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Tolerance Development to the Arousal Effects of Nicotine

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HUBBARD, J. E. AND R. GOHD. *Tolerance development to the arousal effects of nicotine.* PHARMAC. BIOCHEM. BEHAV. 3(3) 471–476, 1975. – To determine if repeated daily doses of nicotine induces tolerance to both its EEG and behavioral activating effects, rats implanted with sets of bipolar cortical electrodes and carotid cannulae received intracarotid injections of either (1) 6 daily doses of nicotine (as tartrate, 30 μg/kg) (Group 1) or (2) 3 daily doses of saline followed by 4 days of nicotine (Group 2). From an exercise-induced resting state, nicotine produced immediate EEG and behavioral arousal, both of which disappeared in Group 1 by Day 6. Saline administered to Group 2 rats produced little or no response but subsequent nicotine resulted in responses similar to those produced by Group 1 animals. It is concluded that tolerance development to nicotine activation is reflected not only in behavior, but also in the EEG.

Nicotine Arousal EEG Behavior Tolerance

NICOTINE administered intravenously at intervals of less than 30 min produces tolerance to its EEG and behavioral arousal effects: this tolerance is not seen when the drug is given at intervals greater than 30 min [8, 31, 35]. Others have demonstrated long term behavioral tolerance to nicotine in terms of spontaneous activity [15, 20, 29] and learned behavior [9,25], but not to the postnicotine arousal effect. Preliminary studies in the authors' laboratory suggested that the effects of single daily doses of nicotine are carried over for at least 24 hr, resulting in tolerance development to both the EEG and behavioral activating effects of nicotine [14]. The present study was conducted to examine this possibility.

METHOD

Animals

Fifteen male Wistar-Lewis rats (Charles River Breeding Laboratory) weighing 250–300 gm were used. They were housed at the Health Sciences Center animal care facility, fed and watered ad lib, and maintained at constant temperature on a regulated 12 hr light/dark cycle.

Surgical Procedures

A set of 4 bipolar cortical electrodes and an indwelling carotid cannula were implanted in each of the rats anesthetized with sodium pentobarbital (Nembutal Sodium, Abbot Laboratories; 35 mg/kg). The cannula, made of PE 50 and Tygon tubing [11], was inserted in the right carotid artery toward the heart, sutured to the surrounding tissue and skin, and exteriorized on the back of the neck. A week following cannula placement, the electrodes were implanted as described elsewhere [12] establishing contact with the left frontal, parietal, occipital and temporal cortices.

Testing Procedure

Testing began 3–5 days following electrode placement and was conducted in a small ventilated soundproof chamber within a Faraday cage. The chamber was equipped with lighting, a Plexiglas viewing window, tethered EEG input leads and tethered small-bore Tygon tubing (Technicon autoanalyzer transmission tubing, Gradko Scientific Co.) leading to a microliter syringe external to the Faraday cage. The tubing was filled with either saline or nicotine solution; a syringe needle was attached at the free end for insertion into the carotid cannula. With this arrangement, the rats were free to move about, to be observed, and to have EEG's recorded and injections made with minimal disturbance. To achieve a resting state with synchronized slow wave (high amplitude, low frequency) EEG activity, the rats were fatigued by 3 hr exercise on a motorized activity wheel (1 1/3 rpm) just prior to each test.

The animals were divided into two groups. Rats in Group 1 (n = 10) were fatigued as described and connected to the external tubing and EEG leads. After onset of stable synchronized wave activity, usually within 10–15 min, 30 μg/kg nicotine tartrate (K & K Laboratories, Inc.), equivalent to 9.6 μg/kg nicotine base, in 0.05 ml phosphate buffered saline (PBS) was injected over a 20 sec interval. (Hereafter, unless otherwise indicated, all nicotine doses will be expressed as the tartrate form). This procedure was performed once each day during the early afternoon for 6 days. Rats in Group 2 (n = 5) were treated similarly, but received 0.05 ml PBS on Days 1–3, then 30 μg/kg nicotine

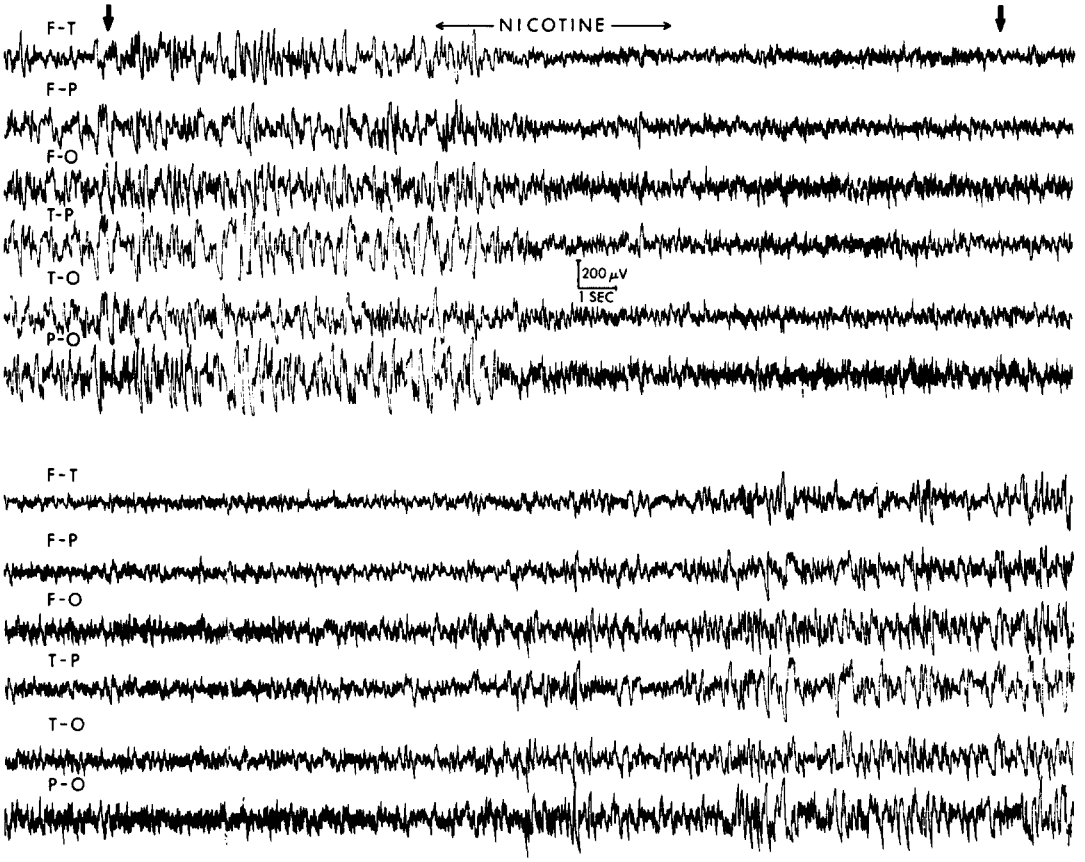

FIG. 1. Bipolar cortical recording of rat EEG showing change of synchronized activity to desynchronization during intracarotid nicotine tartrate (30 μg/kg) administration (vertical arrows). In this study the onset time (between first vertical arrow and start of desynchronization) and the duration of desynchronization were measured; F = frontal, P = parietal, T = temporal, O = occipital).

on Days 4–7. EEG changes were evaluated on the basis of onset interval and duration times of the desynchronized fast wave (low amplitude, high frequency) EEG activity. The behavioral response was scored on the basis of the following criteria: 0 – no arousal, +1 – some head and/or jaw movements, +2 – change of position and other movement for less than 60 sec, +3 – change of position and other movement for 60–120 sec, +4 – change of position and other movement for 120–200 sec. Observations were made for up to 200 sec following onset of desynchronization.

RESULTS

Arousal Response

As seen in Fig. 1, nicotine produced rapid onset of desynchronization, usually within the first 10 sec of the injection (Table 1). Desynchronization began simultaneously and was of equal duration in all leads, indicating uniform response throughout the cortex. Behavioral arousal, if observed, occurred 1–2 sec after desynchronization onset. Responses ranged from slight head movements to a startle response with subsequent walking and exploratory behavior. In some cases. particularly by Day 5 in Group 1, EEG desynchronization was recorded without observable behavioral changes (Tables 1 and 2).

Group 1

When nicotine was administered to Group 1 rats during the 6 day period, EEG desynchronization duration dropped markedly with a slight increase (particularly by Day 5) in onset times (Table 1). With respect to desynchronization duration, the p values of day-to-day differences reflect that responses on Days 1–3 ($p<0.2$) were relatively uniform while the significant drop in responsiveness took place over Days 3–5 ($p<0.001$). There was considerable individual variation in the rate of decrease in responsiveness; this accounted for the comparatively large standard errors (Table 1) during this period. Correspondingly, the degree of behavioral arousal produced by nicotine injection also dropped, with much of the decrease occurring during Days 3–5 (Table 2). This decreased responsiveness was overcome with higher doses of nicotine (60 μg/kg).

Group 2

The purpose of Group 2 was to determine (1) the effect of the saline vehicle and (2) if the decreased responsiveness to nicotine was a result of accommodation or habituation to the experimental procedure. On Days 1–3 there was little or no EEG (Table 1) or behavioral (Table 2) response during the period of saline injections. On Day 4, with first administration of nicotine, both EEG (Table 1) and be-

TIME INTERVAL OF EEG DESYNCHRONIZATION ONSET AND DURATION PRODUCED BY SALINE OR NICOTINE INJECTION

	DAY													
	1		2		3		4		5		6		7	
Animal	OT	D	OT	D	OT	D	OT	D	OT	D	OT	D	OT	D
Group 1														
1	6	200	9	200	14	200	8	200	13	8	–	0	*	*
2	7	200	11	200	10	200	6	105	*	*	*	*	*	*
3	3	157	6	177	6	84	16	14	–	0	–	0	*	*
4	6	200	11	200	4	200	11	50	–	0	–	0	*	*
5	14	186	17	130	9	81	–	0	*	*	*	*	*	*
6	3	200	6	200	3	200	9	200	7	97	–	0	*	*
7	4	200	8	200	9	140	6	125	10	60	–	0	*	*
8	3	120	5	200	11	133	5	62	17	8	8	54	*	*
9	9	90	16	200	8	200	17	46	13	20	–	0	*	*
10	16	130	10	200	8	200	10	200	–	0	–	0	*	*
Mean ± SE	7.1 ± 1.5	168 ± 13	9.9 ± 1.3	191 ± 7	8.2 ± 1.0	164 ± 16	9.8 ± 1.4	100 ± 25	12.0 ± 1.2	24 ± 13	–	7 ± 7	*	*
Group 2														
11	–	0	–	0	–	0	6	200	6	200	6	200	15	130
12	–	0	–	0	–	0	11	171	8	200	5	200	7	2
13	–	0	–	0	–	0	5	200	*	*	*	*	*	*
14	–	0	–	0	–	0	8	160	7	133	*	*	*	*
15	–	0	15	43	–	0	4	190	6	195	5	200	7	140
Mean ± SE	–	0	–	9 ± 9	–	0	6.8 ± 1.2	184 ± 8	6.8 ± 0.5	182 ± 16	5.3 ± 0.3	200 ± 0	9.7 ± 2.7	91 ± 44

*Animal not used due to technical difficulties (#2,5,14 – pulled cannulae; #13 – blocked cannula)
†Saline injected animals represented by Group 2, Days 1–3; Nicotine given as tartrate (30 μg/kg) over 20 sec
OT = onset time (sec); D = duration of desynchronization (sec)

TABLE 2

SCORED BEHAVIORAL RESPONSES TO SALINE OR NICOTINE INJECTIONS

	DAY						
Animal	1	2	3	4	5	6	7
Group 1							
1	+4	+4	+3	+2	0	0	*
2	+4	+4	+3	+1	*	*	*
3	+3	+3	+1	0	0	0	*
4	+3	+3	+4	0	0	0	*
5	+4	+3	+2	0	*	*	*
6	+4	+4	+3	+2	+1	0	*
7	+4	+4	+3	+3	+1	0	*
8	+2	+4	+3	+1	0	+1	*
9	+1	+3	+3	+1	0	0	*
10	+3	+2	+4	+4	0	0	*
Mean	+3.1	+3.3	+2.9	+1.4	+0.6	+0.1	
Group 2†							
11	0	0	0	+4	+4	+4	+3
12	0	0	0	+3	+3	+2	0
13	0	0	0	+4	*	*	*
14	0	0	0	+4	+4	*	*
15	0	+1	0	+3	+4	+2	+2
Mean	0	+0.1	0	+3.6	+3.6	+3.7	+1.6

*Animal not used due to technical difficulties (#2,5,14 – pulled cannulae; #13 – blocked cannula)
†Saline injected animals represented by Group 2, Days 1–3

havioral (Table 2) arousal increased to the levels observed on Day 1 of nicotine treatment in animals of Group 1. By the 4th day of nicotine administration, Group 2 rats, like those of Group 1, declined in EEG and behavioral responsiveness. Since the effects of nicotine administration over the same time course in both groups of animals were not significantly different, the data were pooled and are presented in Fig. 2. In summary, saline administration produced little or no arousal response; the first few nicotine injections resulted in a marked increase of both EEG and behavioral arousal, which, after the 6th nicotine administration diminished in a parallel fashion to approach saline levels.

DISCUSSION

This study reveals that when nicotine is given daily in small amounts, the drug's ability to produce EEG and behavioral activation is rapidly reduced, the effect being observed after only three 9.6 μg/kg doses of nicotine base at 24 hr intervals. This effect probably does not represent accommodation or habituation by the rats to extrinsic stimuli in the experiment since the response of Group 2 animals with first administration of nicotine, having received 3 previous injections of saline, was not significantly different from those of Group 1 rats on the first day of nicotine. It is concluded, therefore, that rats rapidly acquire tolerance to the EEG and behavioral arousal effects of nicotine and that the tolerance lasts at least 24 hr.

Domino *et al.* [8, 31, 35] have demonstrated tachyphylaxis with respect to the behavioral EEG activating effects of nicotine when the drug is given at short intervals; however, when given at intervals greater than 30 min, these investigators found that nicotine produced its usual central effects. In the present study, nicotine administered repeatedly at 24 hr intervals produced tolerance. In addition

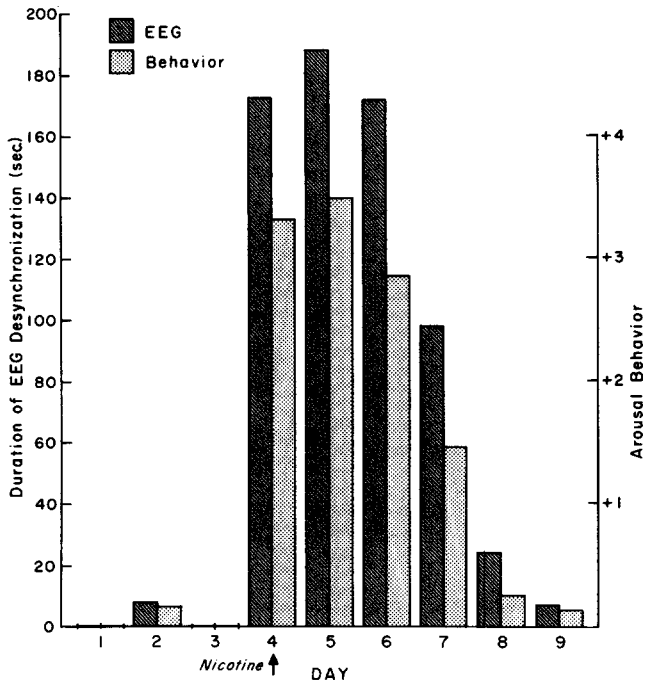

FIG. 2. Graph of the means of pooled data from Groups 1 and 2 (see text) illustrating the development of tolerance to the EEG and behavioral arousal effects of nicotine. By the 6th day of daily nicotine administration (day 9 on the abscissa), the animals' responsiveness to postnicotine EEG and behavioral activation has been reduced to saline levels (Days 1–3 on the abscissa).

to the injection interval, two other differences in experimental design between these two studies may account for the conflicting observations. First, differences in species and type of animal preparation may be involved since Domino worked with brain stem-transected cats and dogs. Secondly, and perhaps most important, the rate and duration of nicotine injection in this study were different from that used by Domino; the administration in this study was 30 μg/kg tartrate over 20 sec while Domino injected 10 μg/kg base over 1 min. Thus the rate in this study was faster while the duration was shorter. Currently, studies are under way to examine the effect of a very slow rate of nicotine administration over a prolonged period (11 μg/kg/min tartrate over 40 min) on tolerance development. In a study which evaluated the chronic effects of nicotine on rabbit EEG activity, Bhattacharya and Goldstein [5] noted that tolerance did not develop. Failure to observe tolerance in this case may be due to the species difference or to the subcutaneous route of administration; in regard to the latter, the nicotine may have been metabolized before sufficient blood levels were reached to induce tolerance.

On the basis of all these observations, it is suggested that two different mechanisms are responsible for these differences in tolerance intervals to the EEG and behavioral activating effects of nicotine. The shorter interval tolerance as reported by Domino and coworkers [8, 31, 35] may be due to prolonged receptor occupation by nicotine, which is known to last for as long as 30 min [22]. Longer interval tolerance as demonstrated here may result from induction by nicotine of its own metabolic enzymes or of other enzymatic systems since both nicotine injections and cigarette smoking have been reported to stimulate metabolic enzyme activity [2, 3, 7, 32, 33, 34].

It is known that nicotine readily crosses the blood brain barrier [21] and concentrates in the molecular and pyramidal cell layers of the hippocampus [26]. Nicotine-induced EEG activation (and seizure activity with sufficient doses) appears to originate from the hippocampus and spread to the cerebral cortex [10, 30, 35]. On the other hand, nicotine produces peripheral effects such as respiratory stimulation and blood pressure elevation [35]. These peripheral and central actions of nicotine have been pharmacologically dissociated from one another. Cholinergic antagonists such as atropine block the peripheral nicotine effects but not CNS activation, while only mecamylamine is able to block EEG activation [8, 16, 35]. This drug analysis has led to the conclusion that the EEG desynchronizing and behavioral arousal effects of nicotine are due primarily to direct CNS actions rather than peripheral afferent stimulation [35]. On the basis of this and the observations that (1) behavioral arousal was always seen after EEG desynchronization onset and (2) EEG activation was observed without behavioral arousal, it is suggested that the arousal and its tolerance development as demonstrated in the present study are central in origin.

Finally, a word should be said with respect to the nicotine induced arousal phenomenon. In most if not all species examined, nicotine produces EEG activation closely resembling normal arousal [6,13] from either the unanesthetized resting state or anesthetized state [1, 6, 8, 13, 17, 23, 24, 27, 28, 31, 35]. Behaviorally, however, the effects of nicotine are not as well defined. While the arousal response in the present study is similar to that described for the cat [8, 27, 28], others have reported a depressant effect of nicotine upon rat [29] and mouse [18] behavior, and, in cats, with depression followed by stimulation [19,20]. These and other reports call attention to the many conflicting results in the nicotine and tobacco literature which stem not only from species, sex and preparation differences, but also from diurnal, inter- and intrastrain, and individual variability. These differences coupled with the multiple actions of nicotine need to be carefully considered when interpreting data related to the effects of nicotine.

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