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BIPHASIC DOSE-RELATED RESPONSES OF THE CNV (CONTINGENT NEGATIVE VARIATION) TO I.V. NICOTINE IN MAN

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1 The effects of intravenous injections of nicotine bitartrate, given as intermittent 'shots', on the magnitude of the contingent negative variation (CNV) were studied in twelve male volunteers.

2 In one series of experiments in five subjects, a fixed dose of nicotine was used. In three of these subjects nicotine 500 μg or 750 μg produced a reproducible increase in magnitude of the CNV. In two subjects nictone 750 μg produced a reproducible decrease in magnitude of the CNV. The direction and magnitude of the CNV changes could be reproduced by cigarette smoking.

3 In another series of experiments in eight subjects, dose-response relationships for the effect of nicotine on the CNV were measured using a range of doses from 12.5 to 800 μg. Individual and mean dose-response curves were found to be biphasic so that whilst smaller doses produced an increase of CNV magnitude (stimulant effect), larger doses produced a decrease of the CNV (depressant effect).

4 The results are discussed in relation to the possible mechanism of action on the brain of nicotine as obtained by inhaling cigarette smokers.

Introduction

A number of studies have demonstrated that the magnitude of the contingent negative variation (CNV) can be altered by drugs. Drugs which increase the size of the CNV in normal subjects include LSD (Walter, 1964), cannabis (Kopell, Tinklenberg & Hollister, 1972; Low, Klonoff & Marcus, 1973), caffeine (Ashton, Millman, Telford & Thompson, 1974), and methylamphetamine (Kopell, Wittner, Lunde, Wolcott & Tinklenberg, 1974). Drugs which increase the size of the CNV in normal subjects include carbon monoxide (Groll-Knapp, Wagner, Hanck & Haider, 1972), ethanol (Kopell, Tinklenberg & Hollister, 1972), nitrazepam (Ashton *et al.*, 1974), diazepam (Ashton, Millman, Telford & Thompson, 1976, 1978), flurazepam (Hablitz & Borda, 1973), quinalbarbitone (Kopell, *et al.*, 1974), chlorpromazine (Tecce, Cole & Savignano-Bowman, 1975), atropine and metoclopramide (Thompson, Newton, Pocock, Cooper, Crow, McCallum & Papakostopoulos, 1976). Cigarette smoking appears to have a biphasic effect on the human brain and may produce both stimulant and depressant effects on CNV magnitude (Ashton, Millman, Telford & Thompson, 1973; Ashton *et al.*, 1974).

If these biphasic effects of smoking are largely due to nicotine obtained from the cigarette it should be possible to produce similar effects by administering nicotine intravenously in doses which approximate to those obtained by smokers when smoking a cigarette. Armitage, Hall & Sellers (1969) have shown in animals that intravenous nicotine can mimic the effects of cigarette smoking but only if the drug is given as intermittent 'shots'; comparable effects are not obtained if the nicotine is administered as a continuous infusion. In the present work the effects of intravenous nicotine on the CNV were studied in human volunteers who received the drug as intermittent 'shots' as used by Armitage and his colleagues (1969) in animals.

Methods

The experiments were carried out on healthy male volunteers who were undergraduates or postgraduates. The project had been approved by the appropriate local Ethical Committee.

Measurements

The CNV was recorded and measured as described previously by Ashton *et al.* (1974). Briefly, subjects were presented with series of paired signals of which

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the first S_1 (warning signal) was a flash of light whilst the second S_2 (imperative signal) 1.25 s later was a tone emitted through a loud speaker. The subject was instructed to press a button as soon as possible after S_2 which stopped the tone. The EEG was recorded between the left mastoid and vertex positions. The averaged response to each series of ten pairs of signals was traced out by an X-Y recorder. The signals were given in series of ten, each of which lasted 1 min 10 s. Throughout each series subjects were instructed to keep their eyes fixed on a mark in order to reduce eye movements to a minimum. In most experiments compensation was made for eye movements as described previously (Ashton, Millman *et al.*, 1978).

The magnitude of the CNV was measured as the area between the negative deflection and the base line and expressed in μV.s. Heart rate was obtained from the ECG recorded via precordial leads.

Intravenous nicotine

Injections of nicotine were given in the form of nicotine bitartrate, measured in terms of base, which was made up from specially prepared sterile solutions of neutralised nicotine bitartrate according to the method of Armitage, Dollery, George, Houseman, Lewis & Turner (1975). A fresh solution was prepared for each experiment by diluting with physiological saline a stock solution which contained either 100 μg/ml or 150 μg/ml. Each total dose of nicotine was given as five equal intermittent 'shots', each of 1 ml, injected at intervals of 1 min. Corresponding injections of physiological saline were given as a control. All injections were made via a 'butterfly' needle inserted into a vein of the forearm opposite to the upper limb being used for button pressing. Throughout the experiment the 'butterfly' needle was continuously flushed with a slow infusion of warmed physiological saline but when nicotine or physiological saline (as control) were given as intermittent 'shots', the rate of the continuous infusion of physiological saline was increased for 20 s before and after each 'shot'. This procedure ensured that each 'shot' was washed through the 'butterfly' needle adequately, a procedure which had been adopted by Armitage and his colleagues (1975).

Procedure

On arrival each subject lay on a couch in the subject room with his head and shoulders propped up on a pillow. His position was adjusted both for comfort and also to ensure that he could see without strain the xenon flash lamp which was positioned to be on a level with his eyes. After cleaning the appropriate areas of skin with spirit, ECG and scalp electrodes were placed in position. A 'butterfly' needle was inserted into a convenient forearm vein and slow infusion of physiological saline started. A medically qualified member of the team sat beside but was screened from the subject throughout the experiment and was responsible for making the injections of nicotine and physiological saline. The other observers sat in the recording laboratory which was situated adjacent to the subject room and from which the experiment could be seen via a 'one-way' glass window. The standard arrangements of closed circuit television and a two-way intercom system between observers and subject were supplemented by a safety feature in the form of a private communication channel via a microphone in front of the observer in the recording room and a miniature earphone worn by the observer who sat beside the subject. This facility enabled the observer in charge of the infusion to be kept fully informed of the subject's responses to it, without the subject's knowledge. A slave cardiac ratemeter was also positioned in the subject room so that it would be seen by the observer but not by the subject.

After the subject had been prepared for the experiment, he was given a brief settling down period. Pre-recorded instructions were played to the subject and the experiment then began. After a practice run, injections of physiological saline or nicotine were given (see '**Results**'). Care was taken to ensure that the subject remained unaware of the nature of the injection. The subject lay quietly and read light literature except when the button was being pressed and the CNV recorded. Some of the subjects took part in repeat experiments and five of them also took part in an additional experiment in which they were asked to smoke a standard cigarette. All experiments occupied approximately 2 h of the subject's time.

Two sets of experiments were carried out. (i) After a number of control injections of physiological saline, five subjects received a fixed dose of nicotine which was repeated and then followed by a second series of injections of physiological saline. (ii) In the second set of experiments eight subjects received a number of different doses of intravenous nicotine alternated with control injections of physiological saline. From the results dose response curves could be constructed.

Results

1 *Experiments using a fixed dose of intravenous nicotine*

In an earlier study of the effects of cigarette smoking on the CNV (Ashton *et al.*, 1974), out of 22 subjects, eleven showed a depressant effect, seven a stimulant effect, whilst four showed a biphasic effect. In the present experiments with intravenous nicotine both stimulant or depressant effects were again observed in five subjects. The dose of nicotine administered to

each subject was estimated to be similar to that which he usually obtained from smoking a cigarette. This estimate was obtained from analysis of the butt from a cigarette smoked in the laboratory on a day prior to the intravenous nicotine experiment.

(a) *Subjects in whom intravenous nicotine produced an increase in magnitude of the CNV* In three of the subjects two doses of 500 μg or 750 μg (each dose divided into five equal 'shots' given once a minute) produced an increase in the magnitude of the CNV which was recorded immediately after the injection had finished. A typical example is shown in Figure 1 (subject 1) where after two saline controls, five 'shots' of 100 μg nicotine increased the size of the CNV to 160% when compared with the mean of the two saline controls. When the dose of nicotine was repeated, the CNV which followed it was increased again although on this occasion to 124% compared with the saline control. During the subsequent post-nicotine saline injections there was a progressive fall in the magnitude of the CNV to values comparable with the original saline control values.

In order to test the reproducibility of these effects, the medical student who acted as the subject for the experiment shown in Figure 1 (subject 1) returned to the laboratory for a repeat experiment 7 weeks after the date of the first experiment and the similar results are shown in Figure 2. The CNVs which were recorded after the control injections of saline were smaller than the control values obtained in the first experiment (see Figure 1). It seems likely that this was due to the fact that the subject was less aroused at the time of the second experiment due to familiarity with the procedure. In spite of this initial difference after five 'shots' of 100 μg nicotine i.v. (total 500 μg) the magnitude of the CNV increased to 155% compared with the mean of the two saline control values. After the same dose of nicotine was repeated, the magnitude of the CNV was a little larger (164%) than after the first dose of nicotine. After each of the post-nicotine saline controls the CNVs showed a slow but definite fall in magnitude although they did not reach control values by the end of the experiment.

In another experiment, the same subject (subject 1) smoked a cigarette and this produced effects on the CNV similar to those produced by intravenous nicotine. After two control CNVs, Figure 3 shows that immediately after finishing a standard cigarette (nicotine yield 2.1 mg) the size of the CNV increased to 245% compared with the mean of the two pre-smoking saline controls and 8 minutes after finishing the cigarette, the CNV had fallen to 133% of control value. This result, which was similar in all three subjects, demonstrates that the effect of smoking a single cigarette and that of a comparable dose of nicotine intravenously (in the form of intermittent 'shots') produce comparable effects both in magnitude and direction.

(b) *Subjects in whom intravenous nicotine produced a decrease in magnitude of the CNV* In two of the five subjects two doses of 750 μg nicotine produced a decrease in magnitude of the CNV. Figure 4 shows the records obtained from a typical experiment (subject 5, Table 1). After stable responses to three control saline injections, five 'shots' of 150 μg were given after which the CNV became immediately depressed to 45% of the mean control value and remained depressed to a lesser degree (60% of mean control) after a second series of five 'shots' of 150 μg. This subject had not smoked a cigarette on the day of the experiment and stated spontaneously that he experienced a transient lightheadedness after the third intravenous shot of nicotine and at the same time was observed to exhibit swallowing movements of which he was unaware as revealed by subsequent questioning. After each of the post-nicotine saline injections there was a progressive recovery of the CNV control values. This experiment was repeated seventeen days later using the same dose of nicotine and gave results comparable to the first experiment.

(c) *Comparison between the effects of intravenous nicotine and cigarette smoking* The direction and magnitude of the changes in the CNV caused by intravenous nicotine in each subject could be reproduced by cigarette smoking. As well as subject 1 (see Figures 1, 2 and 3) each of the other four subjects took part in an additional experiment in which a standard cigarette was smoked (nicotine yield = 2.1 mg) under the same experimental conditions as those used in an earlier study in which the effect of cigarette smoking and other drugs were tested on the CNV (Ashton *et al.*, 1973, 1974). In all five subjects, smoking a single standard cigarette produced a change in the CNV which was similar both in magnitude and direction to that produced by intravenous nicotine.

From these results it was therefore concluded that the effects of the CNV of a fixed dose of intravenous nicotine and smoking a standard cigarette were closely similar and so suggested that the effects of cigarette smoking on the CNV were due largely if not entirely, to nicotine absorbed from the tobacco smoke.

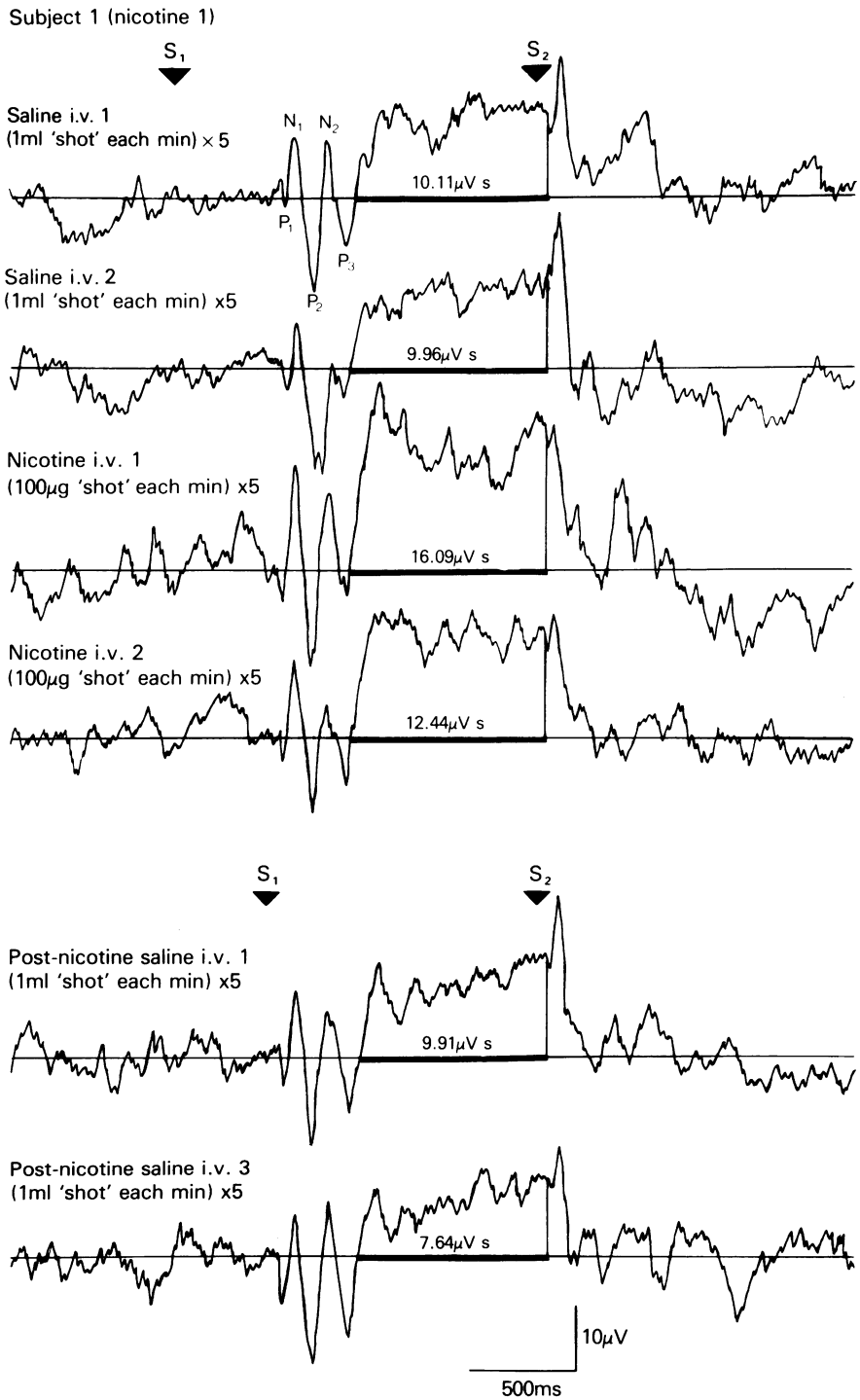

Figure 1 Increase in magnitude of CNV in subject 1 after two doses of i.v. nicotine, 500 μg i.v. (given as 5 shots of 100 μg each min for 5 min). Physiological saline control (5 shots of 1 ml each min for 5 min) given before and after nicotine. S_1 = flash, S_2 = tone, calibration 500 ms and 10 μV; CNV measured as area under curve in μV.s.

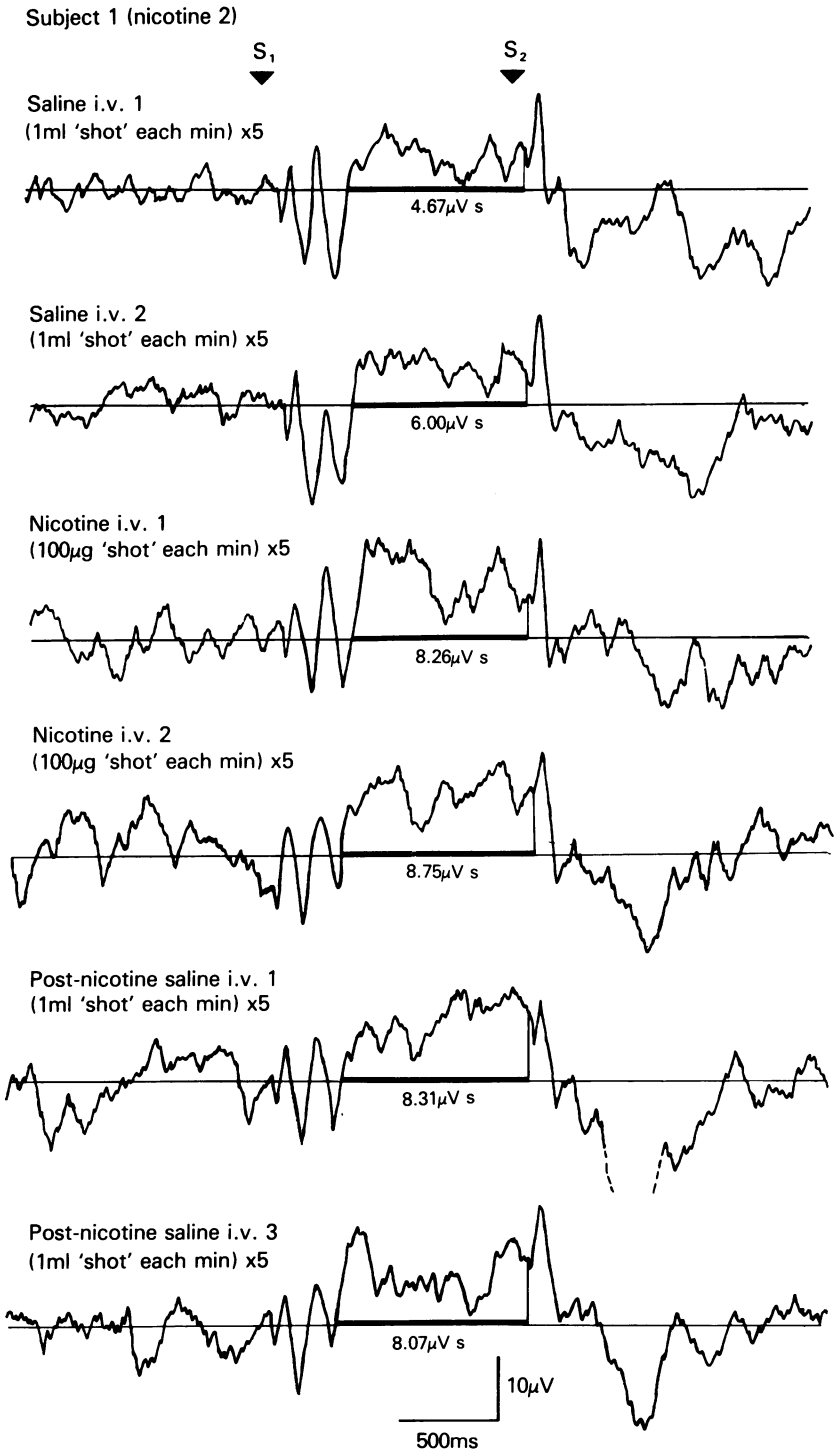

Figure 2 Repeat experiment in same subject (subject 1) as in Figure 1 showing increase in magnitude of CNV after two doses of nicotine 500 μg i.v. (given as 5 shots of 100 μg each min for 5 min). Physiological saline control (5 shots of 1 ml each min for 5 min) given before and after nicotine. Symbols and calibration as in Figure 1.

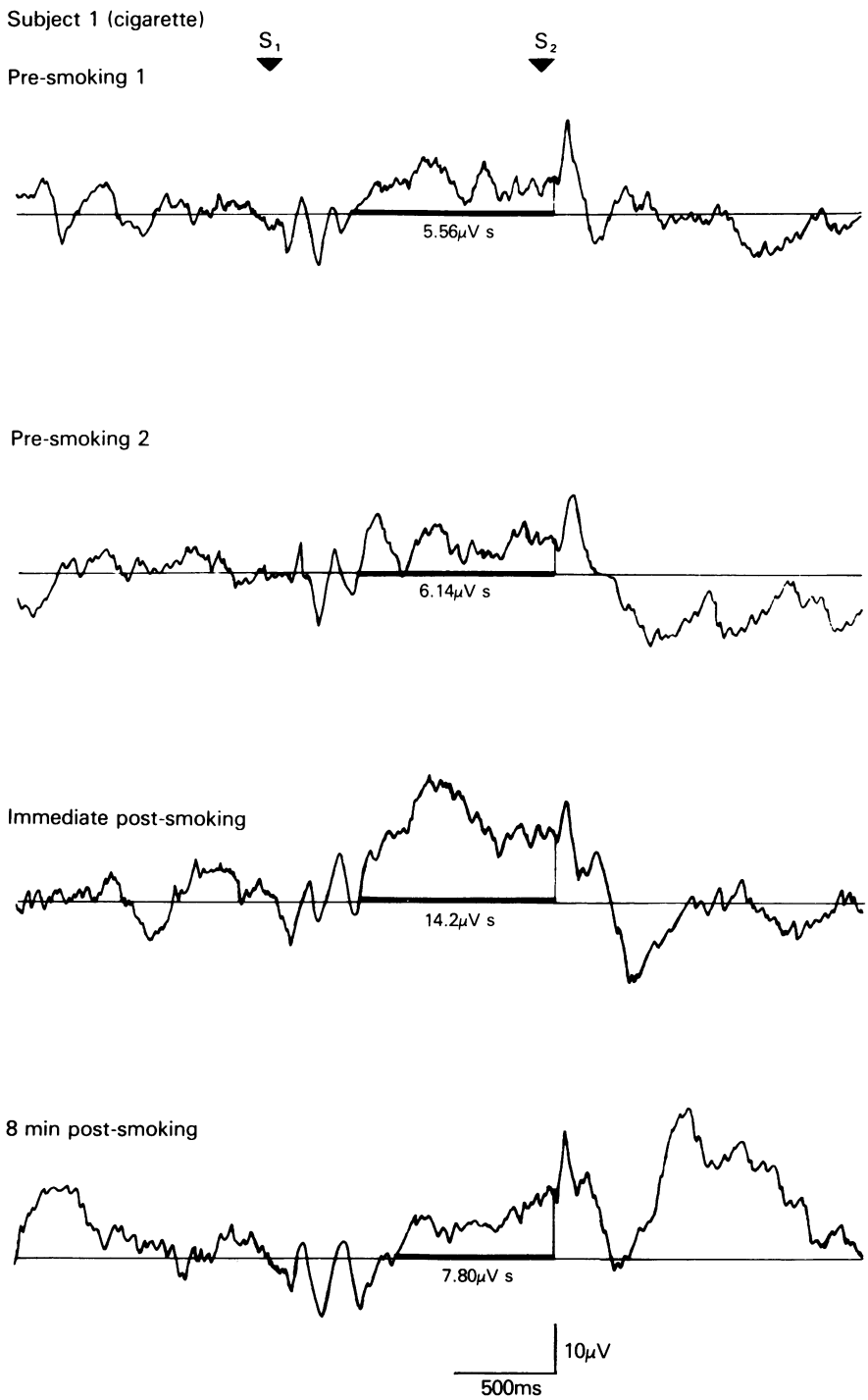

Figure 3 Increase in magnitude of CNV after smoking one standard cigarette in same subject (subject 1) as in experiments of Figures 1 and 2. After two control CNVs note increase in size of immediate post-smoking CNV; 8 min later CNV is still larger than control levels. Symbols and calibration as in Figure 1.

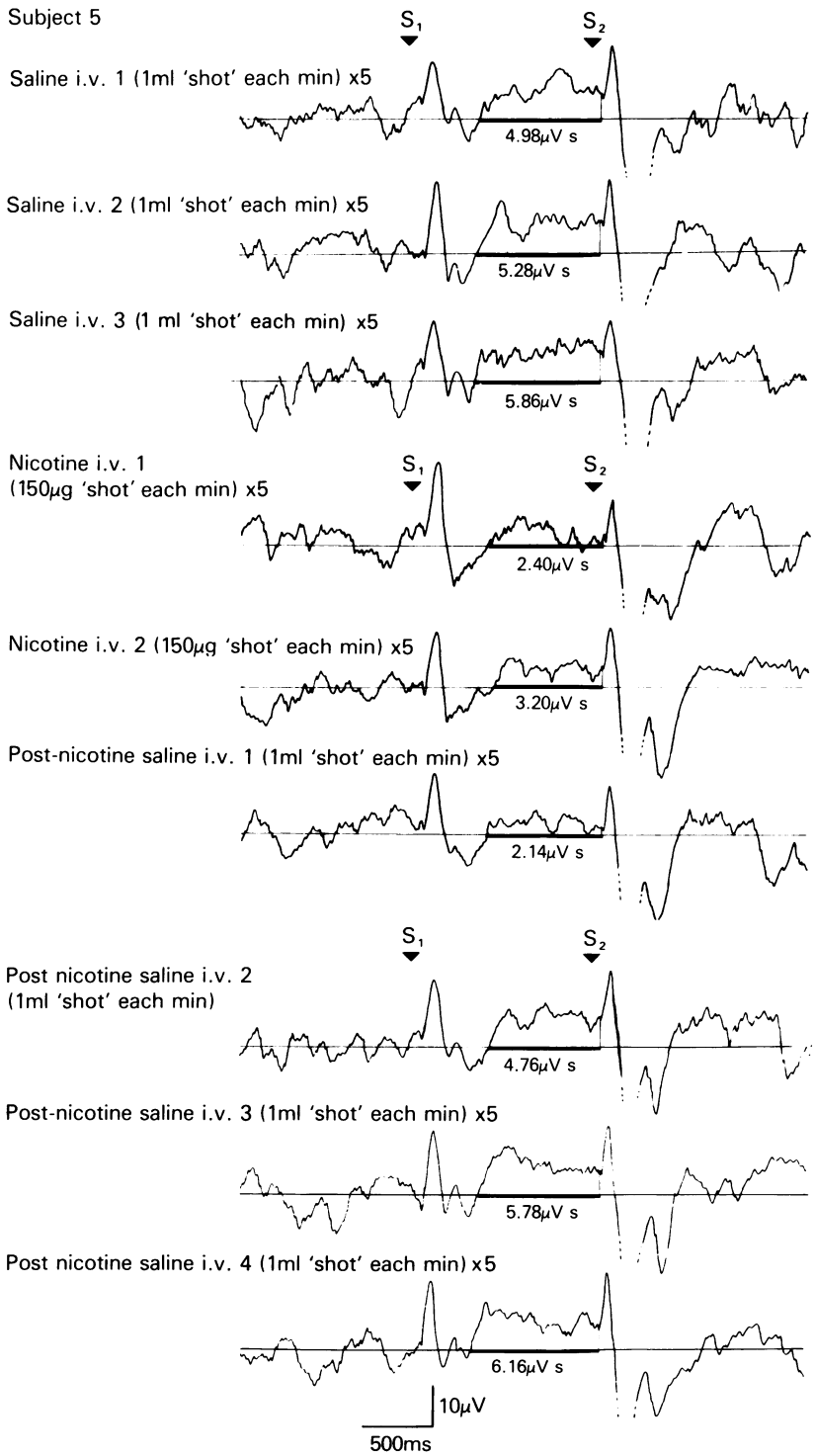

Figure 4 Decrease in magnitude of CNV in subject 5 after two doses of nicotine 750 μg i.v. (given as 5 shots of 150 μg each min for 5 min). Physiological saline control (5 shots of 1 ml each min for 5 min) given before and after nicotine. S_1 = flash, S_2 = tone, calibration 500 ms and 10 μV.

2 *Dose-response relationships of intravenous nicotine*

The results obtained thus far showed that fixed doses of intravenous nicotine mimicked the effects of smoking a cigarette on the CNV. However, whilst some subjects showed an increase in magnitude of the CNV under both conditions, others showed the opposite effect and this raised the intriguing problem as to whether the direction of the effect depended upon the drug or upon the individual who received it. In an earlier study with a group of cigarette smokers (Ashton *et al.*, 1974), it was observed that whereas the majority of smokers showed either an increase or a decrease in magnitude of the CNV following smoking, a small number of subjects showed a biphasic effect. This suggested that the direction and magnitude of the changes in the CNV induced by cigarette smoking or nicotine depended upon the dose of nicotine given to a particular individual. If this hypothesis is correct it ought to be possible to produce both types of effects, i.e. increases and decreases in any individual by giving an appropriate series of doses of nicotine ranging from small to large.

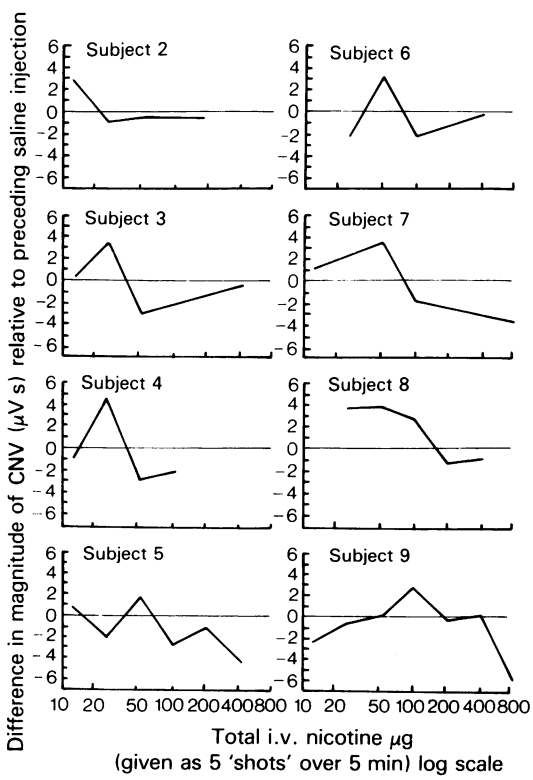

Figure 5 Dose-response curves in eight subjects. Abscissae: total i.v. nicotine μg (given as 5 'shots' over 5 min.) log scale; ordinates: difference in magnitude of CNV (μVs) relative to preceding saline control.

Therefore dose-response curves for intravenous nicotine were obtained in eight subjects. After a practice run, each subject received alternating injections of physiological saline and intravenous nicotine; after each injection a CNV was recorded. In some experiments the doses of nicotine were increased sequentially, but in others the doses were given in random order. Figure 5 shows dose-response curves from each of the subjects illustrating that they are of a novel form so that with small doses of nicotine there is an increase in magnitude of the CNV with increasing dose (compared with the effect of the saline injection which preceded each dose of nicotine) whilst with larger doses the magnitude of the CNV decreases with increasing dose and ultimately becomes smaller than the control values, reaching a *minimum* with the highest dose of nicotine. The dose-response curves of all eight subjects were similar in shape but crossed the saline control baseline at different values of nicotine dosage. Biological variation between individual dose-response curves is already well known with other drugs, for example, the effect of isoprenaline on heart rate (Rawlins, Davies & Routledge, 1976).

Computation of mean dose-response curve for different subjects

Since the general form of the dose-response curves of the eight subjects was similar, a mean dose-response curve was prepared. For each of the seven doses of nicotine i.e., 12.5, 25, 50, 100, 200, 400 and 800 μg, the change in the mean CNV magnitude relative to the preceding saline control was calculated. The seven values were then plotted in the form of a log dose-response curve (Figure 6).

This mean curve is clearly biphasic but gives a distorted picture of the results because both the shape of the curve and the crossover point for each subject differs. In order to summarise the most important features of the individual dose-response curves, the graph shown in Figure 7 (Table 1) was constructed as follows. For each subject four points were determined (i) change in CNV magnitude at the minimum dose of nicotine given, (ii) the zenith, i.e. change in CNV magnitude at the dose which produced the largest *increase* in the CNV magnitude, (iii) the dose at which the curve crossed the baseline and (iv) the nadir, i.e. change in CNV magnitude at the dose (within the range of doses which could be fitted into each experiment) which produced the largest *decrease* in CNV magnitude. The mean and s.e. mean for each of these four sets of values were then calculated both for dose and response except for (iii) where only the mean and s.e. mean for the dose could be calculated. All the individual values for change in CNV magnitude in (i), (ii) and (iv) were expressed as differences from their respective preceding salines before calculating the mean values. The graph confirms the biphasic res-

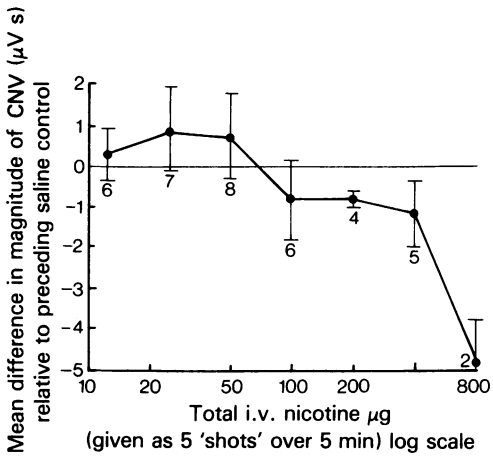

Figure 6 Mean dose-response curve of i.v. nicotine on CNV magnitude in eight subjects. Abscissae: total i.v. nicotine μg (given as 5 'shots' over 5 min) log scale; ordinate: Mean difference in CNV magnitude (μV.s ± s.e. mean) relative to preceding saline control. Numbers indicate number of individual values at each dose.

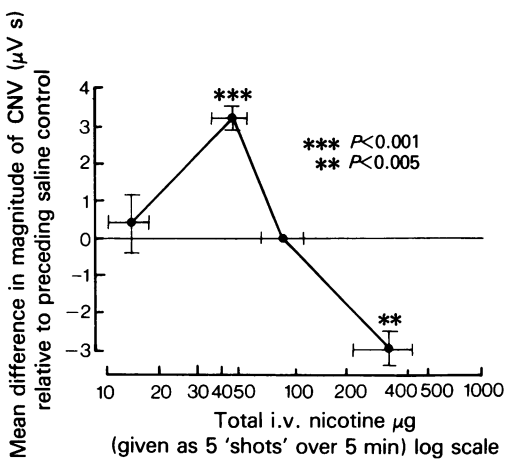

Figure 7 Graph of effect of i.v. nicotine on CNV magnitude in terms of mean minimum dose response, mean zenith, mean crossover point and nadir in eight subjects. Minimum, zenith, crossover point and mean nadir as in Table 1. Mean and s.e. mean given for dose and response, except for crossover point where s.e. mean given for dose only. CNV magnitude at zenith and nadir points are significantly different from their preceding saline values ($P < 0.001$ and $P < 0.005$, respectively; t-tests). Nicotine dosage range at zenith is significantly different from that at nadir ($P < 0.025$, t-test).

ponse of the CNV to nicotine and a t test of the zenith and nadir points shows them to be significantly different from their preceding saline values. In addition, the range of doses which produced an increase in magnitude of the CNV is significantly different from the range which produced a decrease in magnitude ($t = 2.36$ $P < 0.025$).

Heart rate

In this series of experiments the heart rate, which was monitored for medical reasons, was observed to increase during the administration of nicotine. The results obtained from the eight subjects showed that the direction of change of the CNV was independent of the change in heart rate. These results have been published elsewhere (Ashton, Stepney & Thompson, 1979).

Discussion

The results obtained in the present study show that

Table 1 CNV response to different doses of i.v. nicotine in eight subjects. Each nicotine dose (μg) given as five 'shots' at 1 min intervals. Δ CNV: change in CNV magnitude (μV.s) from preceding saline control. Minimum: Δ CNV at the minimum dose of nicotine given; zenith: Δ CNV at the dose which produced the largest increase in CNV magnitude; crossover: the dose at which the curve (Figure 7) crossed the baseline; nadir: Δ CNV at the dose which produced the largest decrease in CNV magnitude.

	Minimum		*Zenith*		*Crossover*	*Nadir*	
Subject	*Dose*	*Δ CNV*	*Dose*	*Δ CNV*	*dose*	*Dose*	*Δ CNV*
2	12.5	+2.8	12.5	+2.8	21.5	25	−0.08
3	12.5	+0.33	25	+3.56	36.5	50	−3.06
4	12.5	−0.95	25	+4.42	38.0	50	−2.87
5	12.5	+0.75	50	+1.75	65.5	400	−4.32
6	25.0	−2.05	50	+3.26	76.0	100	−2.10
7	12.5	+1.15	50	+3.45	78.0	800	−3.70
8	25.0	+3.75	50	+3.84	158.0	200	−1.30
9	12.5	−2.14	100	+2.64	185.0	800	−5.99
Mean	13.13	+0.46	45.31	+3.22	82.31	303.13	−2.93
s.e. mean	3.02	0.76	9.43	0.29	20.86	116.54	0.46

intravenous injections of neutralised nicotine administered as intermittent 'shots' can mimic the effects of cigarette smoking on the CNV. Both nicotine and cigarette smoking (Ashton *et al.*, 1973) produce biphasic effects on CNV magnitude which are dose-related. Although the genesis of event-related slow potentials of the brain, of which the CNV is the archetype (Walter, Cooper, Aldridge, McCallum & Winter, 1964), remains to be elucidated, previous studies have shown that the magnitude of the CNV can be used as a sensitive, objective and reproducible method to detect the effects of stimulant and depressant drugs on the human brain (Ashton *et al.*, 1973, 1974, 1976). Thus the results suggest that the biphasic effect of nicotine on the CNV reflects its ability to produce both central stimulant and depressant effects.

Armitage and his colleagues (Armitage, Hall & Morrison, 1968; Armitage *et al.*, 1969) also obtained both stimulant and depressant effects of nicotine in animals and demonstrated that intravenous nicotine imitated the effects of puffs of inhaled tobacco smoke only if administered as a series of 'shots' and not if infused continuously. The need to administer nicotine intermittently in order to imitate the effects of cigarette smoking may reflect subtle and unusual pharmacological properties of nicotine. In the superior cervical ganglion of the cat, nicotine produces a biphasic response, first stimulating and then paralysing nerve cells (Langley & Dickinson, 1889; Paton & Perry, 1953). The initial stimulant effect is due to depolarisation of the ganglion cells, while the paralytic effect is due to a prolonged depolarisation which may not, however, persist for the total duration of the block. It has been suggested (Ginsberg, personal communication) that the latter part of the paralytic phase is accompanied by certain reversible conformational changes in the nerve cell membrane. During the paralytic phase, the ganglion responds neither to electrical stimulation nor to a second dose of nicotine (Feldberg & Vartianen, 1935). Thus the biphasic action of nicotine depends upon both the pharmacodynamic and pharmacokinetic properties of nicotine. Since the present study is concerned principally with the central actions of nicotine, it is important to note that nicotine, which may enter the circulation via the lungs (as in smoking) or via a vein (as under experimental conditions), rapidly crosses the blood-brain barrier to enter the brain; conversely, when the concentration gradient is reversed, nicotine will rapidly leave the brain (Schmiterlow & Hansson, 1962, 1965).

When all these facts are considered together it is reasonable to postulate that the puff of cigarette smoke or the 'shot' of intravenous nicotine initiates the following sequence of events. The dose of nicotine enters the circulation, rapidly passes into the brain and acts upon susceptible areas. At any specific site, nicotine will cause initial stimulation followed by a period of paralysis, the duration of which is related to the dose of nicotine or probably more directly to the maximum local concentration achieved together with its rate of rise and fall. After a small dose of nicotine there will be a brief stimulant effect followed by a brief paralytic phase, at the end of which the receptors are able to respond again to a further dose of nicotine. After a large dose of nicotine the stimulant effect is followed by a longer paralytic effect, during which an additional dose of nicotine is unable to produce excitation. Thus, it seems probable that if a smoker desires a stimulant effect he can achieve it by manipulating each inhalation of cigarette smoke so that a 'shot' of nicotine with characteristics necessary to produce maximal stimulation followed by minimal paralysis of the receptor enters the circulation. Conversly, if a depressant or paralytic effect is required, each inhalation will need to be manipulated to produce a 'shot' which causes minimal stimulation and maximal paralysis. Experimental evidence which supports the hypothesis that smokers modify their smoking behaviour so as to alter the rate of nicotine intake comes from the results of two other studies (Ashton & Watson, 1970; Ashton *et al.*, 1979).

If the action of nicotine on the brain, as determined by the CNV, represents the resultant of a stimulant and a depressant effect, which neuroanatomical systems mediate the effect? The simplest possibility is that nicotine modulates a single system through its dual ability to excite and paralyse that system. Another possibility is that nicotine acts simultaneously upon two systems which have different dose-effect relationships and which themselves interact. Under these conditions, fine adjustment of the balance between these two systems could be achieved from moment to moment by delivery to the brain of precisely determined 'shots' of nicotine. Such a possibility would be in accord with the two-arousal hypothesis suggested by Routtenberg (1968) where the reticular activating system (which has already been implicated in the genesis of the CNV; Rebert, 1972) and the limbic system may represent the two main elements which are concerned with general arousal and goal-directed behaviour, respectively. Other possibilities obviously exist but it seems clear that whatever mechanism actually takes place, it must produce some desirable change in brain function or there would be no smokers.

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