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From: W. L. Dunn CONFIDENTIAL

Subject: Behavioral Research Accomplishments - 1979

THE ELECTROENCEPHALOGRAPHY PROGRAM [Gullotta & Frankovitch]

The Effects of Cigarette Smoking on the Early, Late and After-Discharge Components of the Visual Evoked Response

The major purpose of this study was to investigate the effects of cigarette smoking on the visual evoked response as observed via electroencephalography. Based upon the results of earlier studies, we hypothesized that: 1) cigarette smoking would augment the peak to peak amplitudes of the late VER components, without altering the amplitudes of the early components; 2) the VER after-discharge would be enhanced following cigarette smoking; and 3) cigarette smoking would not affect VER latencies.

CONNECTION WITH

We found that under short-term smoke deprivation conditions (1 hr.) cigarette smoking resulted in an enhancement of the peak to peak amplitudes of three of the four late components, and that there was a trend toward enhancement in the fourth. As hypothesized, cigarette smoking did not alter the amplitudes of the early VER components. Contrary to our hypothesis, however, cigarette smoking did not affect the VER after-discharge. Cigarette smoking following overnight deprivation failed to alter any of the measures.

The results of this study are at odds with two earlier reports on the effects of cigarette smoking on the VER. Hall, et al. (1973) found that the amplitude of only one VER component was enhanced following cigarette smoking. Friedman, et al. (1974) found that the enhancement was restricted to a different single component.

In contrast to the rather marginal results of the studies cited above, our findings are quite robust. Under short-term smoke deprivation conditions much of the late VER amplitude is enhanced. This enhancement suggests that following smoking, individuals are more receptive to visual stimulation. These data, therefore, may be relatable to smokers' subjective reports of increased alertness and ability to concentrate following cigarette smoking.

VER II

This study was designed to answer questions arising from the first VER experiment. Specifically, we were interested in finding out why cigarette smoking affected VER amplitudes when subjects were 1 hr. smoke deprived, but not overnight deprived. We hypothesized that following overnight smoke deprivation, brain levels of CNS-active smoke constituents would be so low that smoking a single cigarette would not appreciably alter the evoked response. We further speculated that if this were true, significant changes in the VER could be obtained by having subjects smoke several cigarettes.

In this experiment VERs were recorded four times in one session on overnight smoke deprived subjects. The first recording yielded pre-smoking baselines. Then recordings were repeated after subjects smoked each of three cigarettes.

The results of this study failed to support our hypothesis. A significant depression in the amplitudes of most VER peaks was obtained both post-smoking and in the dry-puff control condition. These data indicate that repeated testing, in and of itself, has a depressant effect on the amplitudes of VER peaks. They further indicate that cigarette smoking under overnight smoke deprivation conditions do not reverse this depressant effect.

The results of this study point out that degree of deprivation is a critical variable in studies of the VER. In order for the VER to be employed as a sensitive index of cigarette smoking, the period of smoke deprivation must be short.

Long-Term Smoke Deprivation and the Electrical Activity of the Brain

The purpose of this study was to assess the effects of the cessation of cigarette smoking on the visual evoked response (VER). Two previous studies (Hall, et al., 1973; and Friedman, et al, 1974) reported that under conditions of smoke deprivation ranging between 12 and 36 hr., peak to peak amplitudes of some VER components were depressed, and that this depression was reversed after smoking a cigarette.

These observations led us to speculate about what would happen to VER peak to peak amplitudes if smoking was discontinued for days or months. If the cessation of cigarette smoking resulted in a permanent reduction in VER amplitude this would indirectly imply a return to some kind of pre-smoking baseline. If, on the other hand, the VER remained depressed for some period of time and then gradually recovered to the pre-quitting level, this would suggest a withdrawal effect.

The results of this study failed to replicate the earlier reports of an amplitude depression of components of the VER under 12 and 36 hr. deprivation. Instead, VER peak to peak amplitudes remained at pre-quitting baseline levels throughout the study (as long as 3 mo. for some subjects). Therefore, we must conclude that, under the conditions of our experiment, the cessation of cigarette smoking has no effect on VER amplitudes.

We found that there was a large increase in the latency of one VER component one day post-quitting. This increase was only transient, however, and baseline levels were obtained by two days post-quitting.

As expected, heart rates decreased after quitting. This decrease was maximal 36 hr post-quitting and represented about a 12 BPM decrease. Beyond 36 hr. no further changes were observed.

Post-Quitting, the most dramatic changes in behavior that were observed were disturbances in sleep and an increase in food consumption, particularly of foods high in sugar. We observed no changes in mood.

These data, taken as a whole, suggest that with respect to the VER, the cessation of cigarette smoking results in changes that are both transient and slight. The results of our study, then, are more consistent with the notion that the behavioral disruptions following the cessation of smoking are due to the interruption of a well-learned habit and not physiological withdrawal.

The Effects of Cigarette Smoking on the Auditory Evoked Response

Guha and Pradhan (1976), employing cats as subjects, showed that low doses of nicotine augmented the auditory evoked response (AER), while higher doses depressed the response. In humans, Friedman, et al. (1974) failed to find statistically significant effects of cigarette smoking on the AER. They noted, however, that the response tended to be reduced. Incidentally, using the same subjects, Friedman, et al. showed that VERs were augmented following cigarette smoking.

If it is true that cigarette smoking augments VERs, but depresses AERs, this would mitigate against a generalized, stimulant effect of smoking on the CNS. Rather, data such as these would suggest that smoking has selective effects on specific neural systems. One could postulate, for example, that cigarette smoking (via its centrally active components) could participate in selective attention.



Although the data have not been completely analyzed, it does appear that cigarette smoking depresses AERs in a reliable and predictable fashion. Specifically, preliminary analyses show that smoking results in a 36% decrease in the amplitude of an AER component which occurs with a latency of 200-350 msec.

These results, together with the results of studies on the VER suggests that: 1) cigarette smoking has specific influences on CNS responses to sensory stimulation; and 2) cigarette smoking may somehow participate in the gating, by the CNS, of incoming sensory information.

Computer Programs for the Analyses of Sensory Evoked Responses (with S. Osborne & N. Nunnally)

A basic problem in all research on evoked responses is the objective and reliable identification and measurement of the waveforms which are generated. In order to minimize these difficulties we chose to develop computer programs that would aid us in this task.

Thus far, a total of three programs have been completed. Two of these programs are digital applications of algorithms for peak identification in visual and auditory evoked responses. The program used to measure visual evoked responses does so with about an 80% accuracy rate. The accuracy rate for the program used to measure auditory evoked responses is even better.

The third program was designed to yield power spectral density values for visual after-discharge responses. This program performed well, and it is currently being modified for spectral analyses of ongoing EEG data.

Pilot Studies for Sensory Evoked Responses

In order to successfully conduct research on the effects of cigarette smoking on sensory evoked responses, a large number of methodological variables had to be considered. Each of these variables was treated as a "mini experiment," so that its possible contribution to our findings could be assessed. These mini experiments included:

1) The effects of varying interstimulus intervals.
2) The effects of varying stimulus intensity.
3) The effects of varying deprivation conditions.
4) The effects of varying nicotine deliveries.
5) The effects of varying electrode locations.
6) Intra vs. inter subject comparisons.
7) Directed vs. nondirected attention.
8) Random vs. fixed stimulus delivery rates.
9) Binaural vs. monaural stimulation.



We believe that the results of these experiments have laid a solid foundation which to conduct future studies on smoking effects on sensory evoked responses.

THE COMPARATIVE PROGRAM [Levy, Carron, Allen (Young & Rowsey)]

Nicotine Discrimination Studies

We have been training rats to discriminate between injections of nicotine and injections of saline. During 1979 a major study of the cuing properties of 6-Methylnicotine was completed, several nicotine analogues were screened and a study of muscarinic cholinergic discrimination was begun.

The study of 6-Methylnicotine, which included the production of dose-response curves for both nicotine and 6-Methylnicotine, showed that adding a methyl group to the pyridine ring at the 6 position does not substantially change the ability of the analogue to produce a nicotine cue. Addition of an ethyl group at the 6 position, on the other hand, does result in a slight reduction in potency. Analogues with alkyl groups added at the 5 position are less active than nicotine, but still retain some activity, while 4-Methylnicotine is apparently quite inactive. [These data can all be found in the Behavioral Research Laboratory 1979 Annual Review - Part 1 and a memo from Levy and Carron to Dunn, dated Dec. 17, 1979.]

Previous research from other laboratories has shown that rats trained to discriminate nicotine from saline do not accept muscarinic compounds as being like nicotine. In addition, rats trained to discriminate arecoline (a muscarinic agonist) from saline do not accept nicotine as being like arecoline. The obvious implication of these studies is that the receptors and neural pathways which mediate the nicotinic and muscarinic cues do not overlap.

We have trained five rats to make a pilocarpine-saline discrimination (pilocarpine hydrochloride is a muscarinic cholinergic agonist). When these rats are injected with nicotine, they respond as if they had been injected with pilocarpine. However, when our nicotine trained rats are injected with pilocarpine (and the peripheral m-cholinergic receptors are blocked by atropine methylnitrate) they respond as if they had been injected with saline. We are currently conducting additional tests using a variety of blocking agents in order to help us explain these findings.

The discrimination paradigm is, to date, our most effective and efficient test for screening nicotine analogues.

Activity Monitoring

We have been monitoring the locomotor activity of rats following the injection of nicotine and nicotine analogues. During 1979 seven studies were conducted to determine if this test would be useful as an initial screen for nicotine analogues. Our major findings are given below:

1) Doses of nicotine ranging from 0.05 to 0.40 mg/kg all produce significant depression of locomotor activity relative to the saline controls, whether the tests are conducted in an illuminated or darkened chamber.

2) Two nicotine analogues which suppress locomotor activity (5,6-Dimethylnicotine, 0.8 mg/kg; 6-Ethylnicotine, 0.4 mg/kg) also produce a nicotine cue in the nicotine-saline discrimination task.

3) Three nicotine analogues which do not suppress locomotor activity (4-Methylnicotine, 0.4 mg/kg; 5-Isopropylnicotine, 1.6 mg/kg; 5,6-Tetramethylene nicotine, 0.8 mg/kg) produce a saline cue in the nicotine-saline discrimination task.

4) Nicotine-experienced rats do not show reliable suppression of locomotion following nicotine injection.

Thus, we have found that activity monitoring can be used effectively to screen nicotine analogues since the reuslts agree with results from the discrimination task. However, because of the amount of time and effort required to collect the data, as well as the cost of the large number of animals required, this test is not an efficient screen for analogues.

Nicotine Self-Administration

Rats can be taught to self-administer nicotine via an indwelling intravenous catheter, thus demonstrating that the nicotine is reinforcing to them (Lang, Latiff, McQueen and Singer, 1977). In our Plans and Objectives for 1979 we proposed to conduct studies involving self-administration of nicotine. In April, 1979, C.Levy went to the University of Minnesota to learn how to perform the necessary surgical procedures. However, the initiation of these studies was postponed until completion of the construction of additional laboratory space.

CONFIDENTIAL

Tail Flick

Nicotine has been shown to have analgesic properties as measured by the tail flick test (Sahley and Berntson, 1978). We have done extensive testing of (+)- and (-)-nicotine using the tail flick test in an effort to determine if we could use this measure as a screen for nicotine analogues. During 1979 five studies were conducted, all of which confirmed that injection of (-)-nicotine produces an increase in the rats' tail flick latencies. However, a major problem with all of these studies was that the injections also produced severe agitation of the rats. Their twisting and turning in the restraining tubes made reliable data collection difficult, if not impossible.

In our first four studies we compared the analgesic properties of (-)- and (+)-nicotine. The results of these studies showed that (-)-nicotine, 0.8 mg/kg and (+)-nicotine, 24.0 mg/kg, both significantly increased the rats' tail flick latencies. On the other hand, doses of (+)-nicotine at 0.8, 8.0 and 16.0 mg/kg did not produce a significant elevation in the rats' pain threshold. Thus, only a dose of (+)-nicotine which was thirty times greater than the effective dose of (-)-nicotine produced comparable results. Unfortunately the rats often convulsed following this high dose of (+)-nicotine, introducing unknown complications into the interpretation of the data.



Our fifth and final study was designed to determine if we could minimze the nicotine induced agitation which we had previously observed following doses of (+)- and (-)-nicotine which increased tail flick latencies. Two different strategies were tried: 1) lower the dose of (-)-nicotine to 0.6 mg/kg and 2) inject a "cocktail" of hexamethonium chloride, 10 mg/kg, and (-)-nicotine, 0.8 mg/kg. Both of these injection conditions produced increased tail flick latencies, but the agitation still remained.

We do not recommend the tail flick test as a screen for nicotine analogues if the injections are administered subcutaneously. Dr. Berntson (personal communication) has informed us that central administration of nicotine produces analgesia without agitation. We therefore have proposed that central infusion of nicotine be used for tail flick testing during 1980.

Prostration Syndrome

Abood and his coworkers (1978) have reported that rats become temporarily prostrate following the infusion of 2-10 µg of (-)-nicotine bitartrate into the lateral ventricle. From their data they concluded that this prostration syndrome was mediated via central noncholinergic nicotinic sites. We have been interested in the prostration syndrome as a screen for nicotine analogues since this behavior may be mediated by receptors which are quite different from those mediating the nicotine cue or nicotine-induced analgesia. During 1979 over fifty rats were implanted with cannulae, and four major studies were conducted.

Our first study demonstrated conclusively that pretreating rats with mecamylamine hydrochloride, but not hexamethonium chloride, substantially reduced the severity of their response to nicotine. These results strongly suggest that the prostration syndrome is mediated by nicotinic cholinergic sites in the brain, rather than by noncholinergic sites as suggested by Abood and his coworkers.

Our next two studies examined the difference in potency between (-)- and (+)-nicotine. These studies were important because Abood had reported a 100-fold difference in potency, and this high degree of stereospecificity could be used in further support of his contention that the receptors mediating the prostration syndrome were not nicotinic cholinergic receptors. Our results showed that (+)-nicotine is ten to twenty times less active than (-)-nicotine in producing the prostration syndrome. This difference in potency between the two stereoisomers of nicotine falls within the range of values reported in the literature for a variety of tests (Aceto and coworkers, 1979). Thus, these data again support the position that the receptors mediating the prostration syndrome are nicotinic cholinergic.

Our fourth study compared the potency of (-)-nicotine with the racemic mixture (+),(-)-nicotine. The results of this study appeared to be consistent with our previous findings. For example, 5 µg of (+),(-)-nicotine produced a slightly more severe prostration syndrome than 2.5 µg (-)-nicotine, but less severe than 5 µg of (-)-nicotine. An interesting question arose out of this study: What is the best procedure to use when conducting a dose-response study? Is it better to hold concentration constant and let volume vary, or vice versa? We are presently conducting a study in which volume is held constant, since we have previously let volume vary, as did Abood.

We anticipate that the prostration syndome will be a very useful test for screening nicotine analogues.



THE EXPERIMENTAL PROGRAM [Ryan, Jones (Faust)]

Annual Monitoring

The third annual monitoring of cigarette acceptability was conducted.

Twenty-six hundred twenty-two panelists rated five cigarettes - with nominal deliveries of 5, 9, 13, 17, and 21 mg tar - for strength and acceptability. The test cigarettes - all Marlboro-like blends with delivery differences created by dilution, filtration, and paper changes - were two years old, had been kept in cold storage since manufacture, and showed little change in delivery, appearance, or acceptability attributable to aging.

Acceptability and strength ratings resembled those from prior years: the panel as a whole gave its highest ratings to the 13 and 17 mg models, lower ratings to the 9 and 21 mg models, and the lowest rating to the 5 mg model. The perceived strength ratings increased as model delivery increased, with the 5 mg considered weakest and the 21 mg model strongest.

Full flavor smokers gave high ratings to the 13, 17, and 21 mg models, lower ratings to the 9 mg, lowest rating to the 5 mg cigarette. Low delivery smokers gave high ratings to the 9 and 13 mg model, a lower rating to the 17 mg model, lowest ratings to the 5 mg and 21 mg model. Ultra Low ([redacted]) smokers

gave highest ratings to the 5 mg model and progressively lower ratings to the higher delivery cigarettes. This is the third time we have made the observation that the more the test model resembles the cigarette usually smoked, the higher its acceptability will be.

Examining the smaller group of people who have switched delivery levels - from full flavor to low delivery - since monitoring began, we see little evidence that switching can be predicted from acceptability ratings. Although the data are not conclusive, because of variability and scarcity of cases, once the switch has occurred, there are shifts in the ratings. Thus, full flavor smokers who shifted to low delivery between '77 and '78 or '78 and '79, originally gave their highest ratings to the 17 mg model but after their switch they gave their highest ratings to the 13 mg model. At the same time, they increased their ratings of the 5 mg model and decreased their ratings of the 21 mg model.

None of this data provides any support for the idea that today's smokers would currently like a cigarette like the one their fathers used to smoke. At the same time, it also provides little support for the idea that they would currently like a cigarette like the ones their sons will smoke a few years from now.

Nicotine Discrimination

A panel of 20 employee smokers compared a set of 5 low tar cigarettes (about 6 mg FTC tar) which differed only in nicotine deliveries. Nicotine delivery was varied by mixing denicotinized tobacco with regular tobacco. Panelists were asked to tell which of two cigarettes had more nicotine in the smoke, using harshness, impact, taste, or other cues as the basis for their judgments. Each smoker evaluated all 10 possible pairs of five nicotine deliveries.

Although some smokers were better discriminators than others, no differences were consistently detected correctly by all, and no smoker was always correct. The results indicated that about 9 out of 10 panelists could correctly identify the stronger cigarette if the stronger contained 50% more nicotine than the weaker. When delivery differences were smaller, the proportion of correct judgments dropped towards the chance level.

Salivation Studies

We have begun a research program into the relationship between saliva flow and smoking behavior. Still in its formative stages, we are beginning to see some interesting data on how smoking affects flow.

In one portion of the project we collect saliva on rolls of cotton held under the tongue for two-minute periods. When measures of saliva flow were made before and after smoking full flavored [redacted] or ultra low delivery [redacted], the post-[redacted] flows were about 20% greater than the post-[redacted] flows. Whether this is related to the often heard comment that low delivery cigarettes leave the mouth hot and dry is debatable, but possible.

We are currently measuring saliva flows before, during, and after smoking and/or dry puffing among smokers and nonsmokers (who only dry puff). The data are as yet incomplete, but we already see that the act of puffing alone, whether the cigarette is lit or unlit, tends to increase flow. Apparently the same suction which pulls in the smoke also tends to pull out the saliva from the salivary storage reservoirs.

Although subjects differ greatly in their salivary flow, the general effects during puffing and dry puffing appear to be the same for almost all those tested.

In a second part of the project we have begun to study the nicotine content of saliva among smokers. Don Magin and George Walker of the Analytical branch have devised a gas chromatographic procedure which reads nicotine in saliva concentration between the range of 0.2 and 30.0 parts/million.

Measurement is based on sputum samples, weighing about 0.5 grams, spit into a vial before and at different times after smoking. Compared to pre-smoking levels, the post-smoking concentrations appear to grow and then diminish. The exact shape of the curve is as yet unknown. To the extent that salivary nicotine reflects levels in other parts of the body, we may be able to relate the level of nicotine in the system to the desire to smoke.

Inhalation Monitoring

An additional year has been devoted to solving instrumentation problems, with substantial progress to report. With the continued help of the Electrical Engineering Lab we have completed feasibility studies of the Respitrace Calibrator and have concluded that the device can be applied to observing the inhalation patterns of smokers over 8-hour periods with minimal intrusiveness upon normal smoking behavior. These preliminary studies were accomplished with the subject wired directly to a console polygraph. In December we ordered the equipment required for mobile recording. Delivery is expected in March, 1980. Thereafter, hopefully, there should be no great delay in initiating the long-promised investigations.

jh

W. L. Dunn