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Protection by Nicotine from Behavioral Disruption Caused by Reticular Formation Stimulation in the Rat[1,2]

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NELSEN, J. M., K. PELLEY AND L. GOLDSTEIN. *Protection by nicotine from behavioral disruption caused by reticular formation stimulation in the rat.* PHARMAC. BIOCHEM. BEHAV. 3(5) 749–754, 1975. – Male Sprague-Dawley rats prepared with chronic electrodes in the mesencephalic reticular formation were trained to perform on a visual attention task. Short trains of electric current delivered to the reticular formation effectively blocked performance in a reversible and reproducible fashion. Subcutaneous administration of 100 μg/kg nicotine (as the base) served to attenuate the behavioral disruption caused by reticular stimulation. The suggestion that it is a nicotine-induced limbic system activation which antagonizes the behavioral disruption caused by electrically-induced reticular over-activation, is discussed.

Nicotine Visual attention task Reticular formation Limbic system Arousal Brain electrical stimulation

ELECTROENCEPHALOGRAPHIC studies in our laboratory have indicated that chronic nicotine treatment results in changes in cortical-limbic-reticular formation relationships which might well modify an organism's functional state particularly in terms of the level and nature of arousal [2,12]. It has been demonstrated that electrical stimulation of the mesencephalic reticular formation (mesencephalic reticular activating system) can produce cortical EEG activation and also, behavioral activation, for example, the awakening of a sleeping or sedated animal [8]. However, it would appear that the reticular formation (RF) is not unique in its capacity to induce and maintain arousal. First, the EEG state of sleep which follows mid-collicular section is not necessarily permanent. Batsel [1] and Villablanca [16,17] have described patterns of spontaneous EEG arousal followed by alternating states of sleep and wakefulness, emerging 10 to 15 days after such surgical lesioning. Further, studies have shown that electrical stimulation of portions of the limbic system, particularly the amygdaloid nucleus and hippocampus can produce cortical EEG activation even in "cerveau isolé" preparations [3]. This implies that arousal may be mediated by structures other than the reticular activating system, specifically limbic structures.

It has been suggested that the arousal mediated by the RF is nonspecific or generalized in nature while that mediated by the limbic system allows for more selectively motivated or goal-directed behaviors and, further, that the RF and limbic systems are mutually inhibitory [14]. The EEG studies conducted in our laboratory have indicated that chronic nicotine treatment induces a state of heightened limbic arousal or relatively greater limbic influence and lesser RF influence in the production of cortical activation [2,12]. From this finding, it was predicted that such a chronic nicotine state would be beneficial for performance of behaviors requiring focused attention or arousal and discrete goal-directed responses. Direct behavioral testing of this prediction confirmed that chronic nicotine treatment did improve performance of a visual attention task by rats [10,11].

At appropriate current levels, delivery of short trains of electrical current to the RF disrupts on-going conditioned behavior [7] presumably because the induced state is one of generalized over-arousal. If the RF and limbic system are mutually inhibitory, then one could hypothesize that the over-arousal or hyperstimulated state resulting from increased RF activation could be counteracted by increased limbic activation. The current study represents the preliminary investigation of nicotine's efficacy (via its proposed actions on limbic structures) as an antagonist of the behavioral disruption resulting from RF stimulation.

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METHOD

Animals and Surgery

The experimental animals were male, adult Holtzman (Sprague-Dawley) rats. They had been prepared surgically with chronic bipolar electrodes including one placed in the mesencephalic reticular formation (8.0 mm posterior from Bregma, 2.0 mm lateral of midline, and 7.0 mm down from top of skull, placed with the animal's head oriented such that the top of the skull was parellel to the horizontal plane). The surgical procedure was essentially the same as has been described previously [12] with the exception that Winchester connectors were used and the electrodes were manufactured from twisted 30 ga, double formvar coated stainless steel wire. The rats earlier had undergone a course of chronic nicotine treatment but had not received any drug administrations for a month preceding the training and testing related to the present study.

Training

The rats were trained to perform on a visual attention task which has been described previously [11]. Briefly, food deprived animals were taught to press a lever for a food-pellet reinforcement following the presentation of a white cue light in a standard operant conditioning chamber. In the task's final form, the conditional stimulus (CS) which had a 0.2 sec duration, signaled the start of an available response time (ART) of 5.0 sec during which the first and only the first lever press was reinforced and terminated the ART. Failure to respond within the 5.0 sec allowed was scored as an omission error (OE). The ART, whether it was terminated by a correct response or at the end of 5.0 sec (an OE), was followed by a variable intertrial interval (ITI) whose mean length was 10.0 sec. A lever press during the ITI was scored as a commission error (CE) and was punished by a 30.0 sec dark period. Presses during the dark period were also scored as CE's and resulted in the restarting of the 30.0 sec punishment period. Thus, effective performance of this task required the animal to learn both to make correct responses and inhibit incorrect responses. The criteria for successful acquisition of the task were CE and OE socres both equal to or less than 30 percent, that is, no more than 30 OE's per 100 reinforcements and no more than 30 CE's per 100 reinforcements. Sessions were terminated when 100 reinforcements had been obtained.

Procedure

After several months of training when the task had been well learned, the rats were tested to determine for each animal the current level of reticular formation stimulation (RFS) necessary to block performance on the task. Current was delivered 6.0 sec after the onset of a CS independent of whether the rat had responded or failed to respond. By choosing this schedule for current delivery, it was assured that RFS would not occur during an ART or simultaneously with the next CS, but rather during an ITI. The stimulation consisted of 500 msec trains of symmetrical, biphasic constant current pulses which were produced by Grass stimulators (Models S4 and S6) yoked together and used in conjunction with Grass photoelectric stimulus isolation units (Model PSIU6). The frequency was 100 pulse pairs per sec and the pulse width was 0.2 msec with a 1.0 msec delay between the two pulses of a pair.

The appropriate pulse height or current level for each individual was determined by testing while the rat was performing on the task. The initial RFS level was 10 μA. This level was increased by 5 or 10 μA increments in successive daily sessions until the intensity which arrested responding completely was found. When the apparent effective level was determined, the stimulation intensity was reduced to the next lower step for a test session with the contingency that this level should not arrest responding completely if the next higher level was to be considered the appropriate one. This current level was then tested at least 5 times over a two-week period to assure that the disruption of responding was a stable one, reproducible over time. Stimulation current levels were monitored routinely during all sessions with a differential input oscilloscope which measured the current flowing in the series loop formed between the current source and the stimulating electrode. The reliability of this monitoring procedure was verified by measuring directly the voltage drop (at the terminal end of the electrode cable) across a resistance which approximated the impedance of the rat's brain at the RF electrode site.

A session was divided into successive blocks of 10 reinforcements except in the case where RFS was being delivered. In this instance, a block was terminated when an animal had made 10 consecutive OE's (and hence, had received 10 consecutive brain stimulations). Thus, if under certain conditions RFS were to cause only a partial disruption of performance and the rat responded intermittently, it would receive more than 10 brain stimulations per block, but the block was always terminated when the rat had received 10 consecutive RFS's without responding. RFS was delivered during Blocks 2, 4, 6 and 8 but no stimulation was applied during Blocks 1, 3, 5, 7, 9, 10 and so on. A session was ended when 100 reinforcements had been obtained or after 90 minutes had elapsed. Readings of OE and CE scores were taken for each block.

Four critical types of sessions were carried out. These were saline treatment (SAL), nicotine treatment (NIC), saline plus RFS (SAL + RFS) and nicotine plus RFS (NIC + RFS). All injections were given subcutaneously between Blocks 3 and 4 of each session. Nicotine was administered at a dose of 100 μg/kg as the base in saline (400 μg/ml); saline was administered in equivalent volumes.

RESULTS

The parameters of stimulation were the same across animals except for current intensity. The effective level for arrest of responding varied between 40 and 90 μA for the 5 rats who completed behavioral training and testing with their electrode platforms intact. An example of the pattern of RFS disruption of behavior on the attention task is shown in Fig. 1. This pattern was typical of all animals; that is, at the appropriate current intensity responding was effectively arrested by 0.5 msec trains of stimulation delivered on the average only once every 10 sec. During the stimulation blocks (2, 4, 6 and 8), rats were relatively immobile, sitting or standing in one location and emitting far fewer random behaviors than under normal, nonstimulation conditions. The selected current for an individual remained effective as a disrupter of performance across sessions. It is noteworthy that there was poststimulation impairment in blocks which immediately followed stimulation blocks (3, 5, 7 and 9). As can be seen in the cumulative

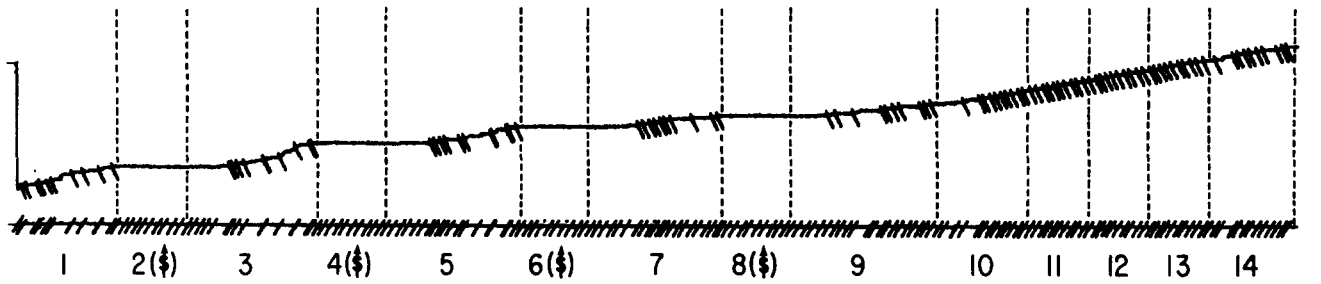

FIG. 1. Cumulative recording of events and responses in a session during which RFS (90 μA) was delivered to Rat No. 3. A deflection on the horizontal line signals the presentation of a CS. A deflection on the rising line indicates a correct response to the CS. The slope of the rising line reflects total responses, both correct and incorrect. RFS was delivered during Blocks 2, 4, 6 and 8 as indicated by the symbol and it effectively arrested responding during these blocks. The carry-over effect into poststimulation Blocks 3, 5, 7 and 9 was characterized by a period of behavioral silence followed by reinitiation of performance which was generally less discrete than during control periods.

record, there was a carry-over of behavioral silence after stimulation had ceased. This was the case for all animals. Further, RFS carry-over effects frequently manifested themselves in uneven performance with rats making more CE's together with more OE's after the re-initiation of responding in a poststimulation block. Blocks 11 through 14 on the sample record illustrate the normal performance pattern, characteristic of the rat under no treatment conditions.

Because the stimulation and poststimulation blocks of trials which followed the injections are the critical ones for between treatment comparisions, Blocks 4 through 9 are the focus of major interest. Shown in the left portion of Fig. 2 are the means and standard errors of OE scores for these 6 blocks of trials under the 4 conditions. An overall analysis of variance (two-way classification, n = 1, mixed model with conditions taken as the fixed variable and subjects, the random variable; cf. [5] p. 254) indicated that the conditions did differ significantly, $F(3,12) = 5.30$, $p<0.05$. Indeed, paired t tests of saline versus each of the treatments confirmed that omission errors were significantly higher under the 3 treatment conditions compared to the saline level: SAL vs. NIC ($t = 2.110$, $p\approx0.05$); SAL vs. RFS ($t = 4.92$, $p<0.005$); SAL vs. NIC + RFS ($t = 3.36$, $p<0.025$).

The scores on the commission error index for the 4 conditions were tested in the same manner, but the analysis of variance did not indicate a significant, between conditions difference. It should be recalled that the RFS level was chosen to arrest responding and thus, CE scores were very low throughout Blocks 4 through 9. (See right portion of Fig. 2.)

Of primary experimental interest was the RFS vs. NIC + RFS comparison. When nicotine treatment was combined with RFS, rats made fewer OE's during the stimulation and poststimulation blocks (Blocks 4 through 9) than when saline was administered prior to RFS. Shown in Fig. 3 are the OE scores for each of the five rats during the SAL + RFS and NIC + RFS conditions. In every case, fewer omissions were made in the NIC + RFS conditions. The group means and standard errors are also shown; the reduction in OE's was significant at less than the 0.05 level (two-tailed t test, $t = 3.18$).

Examination of the cumulative records (see, for example, Fig. 1) suggested that 2 types of RFS effects on behavior could be separated out within the critical Blocks 4

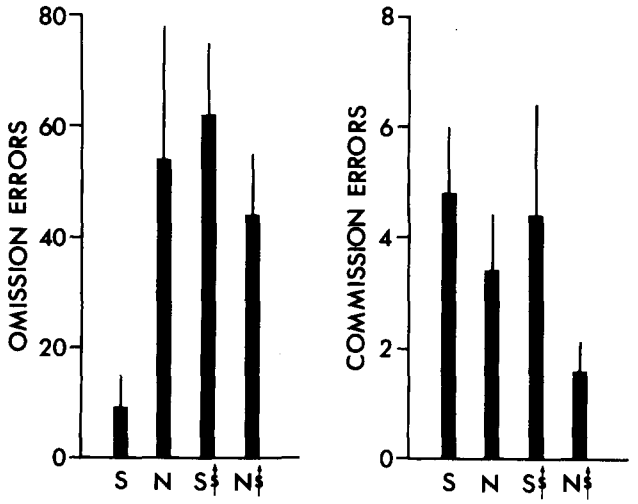

FIG. 2. Means and standard errors of omission error scores (on the left) and commission error scores (on the right) for Blocks 4, 5, 6, 7, 8 and 9. (S = saline; N = nicotine; S⚡ = saline plus reticular formation stimulation; N⚡ = nicotine plus reticular formation stimulation.)

through 9. The first was the direct or immediate disruptive effect on behavior during the periods of stimulation (Blocks 4, 6 and 8). The second was the carry-over disruption into poststimulation blocks (5, 7 and 9). Because this pattern of response emerged, statistical comparisons between Blocks 4, 6 and 8 and Blocks 5, 7 and 9 within conditions were made. The means and standard errors of OE scores which entered into the within condition comparisons are represented in Fig. 4. As would be expected, no significant difference was found between Blocks 4, 6 and 8 and Blocks 5, 7 and 9 in the saline control condition (no stimulation). During the nicotine control (no stimulation) significantly more omissions were made during Blocks 4, 6 and 8 than during Blocks 5, 7 and 9 ($t = 2.62$, $p<0.05$), because the preponderance of OE's occurred immediately after nicotine injection, i.e., in Block 4. The carry-over disruption of RFS was so marked that statistical testing indicated no significant difference in the OE scores from stimulation blocks (4, 6 and 8) and from poststimulation blocks (5, 7 and 9). However, when nicotine was administered (together with

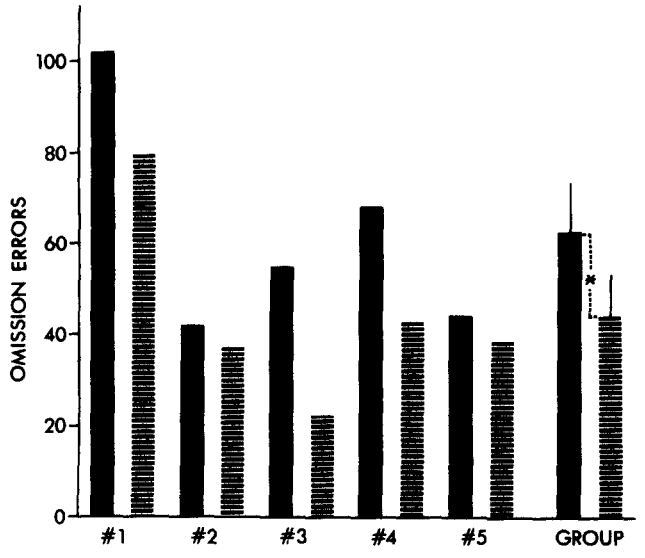

FIG. 3. Omission error scores for individual rats in SAL + RFS sessions (solid bars) and NIC + RFS sessions (striped bars) during Blocks 4, 5, 6, 7, 8 and 9. At the extreme right of the figure are the means and standard errors of OE scores for all rats under the two conditions. The asterisk denotes that the group difference was significant at less than the 0.05 level (two-tailed t test).

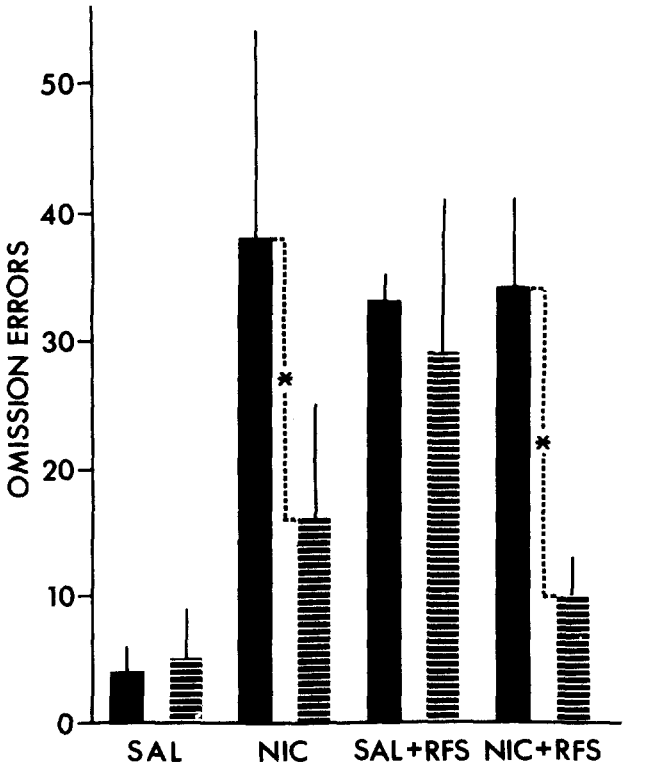

FIG. 4. Means and standard errors of omission error scores for Blocks 4, 6 and 8 (solid bars) and Blocks 5, 7 and 9 (striped bars) during the four treatment conditions. The asterisks indicated the statistically significant (0.05 level) differences for between sets of blocks, within treatment comparisons. Between treatment comparisons are described in the text.

RFS), rats made statistically more omissions during stimulation blocks than during poststimulation blocks indicating that nicotine protected against poststimulation deterioration in performance ($t = 5.02, p<0.005$).

Between treatment comparisons were also made for Blocks 4, 6 and 8 and Blocks 5, 7 and 9, independently. The overall analyses of variance for OE scores during Blocks 4, 6 and 8 and Blocks 5, 7 and 9 indicated significant differences between conditions for each set of blocks, $F(3,12) = 3.70$, $p<0.05$ and $F(3,12) = 3.81$, $p<0.05$, respectively. Paired t tests indicated no significant differences between NIC, RFS and NIC + RFS during Blocks 4, 6 and 8 but OE performance under each of these three conditions deteriorated compared to the saline control level ($t = 2.30$, 8.60 and 4.34 and $p<0.05$, 0.005 and 0.01, respectively).

During Blocks 5, 7 and 9 (that is, the poststimulation blocks for conditions when RFS was delivered), performance deteriorated under the NIC and RFS conditions compared to saline ($t = 1.54$ and 2.01, respectively and $0.05<p<0.10$) but the NIC + RFS omission scores were not significantly different from saline scores. More omissions were made in the RFS condition than either the NIC or the NIC + RFS condition ($t = 2.90$ and 2.10; $p<0.025$ and $0.05<p<0.10$, respectively).

The means upon which the above between treatments, within sets of blocks comparisons were made, are represented in Fig. 4.

Figure 5 summarized the treatment effects in terms of actual cumulative recordings of events and responses for one of the rats during the four critical sessions. These recordings illustrate the protective action or partial antagonism which nicotine exerted against the behavioral disruption caused by RFS. For this rat, not only did nicotine antagonize the carryover effect of RFS (reducing the latency to reinitiation of responding following cessation of RFS blocks), but also it antagonized the direct effect of RFS. Note that in Block 8 of the NIC + RFS session when RFS was being delivered, the rat maintained correct responding through the period.

DISCUSSION

Nicotine was found to provide partial protection against the behavioral disruption caused by electrical stimulation of the reticular formation. This protection was characterized by an attenuation of the behavioral impairment during the stimulation and poststimulation recovery blocks overall (Blocks 4 through 9), but, more consistently across animals, by the attenuation of the poststimulation carryover disruption (Blocks 5, 7 and 9). In fact, performance during these recovery blocks in sessions where nicotine treatment was combined with RF stimulation, was not significantly different from performance exhibited during the same blocks in saline control (no stimulation) sessions. Nicotine's action could be discriminated by visual comparison of cumulative records without need for statistical analysis; most easily observable were the instances of antagonism of the direct effect on RFS, that is, where nicotine-treated rats responded correctly during specific blocks of reticular stimulation. These instances were not consistent enough across animals to be statistically significant (perhaps because of certain aspects of the design of the study which will be discussed), but the observed occurrences deserve consideration.

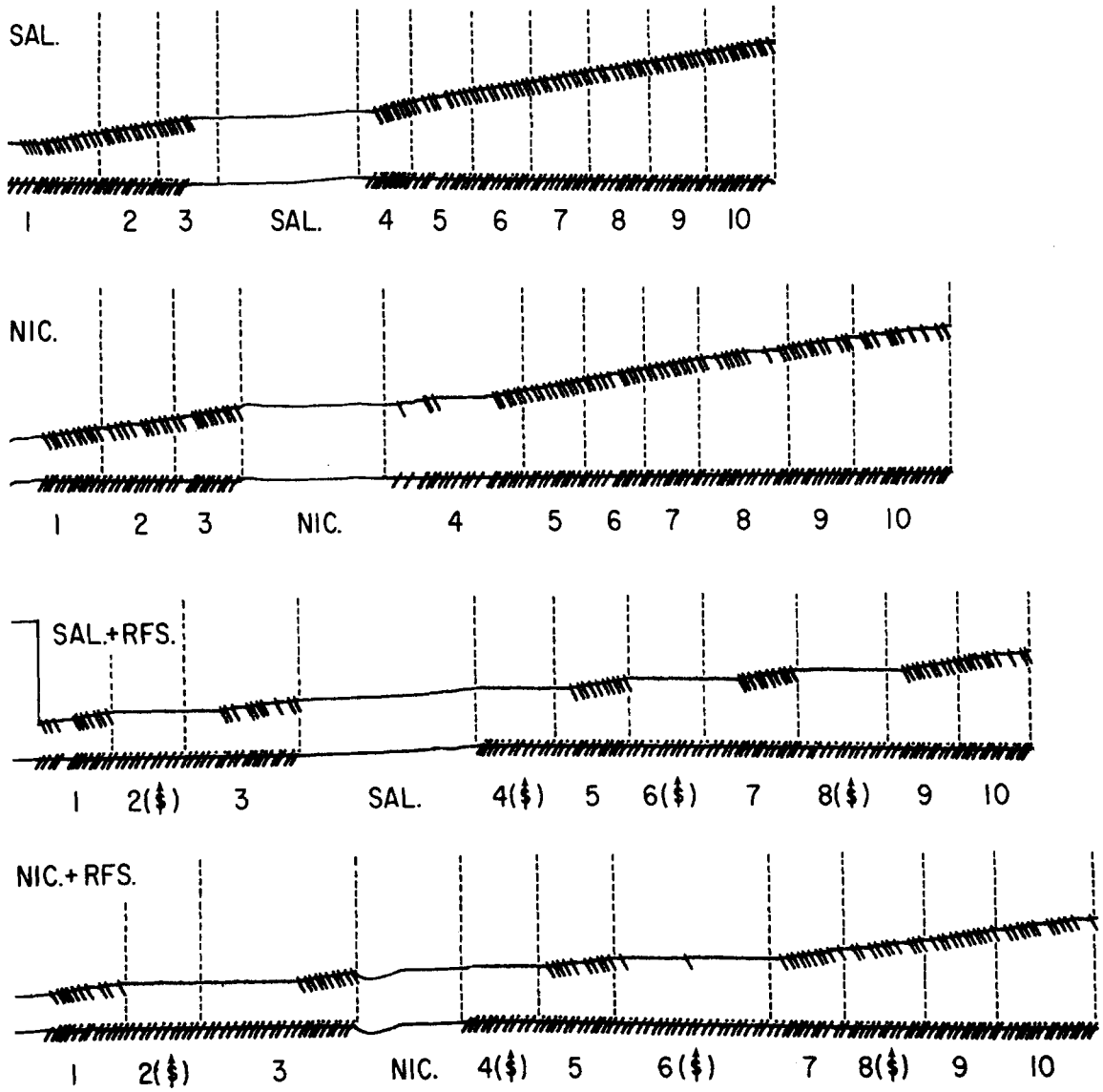

FIG. 5. Cumulative recordings of events and responses of Rat #1 during the four treatment conditions. See Figure 1 for the explanation of the meaning of the tracings. The symbol § denotes those blocks of a session during which RFS was delivered. Note that the rat's latency to reinitiation of responding after RFS was reduced when nicotine was administered (compared to the SAL + RFS condition) and, in fact, the animal responded correctly during Block 8 when RFS was being delivered.

The fact that a single administration of nicotine elicits multiple behavioral and electrophysiological responses has been established [9, 11, 12] and is a crucial, although sometimes confounding, factor in the interpretation of experiments with the drug. In this study, the first response to nicotine was characterized by behavioral disruption occurring almost immediately after an injection and lasting only a few minutes. The protection against the RF stimulation-induced impairment upon which we have focused, was manifested following the first few minutes of nicotine-related disruption and appeared to become more effective as the session continued. (See Fig. 5 in which it is illustrated that fully effective antagonism of impairment during RFS delivery occurred only during Block 8 of the session.) Rosecrans (13) has reported that peak brain concentrations occur 15 minutes after subcutaneous injection of 400 μg/kg of nicotine in the rat. Thus, in our experiments, it is likely that peak brain nicotine levels were only being approached during Block 8 of a session when the last series of reticular stimulations was being delivered. This is a probable explanation for the failure to demonstrate a statistically significant nicotine antagonism of impairment during RFS delivery.

Responses elicited by acute doses of nicotine are often quite different from the responses elicited during a chronic treatment regime [11]. Because the experimental rats had

undergone a course of chronic nicotine treatment which was terminated several months before the current studies were conducted, nicotine's protective action may not have been typical of an acute administration in the strict sense. Recent EEG findings in our laboratory and reports of other behavioral studies indicate that chronic nicotinization may produce some changes in sensitivity to nicotine that persist long after cessation of chronic treatment. For example, Stolerman *et al.* [15] using measures of motor activity in rats, found that tolerance to a challenge dose of nicotine persisted even 90 days after termination of a chronic treatment regime; i.e., rats responded to the challenge dose more as they did to chronic treatment than they did to the initial acute dose. Thus, certain sustained changes in sensitivity to nicotine may have played a role in determining the responses to the acute doses administered in the present study. It remains to evaluate these possible influences and such studies are on-going in our laboratory. Presently, we can conclude only that a single dose of nicotine protects against RFS effects in animals which have undergone previous chronic nicotine treatment.

It would be difficult to explain the mechanism by which nicotine antagonized the impairment of attention performance produced by RF stimulation in terms of a direct effect on the reticular activating system unless that action were depressant. Arguing against this is the work of Domino and coworkers [4] who have reported that the direct action of acute nicotine on the RF is stimulant. The antagonism of the effects of electrically-induced hyperactivation of the RF, therefore, likely involves nicotine's action on another brain substrate. Our EEG studies of the chronic nicotine state indicate that nicotine produces decreases in hippocampal amplitudes and in the frequency of occurrence of theta waves [2,12]; i.e., activity is shifted toward desynchronization. Grastyán *et al.* [6] have reasoned that theta activity reflects the inhibited state of the hippocampus and hippocampal desynchronization, its activated state. They concluded that in its activated state, the hippocampus acts to inhibit the RF, thus providing an important mechanism for the mediation of attention. In light of this proposition, our previous EEG findings [2,12] and our demonstration of the nicotine-related enhancement of attention performance, the view that nicotine counteracts the effects of RF-mediated hyperactivation indirectly, by its action on the limbic system, specifically, the hippocampus, finds support.

The extension of these findings to human smoking behavior suggests a physiological mechanism to account for nicotine self administration: the smoker may be attempting to manipulate his relative arousal state. It is not inconceivable that one of the motivations underlying smoking behavior is the desire to reduce an RF activation level which is manifested in an hyperstimulated or anxious state inappropriate for effective behavior and to engender what might be considered a state of useful behavioral arousal.

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