

# The effect of filtrate fractions of tobacco smoke on the avoidance behavior of rats under extinction procedure<sup>1</sup>

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## Summary

The avoidance response times for 4,400 runs in a shuttlebox under extinction procedure were measured for 22 rats, 20 minutes after exposure to filtered and unfiltered cigarette smoke. The test conditions of full smoke (5 and 10 puffs) and charcoal-filtered smoke (10 puffs) differed significantly from the control and Cambridge-charcoal filter conditions (10 puffs) in the direction of inhibiting extinction (i. e. shortening of the response times). As the test conditions of full smoke and charcoal-filtered smoke were the only ones which contained the particulate phase, of which nicotine is pharmacologically by far the most active ingredient, this stimulating effect was attributed to nicotine. The stimulating effect commonly observed in behavioral experiments through nicotine injection has therefore been demonstrated here through the medium of small controlled doses of cigarette smoke, comparable to the non-toxic intake of this substance by humans.

Although a stimulating effect from nicotine has been demonstrated in many types of behavior experiments with rats in recent years, a stimulating effect in behavioral tests has not been described for awake laboratory animals which have inhaled small controlled doses of filtered or unfiltered cigarette smoke. Some older studies have indicated that small amounts of tobacco smoke may act as a stimulant, for example in maze learning (Pechstein and Reynolds, 1937), but the intake and nature of the smoke, as well as other aspects of the experiments, were only vaguely defined.

A recent study has demonstrated an inhibiting effect exerted by unfiltered and several types of filtered cigarette smoke on a swimming endurance test in rats (Bättig and Hrubes, 1971), with unfiltered smoke and charcoal-filtered smoke having the strongest negative effect on the test. In the light of this finding, it was decided to determine in this present study whether similar results would be obtained by testing the effect of filtered

and unfiltered cigarette smoke on the avoidance behavior of rats under extinction procedure. This test was used because it had already been shown to be an effective medium for studying the influence of nicotine and total alkaloids extracted from cigarette smoke upon conditioned behavior (Driscoll and Bättig, 1970). In that experiment it was demonstrated that small injected doses of these two substances stimulated the rats to approximately the same, highly significant degree.

## Method

**Animals:** 22 male albino rats, about 10 months old and weighing between 460 and 640 grams at the start of the experiment, were used. Each rat had undergone several months of training in the shuttlebox in prior studies, and had not previously been exposed to cigarette smoke.

**Treatments:** The smoking machine used was developed by Davis et al. (1970). The rat was kept immobilized in a restraint compartment, with its head protruding from an opening in one end. Its nose projected through an airtight, membranous rubber collar, into one end of a smoke cylinder. A controlled air-cigarette smoke mixture was sucked into the cylinder once every minute and remained there for 15 seconds. During the remaining 45 seconds the cylinder was ventilated with fresh air. This rotation was maintained by an electric timing device, operating together with a vacuum pump leading to a diaphragm at the opposite end of the cylinder. A filterholder was located between the cigarette and the vents at one side of the cylinder. The gas phase of smoke was introduced into the smoke cylinder by fitting into the filterholder a "Cambridge" filter and behind this adding one gram of activated charcoal (Norit I). The gas phase was presented to the rats in doses of 10 and 20 puffs (1 and 2

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cigarettes), and is represented here by the symbols G 10 and G 20. By inserting the Cambridge filter only, the combined vapor and gas phase of the smoke was obtained (VG 10 and VG 20). By putting in the activated charcoal alone, the combined particulate and gas phase was obtained (PG 10 and PG 20). Control conditions consisted of 10 and 20 minutes in the restraint compartment without a cigarette (N 10 and N 20), whereas full (unfiltered) smoke was presented in doses of 5 and 10 puffs ( $\frac{1}{2}$  and 1 cigarette), referred to here as S 5 and S 10. "King-size", non-filter tip American blend cigarettes were used, with an approximate yield of 1.8 mg nicotine and 30 mg particulate matter in the mainstream smoke of each cigarette. Ten groups formed of two or three animals each received, in a latin square sequence, each of the ten conditions two times. This comprised the two "parts" of the experiment, the second of which replicated the first. All rats were treated and tested three times during the week, on alternating days, and each group was exposed to a different condition on the first day of the experiment.

*Test:* 20 minutes after every smoking machine exposure, during both parts of the experiment, the exposed rat underwent 10 runs in a shuttlebox under extinction procedure. The shuttlebox used in this experiment has already been described in detail (Bättig, 1957). The conditioned stimulus (CS) consisted of light and buzzer signals. The unconditioned stimulus (US), used only during previous training and during a two-week period of gradual adjustment to cigarette smoke which immediately preceded the experiment, was a stabilized AC current of 60 V (shock) applied to the grid floor of the shuttlebox at a definite CS-US interval (.05 or .10 minute). During extinction procedure, the shuttlebox testing consisted of 10 runs, each run preceded by the CS, but without any application of the US whatsoever. During each run the

length of time elapsing between the onset of the CS and the conditioned response (when the rat passed through the door into the other chamber as far as the base of its tail) was recorded. The interval between runs, from the completion of the avoidance response until the onset of the next CS, was .25 minute. Following the CS presentation, the rat was considered to have shown an avoidance failure if it did not respond within .25 minute, this figure being recorded. The CS was discontinued at this point and presented again after the regular .25 minute interval. The results of the 4,400 extinction procedure runs were submitted to an analysis of variance, with statistical evaluation (Duncan multiple-t test) of the partial averages being subsequently performed.

### Results

The only persistent signs of stress in the smoking machine were increased tear secretion and salivation. Increased defecation occurred only in the early days of the experiment. All of the animals lost from 15 to 35 grams of weight during the first week of exposure to smoke, and the new reduced weight levels were maintained or continued to gradually decrease during the seven weeks of the experiment. Two weeks after cessation of exposure to smoke, all of the rats were back to their mature, pre-experimental weights.

In the first part of the experiment, none of the test conditions differed significantly from any of the others. In the second part, none of the test conditions differed significantly from the control (N 10 or N 20) in the direction of accelerating extinction, i. e. lengthening of the response times. However, two conditions differed significantly ( $p < .05$ ) from N 10 in the direction of inhibiting extinction, or shortening of the response times. These were S 10 and PG 10 (fig. 1), and this level of

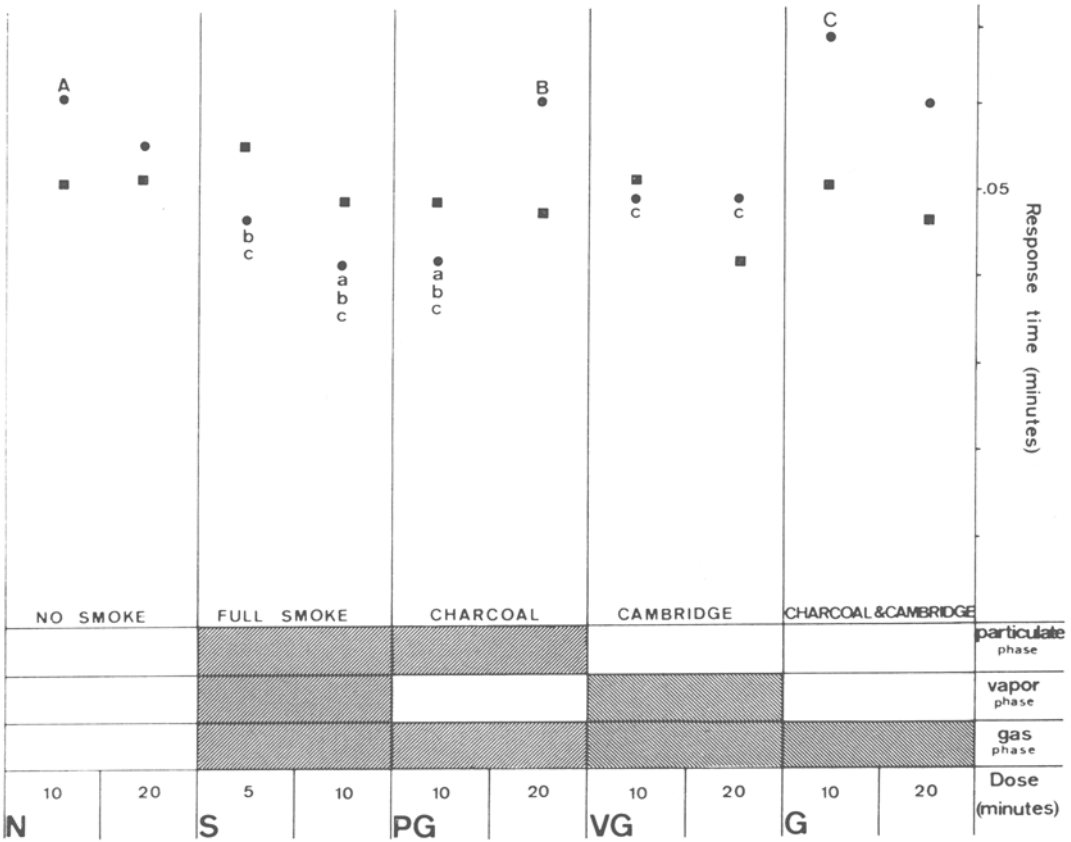


Fig. 1 The effect of filtered and unfiltered cigarette smoke on avoidance response in rats under extinction procedure. ■ = part I, ● = part II Significant differences: A vs. a; B vs. b; C vs. c ( $p < .05$ )

significance was sustained for the extinction procedure as a whole, when the averages of both parts were calculated together. Both of these, plus S 5, also differed to a significant degree from G 10 in the direction of shorter response times. Surprisingly, G 10 differed significantly from VG 10 and VG 20 in the direction of longer response times. The reason for this was readily ascertained, however, and will be discussed. Also misleading was the value for PG 20, which was significant in the direction of longer response times when

compared to S 5, S 10 and PG 10. The reason for this was also apparent, and was related to the reason for the G 10 vs. VG 10/VG 20 result.

#### Discussion

As mentioned, increased tear secretion and salivation were the only persistent signs of smoking machine stress seen. *H.-J. Kuschle et al. (1952)* as cited, stated that these phenomena always occurred in rats exposed to

cigarette smoke, even after months of exposure, and that the rats appeared never to develop a tolerance to the "irritant effect of tobacco smoke" (Larson et al., 1961). Increased defecation, which was a persistent finding in a previous study (Bättig and Hrubes, 1971), did not occur in this present experiment after the first few days of smoke exposure. In the previous experiment, therefore, this finding was more likely related to nervousness of the animals due to immaturity and less extensive handling, rather than to smoking machine stress. The weight losses recorded in the present study were noteworthy and were probably caused by a combination of the effect of the experimental procedures on eating and the effect of the smoke constituents on the metabolism and behavior of the rats, but more measurements would have been necessary to draw any definite conclusions.

Since significant differences among the test conditions were not obtained in the first part of the experiment, discussion of the results will be limited to the second part only. The finding in regard to parts I and II, however, indicated that the influence of different exposure conditions on shuttlebox behavior became prominent only after the rats were sufficiently conditioned to the pharmacological effects of the smoke ingredients, and to the procedure of smoke inhalation.

It has been seen (fig. 1) that the conditions S 10 and PG 10 differed significantly from the control condition N 10 in the direction of shortening the response times and that S 10 and PG 10, in addition to S 5, differed significantly from condition G 10 in the same direction. These results were apparently due to the stimulating effect of nicotine, which was present only in the full smoke and charcoal-filtered smoke conditions, as part of the particulate phase. This experiment, together with a previous extinction procedure experiment (Driscoll and Bättig, 1970), have there-

fore accomplished in behavioral studies with awake rats what has been accomplished in physiological studies with artificially ventilated cats and dogs (Armitage, 1965; Isaac and Rand, 1969), that being the realization of similar behavioral (and physiological) responses through the injection of small doses of nicotine and also through the introduction of that substance into the bloodstream by the inhalation of cigarette smoke. Although the doses of cigarette smoke were correlated as much as possible to the artificial ventilation studies, and therefore to what was considered to be the "normal" intake of that substance in man, it must be stressed that a controlled amount of smoke could only be presented to the rats, knowing that they actually inhaled it to differing degrees and thereby were probably subjected to varying doses of nicotine. It could be safely stated, however, that all of the animals received small non-toxic amounts of nicotine (with the possible but improbable exception of the PG 20 condition), and that nicotine was in all likelihood responsible for the shorter response times seen. In contrast to this result, however, an experiment utilizing 10 puffs of full smoke resulted in a significant depression of swimming endurance performance (Hrubes and Bättig, 1970), further accentuating the mental and physical differences which undoubtedly exist between the two tests, and which have been previously discussed (Bättig and Hrubes, 1971; Driscoll and Bättig, 1970).

The test condition of PG 20 differed significantly from S 5, S 10 and PG 10 in the direction of longer response times (fig. 1), but the reason for this seemingly contradictory result was apparent, and helpful in relation to planning future experiments. Although significantly short response times were actually observed in most of the rats upon exposure to PG 20, some of the rats became obviously very ill during the course of the second cigarette and did not fully recover after 20 min-

utes, thus being unable or almost unable to function in the shuttlebox. This produced very high response times for a few rats, and caused the misleading value seen for PG 20 as a whole. This "illness" occurred only in the PG 20 test condition, and was due largely to differences in breathing characteristics observed among the various filter conditions, which will be discussed in the next paragraph. Other studies conducted in this laboratory have revealed that the LD<sub>50</sub> for full smoke was approximately 25 puffs (2½ cigarettes) for young rats in these smoking machines (Deuber, 1970). Whereas this latter finding was taken into account for the full smoke conditions, in which 5 and 10 puffs were presented as compared to 10 and 20 puffs for the filter conditions, the PG 20 results in this study have made it apparent that in the future a 10 puff maximum should be extended to the filter conditions as well, due to the necessity of carrying out behavior studies with non-toxic amounts of cigarette smoke comparable to human conditions.

G 10 differed significantly not only from the "nicotine" conditions, but from VG 10 and VG 20 in the direction of longer response times. The reason for this result was that the rats breathed more vigorously when the vapor phase (which included all of the irritants) was absent, thus encouraging increased inhalation of the toxic gas phase. This difference in breathing between these two filter conditions has been observed and recorded in other studies in this laboratory, and also played an important part in the difficulties associated with the PG 20 condition, as the charcoal filter there also removed the irritants from the smoke, causing the animals to be much less inhibited in their breathing than with full smoke. The action by charcoal filters in removing the vapor phase from cigarette smoke and thereby greatly reducing its irritant properties has been previously described by H. B. Haag et al. (1959) as cited

(Larson et al., 1961), and has been correlated directly to the use of these smoking machines in a recent study, which included a chemical analysis of the various filtered smoke conditions (Bättig and Hrubes, 1971). The Cambridge filter has been found to remove only a small part, if any, of the vapor phase constituents from cigarette smoke (Williamson and Allmann, 1966), although it causes a substantial reduction in the total amount of smoke due to absorption of the particulate phase. The gas phase alone has been seen to exert a significantly depressant effect in a swimming endurance test using the same cigarettes (Bättig and Hrubes, 1971). This depression was most likely due to CO gas, none of which is removed by Cambridge or charcoal filters, and which comprises roughly 4 to 5% of the total smoke in commonly used tobaccos (Kruszynski and Henriksen, 1969). In a recent study in this laboratory which utilized the same smoking machines and cigarettes, it was found that approximately the same depressant effects in a swimming speed test with rats were achieved with the gas phase and with a comparable % mixture of pure CO (Deuber, 1970).

It is difficult to develop animals' models which resemble human "smoking", due to the anatomical, physiological and motivational differences existing among man and the other species. In relation to "mental efficiency" smoking experiments with human subjects, however, it has been stated that: "Results of tests on non-smokers while smoking, or on habitual smokers in non-smoking periods, would be equally meaningless, either in themselves or compared to tests on non-smokers not smoking or on smokers smoking" (Larson and Silvette, 1968). In addition, the differences existing among individual humans in regard to their smoking characteristics, resulting in variations in the composition of the smoke as well

as in total smoke consumption by different people (Lexington, Ky. Proceedings), along with the impossibility of conducting smoking experiments with humans under really controlled conditions, make it imperative that animals be used in this type of research. In an opinion which is shared by these authors, *R. B. Griffith* has stated that inhalation research is important in that one is working with an intact animal, and if one uses smoke composition equivalent to human exposure, one has the best chance of correlating animal response with human response (Lexington, Ky. Proceedings). Furthermore, the well-developed filtration capacity of the upper respiratory tract of animals usually breathing through their nose does not exclude the possibility of making fruitful inhalation experiments with rodents (*Dontenwill et al.*, 1966). Some very interesting work has been done involving the introduction of controlled amounts of tobacco smoke into the lungs of artificially ventilated cats and dogs, and projecting the physiological calculations onto the probable effect of smoking doses of nicotine in man (*Armitage*, 1965; *Isaac and Rand*, 1969). These exact methods cannot be applied to behavioral research, for obvious reasons, but raise a question which concerns both the use of awake or anesthetised animals, that being: What exactly is tobacco smoke? It is, at best, a highly variable biological material. Different kinds of tobacco are grown in many parts of the world, under various conditions and these tobacco varieties are modified still further by processing and other manufacturing variables (Lexington, Ky. Proceedings). There are substantial differences among the various commercial cigarettes, and even changes within the same brand from batch to batch. The differences involve in part such factors as moisture content, tightness of pack, burning quality of the tobacco, and the manner in which the cigarettes are handled during the experiment

(*Larson*, 1960), all of which influence the distribution of the various substances in the smoke and, subsequently, their effect upon the animal organism. One must also take into consideration the many hundreds of ingredients found in tobacco and in tobacco smoke, some of which, including highly important substances such as nicotine, are present in varying amounts in different tobaccos.

It is therefore important to regard the results of animal behavior/smoking machine experiments in the light of the difficulties of control which still exist. Two of the most important of these are: i) the high degree of variability which may occur among tobacco products used, this being especially important in comparing the results of experiments conducted in different laboratories or at different times, and ii) the problem of producing and operating an animal-model system which is both scientifically accurate and which also provides an adequate (experimental) resemblance to human smoking. The first difficulty may be overcome by the production of a special "research cigarette", with a uniform composition, which will ensure the reliability of the tobacco product used in smoking research (Lexington, Ky. Proceedings). The second problem can only be resolved through continued experimentation with, and perfection of, the systems used to simulate smoking.

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