals, it is reasonable to ask whether or not cotinine may contribute to the cognitive decrements complained of by deprived smokers. As will be described in Chapter 3, there is a tendency for 20 mg oral cotinine to slow RT in nonsmokers. Therefore, the hypothesis of the present study is that larger doses of cotinine will have slowing and possibly other detrimental effects on cognition.

To test cotinine directly, nonsmokers will be used. Because drug effects cannot be directly inferred without sampling drug levels in the body, saliva cotinine samples will be taken before and after drug administration. Saliva

samples are less intrusive than blood samples, and they have been shown to provide equivalent information (Curvall et al., 1990). Although in general nicotine findings have been inconsistent, by using the same RT/ERP paradigm employed by Callaway et al., I will be able to compare cotinine effects to nicotine effects observed by Le Houezec et al. (1994) on the same task, using the same outcome variables. This paradigm will permit exploration of specific loci of information processing effects, as well as an assessment of general speed and accuracy effects on performance.

Much evidence suggests that nicotine has a general attentional effect. This is also true for learning and memory tasks, at least when an effect is demonstrated. The present study will include a verbal recall task, in order to test the possibility of learning and memory effects, aside from effects on RT performance. The memory task will manipulate list length in an attempt to separate attentional effects from those on memory per se, as was done by Rusted and Eaton-Williams (1991) (for a detailed description, see Chapter 4).

A substantial number of nicotine studies have tested men exclusively. Given that some gender differences in smoking behavior and physiological effects of nicotine are known or suspected to exist, it is important that women be included in this study (see Pomerleau et al., 1991 for review on the use of women subjects in nicotine studies). The importance of including women is underscored by evidence that in the last 25 years the prevalence of smoking among women has declined more slowly than among men, and women smokers have lower rates of quitting than do men (U.S Department of Health and Human Services, 1989). Gender differences in the cognitive effects of nicotine have been suggested. For example, there is evidence that women rely more on smoking's distraction-filtering effects than men do (Biener et al., 1987). In another study, preabstinence serum cotinine correlated significantly with degree of difficulty quitting smoking for men but not for women (Zeidenberg et al., 1977). Accordingly, this study will include half men and half women subjects, which will allow testing for gender effects.

Mood measures will be taken to address the question of whether or not cognitive effects are mediated by or otherwise related to changes in mood. Mood changes with tobacco withdrawal are well-documented, and Keenan et al. (1994) have demonstrated effects of cotinine on such changes. In our pilot study,

however, mood was unaffected by cotinine, while RT was not.

In summary, the primary research question in this study is whether or not cotinine affects cognition in nonsmokers. As a larger goal, by measuring RT, errors, ERPs, recall memory (with an attentional component), heart rate, blood pressure, and mood, I hope to assess both general and specific effects of cotinine on cognition and other aspects of functioning, and to relate these effects to what has been observed among smokers suffering from the symptoms of the tobacco withdrawal.

Chapter 2
Pilot Study Methods

Subjects

Subjects were thirteen nonsmoking Caucasian males, ages 20-36 (mean age=27, std. dev.=4.4), recruited by advertisement in the local community and at a local university. Eleven of the thirteen had never smoked on any regular basis; one subject had smoked for four months, one other for two years, and neither had smoked for at least ten years prior to the study. Half of the subjects currently attended college, and all subjects had at least two years of college education. Subjects were examined by a physician for general good health. Exclusion criteria included high blood pressure, heart disease, mental disorders, alcohol abuse, and current drug use. Reimbursement was made at $8.50 per hour, plus a bonus of 25% of the total, received after completion of all sessions.

Design

Each subject was required to attend three sessions on three separate days. The first was a practice session, followed by two test sessions, each spaced a week apart to eliminate any residual effects of cotinine administration. On each test day the subject was given a pretest. Cotinine (as the fumarate salt) or a lactose placebo was given orally, in capsule form, and the subject was tested at one hour and two hours post drug. The order of administration of drug or placebo was assigned randomly and was counterbalanced between subjects. Testing was double-blind. The choice of dose and time between ingestion and testing was based on pharmacokinetic data reported by DeSchepper et al. (1987). In that study, 20 mg of cotinine produced plasma concentrations between 200 and 600 ng/ml (the range found in regular smokers). Plasma concentration peaked at around 45 minutes post-ingestion.

Tasks

1. Stimulus Evaluation/Response Selection (SERS)

The cognitive task used discriminated two information processing stages: stimulus evaluation and response selection (SE/RS or SERS) (Callaway et al., 1985; Naylor et al., 1985). Two levels of stimulus complexity and two levels of response complexity were manipulated. The SERS task target stimulus was an X, appearing on each trial in one of four horizontally-arrayed positions on a video screen. The position of the target varied randomly from trial to trial. In the easy stimulus condition the three nontarget positions each contained a dot. In the hard stimulus condition the three nontarget positions each contained an asterisk, of the same height as the target. The subject responded to stimulus onset by pressing one of four keys on a box, held on his lap. The keys were arrayed horizontally, corresponding to the four possible target positions on the screen. In the easy response condition the subject pressed either the key farthest left or farthest right, depending on whether the target appeared to the left or right of the center of the display. In the hard response condition the subject pressed one of four keys to match the corresponding position in which the target appeared. Eight blocks of 32 trials each were presented; response condition alternated from block to block, beginning with the easy response condition. Within each block stimulus condition varied randomly. The result was 64 trials each of the easy stimulus/easy response (EE) easy stimulus/hard response (EH), hard stimulus/easy response (HE), and hard stimulus/hard response (HH) conditions. Task conditions are depicted in Figure 1.

The subject was seated in a dimly-lit, sound-attenuated room, in a comfortable chair, 144 centimeters from the video screen. The stimulus array appeared in the upper half of the screen and was viewed through artificial 1 mm pupils mounted on a combined chin/headrest. Artificial pupils were used to control for pupil size changes that can occur with drugs that affect the cholinergic system. Prior to each trial a fixation display, a checkerboard pattern of the same size as the stimulus display, appeared on the screen. On each trial the stimulus display remained on the screen until the subject responded, to a maximum of 1852 ms, at which time the fixation display reappeared. The total fixed trial interval (from

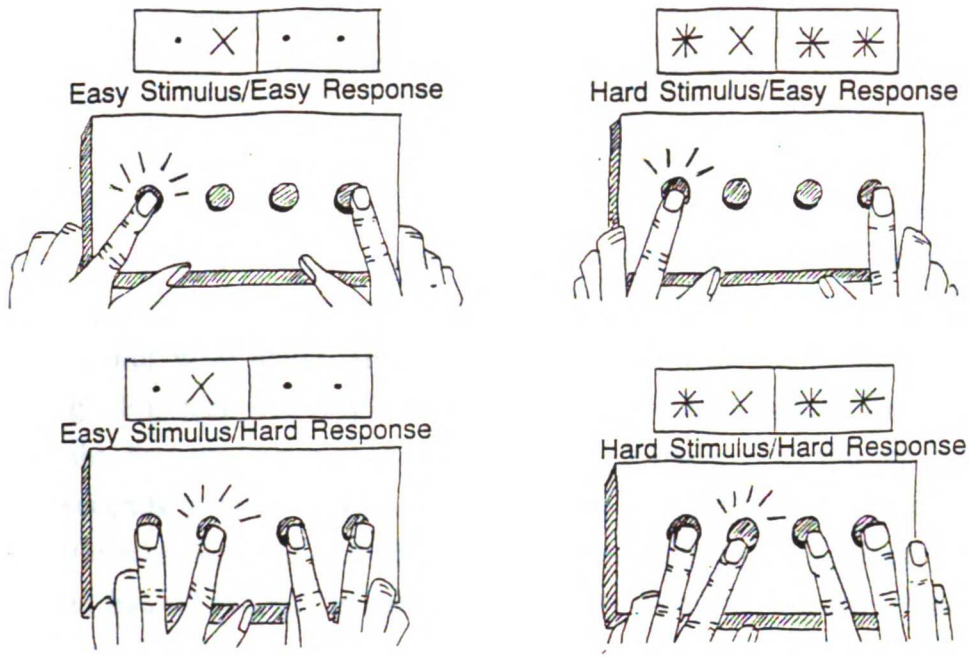


Figure 1. Stimulus Evaluation/Response Selection (SERS) task conditions. The target position in the array varies randomly from trial to trial. The easy stimulus condition contains dots in the nontarget positions, while the hard stimulus condition contains asterisks in the nontarget positions. The easy response condition requires the subject to press one of two buttons, either the outside left or outside right (depending on whether the target is in a position left of center or right of center). The hard response condition requires the subject to press one of four buttons, corresponding to the exact position of the target.

onset of one stimulus display to onset of the next trial's stimulus display) was 2100 ms, plus or minus 100 ms. After each block of 32 trials, there was a pause, while the experimenter instructed the subject to switch to the alternate response condition. The entire test of 256 trials (8 blocks) was completed in about 15 minutes. At the beginning of each test, the subject was instructed to respond as quickly and accurately as possible. RT, errors, ERPs, and eye movements were recorded.

2. Memory Task

Subjects were tested on a selective reminding task that had previously proved sensitive to drug effects on recall in our laboratory (Brandeis et al., 1992). The test, modified from Buschke (1973), involved eight different lists of 17 words each. All words were nouns, and all were matched for frequency and concreteness of meaning. For each test two lists were used. The experimenter read aloud the words on the first list at a rate of one every two seconds. The subject was then given 90 seconds to write down all the words he could remember. The experimenter then repeated only those words the subject failed to recall. The subject then had 90 seconds to recall again all the words on the list. This procedure was repeated with a second list. Different pairs of lists were used for each test (i.e., for each pre and post test on each test day). Though all lists were assumed to be equivalent, the order of lists given was counterbalanced between subjects and between pre and post drug testing. Lists are presented in Appendix A. Each memory task test was completed in approximately 15 minutes. Number of words missed on each trial was scored.

3. Other Dependent Measures

Blood pressure and heart rate were measured. The Profile of Mood States (POMS) (McNair et al., 1971) was also administered. The POMS questionnaire consisted of six subscales: tension, anger, depression, vigor, fatigue, and confusion. Saliva samples for measurement of cotinine concentrations were collected when the subject first entered the lab and at one and two hours post drug, on both test days. Saliva cotinine levels are known to be similar in magnitude, and are highly correlated with, plasma cotinine levels (Curvall et al., 1990; Jarvis et al., 1984). Samples were assayed by gas chromatography, as described by Jacob et al. (1981),

modified for use of a capillary column. Samples were assayed for cotinine and nicotine. The limit of quantitation (as supported by quality control data) was 10.0 ng/ml for cotinine and 0.5 ng/ml for nicotine. Results of the pretest sample assays were used to confirm nonsmoking status. Results of the posttest sample assays were used to document the level of exposure achieved by drug administration.

Procedure

On the initial practice day procedures were explained to the subject, and informed consent was obtained. Each subject was then tested on each task, and blood pressure and heart rate were taken and the POMS was administered. Each subject's EEG was recorded during the SERS task, and averaged ERPs were inspected to insure the presence of identifiable peaks in his EEG. The subject was asked not to consume alcohol for 48 hours before testing and to consume his normal amount of caffeine before arriving at the laboratory on each test morning. The subject was questioned on arrival at the test sessions to confirm compliance with these requests. Testing sessions began between 7:00 and 10:00 a.m. and lasted for about four hours. Baseline saliva sample, blood pressure, heart rate, and POMS were collected. The subject performed the memory task, then the SERS task. The drug or placebo was administered. The SERS task was repeated at approximately one hour post drug, corresponding to a time window in which orally administered cotinine had been shown to reach peak concentrations (Curvall et al., 1990; De Schepper et al., 1987). At about one and one-half hours post drug the subject completed the memory task posttest, followed by a second SERS posttest at two hours post drug. Blood pressure, heart rate, and POMs were collected a total of five times: twice before drug administration, and at 30, 60, and 75 minutes post drug.

Event-Related Potential Recording and Component Identification

During each administration of the SERS task the EEG was recorded, using a modification of the standard 10-20 system (Jasper, 1958), recorded from 16 electrodes embedded in an electrode cap. The electrode sites were Fz, Cz, Pz, Oz,

A1/2, T3/4, C3/4, T5/6, P3/4, and O1/2. In the cap's design, O1 and O2 were each shifted laterally, to be halfway between Oz and T5/6, to approximate equidistance between electrodes. Fz was the reference, and Fpz was the ground. EOG was recorded between the outer canthus and above the left eyebrow. Electrode impedance was kept below 10 k ohm. The EEG was amplified by a Grass Model 12, with filters set at 0.3-100 Hz bandpass. For each trial the total sampling period was 800 ms (100 ms prestimulus and 700 ms post-stimulus), and the sampling rate was every four ms. On line averaging excluded trials in which the EOG peak-to-peak amplitude exceeded 50 microvolts. Averages were inspected after each run. Single trial data were stored on an optical disk.

N100 and P300 latencies and amplitudes were derived by a component identification method, achieved by a topographic component recognition algorithm that looks for the best fit between the subaverage for each experimental condition and a grand average template "map." This method utilizes our knowledge that ERP components occur within a certain time window and have a known scalp distribution. The method is described fully in Brandeis et al. (1992). It computes a spatial map, including all electrodes, for each time point. Map series are transformed to the average reference and normalized to unity. A spatial root mean square (RMS) is used to compute topographic dissimilarity between maps. Component model maps are obtained from the grand mean ERP map series at latencies of interest (around 200 ms and 400 ms poststimulus), where topographic change (sequential dissimilarity) is minimal. Each component map is used to identify in each averaged ERP the latency of the map that most closely resembles (i.e., yields the smallest dissimilarity from) the model map. A stability constraint is computed to reduce spurious findings. For each component, the average of the normalized maps thus identified becomes the updated model map, and the search window is reset around the new mean latency. Iterations of these updated model maps ensure stable estimates. An increase of the model map's global field power (spatial RMS before normalization) over iterations indicates that the (normalized) maps contributing to this average have become more similar. Once a stable solution is reached, this spatial RMS measure is the average correlation between the mean map and its constituent maps.

Data Analysis

Analyses were conduced using repeated measures analysis of variance on thirteen subjects for all outcome measures except ERPs, for which one subject's data were deleted due to excessive eye movements. For comparisons with more than one degree of freedom p values were corrected with the Greenhouse-Geiser estimates of sphericity. Unless otherwise reported, all significant results were $p<.05$.

Chapter 3
Pilot Study Results

SERS Task

For all SERS task dependent variables the within factors included drug (placebo, cotinine), time (pre, post 1 (60 minutes post-drug), post 2 (120 minutes post-drug), and stimulus level (easy, hard). The drug effect was tested by the drug x time interaction. An overall analysis was conducted on all three times, followed by specific analyses for pretest versus post 1 and pretest versus post 2.

1. Reaction Time

Mean RTs at pretest, posttest 1 and posttest 2 are given in Table 1.

Table 1. *Mean Reaction Times (in milliseconds) for Pilot Study (n=13)*

	Placebo		20 mg Cotinine	
	Mean	(S.E.)	Mean	(S.E.)
Pre	524	(14.3)	522	(11.6)
Post 1	505	(10.0)	520	(11.6)
Post 2	516	(13.6)	514	(11.5)

Cotinine slowed RT relative to placebo at posttest 1, that is, it did not speed RT to the degree that placebo did. Speeding of this magnitude (19.5 ms) by placebo from pretest to posttest is not uncommon in choice RT tasks, even after a practice session. For other examples, see Callaway et al. (1985) (14 ms and 20 ms speeding by placebo pre to post on two cognitive tasks) and Halliday et al. (1986) (19 ms speeding by placebo). This speeding presumably reflects a practice or learning effect, or perhaps an arousal change or some other uncontrolled factor that

changes from pre to post. Thus, other things being constant, cotinine "slowed " RT when referenced to the speeding by placebo. On the overall test there was no significant drug x time interaction, but analysis for the pretest versus post 1 showed a near-significant drug x time interaction ($F(1,12)=3.97$, $p<.07$); at posttest 2 compared to pretest it was not significant ($F(1,12)=0$).

2. Accuracy

There were no significant changes in accuracy. The average proportion of errors for any drug condition or time did not exceed five percent, however.

3. Speed-Accuracy Tradeoff

On examination of other drug data on the SERS task, it appears that drugs can affect not only RT, but also a subject's speed-accuracy tradeoff process (Callaway et al., 1994). Subjects can vary their reaction times by trading between accuracy or speed. When asked to respond quickly, subjects make a large number of errors, while instructions to respond accurately result in an increase in mean RT. A plot of accuracy versus speed, termed a speed-accuracy function (SAF), can be approximated by a log function (Wood & Jennings, 1976). When instructions stress speed, subjects' responses are moved to the left along the function. This represents a speeding of RT (increase in the number of fast RTs), but at the expense of increasing errors. Thus, subjects trade speed for accuracy. Due to accuracy instructions in the SERS task, subjects made few errors overall. In order to increase the number of RTs at the fast end of the distribution, to examine whether or not the SAF is affected by the drug, the effects of individual differences and differences between task conditions were removed. RTs were normalized by task and subject, then the normalized RTs were grouped into equal-sized bins. The top portion of Figure 2 re-illustrates the relative slowing of RT by cotinine in this context. From pre to post 1 placebo increased the number of RTs in the fastest bins, while cotinine did not. The bottom portion of Figure 2 shows that accuracy did not change much in any of the bins for placebo, while for cotinine error frequency increased in the second-fastest bin. Along with the finding that overall accuracy was not changed by cotinine, this observation indicates that slowing by cotinine was not due to a tradeoff between speed and

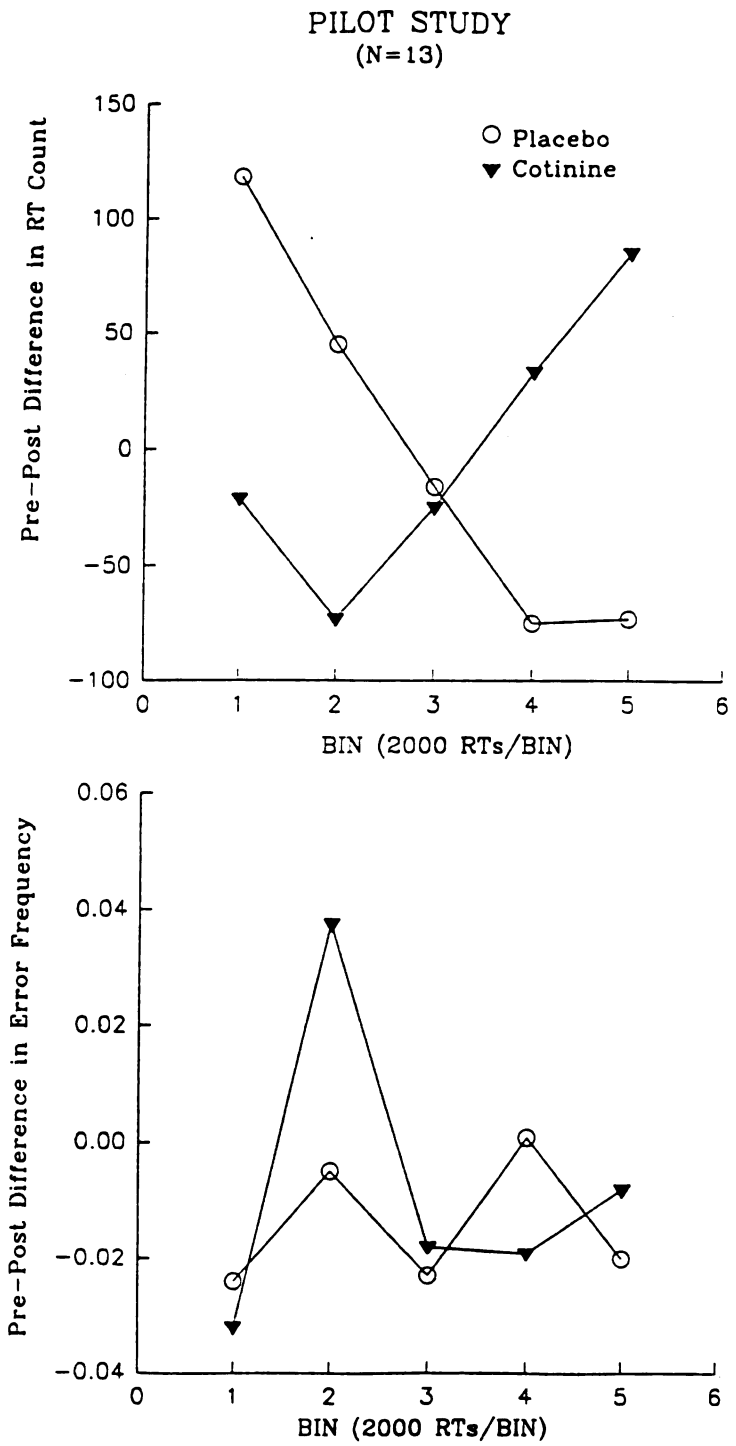


Figure 2. Top figure: Post 1-pre difference in number of RTs per bin for placebo and cotinine. RT bins are numbered from fastest to slowest on the X axis. Bottom figure: Post 1-pre difference in error frequency for placebo and cotinine. The Y axis depicts the proportion of errors.

accuracy (otherwise, subjects would have become more accurate with cotinine). While some drugs appear to change the SAF, as evidenced by increased or decreased accuracy all along the curve (see Callaway, et al., 1994), the present data does not imply any dramatic effect on the SAF by cotinine, since the only difference occurs in the second-fastest bin.

4. N100

There were no significant effects on N100 amplitude. There were no significant drug x time interactions on N100 latency, although there was a significant drug x time x response interaction ($F(2,22)=5.6$, $p<.01$). Mean N100 latencies are presented in Table 2. Cotinine slowed N100 latency relative to placebo for the hard response at post 1 and for the easy response at post 2.

Table 2. *Mean N100 Latencies (in milliseconds) by Response Condition for Pilot Study (n=12)*

	Placebo				20 mg Cotinine			
	Easy Response		Hard Response		Easy Response		Hard Response	
	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E)	Mean	(S.E.)
Pre	218	(5.2)	217	(5.2)	215	(4.4)	214	(4.8)
Post 1	216	(4.8)	212	(4.4)	211	(4.4)	221	(4.8)
Post 2	214	(4.4)	220	(5.2)	221	(4.4)	217	(4.8)

5. P300

There were no significant drug effects on P300, either for amplitude or latency (all $Fs < 1.0$).

Memory Task

A repeated measures ANOVA was conducted with drug, time (pretest, posttest at about 90 minutes post drug), and cue (free recall versus recall after selective reminding) as within factors. Data for the two 17-word lists were

combined. There was a main effect of cue (F(1,12)=87), indicating a difference in number of words missed between free recall and recall after selective reminding. There was no significant drug x time interaction, hence there was no drug effect. There was no significant drug x time x cue interaction, hence there was no drug effect on selective reminding.

Blood Pressure, Heart Rate, and POMS

Cotinine had no significant effects on blood pressure or heart rate. Cotinine had no significant effects on any of the six POMS subscales (tension, anger, depression, vigor, fatigue, or confusion).

Saliva Cotinine

For all subjects on days they were administered cotinine, saliva cotinine reached levels comparable to those found in regular smokers (200-900 ng/ml)(Benowitz et al., 1983). The range of peak cotinine levels achieved was 247-606 ng/ml (mean=409, std.dev.=117). Several subjects had detectable levels (10-50 ng/ml) of cotinine either on the placebo day or at pretest on the cotinine day. (The lower limit of assay quantitation was 10 ng/ml). These amounts of cotinine are less than those found with regular smoking, although they can be found in nonsmokers who have been exposed to cigarette smoke. The subjects who had detectable cotinine had jobs at restaurants and in one case, a music club, where large amounts of smoke were present.

Although all subjects at some point reached desired cotinine levels, there was considerable variance between subjects at post 1 and post 2. The means for cotinine levels at post 1 and post 2 were similar (post 1=312 ng/ml, std.dev.=205; post 2=340 ng/ml, std.dev.=90). Some subjects had their peak cotinine levels at post 1, others at post 2. At post 1 four subjects still had levels below 100 ng/ml. Saliva cotinine levels did not correlate significantly with RT effect sizes at post 1 or at post 2.

Chapter 4
Present Study Methods

Subjects

Subjects for the present study were 16 nonsmokers: eight male and eight female, ages 20-38. Average age was 27 (std.dev.=5.1) for the males and 28 (std.dev.=6.6) for the females. Subjects were recruited from the San Francisco State University "Job Hotline" and were paid $10.00 an hour, with a bonus of 25% of the total earned added on completion of all sessions. All subjects were either undergraduate or graduate students. Twelve were Caucasian, two were Asian, one was Black, and one was Hispanic. Subjects were all "never-smokers," that is, each had never smoked more than five cigarettes in his or her lifetime and had smoked no cigarettes within the past year. Current nonsmoking status was confirmed by taking saliva samples for concentrations of cotinine at the outset of each visit to the laboratory. Exclusion criteria included high blood pressure, heart disease, current or previous psychiatric disorders or use of antidepressants, alcohol abuse, current drug use, regular exposure to environmental tobacco smoke, and extreme deviation from normal height and weight. Average daily caffeine consumption was obtained and subjects were excluded if they drank more than three cups of coffee or tea a day or more than six colas a day. (Subjects were requested to consume their usual amount of caffeine on the morning of each test session and were asked about their consumption that day when they arrived. Subjects were also requested not to consume alcohol for 48 hours before each test session and were asked about alcohol consumption when they arrived.) Women were excluded from the study if they suffered from severe premenstrual symptoms or if they had irregular menstrual cycles. Each woman accepted into the study reported where she was in her cycle at each testing session. Women were not tested during the five-day premenstrual period. All subjects were screened for general good health.

The choice of 16 subjects was based on a power analysis. Because the doses to be used here were higher than those of the pilot study, power was not calculated using the pilot study data. Instead, since I predicted that cotinine would

have slowing effects, the power analysis utilized data from another drug with slowing effects previously tested in our laboratory. On the same RT task used here a 61 ms slowing of RT by clonidine was observed, which translates into an effect size of 1.7. For this effect size, the probability of finding a slowing effect of that magnitude or greater is 98% (power = .98), with 16 subjects.

Design

Each subject was tested on four separate occasions--a practice session followed by three test sessions, each a week a part. On each test day each subject was tested pre-drug and one hour post drug. The posttest was timed at one hour based on results from the pilot study.

A subgroup of eight subjects (four women, four men) were assigned to receive doses of 0 (placebo), 30, and 60 mg cotinine; the other eight were to receive 0, 60, and 90 mg. This design was conceived to permit comparisons of 0 and 60 mg doses in all subjects, while permitting the economical acquisition of additional dose/response information. Due to an error in drug preparation, nine subjects (five women, four men) actually received 0, 30, and 60 mg, and the remaining seven received 0, 60, and 90 mg doses. Doses were adjusted for subjects' individual weights, that is, each subject received 0.5, 1.0, and 1.5 mg per kg of body weight (referred to here as 30, 60, and 90 mg doses, respectively). Order of drug administration was counterbalanced between subjects, and testing was double-blind. As in the pilot study, test sessions were held one week apart to eliminate residual effects of cotinine administration.

Tasks

1. Stimulus Evaluation/Response Selection (SERS)

The SERS task was again used, as with the pilot study, described in Chapter 2.

2. Memory Task

A verbal free recall task was used that employed both "short" and "long" word lists. Rusted and Eaton-Williams (1991) suggested that inconsistency of findings in nicotine studies using verbal recall tasks may be due to differing attentional demands of the different tasks and that nicotine primarily affects attention rather than memory per se. Accordingly, they varied the length of word lists on a recall task and found a greater facilitating effect of oral nicotine on longer lists, which purportedly required more sustained attention. They suggested that, although list length also altered general difficulty of the task, another manipulation of difficulty (change in the amount of time each item is presented to the subject) made no difference in nicotine's effect. Rusted and Eaton-Williams interpreted their findings as an effect of nicotine on attention.

The present study employed a modified version of the task used by Rusted and Eaton-Williams. A short list (15 words) was presented for recall four times (four trials) and a long list (30 words) was presented for four trials. To present the lists, each word appeared on the computer screen for two seconds, followed by two seconds of blank screen, then the next word appeared for two seconds, and so on. Subjects were required to complete a written recall following presentation of the entire list (with a time deadline of 90 seconds for the short list and two minutes, 15 seconds for the long list). By random assignment, half the subjects received a short list first every time they did the task, and the other half received the long list first every time. All items on the short lists were matched for number of syllables, frequency, and concreteness of meaning. Items on the long lists were also so matched (source of words: Togia and Battig, 1978, Handbook of Semantic Word Norms, Lawrence Erlbaum & Associates). No items were repeated between lists. A different list was used for each test (word lists used are presented in Appendix B). Number of words recalled and number of intrusion words, incorrect words, and repetitions were scored on each trial. The task allowed assessment of immediate recall effects as well as effects on learning rate, forgetting, and interference (from previous lists).

3. Other Dependent Measures

As with the pilot study, blood pressure and heart rate were taken, and the

POMS was administered. In addition, at the end of each test session the subject was asked his or her impression of the drug dose he or she had received during that session. The subject marked a vertical line on a visual analogue scale. The scale was a 100-millimeter line, with the left end of the line labeled "weak" and the right end labeled "strong." The impression of dose score was measured as the distance of the mark from the left end of the line, in centimeters.

Procedure

Subjects were given extensive practice on the SERS task and the memory task on a separate practice day. On the practice day subjects were also weighed so their drug dosages could be prepared. On each test day the subject performed the memory and SERS tasks. He or she was then administered the capsule (cotinine or placebo) The SERS task was repeated at approximately one hour post-drug (post-capsule, cotinine or placebo), followed by the memory task. Blood pressure, heart rate, and POMS were collected at the outset, and at 30 and 60 minutes post-drug. Saliva samples were collected pre-drug and at approximately one and one-half hours post-drug. Samples were assayed for nicotine and cotinine concentrations. At the end of the session the subject marked the visual analogue scale question regarding his or her impression of dose received.

ERP Recording

ERPs were recorded and components selected in the same manner as described for the pilot study in Chapter 2.

Data Analysis

Analyses were conducted using repeated measures analysis of variance (ANOVA). For the SERS task within factors included drug, time (pre, post), stimulus complexity (easy, hard), and response complexity (easy, hard). Separate analyses were computed on RT, N100 and P300 latencies and errors. Significance

of the drug effect was tested by the drug x time interaction. Gender was a between subjects factor. For the memory task, a repeated measures ANOVA was computed on number of words recalled (and errors), with within factors including drug, time, and trial. Between factors included gender and list order (short versus long first). For all dependent variables analyses were conducted on all subjects, comparing data for the placebo and 60 mg dose. Separate analyses were done, for descriptive purposes, comparing 0, 30, and 60 mg doses for the low dose subgroup (n=9) and comparing 0, 60, and 90 mg for the high dose subgroup (n=7). Greenhouse-Geiser estimates of sphericity and adjusted p values were used for comparisons with more than one degree of freedom.

Chapter 5
Present Study Results

SERS Task

1. Reaction Time

Mean RTs for all subjects (n=16) at 0 and 60 mg doses are shown in Table 3 and depicted in Figure 3. As in the pilot study, cotinine "slowed" RT relative to placebo; it did not speed from pre to post to the same extent placebo did. The difference was not statistically significant, however. There were main effects of time, stimulus, and response, as is always seen with the SERS task. RT speeded an average of 10 ms pre to post ($F(1,14)=11.5$, $p<.004$). There was an average difference of 42 ms between the easy and hard stimulus conditions ($F(1,14)=120$, $p<.0001$) and an average difference of 60 ms between the easy and hard response conditions ($F(1,14)=151$, $p<.0001$). There was no effect of gender and no interaction of gender and drug effect.

Mean RTs for the low dose (n=9) and high dose (n=7) subgroups are shown in Table 3 and depicted in Figure 3. In both subgroups there was a dose-related decrease in speeding from pre to post, with greater decreases occurring at larger doses, relative to placebo. Again, however, these differences were not statistically significant. Again, there were no significant gender effects.

To obtain more power to detect cotinine's apparent slowing effect, data from the 20 mg pilot study (n=13) were merged with the 30 mg data from the present study (n=9). Saliva cotinine levels achieved were similar for both groups. An ANOVA showed no significant drug x time interaction, but there was a significant drug x time x response interaction ($F(1,20)=5.67$, $p < .03$). There was no effect of study (pilot versus present). Table 4 illustrates that the "slowing" by cotinine relative to placebo was greater for the hard response condition. This interaction was not significant for either study alone.

2. Accuracy

As with the pilot study, there were no significant changes in overall

accuracy. The average proportion of errors for any drug condition or time did not exceed five percent.

Table 3. *Mean Reaction Times (in milliseconds)*

All Subjects (n=16)

	Placebo		60 mg Cotinine	
	Mean	(S.E.)	Mean	(S.E.)
Pre	496	(9.7)	492	(10.9)
Post	483	(9.9)	484	(9.7)

Low Dose Subgroup (n=9)

	Placebo		30 mg Cotinine		60 mg Cotinine	
	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)
Pre	512	(12.3)	513	(14.2)	511	(15.0)
Post	502	(13.5)	501	(13.8)	502	(12.6)

High Dose Subgroup (n=7)

	Placebo		60 mg Cotinine		90 mg Cotinine	
	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)
Pre	468	(13.9)	468	(14.9)	463	(12.9)
Post	459	(13.6)	461	(14.4)	460	(12.1)

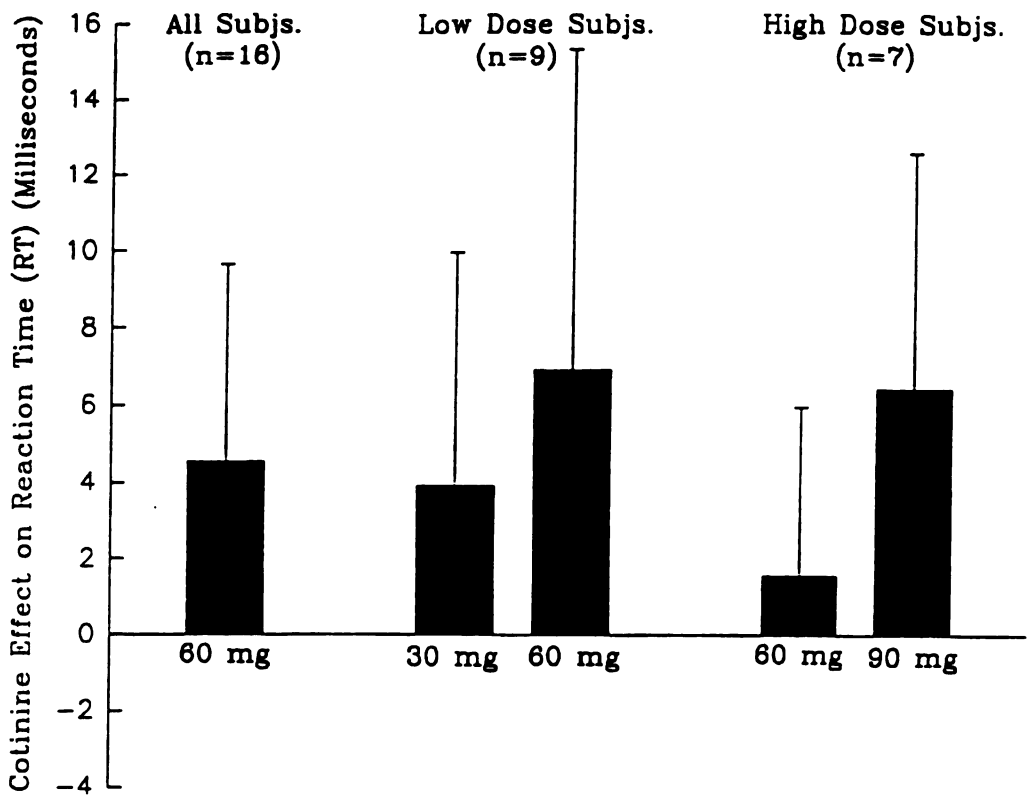


Figure 3. Effects of cotinine on RT. The size of the drug effect is calculated as the difference between the pre-post change in the drug condition and the pre-post change in the placebo condition. Positive values indicate slowing by the drug relative to placebo. Effects seen are not statistically significant.

Table 4. *Mean Reaction Times (in milliseconds) for 20 & 30 mg Doses Combined from Pilot Study and Present Study. Interaction with Response Complexity (n=22)*

Both Studies

	Placebo				Cotinine			
	Easy Response		Hard Response		Easy Response		Hard Response	
	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)
Pre	488	(9.0)	556	(10.5)	489	(9.4)	547	(10.6)
Post	473	(9.7)	534	(9.0)	482	(9.5)	543	(9.5)

Pilot Study (n=13)

	Placebo				20 mg Cotinine			
	Easy Response		Hard Response		Easy Response		Hard Response	
	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)
Pre	491	(12.2)	557	(13.3)	495	(10.5)	549	(11.9)
Post 1	475	(10.6)	534	(10.2)	493	(10.6)	547	(10.2)

Present Study - Low Dose (n=9)

	Placebo				20 mg Cotinine			
	Easy Response		Hard Response		Easy Response		Hard Response	
	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)
Pre	483	(13.4)	554	(17.3)	481	(17.7)	545	(20.0)
Post 2	470	(18.8)	534	(16.7)	465	(20.0)	537	(17.0)

3. Speed-Accuracy Tradeoff

An examination of speed-accuracy function (SAF) data as described for the pilot study was also made in the present study. As Figure 4 illustrates, there is some apparent decrease in the number of RTs at the fast end with 60 mg pre to post relative to placebo, which would be consistent with the small slowing of RT by cotinine. Figure 4 shows that, although cotinine decreased accuracy relative to placebo for a number of RT bins, cotinine does not seem to produce any dramatic change in accuracy that would suggest any significant effect on the SAF.

4. N100

There were no significant drug effects on N100 amplitude. There were no significant drug effects on N100 latency. Mean N100 latencies for all subjects' 0 versus 60 mg doses and for the two dose subgroups are shown in Table 5 and depicted in Figure 5. For the low dose subgroup there was a nonsignificant tendency to slow N100 latency by the 60 mg dose ($F(2,14)=3.35$, $p<.09$).

5. P300

There were no significant drug effects on P300 amplitude. As with the RT results (and the N100 latency results within each subgroup), there was (except for 60 mg in the high dose subgroup) a dose-related "slowing" of P300 by cotinine relative to placebo, which was statistically nonsignificant. Mean P300 latencies are shown in Table 6 and Figure 6. Figure 7 demonstrates the stimulus complexity effect on P300 latency that is consistently observed for the SERS task. For all subjects (n=16), comparing the 0 and 60 mg doses pre and post, mean P300 latency was 36 ms faster for the easy stimulus than for the hard stimulus ($F(1,14)=15$, $p<.001$). (The response complexity effect was not significant, as would be expected.) A comparison of Figures 7 and 8 illustrates the contrast between the large effect of stimulus complexity and small, nonsignificant effect of 60 mg cotinine on P300 latency.

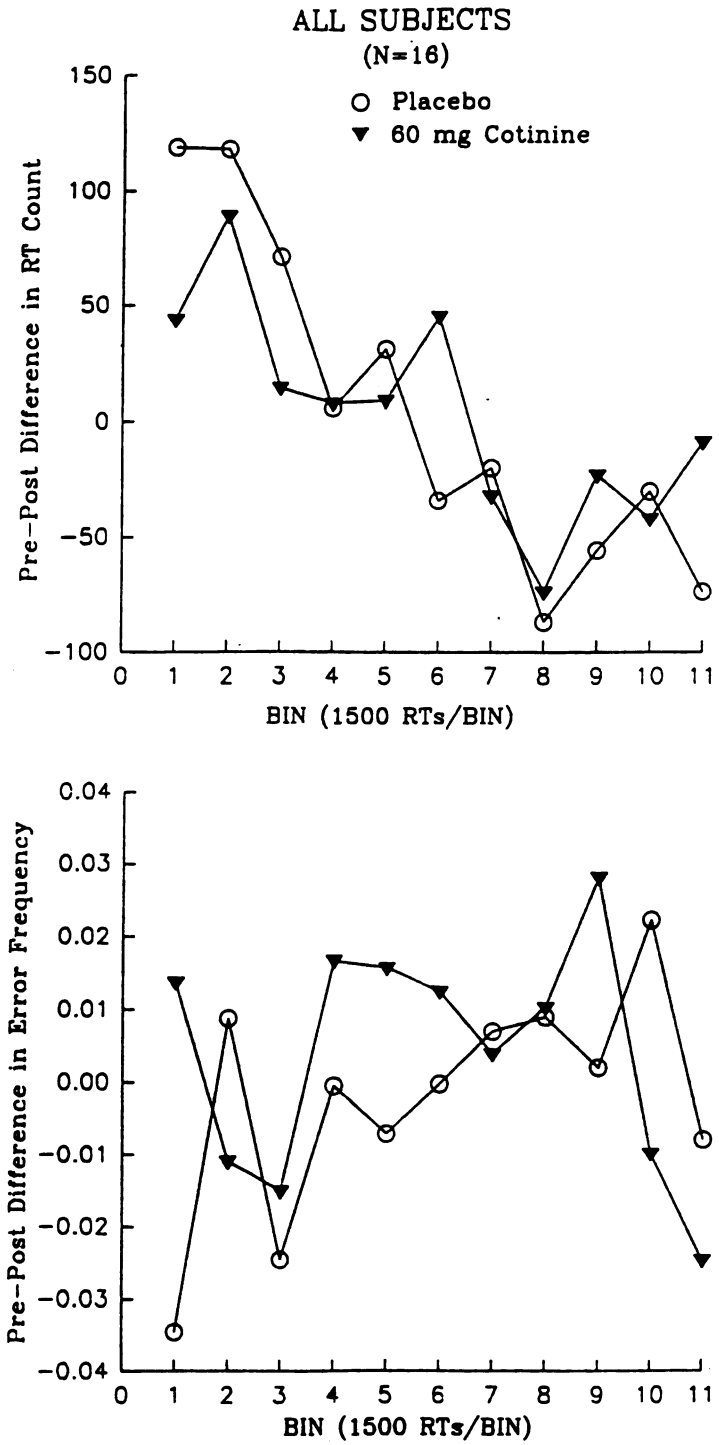


Figure 4. Top figure: Post-pre difference in number of RTs per bin for placebo and 60 mg cotinine. RT bins are numbered from fastest to slowest on the X axis. Bottom figure: Post-pre difference in error frequency for placebo and 60 mg cotinine. The Y axis depicts the proportion of errors.

Table 5. *Mean N100 Latencies (in milliiseconds)*

All Subjects (n=16)

	Placebo		60 mg Cotinine	
	Mean	(S.E.)	Mean	(S.E.)
Pre	218	(2.6)	214	(2.6)
Post	212	(2.4)	213	(2.9)

Low Dose Subgroup (n=9)

	Placebo		30 mg Cotinine		60 mg Cotinine	
	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)
Pre	222	(3.6)	219	(3.9)	211	(3.8)
Post	213	(3.7)	210	(3.6)	218	(4.4)

High Dose Subgroup (n=7)

	Placebo		60 mg Cotinine		90 mg Cotinine	
	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)
Pre	214	(3.8)	217	(3.4)	216	(3.5)
Post	210	(3.0)	207	(2.7)	214	(3.8)

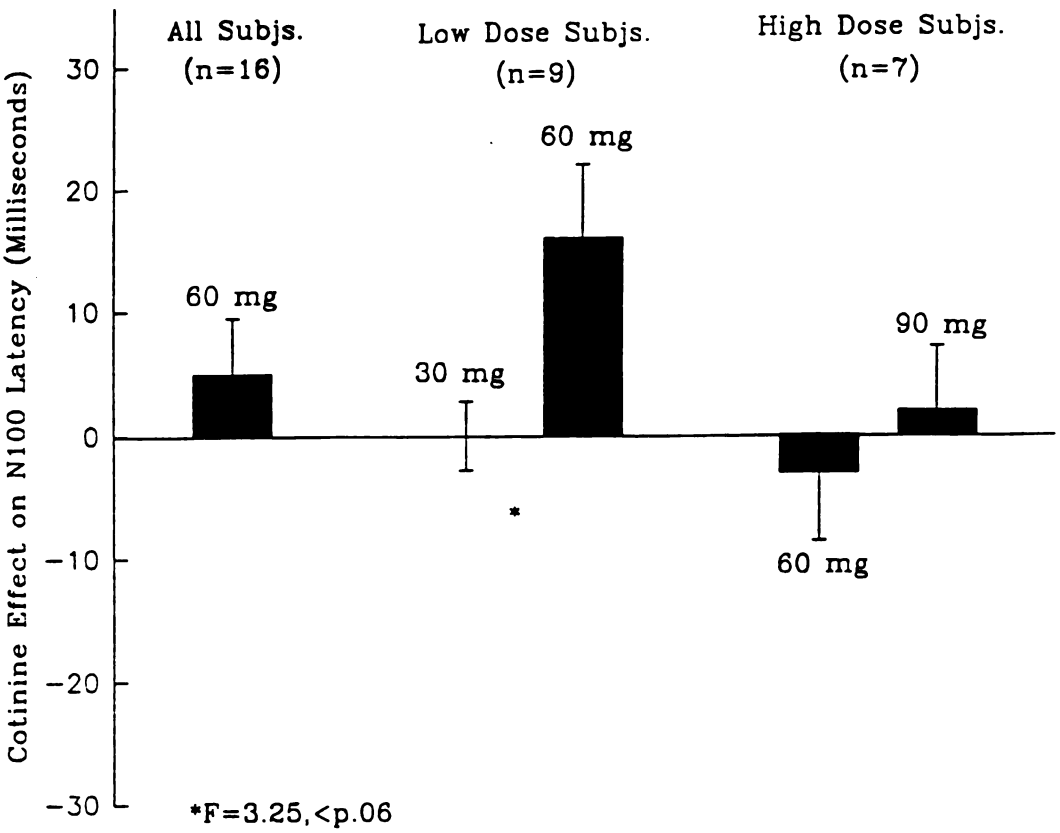


Figure 5. Effects of cotinine on N100 latency. The size of the drug effect is calculated as the difference between the pre-post change in the drug condition and the pre-post change in the placebo condition. Positive values indicate slowing by the drug relative to placebo; negative values indicate speeding by the drug vs. placebo.

Table 6. *Mean P300 Latencies (in milliseconds)*

All Subjects (n=16)

	Placebo		60 mg Cotinine	
	Mean	(S.E.)	Mean	(S.E.)
Pre	450	(6.0)	453	(6.4)
Post	438	(5.6)	446	(6.8)

Low Dose Subgroup (n=9)

	Placebo		30 mg Cotinine		60 mg Cotinine	
	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)
Pre	457	(8.0)	446	(9.6)	454	(8.4)
Post	436	(7.6)	443	(10.0)	453	(9.6)

High Dose Subgroup (n=7)

	Placebo		60 mg Cotinine		90 mg Cotinine	
	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)
Pre	441	(8.4)	452	(9.6)	443	(9.2)
Post	442	(8.8)	436	(10.0)	449	(8.8)

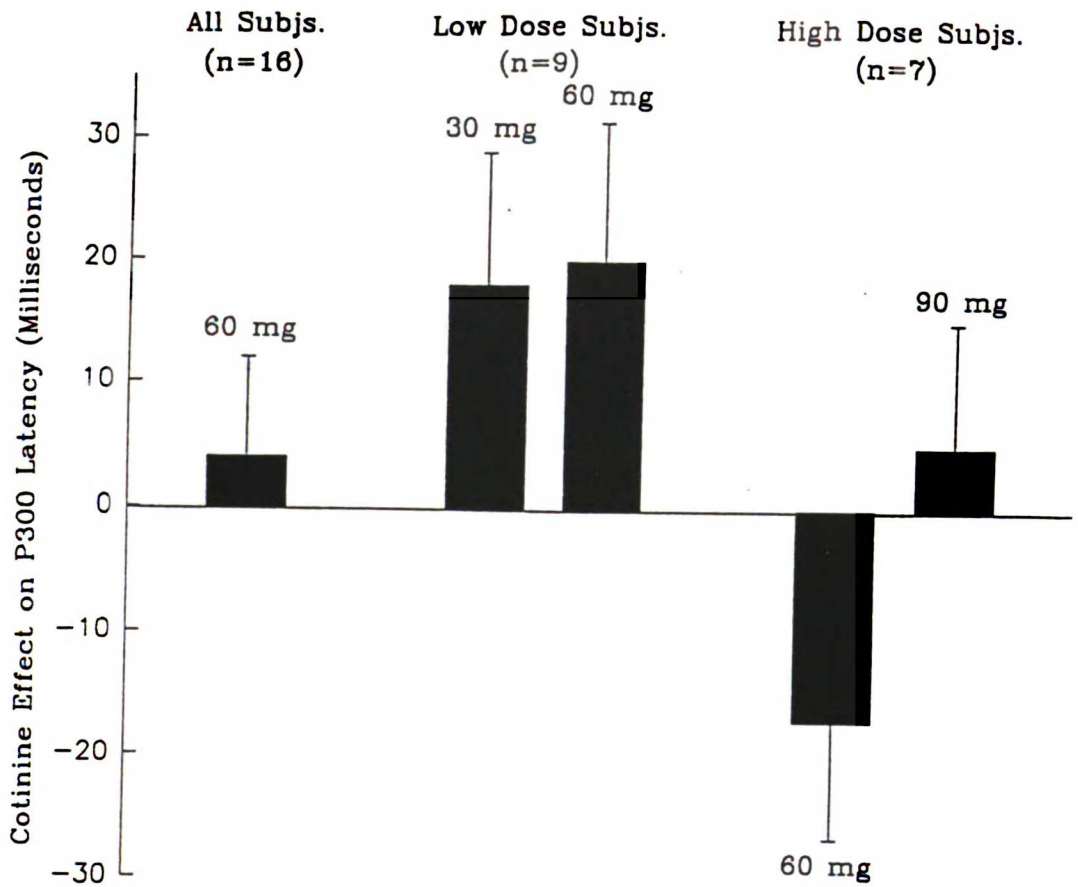


Figure 6. Effects of cotinine on P300 latency. The size of the drug effect is calculated as the difference between the pre-post change in the drug condition and the pre-post change in the placebo condition. Positive values indicate slowing by the drug relative to placebo; negative values indicate speeding by the drug relative to placebo. Effects seen are not statistically significant.

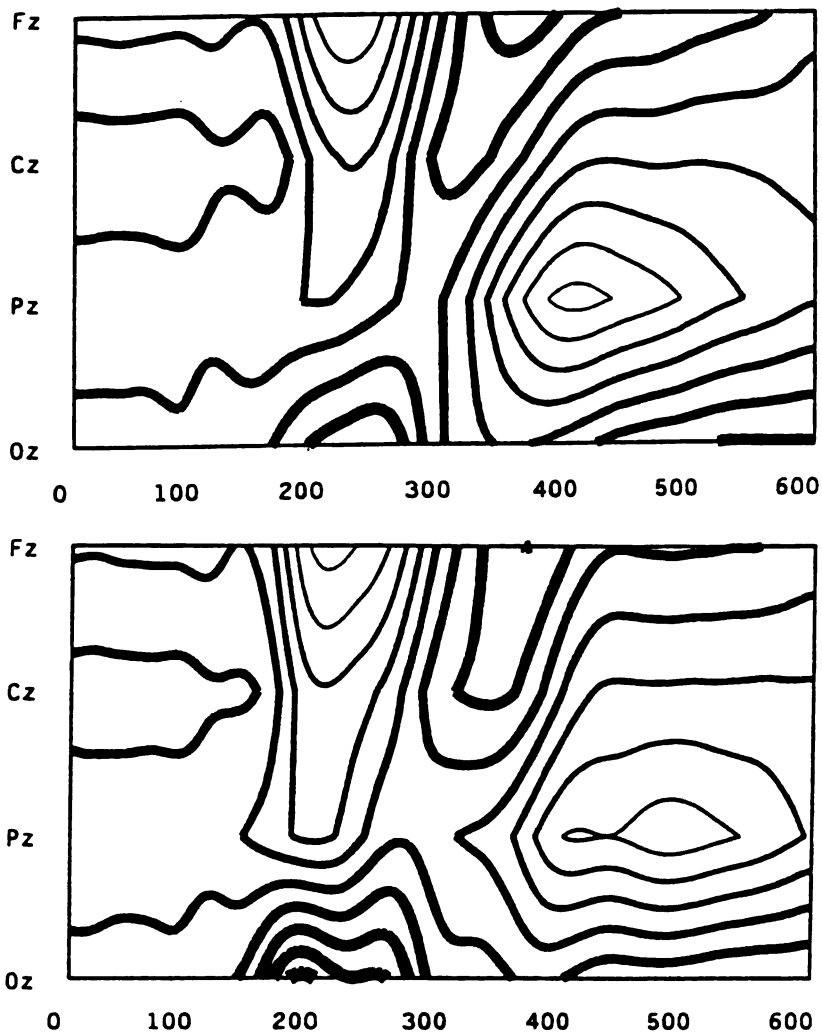


Figure 7. Chronotopograms for the easy stimulus and hard stimulus conditions for midline electrodes. Horizontal axis shows time in milliseconds. Isovoltage lines are in 1 uV increments. Negative potentials are thick lines; positive potentials are thin lines. V+ and V- are the maximum and minimum potentials in microvolts for each condition.

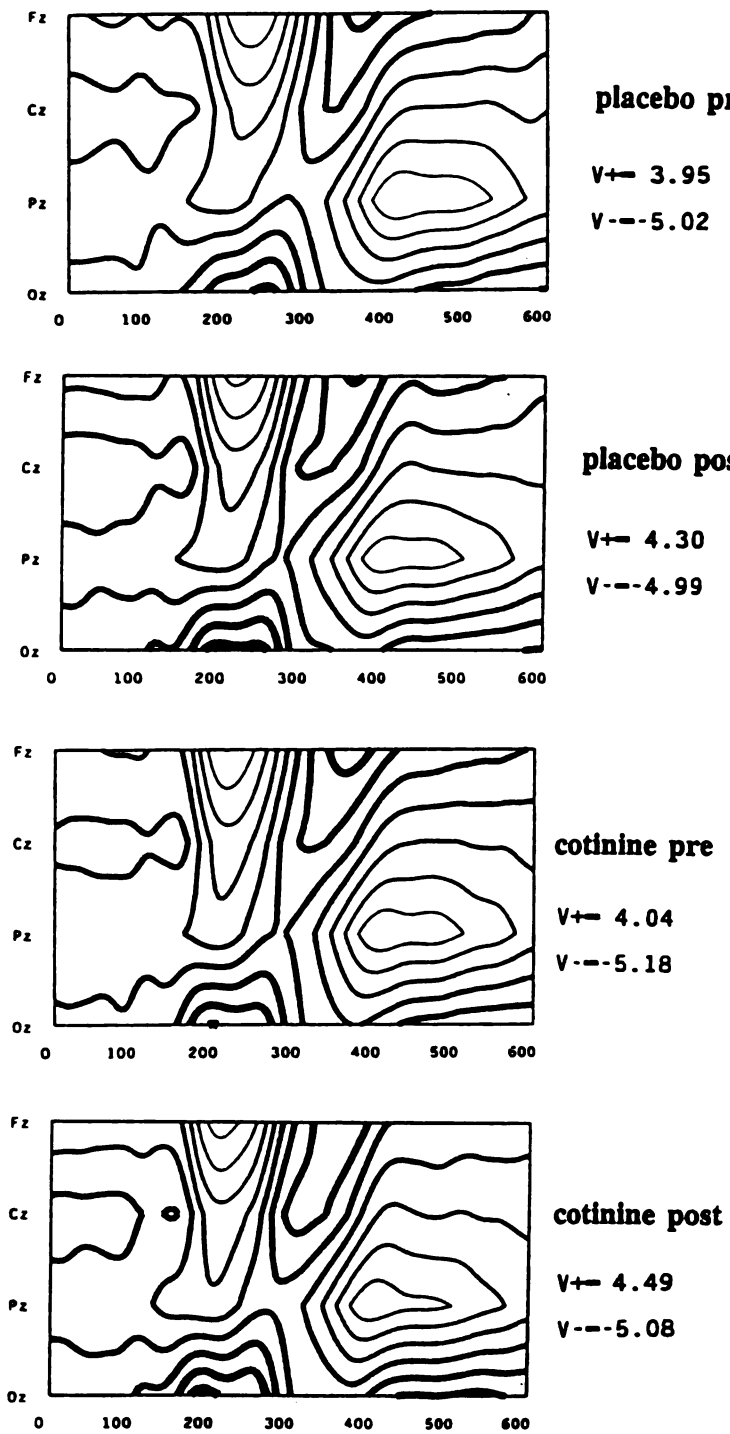


Figure 8. Chronotopograms for each drug by time condition for all subjects, comparing placebo and 60 mg cotinine. See Figure 7 for description.

Memory Task

1. Short List

For all subjects' 0 versus 60 mg data on the 15-word list, repeated measures ANOVA yielded no significant drug effects on number of words recalled. There was a main effect of trial ($F(3,36)=57, p<.0001$); as would be expected, subjects increased the number of words recalled across the four trials. (For each trial means were 9, 12, 13, and 14 words, respectively.) There was no drug x time x trial effect, therefore cotinine did not affect the learning rate. There was no effect of gender or order.

For the low dose subgroup there was also a significant effect of trial ($F(3,15)=49$, $p<.0002$). There was also a drug x prepost effect ($F(2,10)=5.85$, $p<.05$). For the high dose subgroup there was a significant trial effect ($F(3,9)=17$, $p<.01$), but no other significant effects.

The means for the total number of words recalled per test (over four trials) are shown in Table 7 and Figure 9. For all subjects combined, the 60 mg dose decreased the number of words recalled. For the low dose group, however, 30 mg and 60 mg doses increased the number of words recalled pre to post, relative to placebo. For the high dose subgroup the only significant effect was the main effect of trial ($F(3,9)=17$, $p<.0004$). In the high dose subgroup the 60 and 90 mg doses decreased the number of words recalled relative to placebo. In each subgroup, the higher the dose, the fewer the number of words recalled. Figure 10 illustrates the post-pre difference in scores over the four trials.

2. Long List

For all subjects' 0 versus 60 mg dose data on the 30-word list, cotinine significantly decreased the number of words recalled (on average 5.5 fewer words pre to post, relative to placebo, over the four trials). (Drug by time interaction yielded $F(1, 12)=5.67$, $p<.03$). There was a main effect of trial ($F(3,36)=134$, $p<.0001$). Subjects improved across trials (means are 14, 20, 24, and 26 words recalled for the four trials, respectively). None of the other interactions with drug were significant. There were no effects of gender or order. For the low dose and high dose subgroups there were main effects of trial (low group $F(3,15)=170$,

Table 7. *Total Words Recalled from Short List (15 Words x 4 Trials = 60 Possible)*

All Subjects (n=16)

	Placebo		60 mg Cotinine	
	Mean	(S.E.)	Mean	(S.E.)
Pre	51.7	(1.5)	50.1	(1.9)
Post	51.2	(1.8)	48.9	(2.4)

Low Dose Subgroup (n=9)

	Placebo		30 mg Cotinine		60 mg Cotinine	
	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)
Pre	52.8	(1.5)	50.0	(2.2)	50.0	(2.6)
Post	50.9	(2.6)	50.9	(2.3)	49.0	(3.6)

High Dose Subgroup (n=7)

	Placebo		60 mg Cotinine		90 mg Cotinine	
	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)
Pre	50.3	(2.9)	50.3	(3.1)	51.7	(2.5)
Post	51.6	(2.7)	48.9	(3.5)	46.3	(4.1)

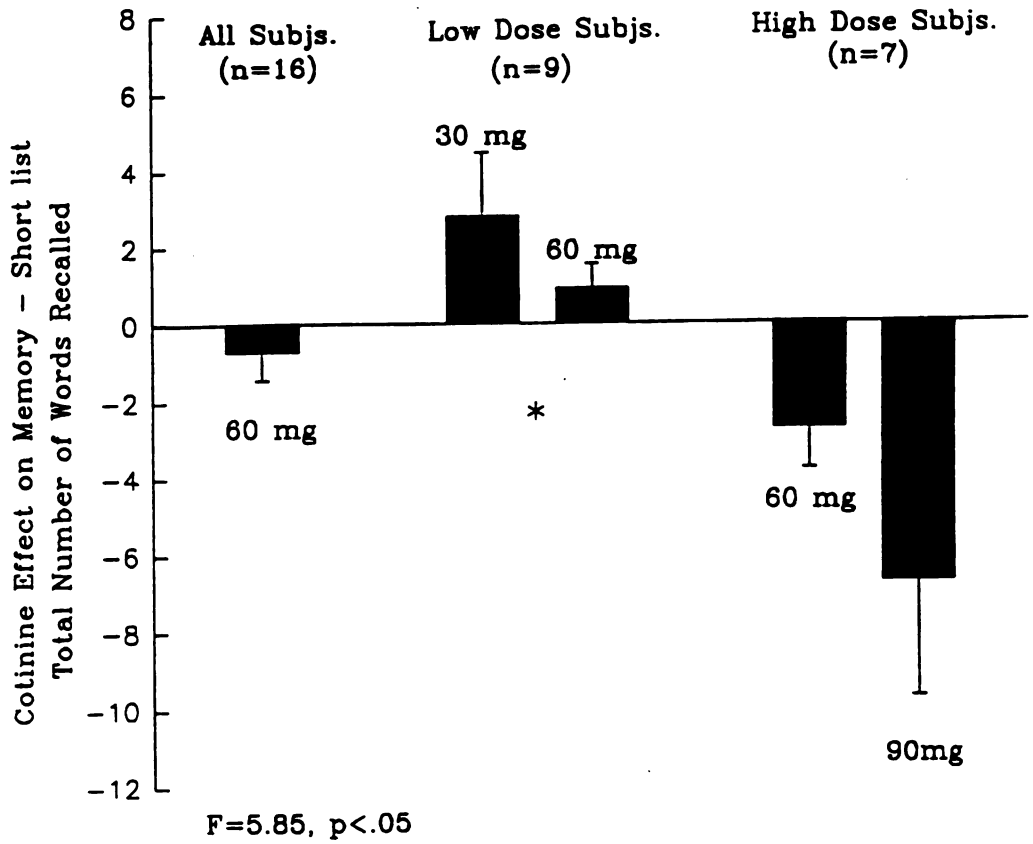


Figure 9. Effects of cotinine on memory task--total number of words recalled from short list (15 words over 4 trials = 60 possible). Size of drug effect is calculated as the difference between the pre-post change in drug condition and the pre-post change in placebo condition. Negative values indicate fewer words recalled for drug relative to placebo; positive valued indicate more words recalled for drug vs. placebo.

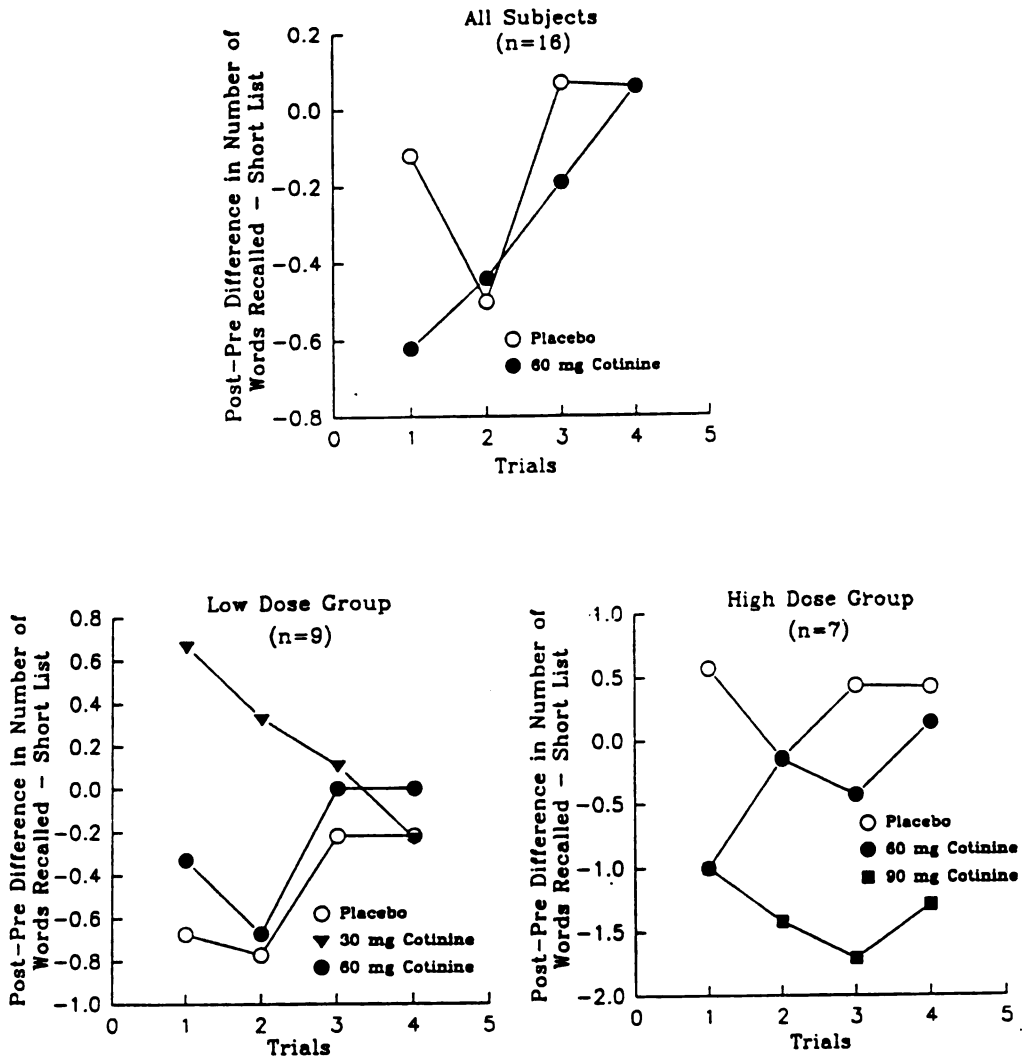


Figure 10. Memory task: post-pre difference in number of words recalled on each of four trials for the short list, for all subjects comparing the placebo change to the 60 mg cotinine change, and for each of the dose subgroups. Drug effect difference across trials (drug x time x trial interaction) was not statistically significant for any group.

p<.001; high group F(3,9)=25, p<.01), but no significant drug effects. Means for total number of words per trial are shown in Table 8 and Figure 11. For the long list, all drug effects were consistent across subgroups and doses in decreasing the number of words recalled, relative to placebo. Figure 12 illustrates the post-pre differences across the four trials.

To see whether the decrease in number of words recalled per trial on the long list depended on the serial position of words in the lists, each list was divided into six blocks of five words each. Subjects' recall scores in each of the six sequential blocks were analyzed, using a repeated measures ANOVA with drug, time and block as within-subject factors. There was a main effect of block (F(5,75)=15, p<.0001), with subjects recalling more words at the beginning and end of the list. There was no significant interaction of block with drug x time, however. Figure 13 illustrates the post-pre differences in scores across the blocks.

<u>3. Errors</u>

Few errors of commission were made on the memory task. For the short list and the long list the difference in the number of errors pre to post was calculated for the 60 mg dose and placebo. T tests were computed on the placebo and 60 mg difference scores for both list lengths. There was no significant difference in number of errors.

Blood Pressure/Heart Rate/POMS

Cotinine had no significant effects on blood pressure or heart rate at any dose. Neither did it affect any of the six POMS subscales (tension, anger, depression, vigor, fatigue, or confusion).

Subjective Impression of Dose

One score of each subject's subjective impression of the strength of dose received was obtained for each test session. That score (number of centimeters from 0 on a 10-centimeter line) did not correlate with actual dose received (r = 0.2, p<.16), although it did correlate significantly with session number (r = 0.33,

Table 8. *Total Words Recalled from Long List (30 Words x 4 Trials = 120 Possible)*

All Subjects (n=16)

	Placebo		60 mg Cotinine	
	Mean	(S.E.)	Mean	(S.E.)
Pre	84.4	(4.8)	87.6	(4.3)
Post	86.6	(5.2)	84.3	(4.8)

Low Dose Subgroup (n=9)

	Placebo		30 mg Cotinine		60 mg Cotinine	
	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)
Pre	82.3	(5.9)	85.9	(5.6)	86.6	(5.6)
Post	85.8	(5.6)	85.2	(7.0)	85.1	(5.3)

High Dose Subgroup (n=7)

	Placebo		60 mg Cotinine		90 mg Cotinine	
	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)
Pre	87.0	(8.5)	88.9	(7.3)	83.1	(8.9)
Post	87.7	(10.1)	83.1	(9.2)	80.0	(11.7)

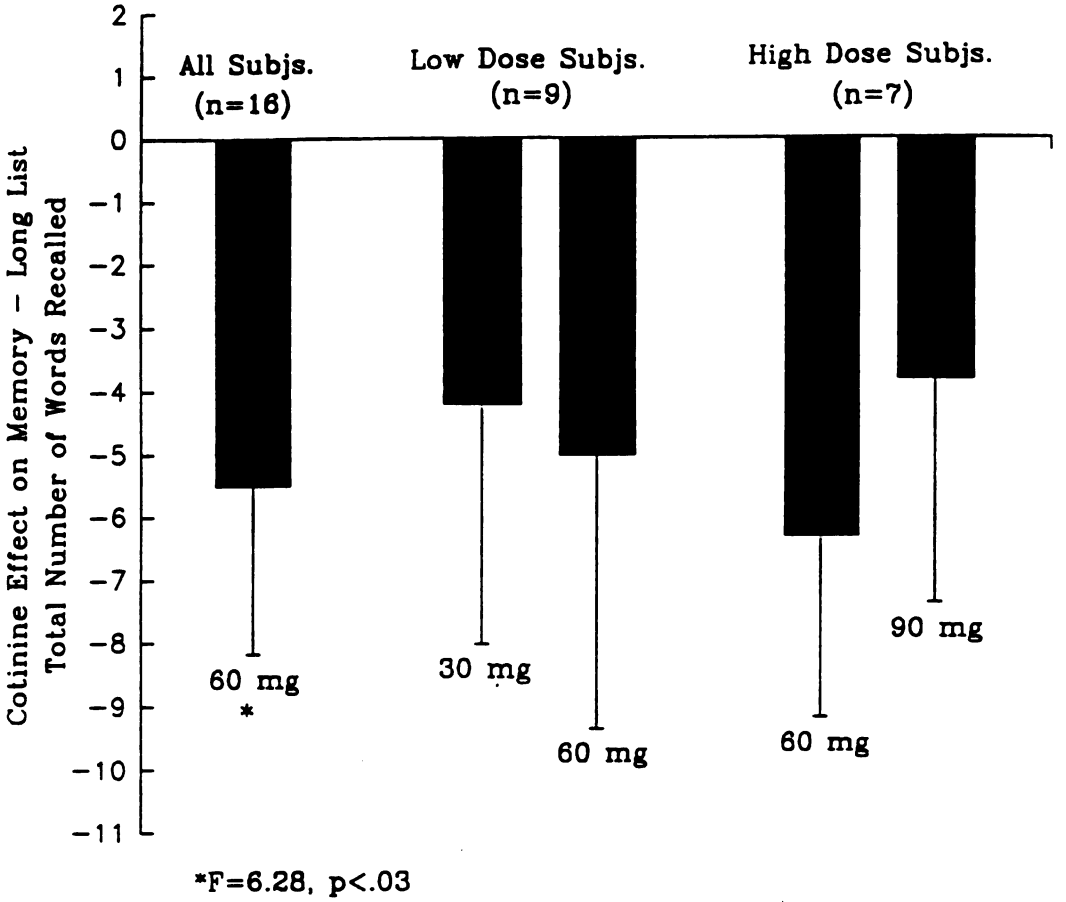


Figure 11. Effects of cotinine on memory task--total number of words recalled from long list (30 words over 4 trials = 120 possible). Size of drug effect is calculated as the difference between the pre-post change in drug condition and the pre-post change in placebo condition. Negative values indicate fewer words recalled for drug vs. placebo.

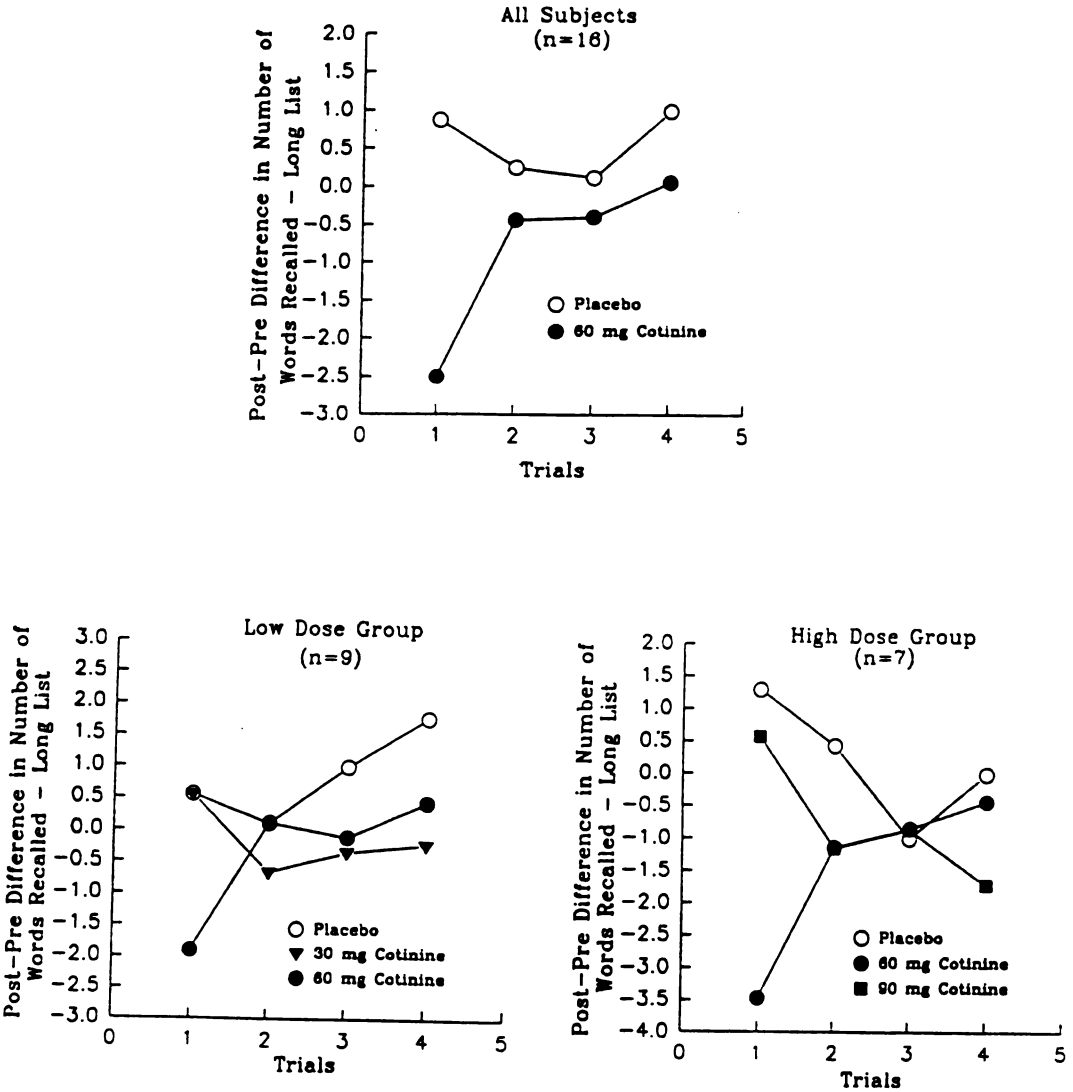


Figure 12. Memory task: post-pre difference in number of words recalled on each of four trials for the long list, for all subjects comparing the placebo change to the 60 mg cotinine change, and for each of the dose subgroups. Drug effect difference across trials (drug x time x trial interaction) was not statistically significant for any group.

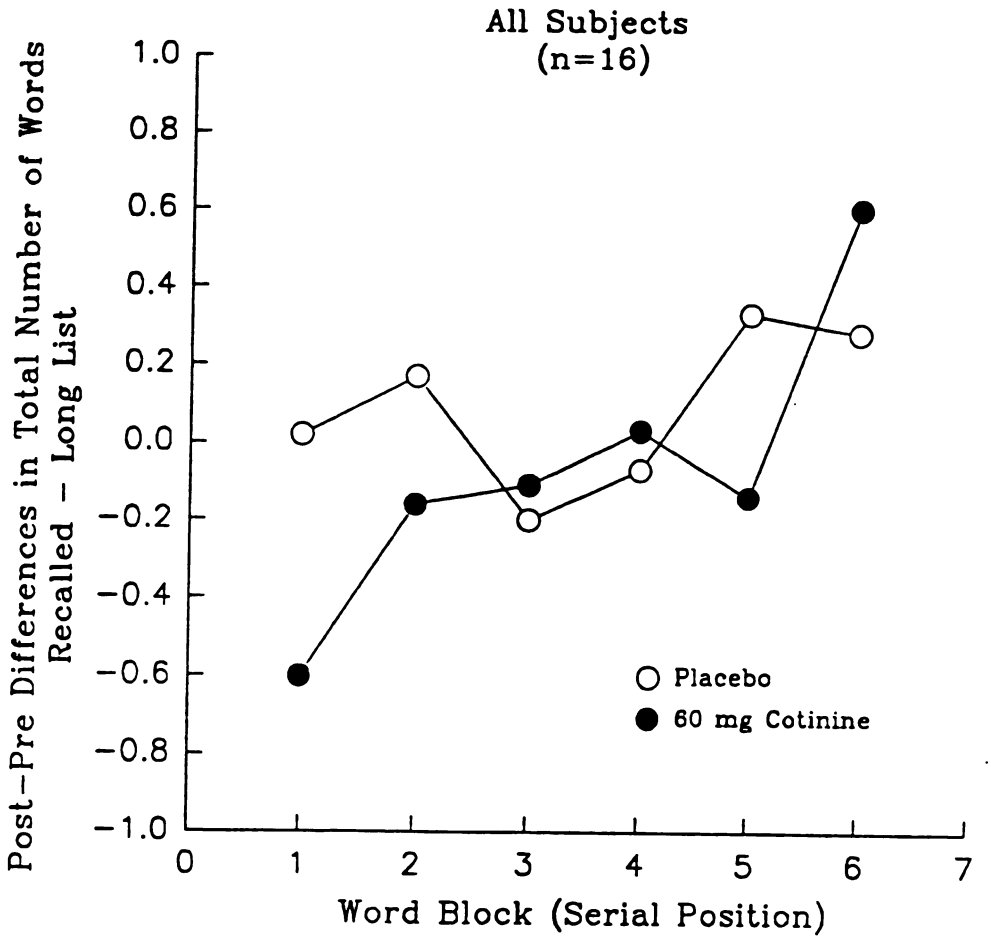


Figure 13. Memory task serial position effects on the long list for all subjects, comparing placebo to 60 mg cotinine. Post-pre difference in the total number of words recalled (over 4 trials) for each block of 5 words. Block 1 is the first 5 words, block 2 is the second 5 words, and so on.

p<.02). Mean score increased as session number increased (means = 1.18(1.2), 2,29(2.0), and 3.12(3.3), for sessions 1-3, respectively).

Saliva Cotinine

Saliva cotinine levels for all subjects verified their nonsmoking status. Saliva cotinine confirmed that 15 of the 16 subjects were administered cotinine in the expected doses, according to their random assignment. For one subject, saliva cotinine levels indicated that she had received 0, 30, and 60 mg doses instead of her assigned 0, 60, and 90 mg doses. The range of saliva cotinine levels and mean cotinine levels for each drug x time condition are shown in Table 9 and depicted in Figure 14. Variability was much less than in the pilot study, possibly because the doses here were larger and were adjusted to subjects' weights.

Saliva cotinine levels across all subjects showed a significant inverse correlation with the memory task effect for the short list, such that the number of words recalled decreased as the level of cotinine increased ($r = -0.54$, $p < .001$). There was no such correlation for the long list ($r = 0.03$, $p<.8$). A supplementary analysis was conducted on saliva cotinine and the short and long list memory task effects for all subjects across the range of doses received. The analysis was done using the NONMEM (Nonlinear Mixed Effects Model) program (Beal & Sheiner, 1990), specifically developed for analyzing population pharmacokinetic and pharmacodynamic data. A nonparametric spline function was used to fit the data. As with the correlational analysis, saliva cotinine was found to have a significant linear (decreasing) relationship to memory effects on the short list, but no significant relationship to memory effects on the long list. Figure 15 illustrates these results. Saliva cotinine was not found to have a significant relationship to RT effects.

Table 9. *Mean Saliva Cotinine (in ng/ml) Post Drug Administration*

	N	Mean	(Standard Error)
Placebo	16	3	(2.3)
30 mg Dose	9	826	(130.2)
60 mg Dose	16	1720	(268.2)
90 mg Dose	7	2745	(140.9)

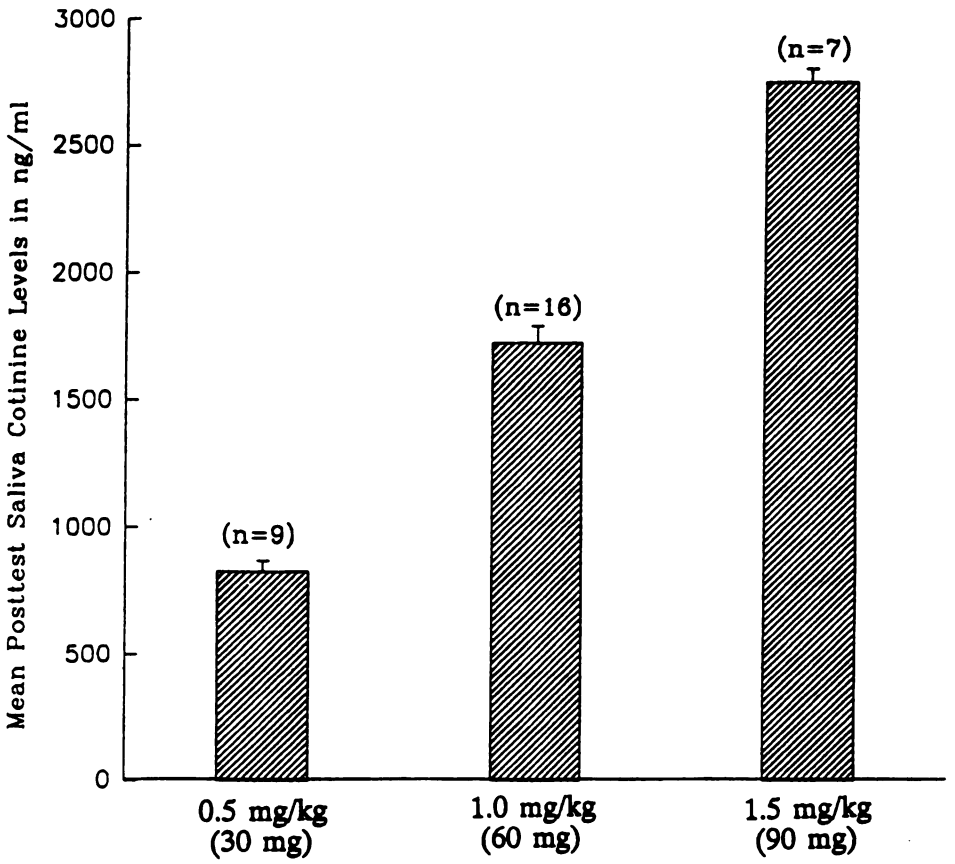
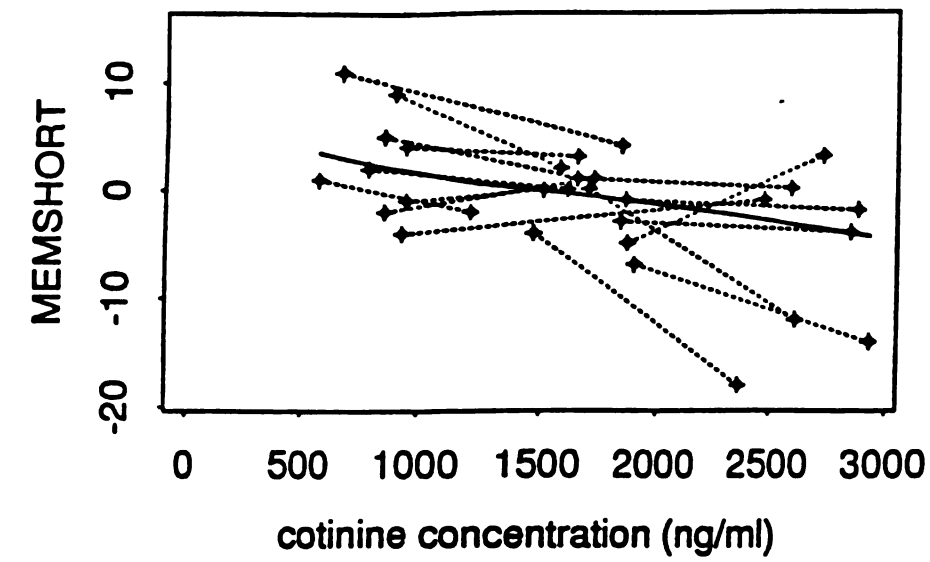


Figure 14. Saliva cotinine levels at posttest for each dose in ng/ml. All pretest scores had a mean of < 10 ng/ml (the lowest level of quantitation). Chronic levels in regular smokers average 300-900 ng/ml. The half-life of an acute dose is 15-20 hours.



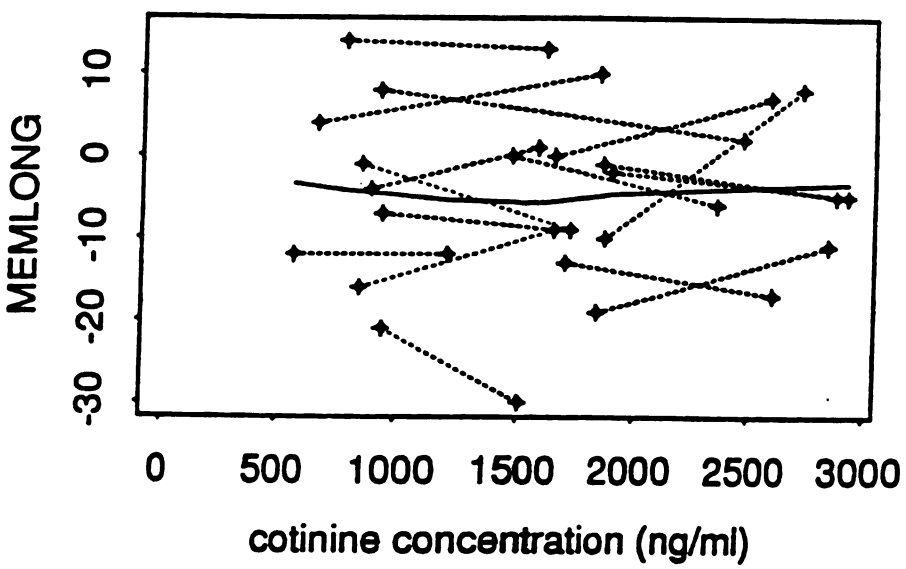


Figure 15. Relationship between saliva cotinine levels and pair of memory task effects (difference between pre-post change with placebo and pre-post change with cotinine for each of two doses) for the short and long lists, using a spline fitting routine in the NONMEM program. Each subject is represented by a connected pair of points, corresponding to each subject's pair of cotinine doses.

Chapter 6
Discussion

In this study multiple acute doses of cotinine administered to nonsmokers produced cognitive effects. In the verbal memory task 60 mg cotinine significantly impaired recall on the long list for all subjects. Cotinine had no effect on the short list for the group as a whole. For the low dose subgroup of subjects, however, cotinine significantly improved recall of the short list, while for the high dose subgroup cotinine showed nonsignificant impairment. For both subgroups effects on the short list were dose-related; for the low dose subgroup the higher dose produced less improvement than the lower, and for the high dose subgroup the higher dose produced more impairment than the lower. As will be discussed, these memory effects, possibly resulting from changes in the serotonergic system, may reflect impairment of long term memory consolidation and either no effect or bidirectional effects on short term memory. Cotinine also displayed a weak, but consistent dose-related slowing of all information processing measures. Although not statistically significant, for RT, N100 latency, and P300 latency, within each dose subgroup, the higher dose produced slower performance than the lower dose, without exception. These slowing effects are consistent in direction with the near-significant slowing of a single dose of cotinine on RT in the pilot study. There were no effects of cotinine on heart rate, blood pressure, mood measures or subjective impression of having taken a drug. Effects did not differ between men and women.

SERS Task

It is perhaps surprising that, although 30, 60, and 90 mg doses in this study slowed RT to increasing degrees, respectively, their effects were not significant, given that the smaller 20 mg dose showed a near-significant slowing of RT in the pilot study. The explanation no doubt lies at least partly with individual variability (in the pilot study 10 of 13 subjects were slowed by cotinine, while in the present study 11 of 16 were slowed). It is also possible that the task was too easy to

permit detection of a drug effect. When data for the 30 mg dose was combined with the 20 mg pilot study data, a significant interaction with response condition was observed. The slowing by cotinine was greater for the hard response condition in the 20 mg data and in fact only occurred for the hard response condition in the 30 mg data. Although this interaction was not apparent on overall analysis of the present study data, it is possible that a task that manipulates response complexity in a variety of ways would reveal a greater and more specific effect by cotinine.

The significant interaction of drug by time by response on N100 latency in the pilot study was not replicated in this study. In the pilot study cotinine slowed N100 latency for the hard response at posttest 1 and for the easy response at posttest 2. The present study paradigm included only the equivalent of posttest 1, but there was no interaction of drug effect with response complexity. The near-significant slowing of N100 latency by 60 mg in the low dose subgroup is at least consistent with the pilot study effect in direction, however. And it is consistent to the extent that both studies showed more evidence for an effect on N100 latency than P300 latency, for which there were no significant or near-significant results.

It is perhaps interesting to note that nearly every drug tested thus far in the SERS task paradigm, other than cotinine or nicotine, has demonstrated a significant effect on N100 latency (Le Houezec et al., 1994-no effect of nicotine; Naylor et al., 1993; Halliday et al., 1994). It may be that cotinine is less psychoactive than the other drugs tested, although that does not seem likely to be true of nicotine. A lack of effect is contrary to what one might expect if N100 latency reflects early stimulus processing, which nicotine arguably should influence as a cholinergic agonist (see Brandeis et al., 1992). If N100 latency reflects the level of some general attentional state, one might also expect cotinine (and nicotine) to affect it, given existing evidence that nicotine facilitates attention. Again, it is possible that a harder task could reveal such an effect with cotinine, given the interaction with response complexity in the pilot study, and the nearly-significant slowing by one subgroup in the present study. Or it may be that, as attention is a multifaceted concept, cotinine (and nicotine) may affect some other aspect or type of attention, better reflected in other measures.

There was no significant or near-significant effect of cotinine on P300 latency. Except for the 60 mg dose in the high dose subgroup, cotinine had weak slowing effects relative to placebo. This is in contrast to Le Houezec et al., (1994), in which nicotine speeded P300 latency in the hardest task condition, which is consistent with the idea that nicotine affects stimulus processing. In that study nicotine actually slowed P300 latency for the easier task conditions, however. The authors had no ready explanation for that finding. We might note that this is another example of task difficulty as a determining factor for demonstrating drug effects. In the present study cotinine did not show a significant interaction with task condition that might correspond to (or be in the opposite direction of) the nicotine effect, that is, cotinine did not selectively slow P300 latency in the hardest task condition.

Memory Task

Cotinine produced significant and specific effects on verbal recall. Just as the lack of RT effects was surprising, so was the presence of cotinine effects on the memory task. Nicotine findings from learning and memory experiments are generally less reliable than findings of its effects on RT (see review in Chapter 1). Similarly, the pilot study results suggested effects of cotinine on RT, but not recall memory. The memory task used in the pilot study was different than the one used in the present task; the pilot study list length was not manipulated, subjects were given two trials rather than four, between the first and second trials only the words they had missed were presented again, and the words were read aloud instead of being presented visually. It is not obvious which of these differences would have lead to such different results for the two studies. The pilot study list length, at 17 words, was closer to the short list in the present study. The pilot study task was used because it had been used previously to demonstrate effects of scopolamine on memory (Brandeis et al., 1992). As other authors have noted, however, in nicotine studies effects are seen more often when longer lists are used, arguably because longer lists require greater sustained attention (Colrain et al., 1992; Warburton et al., 1992).

In the present study task different list lengths produced different results. On the long list 60 mg of cotinine significantly reduced the number of words recalled, relative to placebo, on analysis of all subjects. By contrast, on the same analysis for the short list, 60 mg showed no effect. Rusted and Eaton-Williams (1991), who used a similar task, found the same pattern of effect, though in the opposite direction, with nicotine--nicotine improved recall significantly for the long list, but not the short one. They interpreted this finding as indicating that nicotine improved attention rather than memory per se. They argued that they had previously manipulated stimulus exposure time (a measure of encoding difficulty) on word lists and found no nicotine effect (unpublished data).

In the present study, then, one could argue that cotinine, too, may primarily affect sustained attention, although by impairing rather than facilitating it. There are several findings that argue against an attentional interpretation, however. Cotinine did not interact with block (serial position); its effect was not greater for the items at the end of the long list, as Warburton et al. (1992) argue would be the case if it were affecting sustained attention. In fact, though not significant, from Figure 13 it appears as though cotinine had a greater effect at the beginning of the list, which Warburton et al. contend exhibits an effect on memory processing. The commonly-held view is that primacy items (early blocks) reflect long term memory involvement (see Klatsky, 1980). Therefore an effect of cotinine on the earliest blocks further suggests an effect on memory rather than attention.

The bidirectional effect of cotinine on the short list, depending on dose, also prevents a straightforward interpretation of an attentional effect. Figure 9 illustrates the improvement of short list recall at 30 mg, less improvement from 60 mg in the low dose subgroup, impairment from 60 mg in the high dose subgroup, and the most impairment at the 90 mg dose. (Subjects in the two subgroups did not have significantly different saliva cotinine levels at the 60 mg dose, so the differential effect of 60 mg was due to some undetermined difference between the subgroups.) For the long list, by contrast, as Figure 11 illustrates, all doses produced impairment of recall, although the effect was greatest at 60 mg. The correlational and NONMEM analyses showed cotinine effects to be significantly inversely related to saliva cotinine levels for the short list, but not the long list. This may be because with the short list there was a bigger difference in effect

from the 30 mg to the 90 mg dose (from average improvement to great impairment on average, at 90 mg, though the variability prevented this impairment from reaching significance on ANOVA). For the long list the dose effect was not linear; the impairment seen at all doses bottomed out at the 60 mg dose. The long list would also have created more variability, because as memory requirements increased, individual differences in memory abilities and strategies would have been more likely to hide drug effects. Only at 60 mg was the drug effect larger than the individual differences, hence, the significant ANOVA result for that dose.

There was no significant interaction with trial on either list. Thus, cotinine apparently does not change fatigue levels, since it does not change performance on Trial 4. If cotinine were affecting sustained attention, one might expect to see such a result. The lack of an interaction with trial also implies a lack of effect on the learning curve. As Figures 10 and 12 illustrate, however, much of the (significant) effect of 60 mg on the long list and 30 mg on the short list appear to occur on Trial 1, suggesting a nonsignificant slowing of acquisition of information on the long list and a nonsignificant speeding of acquisition on the short list. The first trial has also been interpreted as measuring "immediate memory span," with subsequent trials providing the learning curve (Durwen et al., 1992). In any case, sustained attention does not explain the results, and it is not obvious why cotinine should have different effects on the two lists.

Other than attention requirements, what is different about the two lists lengths? The most obvious distinction is difficulty. Perhaps for some reason cotinine impairs more difficult tasks while small doses improve and larger doses impair easier ones. This appears to be consistent with the findings of Kleinman et al. (1973), in which smoking deprivation improved performance in an easier verbal paired-associates learning task and impaired a more difficult version of the task. Those authors explain their results in terms of increased arousal (from anxiety) occurring during smoking deprivation, arguing that high arousal has differential effects on easier versus harder tasks. They did not provide any measures of arousal, however, and in the present study none of the measures of arousal (POMS, subjective impressions, heart rate, or blood pressure) were affected by cotinine.

Even though the task involved immediate recall, it is possible that the distinction between the long and short lists implies a difference in the involvement

of short-term (STM) versus long-term memory (LTM). Mewaldt et al. (1983) propose that, because STM has a limited capacity, as the length of a list is increased, the relative proportion of items that must be transferred into LTM for retention increases. Accordingly, if a drug impedes the transfer of information into LTM, leaving STM unaffected, they argue that drugged subjects would show proportionately larger deficits for each increment in list length. One might argue, then, that cotinine selectively impairs the transfer of information into LTM, because it significantly impairs performance only on the longer list. Consistent with this argument is the finding that 60 mg cotinine appeared to impair (though not significantly) primacy items (early blocks on the long list), which are said to reflect LTM, but not recency items (last block), which are said to reflect STM. Effects on the short list also suggest that not only does cotinine not impair STM, but it may improve it (at least at the 30 mg dose).

Rusted and Warburton (1989) postulate that immediate recall type tasks that differ in amounts of delay or processing loads tap into different aspects of immediate memory. They refer to the model of Baddeley and Hitch (1974) and Baddeley (1986), which describes separable components of working memory, responsible for distinct functions. The "central executive mechanism" is in charge of allocating resources for completion of mental operations that include sorting, holding, or analyzing information, and mediating the exchange of information between STM and LTM storage (see also Curran et al., 1991 for application of this model to interpret drug effects). Two "slave" systems: the "articulatory loop" for verbal material and the "visuospatial scratchpad" for nonverbal material, can perform very rapid tasks involving small amounts of information on their own. With greater processing loads, however, central executive resources must be allocated to the task. Pursuant to this view, one could argue that the shorter verbal recall list does not tap central executive functions to the same degree that the long list (with its heavier processing load) does. So cotinine might impair the central executive function (via the long list) at all doses, with differential effects, depending on dose, on the articulatory loop (via the short list). According to this idea, selective impairment of the central executive function is not reflected in list length differences per se; it could be demonstrated on the shorter list in a paradigm using concurrent secondary tasks, for example.

Either possibility--an effect of transfer of information to LTM, leaving STM intact or even improving it, or differential effects on the central executive versus articulatory loop functions of immediate working memory--argue against a non-specific, generalized drug effect on memory performance. These possibilities instead suggest selective effects, which could be investigated in future studies, using paradigms that dissociate specific memory components. The central executive versus articulatory loop tests just described is one example. It is particularly interesting, however, to note that Mewaldt et al. (1983) found results with the benzodiazepine diazepam that look very similar to the cotinine results seen here. As with cotinine, diazepam decreased immediate recall across all serial positions, and particularly the earlier blocks, but not the final block. Also, as with cotinine, they found more impairment with longer word lists--although they used several list lengths rather than just two. In addition, they found that diazepam showed even larger impairment relative to placebo on delayed recall, where there is no STM involvement. They interpreted these effects as indicating a decrement in the transfer of information from STM to LTM. Also, that there was no drug effect on recall of a pretest list further supported the idea of an impairment of acquisition of new information, not retrieval of information already in LTM.

As a further test of the idea that cotinine might selectively impair the process of STM-LTM transfer or consolidation, a variety of list lengths, delayed recall, and delayed recall of a pretest list could be employed, as was done in the Mewaldt et al. study. Increments in list length should then produce corresponding increments in cotinine effects. Delayed recall of pretest lists should not be affected by cotinine. Other manipulations suggested to affect LTM but not STM include rate of stimulus presentation, difficulty of words, and instructions for mental rehearsal of items. Beyond the comparison with diazepam, the use of tasks that tap STM and do not require LTM (rapid, immediate memory scan tasks), would clarify whether or not lower doses of cotinine benefit STM as is suggested by the 30 mg dose effect on the short list. Such attempts to pinpoint particular memory processes affected by cotinine, tested within the framework of specific memory models, would seem an appropriate next step, given the pattern of cotinine results seen here.

Comparison with Other Findings

It appears from these findings, then, that cotinine has specific effects on memory and a weak, generalized slowing effect on performance. These effects are not mediated by mood, physical sensations, or physiological measures, and they are apparently the same for men and women. Although there is no strong evidence for an attentional effect, the suggestion of an effect on N100 latency but not P300 latency, and the memory detriment by 60 mg on the long but not the short list, warn against the outright rejection of an attentional effect. Nonsignificant slowing by cotinine on the SERS task variables can be contrasted with those of nicotine. Le Houezec et al. (1994) demonstrated a speeding of RT and of P300 latency for the hardest task condition. Nicotine also showed a number of effects not seen (even in the opposite direction) with cotinine. For example, nicotine seems to change the speed-accuracy function, and cotinine apparently does not. Nicotine also significantly speeded heart rate, while cotinine had no physiological effects. Nicotine subjects could ascertain when they had received a drug instead of a placebo, whereas cotinine subjects could not. This comparison is analogous to animal work showing cotinine to be weaker than nicotine in effect (Kim et al., 1968) and to tend to impair performance while nicotine significantly enhances it (Brioni & Arneric, 1993). It is also consistent with Hughes et al. (1989), in which deprived smokers showed only a tendency to slow RT.

The significant effect of cotinine on memory, to the extent that it reflects an attentional decrement, may represent an opposing action to that of nicotine (see Rusted & Eaton-Williams, 1991). To the extent cotinine impairs immediate verbal recall, it is consistent with scopolamine (Rusted et al., 1991), benzodiazepines, such as diazepam and lorazepam (Curran, 1991), and mecamylamine, the nicotinic antagonist (Newhouse et al., 1992). One important contrast with scopolamine, however, is that in a task similar to the one used here with cotinine, scopolamine impaired both the short and long list to the same degree (Rusted & Eaton-Williams, 1991). Cotinine effects seem more consistent with those of diazepam, effects, although benzodiazepines have not been observed to improve recall on

shorter lists, as 30 mg cotinine did here. Any finding that cotinine impairs consolidation of LTM storage would be in the opposite direction of some recent nicotine findings (Colrain et al., 1992; Rusted & Warburton, 1992). It is unfortunate that studies of smoking deprivation have largely failed to employ verbal memory tasks. The Kleinman et al. (1973) study, described earlier, did show deprived smokers' improvement and impairment on an easy and hard verbal recall task, respectively. Congruency between their findings and cotinine results here is consistent with the idea that cotinine might contribute to tobacco withdrawal effects. Snyder et al. (1989) found a withdrawal effect of accuracy on the digit recall and arithmetic tasks, both of which involved utilization of working memory. Although these were more immediate memory tasks than those used in the present study, and, although they were nonverbal, showing withdrawal effects on memory supports the notion of cotinine involvement. A study with deprived smokers and nonsmokers given cotinine, employing a verbal recall task, would directly address that question.

Possible Physiological Mechanisms

The cognitive effects of cotinine observed here were not mediated by mood or by any sedating effect, since there were no effects on the POMS, heart rate, blood pressure, or subjective sensations. Unlike nicotine, cotinine is known to have poor affinity for cholinergic receptors (Abood et al., 1981), and cotinine effects in animals are not blocked by the nicotinic antagonist mecamylamine (Goldberg et al., 1989).

Fuxe et al. (1979) demonstrated that cotinine affects serotonin turnover rate and uptake and retention of serotonin more than does nicotine. Bowman et al. (1963) found cotinine to be active in the cerebellum, which contains serotonergic pathways. Interestingly, there is a growing body of evidence implicating cerebellar involvement in higher cognitive functions, specifically including language functions (Leiner et al., 1993) and spatial memory (Middleton & Strick, 1994). In addition to ACh abnormalities, Alzheimer's Disease also involves abnormal serotonin levels (see McEntee & Crook, 1991 and Patel & Tariot, 1991, for reviews). Serotonin manipulations have been shown to affect learning and memory in animal work

(McEntee & Crook, 1991) and with humans (Park et al., 1994). Serotonin and ACh appear to be functionally interactive, with an imbalance disrupting learning and memory (Markowska et al., 1991). In addition, nicotinic drugs can stimulate or suppress dopamine levels, which can facilitate or impair learning and memory (Levin et al., 1994). In some situations serotonin can reverse nicotinic effects on dopamine (Costall & Naylor, 1992). Taken together, there is enough indirect evidence to suggest a serotonergic mechanism for cotinine that could function to counteract nicotine effects, especially in the area of learning and memory. Disentangling cotinine from nicotine effects in order to test this idea in humans could be accomplished by testing nonaddicted "chippers" (smokers who regularly smoke a small number of cigarettes per day or week, who do not have chronically high cotinine levels as addicted smokers do) on both nicotine and cotinine.

Implications for Smokers and Concluding Remarks

The effects observed here with acute cotinine in nonsmokers imply the possibility of more significant cognitive effects in regular smokers, going through withdrawal, who have had chronically high levels of cotinine in their bodies, perhaps for many years. Chronic effects may be very different than acute effects. Nicotine effects on cognition have been much easier to demonstrate in smokers than in nonsmokers, so the subtle cotinine effects obtained here might be amplified if smokers were tested.

The doses used in this study produced higher saliva cotinine levels than those typically seen in regular smokers. Because we don't know how saliva cotinine relates to brain levels of cotinine, and because we don't know how acute dose effects relate to chronic ones, it is difficult to make inferences to actual effects in smokers. That Keenan et al. (1994) found significant mood effects by cotinine while the present study did not provides at least one example of differential effects of cotinine in smokers versus nonsmokers.

Because the slowing effects seen in the present study constitute a replication of the pilot study, and because they are consistent in direction and dose-related, an assumption that the effects are real seems justified. Presumably the inclusion of more subjects would have yielded more significant results. Clearly replication is

needed, with more subjects, with smokers and nonsmokers, and perhaps with the employment of some methods for further specifying memory effects, as discussed above.

Snyder and Henningfield (1989) and Snyder et al. (1989) demonstrated significant slowing of performance on RT tasks following 12 and 24-48 hours of smoking deprivation. That cotinine in nonsmokers showed weak but consistent slowing effects in the two studies reported here implies some contribution by cotinine to this effect. The significant effects of cotinine on verbal recall memory confirm that it is cognitively psychoactive. Given the difficulty so many people have in quitting smoking, it is surely worthwhile to investigate further the role of cotinine in tobacco withdrawal. It may be that smokers who are particularly sensitive to cognitive withdrawal symptoms might succeed in giving up their cigarettes if provided treatments, perhaps pharmacological, that counteract or modify the cognitive deficits produced by cotinine.

Beyond implications for smokers, we now have evidence that cotinine has not only general effects on information processing, but specific effects on memory, and we can do experiments that further clarify those effects. As has been done by some investigators with the use of ERPs and performance measures in RT paradigms to test effects on separable components of information processing, experimental methods for parsing memory into its hypothetical components can further our understanding of the role of cotinine and other drugs (and their transmitter systems) in human memory. Our paradigms can incorporate and test specific theoretical cognitive models. By integrating findings and ideas from neuropharmacology and cognitive psychology, the use of cotinine and other drugs can become powerful tools in the identification of specific cognitive processes involved in human memory and the biology behind them.

Bibliography

Abood, L.G., Reynolds, D.T., Booth, H., & Bidlack, J.M. (1981). Sites and mechanisms for nicotine's action in the brain. Neuroscience and Biobehavioral Reviews, 5, 479-486.

Andersson, K. (1975). Effects of cigarette smoking on learning and retention. Psychopharmacologia, 41, 1-5.

Andersson, K. & Hockey, G.R.J. (1977). Effects of cigarette smoking on incidental memory. Psychopharmacology, 52, 223-226.

Andersson, K., Jansson, A., Kuylenstierna, F., & Eneroth, P. (1993). Nicotine and its major metabolite cotinine have different effects on aldosterone and prolactin serum levels in the normal male rat. European Journal of Pharmacology - Environmental Toxicology and Pharmacology Section, 228, 305-312.

Ashton, H. Marsh, V.R., Millman, J.E., Rawlins, M.D., Telford, R. & Thompson, J.W. (1978). The use of event-related slow potentials of the brain as a means to analyze the effects of cigarette smoking and nicotine in humans. In Thornton, R.E. (Ed.), Smoking Behavior: Physiological and Psychological Influences, New York: Churchill Livingston, pp. 54-68.

American Psychiatric Association (1987). Diagnostic and Statistical Manual of Mental Disorders (Third edition, Revised). Washington D.C.

Baddeley, A.D. (1986). Working Memory, Oxford: Clarendon Press.

Baddeley, A.D. & Hitch, G.J. (1974). Working memory. In Bower, G. (Ed.), The Psychology of Learning and Motivation, Vol. 8, New York: Academic Press, pp. 47-89.

Beal, S.L. & Sheiner, L.B. (1990). NONMEM Users Guides, NONMEM Project Group, San Francisco: University of California, San Francisco.

Benowitz, N.L., Jacob, P.III., Denaro, C., & Jenkins, R. (1991). Stable isotope studies of nicotine kinetics and bioavailability. Clinical Pharmacology and Therapeutics, 49, 270-277.

Benowitz, N.L., Jacob, P.III., Jones, R.T., & Rosenberg, J. (1982). Interindividual variability in the metabolism and cardiovascular effects of nicotine in man. Journal of Pharmacology and Experimental Therapeutics, 221, 368-372.

Benowitz, N.L., Kuyt, F., Jacob, P.III., Jones, R.T., & Osman, A.L. (1983). Cotinine disposition and effects. Clinical Pharmacology and Therapeutics, 34, 604-611.

Biener, L. (1987). Gender differences in the use of substances. In Barnett, R.C., Biener, L., & Baruch, G.K. (Eds.), Gender and Stress, New York: The Free Press, pp. 330-349.

Brandeis, D., Naylor, H., Halliday, R., Callaway, E., & Yano, L. (1992). Scopolamine effects on visual information processing, attention, and event-related potential map latencies. Psychophysiology, 29, 315-336.

Brioni, J.D. & Arneric, S.P. (1993). Nicotinic receptor agonists facilitate retention of avoidance training: Participation of dopaminergic mechanisms. Behavioral and Neural Biology, 59, 57-62.

Callaway, E., Halliday, R., & Naylor, H. (1992). Cholinergic activity and constraints on information processing. Biological Psychology, 33, 1-22.

Callaway, E., Halliday, R., Naylor, H., & Schechter, G. (1985). Effects of oral scopolamine on human stimulus evaluation. Psychopharmacology, 85, 133-138.

Church, R.E. (1989). Smoking and the human EEG. In Ney, T. & Gale, A. (Eds.), Smoking and Human Behavior, Chichester, New York: John Wiley & Sons, pp. 115-140.

Colrain, I.M., Mangan, L., Pellett, O.L., & Bates, T.C. (1992). Effects of post-learning smoking on memory consolidation. Psychopharmacology, 108, 448-451.

Corrigal, W.A. (1991). Understanding brain mechanisms in nicotine reinforcement. British Journal of Addiction, 86, 507-510.

Costall, B. & Naylor, R.J. (1992). Serotonin and psychiatric disorders. Arzneim-Forsch/Drug Research, 42, 246-249.

Curran, H.V. (1991). Benzodiazepines, memory, and mood: A review. Psychopharmacology, 105, 1-8.

Curran, H.V., Shifano, F., & Lader, M. (1991). Models of memory dysfunction? A comparison of the effects of scopolamine and lorazepam on memory, psychomotor performance, and mood. Psychopharmacology, 103, 83-90.

Curvall, M., Elwin, C.E., Kazemi-Vala, E., Warholm, C., & Enzell, C.R. (1990). The pharmacokinetics of cotinine in plasma and saliva from non-smoking healthy volunteers. European Journal of Pharmacology, 38, 281-287.

Dominiak, P., Fuchs, G., von Toth, S., & Grobecker, H. (1985). Effects of nicotine and its major metabolites on blood pressure in anaesthetized rats. Klinische Wochenschrift 63, 90-92.

Donchin, E. (1979). Event-related potentials: A tool in the study of human information processing. In Begleiter, H. (Ed.), Evoked Potentials and Behavior, New York: Plenum Press, pp. 13-75.

Donchin, E., Karis, D., Bashore, T., Coles, M.G.H., & Gratton, G. (1986). Cognitive psychophysiology and human information processing. In Coles, M.G.H., Donchin, E., Porches, S. (Eds.), Psychophysiology: Systems, Processes, and Applications, New York: Guilford, pp. 244-267.

Dunne, M.P., MacDonald, D., & Hartley, R. (1986). The effects of nicotine on memory and problem solving performance. Physiology & Behavior, 37, 849-854.

Edwards, J.A., Wesnes, K., & Warburton, D.M. (1985). Evidence of more rapid stimulus evaluation following cigarette smoking. Addictive Behaviors, 10, 113-126.

Elgerot, A. (1978). Psychological and physical changes during tobacco abstinence in habitual smokers. Journal of Clinical Psychology, 34, 759-764.

Frearson, W., Barrett, P., & Eysenck, H.J. (1988). Intelligence, RT, and the effects of smoking. Personality and Individual Differences, 9, 497-517.

Fuxe, K., Everitt, B.J., & Hokfelt, T. (1979). On the action of nicotine and cotinine on central 5-Hydroxytryptamine neurons. Pharmacology, Biochemistry, and Behavior, 10, 671-677.

Goldberg, S.R., Risner, M.E., Stolerman, I.P., Reavill, C., & Garcha, H.S. (1989). Nicotine and some related compounds: Effects on schedule-controlled behavior and discriminative properties in rats. Psychopharmacology, 97, 295-302.

Gorrod, J.W. & Jenner, P. (1975). The metabolism of tobacco alkaloids. In Hayes, J. (Ed.), Essays in Toxicology, 6, New York: Academic Press, pp. 35-78.

Halliday, R., Naylor, H., Brandeis, D., Callaway, E., Yano, L., & Herzig, K. (1994). The effect of d-amphetamine, clonidine, and yohimbine on human information processing. Psychophysiology, 31, 331-337.

Hasenfratz, M., Michel, C., Nil, R., & Battig, K. (1989). Can smoking increase attention in rapid visual information processing during noise? Electrocortical, physiological, and behavioral effects. Psychopharmacology, 98, 75-80.

Hatsukami, D., Fletcher, L., Morgan, S., Keenan, R., & Amble, P. (1989). The effects of varying cigarette deprivation duration on cognition and performance tasks. Journal of Substance Abuse, 1, 407-416.

Heimstra, N., Bancroft, N., & De Kock, A. (1967). Effects of smoking upon sustained performance in a simulated drug task. Annals of New York Academy of Sciences, 142, 295-306.

Herning, R.I. & Pickworth, W.B. (1985). Nicotine gum improved stimulus processing during tobacco withdrawal. Psychophysiology, 22, 594 (Abstract).

Hillyard, S., Hink, R., Schwent, V., & Picton, T. (1973). Electrical signs of selective attention in the human brain. Science, 182, 177-180.

Hindmarch, I., Kerr, J.S., Sherwood, N. (1990). Effects of nicotine gum on psychomotor performance in smokers and nonsmokers. Psychopharmacology, 100, 535-541.

Houston, J.P., Schneider, N.G., & Jarvik, M.E. (1978). Effects of smoking on free recall and organization. American Journal of Psychiatry, 135, 220-222.

Hughes, J.R., Gust, S.W., Skoog, K., Keenan, R.M., & Fenwick, J.W. (1991). Symptoms of tobacco withdrawal--A replication and extension. Archives of General Psychiatry, 48, 52-59.

Hughes, J.R., Higgins, S.T., & Hatsukami, D. (1990). Effects of abstinence from tobacco. A critical review. In Kozlowski, L.T., Annis, H.M., Cappell, H.D., Glaser, F.B., Goodstadt, M.S., Israel, Y., Kalant, H., Sellers, E.M., Vingilis, E.R. (Eds.), Research Advances in Alcohol and Drug Problems, 10, New York: Plenum Press, pp. 319-398.

Hughes, J.R., Keenan, R.M., & Yellin, A. (1989). Effect of tobacco withdrawal on sustained attention. Addictive Behaviors, 14, 577-580.

Jaffe, H.H. (1990). Drug addiction and drug abuse. In Goodman, A.G., Rall, T.W., Nies, A.S., & Taylor, P. (Eds.), Goodman and Gilman's The Pharmacological Basis of Therapeutics, 8th Edition. New York: Pergamon Press, pp. 522-573.

Keenan, R.M., Hatsukami, D.K., Pental, P.R., Thompson, T.N., & Grillo, M.A. (1994). Pharmacodynamic effects of cotinine in abstinent cigarette smokers. Clinical Pharmacology and Therapeutics, 55, 581-590.

Kim, K.S., Borzelleca, J.F., Bowman, E.R., & McKennis, H. Jr. (1968). Effects of some nicotine metabolites and related compounds on isolated smooth muscle. The Journal of Pharmacology and Experimental Therapeutics, 161, 59-69.

Klatsky, R.L. (1980). Human Memory: Structures and Processes, Second Edition. San Francisco: W.H. Freeman and Company.

Kleinman, K.M., Vaughn, R.L., & Christ, T.S. (1973). Effects of cigarette smoking and smoking deprivation on paired-associate learning of high and low meaningful nonsense syllables. Psychological Reports, 32, 963-966.

Knott, V.J. (1985). Effects of tobacco and distraction on sensory and slow cortical evoked potentials during task performance. Neuropsychophysiology, 13, 136-140.

Knott, V.J. (1989). Brain event-related potentials in smoking performance research. In Ney, T. & Gale, A. (Eds.), Smoking and Human Behavior, Chichester, New York: John Wiley & Sons, pp. 93-114.

Kutas, M., McCarthy, G., & Donchin, E. (1977). Augmenting mental chronometry: The P300 as a measure of stimulus evaluation time. Science, 197, 792-795.

Le Houezec, J. & Benowitz, N.L. (1991). Basic and clinical pharmacology of nicotine. Clinics in Chest Medicine, 12, 681-699.

Le Houezec, J., Halliday, R., Benowitz, N.L., Callaway, E., Naylor, H., & Herzig, K. (1994). A low dose of subcutaneous nicotine improves information processing in nonsmokers. Psychopharmacology, 114, 628-634.

Leiner, H.C., Leiner, A.L., & Dow, R.S. (1993). Cognitive and language functions of the cerebellum. Trends in Neuroscience, 16, 444-447.

Levin, E.D., Briggs, S.J., Christopher, N.C., & Auman, J.T. (1994). Working memory performance and cholinergic effects in the ventral tegmental area and substantia nigra. Brain Research, 657, 165-170.

Levine, J.M., Kramer, G.G. & Levine, E.N. (1975). Effects of alcohol on human performance: An integration of research findings based on abilities classification. Journal of Applied Psychology, 60, 285-293.

Luck, S.J., Heinze, H.J., Mangun, G.R., & Hillyard, S.A. (1990). Visual event-related potentials index focused attention without bilateral stimulus arrays: II. Functional dissociation of P1 and N1 components. Electroencephalography and Clinical Neurophysiology, 75, 528-542.

Magliero, A., Bashore, T.R., Coles, M.G.H., & Donchin, E. (1984). On the dependence of P300 latency on stimulus evaluation processes. Psychophysiology, 21, 171-186.

Markowska, A.L. & Wenk, G.L. (1991). Serotonin influences the behavioral recovery of rats following nucleus basalis lesions. Pharmacology, Biochemistry, and Behavior, 38, 731-737.

McCarthy, G. & Donchin, E. (1981). A metric for thought: A comparison of P300 latency. Science, 211, 77-80.

McEntee, W.J. & Crook, T.H. (1991). Serotonin, memory, and the aging brain. Psychopharmacology, 103, 143-149.

Mewaldt, S.P., Hinrichs, J.V., & Ghoneim, M.M. (1983). Diazepam and memory: support for a duplex model of memory. Memory and Cognition, 11, 557-564.

Michel, C. & Battig, K. (1989). Separate and combined psychophysiological effects of cigarette smoking and alcohol-related consumption. Psychopharmacology, 97, 65-73.

Michel, C., Nil, R., Buzzi, R., Woodson, P.P., & Battig, K. (1987). Rapid information processing and concomitant event-related brain potentials in smokers differing in CO absorption. Neuropsychobiology, 17, 161-168.

Middleton, F.A. & Strick, P.L. (1994). Anatomical evidence for cerebellar and basal ganglia involvement in higher cognitive function. Science, 266, 458-461.

Naylor, H., Halliday, R., Callaway, E. (1993). Biochemical correlates of human information processing. In Vernon, P.A. (Ed.), Biological Approaches to the Study of Human Intelligence, Norwood, New Jersey: Ablex, pp. 333-373.

Newhouse, P.A., Sunderland, T., Tariot, P.N., Blumhardt, C.L., Weingartner, H., Mellow, A., & Murphy, D.L. (1988). Intravenous nicotine in Alzheimer's Disease: A pilot study. Psychopharmacology, 95, 171-175.

Nil, R. & Battig, K. (1989). Smoking behavior: A multivariate process. In Ney, T. & Gale, A. (Eds.), Smoking and Human Behavior, Chichester, New York: John Wiley & Sons, 199-221.

Park, S.B., Coull, J.T., McShance, R.H., Young, A.H., Sahakian, B.J., Robbins, T.W., & Cowen, P.J. (1994). Tryptophan depletion in normal volunteers produces selective impairments in learning and memory. Neuropharmacology, 33, 575-588.

Parrott, A.C. & Craig, D. (1992). Cigarette smoking and nicotine gum (0, 2, and 4 mg): Effects on four visual attention tasks. Neuropsychobiology, 25, 34-43.

Parrott, A.C. & Winder, G. (1989). Nicotine chewing gum (2mg, 4mg) and cigarette smoking: Comparative effects on vigilance and heart rate. Psychopharmacology, 97, 257-261.

Patel, S. & Tariot, P.N. (1991). Pharmacologic models of Alzheimer's Disease. The Psychiatric Clinics of North America, 14, 287-308.

Pomerleau, O., Fertig, J., & Shanhan, S. (1983). Nicotine dependence in cigarette smoking: An empirically-based, multivariate model. Pharmacology, Biochemistry, and Behavior, 19, 291-299.

Risner, M.E., Goldberg, S.R., Prada, J.A., & Cone, E.J. (1985). Effects of nicotine, cocaine, and some of their metabolites on schedule-controlled responding by beagle dogs and squirrel monkeys. The Journal of Pharmacology and Experimental Therapeutics, 234, 113-119.

Ritter, W., Simson, R., & Vaughn, H.G. Jr. (1983). ERP correlates of two stages of information processing in physical and semantic discrimination tasks. Psychophysiology, 20, 168-179.

Ritter, W., Simson, R., Vaughn, H.G. Jr., & Macht, M. (1982). Manipulation of ERP manifestations of information processing stages. Science, 218, 904-911.

Russell, M.A.H., Peto, J., & Patel, U.A. (1974). The classification of smoking by factorial structure of motives. Journal of Royal Statistical Society Series A: General, 137, 313-346.

Rusted, J. & Eaton-Williams, P. (1991). Distinguishing between attentional and amnestic effects in information processing: The separate and combined effects of scopolamine and nicotine on verbal free recall. Psychopharmacology, 104, 363-366.

Rusted, J. & Warburton, D.M. (1989). Cognitive models and cholinergic drugs. Neuropsychobiology, 21, 31-36.

Rusted, J. & Warburton, D.M. (1992). Facilitation of memory by post-trial administration of nicotine: Evidence for an attentional explanation. Psychopharmacology, 108, 452-455.

Sanders, A.F. (1980). Stage analysis of reaction processes. In Stelmach, G.F. & Requin, J. (Eds.), Tutorials in Motor Behavior, Elsevier: North-Holland, pp. 331-353.

Schneider, N.J., Jarvik, M.E., & Forsythe, A.B. (1984). Nicotine vs. placebo gum in the alleviation of withdrawal during smoking cessation. Addictive Behaviors, 9, 149-156.

Snyder, F.R., Davis, F.C., & Henningfield, J.E. (1989). The tobacco withdrawal syndrome: Performance and decrements assessed on a computerized test battery. Drug and Alcohol Dependence, 23, 259-266.

Snyder, F.R. & Henningfield, J.E. (1989). Effects of nicotine administration following 12 hours of tobacco deprivation: Assessment of computerized performance tasks. Psychopharmacology, 97, 17-22.

Sternberg, S. (1969). The discovery of processing stages: Extensions of Donders' method. In Koster, W.G. (Ed.), Acta Psychologica, 30, Attention and Performance II., Elsevier: North-Holland.

Stolerman, I.P. (1991). Behavioural pharmacology of nicotine: Multiple mechanisms. British Journal of Addiction, 86, 533-536.

United States Department of Health and Human Services (1988). The Health Consequences of Smoking: Nicotine Addiction. A Report of the Surgeon General. Rockville, Maryland.

United States Department of Health and Human Services (1989). Reducing the Health Consequences of Smoking: 25 Years of Progress. A Report of the Surgeon General. Rockville, Maryland.

Warburton, D.M., Rusted, J.M., & Fowler, J. (1992). A comparison of the attentional and consolidation hypotheses for the facilitation of memory by nicotine. Psychopharmacology, 108, 443-447.

Warburton, D.M. & Wesnes, K. (1985). Historical overview of research on cholinergic systems and behavior. In Singh, M.M., Warburton, D.W., & Lal, H. (Eds.), Central Cholinergic Mechanism and Adaptive Dysfunctions. New York: Plenum Press, pp. 2-35.

Warburton, D.M., Wesnes, K., Shergold, K., & James, M. (1986). Facilitation of learning and state dependency with nicotine. Psychopharmacology, 89, 55-59.

Wesnes, K. & Revell, A. (1984). The separate and combined effects of scopolamine and nicotine on human information processing. Psychopharmacology, 84, 5-11.

Wesnes, K. & Warburton, D.M. (1978). The effects of cigarette smoking and nicotine tablets upon human attention. In Thornton, R.E. (Ed.), Smoking Behaviour. Physiological and Psychological Influences. Edinburgh: Churchill Livingstone, pp. 131-147.

Wesnes, K. & Warburton, D.M. (1984a). The effects of cigarettes of varying yield on rapid information processing performance. Psychopharmacology, 82, 338-342.

Wesnes, K. & Warburton, D.M. (1984b). Effects of smoking on rapid information processing performance. Neuropsychobiology, 9, 223-229.

Wesnes, K., Warburton, D.M., & Matz, B. (1983). Effects of nicotine on stimulus sensitivity and response bias in a visual vigilance task. Neuropsychobiology, 9, 41-44.

West, R. & Hack, S. (1991). Effect of cigarettes on memory search and subjective ratings. Pharmacology, Biochemistry, & Behavior, 38, 281-286.

West, R.J. & Jarvis, M.J. (1986). Effects of nicotine on finger tapping rate in nonsmokers. Pharmacology, Biochemistry, & Behavior, 25, 727-731.

Yamamoto, K.I. & Domino, E.F. (1965). Nicotine-induced EEG and behavioral arousal. International Journal of Neuropharmacology, 4, 359-373.

Zeidenberg, P., Jaffe, J.H., Kanzler, M., Levitt, M.D., Langone, J.J., & Van Vunakis, H.N. (1977). Cotinine levels in the blood during cessation of smoking. Comprehensive Psychiatry, 18, 93-101.

Appendix A
Pilot Study Memory Task Lists

List 1.	List 2.	List 3.	List 4.	List 5.
blood	colony	ticket	leader	skin
hotel	factory	meat	diamond	market
machine	student	string	author	beast
table	tree	corn	valley	friend
lip	master	industry	cabin	arm
slave	dress	flag	girl	soil
dust	mountain	window	railroad	flood
child	product	dollar	person	engine
woods	cat	vessel	storm	palace
committee	judge	boulder	circle	artist
sea	book	hide	hospital	world
butter	winter	brain	ocean	metal
furniture	contract	shoes	coffee	house
steam	water	clock	mother	convention
kiss	gentleman	king	chair	wine
avenue	air	journal	green	plant
cotton	home	potato	wheat	bottle

(continued)

List 6.	List 7.	List 8.	List 9.	List 10.
newspaper	apple	chief	paper	forest
seat	material	lake	ship	boy
baby	horse	board	flower	professor
letter	building	temple	grandmother	shore
flesh	gift	lad	college	wife
street	corner	cattle	pipe	tower
woman	bar	troops	meadow	fire
river	poet	city	cash	instrument
vegetable	sky	fur	animal	army
owner	disease	magazine	car	plain
star	coast	sugar	queen	creature
hall	door	library	property	cottage
earth	clothing	gold	pencil	tool
teacher	garden	square	toy	forehead
pole	coin	bird	prisoner	candidate
grass	body	village	church	party
money	pupil	iron	bowl	doctor

Appendix B
Present Study Memory Task Word Lists

Short Lists

List 1.	List 2.	List 3.	List 4.
tractor	turtle	pineapple	cinnamon
fish	biscuit	blanket	bubble
dancer	quilt	flute	nurse
animal	photograph	mug	lake
paint	engine	road	car
leaf	nightgown	forest	boulder
pearl	artist	silver	whistle
apple	book	berry	lunch
ocean	hair	cloud	necklace
balloon	moose	squirrel	flower
hockey	pants	novel	glasses
iris	mixer	wife	pup
kid	queen	fawn	sleigh
jeep	trout	satin	gym
aunt	cotton	limb	woman

(continued)

List 5.	List 6.	List 7.
shoe	cafe	coin
kite	bath	movie
cabin	nickel	tulip
meat	valley	mouth
orchestra	carrot	horse
candle	lamp	walnut
crown	fur	sunset
sofa	lettuce	peach
kitten	apartment	library
mansion	nose	butter
uncle	mermaid	sea
yacht	pot	station
spruce	icecream	cocktail
smile	paws	wheat
liquor	suede	hawk

(continued)

Long Lists

List 1.	List 2.	List 3.	List 4.
professor	herb	king	ship
cereal	wheel	sugar	tennis
mattress	trumpet	sparrow	orchid
wing	quail	birch	banana
ball	park	owl	moccasin
willow	clown	telescope	pencil
pipe	soil	yard	dress
burro	camera	tooth	rock
jewel	boat	palm	cat
river	stew	coast	nest
hand	candy	drum	doughnut
basketball	beach	lemonade	surf
rose	raspberry	sail	wood
calf	band	pajamas	pudding
lawn	daffodil	lamb	blossom
globe	rocket	bread	rabbit
seed	grape	gown	room
soda	glove	cottage	stove
quarter	magician	shrimp	diamond
soup	lime	world	duck
velvet	fox	crystal	rug
stone	choir	knight	map
orange	hotel	cream	deer
cruiser	bouquet	shoulder	acrobat
mint	chipmunk	tent	head
trip	camp	letter	moon
flame	wallet	ruby	circus
roof	soccer	cranberry	chili
bass	princess	lion	beard
strawberry	leather	chapel	tongue

List 5.	List 6.	List 7.
earth	farm	heart
coffee	print	cloth
zoo	honey	jello
frost	skirt	palace
pie	lantern	grapefruit
sand	gift	bell
crayon	house	rice
juice	team	children
village	pickle	violin
elm	carnation	sandal
train	key	bluejay
boy	tape	light
gallery	china	sailboat
couch	eagle	pet
bird	lily	magazine
earring	face	fruit
lemon	tangerine	sheets
tool	brain	ham
spice	gem	whale
daisy	priest	emerald
marshmallow	neck	cheeks
blouse	pear	pony
plane	supper	bracelet
robin	parade	date
chocolate	chest	volleyball
phone	soap	tiger
butterfly	organ	ski
baseball	screwdriver	pine
oak	football	toy
clover	saxophone	gold