PHILIP MORRIS U. S. A.

INTER-OFFICE CORRESPONDENCE

RICHMOND, VIRGINIA

CONFIDENTIAL

To: Dr. T. S. Osdene

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From: W. L. Dunn

Subject: Behavioral Research Accomplishments - 1980

EXPERIMENTAL PSYCHOLOGY PROGRAM [Written by Frank Ryan]

1. Two Studies of the Effects of Smoking on Salivary Flow....

In the first study we found that smoking by itself, whether high or low delivery, regular or menthol cigarettes were used, increased salivation dramatically compared to a non-smoking situation. Part of the increase was attributable to the act of puffing (smokers dry puffing unlit cigarettes increased their flow about half as much as when smoking) and part was due to unknown smoke components.

In the second study we examined the effects of WS cigarettes on saliva flow, and found the WS cigarettes behaved like regular or menthol, neither enhancing nor inhibiting salivation more than other cigarettes.

2. A Basic Study of the Smoker's Ability to Detect Differences in Nicotine Delivery at the Marlboro Tar Level (Last Year We Had Done Such a Study at the Merit Level)....

An anomalous response, in which a very small difference was easier to detect than a large difference, was observed. Although it is possible that further analysis may make the observation understandable, we are ready to write off the study's results as "unclear and confusing".

3. The Gas Chromatographic Analysis of Salivary Nicotine....

Preliminary data are suggesting that saliva nicotine concentration increases, then decreases, following smoking, with the peak concentration being somewhere between 5 and 15 minutes following smoking.

4. The Annual Monitoring of Cigarette Acceptability....

The fourth annual monitoring of cigarette acceptability was conducted, yielding results similar to the first three testings. Smokers give higher acceptability ratings to cigarettes most like their own brand (in delivery) and lower ratings to cigarettes which differ from their own brand.

5. Subjective Rod Firmness....

A trained panel evaluated the firmness of cigarette rods along a rating scale from 20= mushy, to 40= very firm. The panel's firmness judgments of 9 different cigarettes were then compared to compacimetric firmness scores and data from Chris Irving's firmness-while-smoking (FWS) machine. The FWS machine yielded correlations of -.99 with the raters judgments, while the compacimeter correlated -.88 with the raters. There is little doubt that the FWS machine, and to a slightly lesser extent the compacimeter, measure the same thing humans measure when they evaluate firmness.

THE INHALATION MONITORING PROGRAM [Written by Jan Jones]

1. Instrumentation....

During the first 2 quarters of 1980 our instrumentation for monitoring inhalation patterns restricted us to laboratory research. Tests were conducted using the respitrace transducer to compare several calibration procedures, to assess the accuracy of the system, and to select the parameters of smoke-laden inhalation patterns which will be our dependent variables. We were able to isolate and control several extraneous variables such as tunic slippage, positioning of the coils on the torso, and restricted movement caused by tight garments.

The mobile Medilog system for monitoring inhalation patterns arrived in June of 1980. Because this was the first unit of its kind on the market, tests of the system's capabilities were initiated, followed by procedural refinements. We evaluated the system's ability to record respiration data as a subject varies body position and performs various normal daily activities (walking, reaching, bending, etc.). The apparatus was found to automatically readjust after abrupt baseline shift to produce an uninterrupted representation of the respiration patterns accompanying physical activity. Based on the results from pilot research we were able to refine the procedure for applying and calibrating the instrumentation. We have determined that our present procedure yields calibration values which are reliable throughout a day and across days for a given individual, and that the calibration factors accurately represent the relative contributions of rib cage and abdomen to the change in total inhalation volume.

2. System Accuracy....

A test of system accuracy was run for the respitrace transducer unit in the first quarter of 1980, and for the mobile Medilog system subsequent to its arrival. We were interested in determining the correlation between the volumes measured by our calibrated instrumentation and the known volumes of a referent.

Volumes measured by the respitrace transducer were compared with those of the same breaths simultaneously measured on a spirometer. Results showed a consistently high correlation, with a mean of +0.94 (±0.056 s.d.). The accuracy of the calibrated Medilog ambulatory unit was derived by comparing respiratory volume, as measured by excursions of the respibands, with a closed-system referent of 1.28 liters. Results indicated an error of less than 5% on the average. Overall the accuracy of the system appears to be highly satisfactory.

3. Criteria Selection for Stable Baseline Behavior....

Ongoing research involves a study of inhalation patterns on a smoker's own cigarette. Our immediate interest is in establishing valid and reliable criteria for determining when a subject's inhalation patterns have stabilized. We are currently looking at data on the normal variability in baseline behavior over time, which wil be used for establishing criteria for stabilization.

THE BEHAVIORAL PHARMACOLOGY PROGRAM [Written by Victor DeNoble]

1. Nicotine Self-Administration....

A major objective of the behavioral research program is to evaluate the reinforcing properties of nicotine and nicotine analogues. This is being accomplished by utilizing intravenous self-administration techniques. Intravenous self-administration has proven to be one of the most useful and powerful techniques devised for compound evaluation and comparison. This is a critical test for the nicotine analogues because it has been shown that reinforcing efficacy does not always correlate with interoceptive generalization.

Rats were prepared with an indwelling venous catheter made of siliconized rubber. The catheter was anchored in the external jugular vein and passed subcutaneously until it exited through the animals back. This was connected via protective tubing and swivel joints to a remote injection pump. Responding on one arbitrarily selected response lever was automatically programmed to activate the injection pump for 6 seconds, delivering an injection of 0.137ml of solution directly into the animals blood stream. Responses on the control lever were recorded but had no programmed consequence.

Nicotine self-administration was initially established at 32ug/kg/injection. Nicotine was available 24 hours per day, under a Fixed Ratio 1 (FR-1) schedule (the injection/response ratio is fixed at one to one). Generally 10 to 14 sessions are necessary for responding to stabilize. After stable behavior is obtained, changes are made in the nicotine delivery procedure to determine if lever pressing is maintained by the contingency

established between lever pressing and nicotine delivery. Changes include substitution of saline for nicotine, reversal of nicotine lever and control lever functions, and automatic non-contingent nicotine injections. The results from a small number of animals show that nicotine self-administration by rats is maintained by the response-nicotine contingency, rather than by other behavioral effects of the drug. Substitution of saline for nicotine failed to maintain responding. When nicotine (32ug/kg/injection) was reintroduced, the number of injections rose to previous levels. During the self-administration, responding occurred almost entirely on the lever delivering nicotine. Control lever responses were less than 10% of the total number of responses. When nicotine injections were delivered non-contingently responding decreased as a function of the frequency of the non-contingent injection.

These results show clearly that nicotine can function as a positive reinforcer for rats.

We also investigated the effects of varying dose on response rate and nicotine intake. Injection doses were presented in descending order (64,32,16,8, 4, and 2 ug/kg/injection). Rats were tested for a minimum of 7 days at each dose. The result shows that as the dose of nicotine was decreased from 64 to 4 ug/kg/injection, the response rate increased. The mg/kg/intake was highest at 64 ug/kg/ injection (2.5mg/kg/day) and decreased with decreasing doses.

2. Effects of Chronic Nicotine Administration and its Termination on Behavior Maintained Under a Multiple Fixed Ratio - Fixed Interval Schedule of Reinforcement...

There is considerable disagreement among researchers as to whether the termination of chronic nicotine administration produces a withdrawal syndrome. Data accumulated from one of our studies indicated that termination of chronic nicotine self- administration produced only a mild transient alteration in ongoing behavior. It is our belief that the magnitude of change observed on nicotine cessation reflects behavioral adjustments to the removal of a positive reinforcer, not a withdrawal syndrome. In order to further investigate this phenomenon we have initiated a study in which the effects of nicotine administration and its termination are being assessed by a rat's performance on a complex multiple Fixed Ratio Fixed Interval schedule. Changes in performance under this schedule reflect changes in CNS integrity. Therefore by using this schedule it may be possible to assess changes, if any, that occur in the CNS upon termination of chronic nicotine administration.

3. Prostration Syndrome...

The laboratory has been able to demonstrate the prostration syndrome reliably with both (-) nicotine and (+)-nicotine, with the latter being about 1/10 to 1/20 as active. Having gained a reliable data base with both (-)-nicotine and (+)-nicotine, it is now essential to examine relative potency of the nicotine analogues, most importantly analogues that have been shown to be nicotine-like in the discrimination tests. In addition, studies to locate sites of action and to determine the extent of the behavioral prostration are now in progress. Even without detailed knowledge of the underlying physiological mechanisms, the use of behavioral measures will provide important information about the sites of action in the brain. We are currently using scheduled controlled behavior to evaluate the effects of intraventricular injections of nicotine. Schedule-controlled behavior is a research technique that is based upon principles of operant conditioning. This technique produces a highly stable and reproduceable baseline of behavior which has been shown to be dependent on the integrity of the CNS. (DeNoble & Begleiter, 1976, DeNoble & Caplan, 1977, Bowman, 1980, Mele and Caplan, 1980).

Using schedule-controlled behavior as a measure, rats continued to display behavioral disruptions 10-12 minutes post infusions. Observation of these animals via a one-way mirror revealed that typical locomoting and grooming behaviors were displayed 5 to 7 minutes before recovery under the FR schedule. The duration of suppression in response rate was approximately 110% longer than that observed with Dr. Abood's scale where recovery from the prostration effects occurs 3-5 minutes post infusion.

Electroencephalographic recordings taken by Dr. Abood after intraventricular injections of nicotine into rats have demonstrated that recovery of baseline hippocampal activity occurs 10-12 minutes post infusion. These latencies in conjunction with the latencies found in the schedule-controlled behavioral task demonstrate that prolonged CNS changes are taking place.

As a continuation of that study these animals were then tested with double their original dose of ℓ-nicotine (5ug to 10ug in 5ul). The time between infusions for all animals was no less than 7 days and daily response rate showed less than a 15% variance from day to day. Surprisingly, the duration of the suppression in response rate produced by the infusion of 10ug of ℓ-nicotine was shorter than that produced by 5ug of ℓ-nicotine. There are several possible explanations of this result which we are now investigating.

We demonstrated that 2'-methylnicotine was behaviorally active in discrimination and prostration tests and is equally as potent

as (-)-nicotine. Using Dr. Abood's rating scale, 2'-methylnicotine did not produce some of the peripheral signs of prostration (less motor control loss, no hyperventilation, no piloerection, no excess urination or defecation). In view of these findings, we tested 2'-methylnicotine using scheduled controlled behavior. Under FR 32, the rats were tested first with 5ug of ℓ-nicotine in 5ul then 5 days later with 5ug of 2'-methylnicotine in 5ul. The data shows that the 2'-methylnicotine produced less suppression of response rate under the FR 32 schedule. This result could reflect 2'-methylnicotine's cross tolerance to (ℓ)- nicotine and/or diminished peripheral effects. In the next series of studies we will separate these events.

In the future we will be conducting studies in which the selective blockade of neural structures will be evaluated in terms of the behavioral components of prostration. We think that this will provide evidence for the sites of action of nicotine and nicotine analogues.

4. Discrimination Studies....

As part of the ongoing nicotine discrimination program, we have completed initial testing on a series of additional compounds. These compounds included various dialkylaminomethylpyridines, metanicotine, its dihydro derivative, and several isomeric nicotines.

Addition of the dimethylaminomethyl substituent to the pyridine ring in the third position produced "nicotine-like" responses from the animals whereas the isomeric 2- and 4- substituted compounds gave no indication of activity. The 3-pyridyl derivatives yielded "nicotine" responses in 55% of the animals at a 4.0 mg/kg dose. (This level represents 10x that of the training dose used in the nicotine discrimination task.) When the dose was increased to 8.0 mg/kg, 4 animals responded on the nicotine correct lever and 3 animals gave incomplete tests.

Metanicotine, unlike its dihydro derivative, showed nicotinic activity in the animals tested. Apparently, the unsaturation in the side chain, in this compound, is necessary to produce activity, since metanicotine at a dose of 4.0mg/kg produced nicotine cues in approximately half of the animals. When the dose was increased to 8.0mg/kg, 100% of the animls responded on the nicotine correct lever.

Of the isonicotine derivatives, the 3,3'substituted N- methylpyrrolidine showed activity while the 2,3-isonicotine and the 4,3'- isonicotine did not. At a dose only 5x that of the normal discrimination training dose of (-)-nicotine (2.0 mg/kg), the 3,3'- isonicotine was active in 9 out of 11 animals. When the

dose was reduced to 1.0 mg/kg, 63% of the animals tested responded with the nicotine correct lever.

A series of open-chain nicotines were synthesized. A methylethylaminomethyl substituent or a diethylaminoethyl substituent was added to the pyridine ring in the third position. Animals injected with a wide range of doses of each compound did not respond on the nicotine correct lever. However, one open-chain compound, 3-dimethylaminomethyl pyridine, did produce behavioral activity. At a 4.0 mg/kg/body weight dose, 75% of the animals responded on the nicotine correct lever. Only 40% of the animals tested at a dose of 2.0 mg/kg/body weight emitted a nicotine response. Note that these doses are 5 to 10 times higher than the daily dose of (-)-nicotine used in the discrimination task.

The most interesting finding was with the 2'-methylnicotine. At a 0.4 mg/kg/body weight dose, 100% of the animals tested responded on the nicotine correct lever. This is the same dose used during the daily nicotine training sessions. Doses of the 2'-methylnicotine higher than 0.4 mg/kg/body weight produced incomplete responding.

Presently, we are preparing to do a dose-response curve using the 2'-methylnicotine. A dose response curve will allow us to assess 2'-methylnicotine's relative potency to dℓ-nicotine and ℓ- nicotine. The effectiveness of 2'-methylnicotine in the discrimination task is now being tested with preinjections of mecamylamine and hexamethonium. These results should indicate whether the discrimination of the 2'-methylnicotine is centrally or perpherally mediated.

THE PSYCHOSOCIAL PROGRAM [Written by Sandra Dunn]

The first efforts toward developing a social psychology program disclosed that current explanations of the influence of psychosocial factors on smoking behavior are limited. Therefore, it was decided the program's initial project would be an exploratory study to estalish a data base for the ultimate development of a generalized model of psychosocial factors and smoking behavior. In order to develop this model it was proposed to examine five classes of variables, all suggesting social psychological correlates of smoking behavior as obtained from the scientific literature.

An instrument was developed for measuring these five variable classes. This instrument, which utilizes a two hour face-to-face interview format, consists of over 200 structured and open ended questions.

A sample of 100 white college educated females, 45 years old and over was obtained for the study. Fifty of the sample were required to smoke cigarette brands delivering less than 6 mg. of tar and the other 50 were required to smoke cigarette brands delivering more than 15 mg. of tar. This sample was selected for the project because these women represented the demograph group identified as the most frequent consumers of ultra-low delivery brands. It is believed that the psychosocial factors influencing smoking behavior are most operative among smokers of ultra-low delivery brands since for these smokers nicotine seeking is most likely not the primary motivation for smoking.

Subject recruitment was a major effort. Thirty-one of the required one hundred participants were obtained through oral and written solicitation of women's organizations in the Richmond metropolitan area and through the use of the services of a local marketing research firm. The remaining participants were obtained by canvassing and screening by mail POL panelists in the Northern Virginia area where the market penetration by ultra-low delivery brands is higher than it is in Richmond.

Interview sites, including all necessary facilities, were acquired and interviewing completed in both Metropolitan Richmond and Northern Virginia.

Work is currently underway in the coding and reduction of the data in preparation for the data analyses.

THE ELECTROPHYSIOLOGY PROGRAM [Written by Frank Gullotta]

I. The Effects of Cigarette Smoking on Auditory Evoked Potentials....

We have previously demonstrated that cigarette smoking augments the amplitudes of the late components of the visual evoked potential to repeated flash stimulation. These findings are consistent with the notion that smoking acts as a generalized central nervous system (CNS) stimulant.

If, indeed, cigarette smoking produces generalized CNS arousal, one would predict that other evoked potentials would also be augmented. Therefore, we tested this hypothesis employing auditory evoked potentials to pure tone stimulation.

We found that, although smoking did not enhance auditory evoked potentials, it did protect them against the normal tendancy of the response to habituate. This suggests that either cigarette smoking is having selective rather than generalized effects upon the CNS, or that the auditory system is less influenced by the stimulant effects of centrally active smoke constituents.

2. The Effects of Cigarette Smoking on the Electroencephalogram....

A number of studies have investigated the effects of cigarette smoking and/or nicotine administration on the electroencephalogram of man and other animals. The results of these investigations, however, have not been consistent. This has been particularly true with regard to the human experiments. We have proposed that a more systematic analysis of the human EEG under varying combinations of smoking and deprivation conditions might yield valuable and reliable results. We have undertaken such studies employing spectral analysis techniques.

Our data, although quite preliminary, indicate that cigarette smoking produces differential effects on EEG power spectra, which depend upon degree of deprivation, nicotine delivery and electrode location.

3. Cigarette Smoking and Pattern Reversal Evoked Potentials....

We have nearly completed a study on the effects of cigarette smoking on the evoked potential to pattern simultation (PREP). PREPs, as oposed to evoked potentials to flash, primarily activate visual cortex. Hence, this technique allows us to study the effects of smoking on visual information processing.

Our data collection has nearly been completed and some tentative conclusions can be drawn. Smoking appears to decrease the latency of the principal component of the PREP. Furthermore, it appears to do so in a deprivation/nicotine dependent manner. These data, then, suggest that cigarette smoking quickens the rate at which the visual system processes information.

4. Cigarette Smoking and the Brainstem Auditory Evoked Potential....

We have begun to assess the possible influences of cigarette smoking on a new type of response -- the brainstem auditory evoked potential (BAEP). The advantage of the BAEP relative to cortical evoked potentials is that the neural generators of the response are better known.

The BAEP contains seven components, or peaks, which represent neural activity from the level of the receptor up to level of the diencephalon. Changes in any of these components following smoking would provide information regarding central nervous system sites of action of smoke constituents.

A recent paper demonstrated alterations in BAEP peaks following systemic nicotine adminstration in rats. It is important to know whether such changes also occur following smoking.

To date, we have been developing our recording techniques and have been conducting preliminary investigations. We are not yet in a position to determine whether smoking affects the BAEP. This information should be available by mid 1981.

5. Habituation of Pattern Reversal Evoked Potentials....

It is commonly reported that cigarette smoking facilitates one's ability to concentrate. Concentration implies sustained attention to stimulation. We are interested in the possibility that we might gain insight into the process involved by employing evoked potential techniques.

When, within a given session, sensory evoked potentials are repeatedly measured, there is a decrement in the response over trials. We interpret this decrement as a decrease in the sensitivity of the system to incoming sensory information. We can then ask whether cigarette smoking alters the rate at which this decrement occurs. If smoking retards the rate at which the evoked potential decreases in amplitude over trials, we will have demonstrated one manner in which concentration might be facilitated by cigarette smoking.

We have recently been gathering data on this subject employing pattern reversal evoked potentials (PREPs). Thus far we have studied the effects of smoking and smoke deprivation on the PREPs of seven smokers. It is too soon to ascertain whether smoking indeed retards habituation, but early results are encouraging. If, when the data are analyzed we appear to have a "real" effect, we will mount a full-scale investigation.