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Individual self-administration of nicotine by rats

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Abstract

Self-administration (SA) of nicotine (N) was studied in 20 male and 19 female N:NIH rats using the two-bottle method. The experimental protocol consisted of seven consecutive periods each lasting 6 days: Period (P)1, choice of water (W) and 0.003% N; P2, choice of W and 0.006% N; P3, choice of W and 0.012% N; P4, W only; P5, choice of W and 0.006% N; P6, 0.006% N only; and P7, choice of W and 0.006% N. Group means showed that males and female rats consumed similar amounts of N during Ps 1–3. After an N-free period (P4), a small decline was observed in the subsequent voluntary intake of N (P5). Forced N (P6) exposure did not affect a subsequent N intake (P7) in males but increased it slightly in females. A survey of individual animals, however, showed that the voluntary N consumption varied greatly among animals, but was quite consistent for a particular rat. Values ranged from 0.43 to 7.59 for males and from 0.35 to 4.69 mg/kg/day for females for Ps 1–3. The N-free (P4) and the forced-N (P6) periods each affected a subsequent voluntary N intake (P5, P7) of the rats very differently, but again consistently, in that some rats decreased, some increased and some did not change their N choice. The results indicate that group means can be misleading in their conclusions and strongly support the assumption that the response of an individual animal to N, and not N per se, is the determining force of its SA.

Keywords: Rats; N:NIH rats; Nicotine; Self-administration; Individual differences

1. Introduction

The exact causes of heavy smoking or tobacco use and abuse are unknown, although nicotine has been identified as an important agent involved in the initiation and maintenance of, as well as the inability to stop, this behavior. Thus, nicotine has been labeled and is commonly referred to as an "addictive" substance, which when acting on the brain of the user causes an uncontrollable craving for this substance. To better understand the underlying causes of this craving for nicotine, the self-administration (SA) of this substance has been studied extensively in rats (Brower et al., 2002; Cabeza de Vaca and Carr, 1998; Corrigall and Coen, 1989; De la Garcia and Liu, 2002; Donny et al., 1998, 1999, 2000; Glick et al., 1996; Horan et al., 1997; LeSage et al., 2002; Maehler, 1999; Maehler et al., 2000; Shoaib et al., 1997; Smith and Lang, 1980; Todte et al., 2001; Valentine et al., 1997; Wilmpouth and Spear, 2002). These studies have shown that animals readily self-administer nicotine. However, many of these studies have used only male rats of an older age, although in the human situation smoking can be found in both sexes and usually starts early during puberty (DiFranza et al., 2000; Eisenberg and Balster, 2000; Griffin et al., 1999; Koplan, 2002; Satcher, 2002; Unger et al., 1987, 2002). In addition, these studies have focused mostly on group means of nicotine self-administered under various experimental conditions. Little attention has usually been paid to individual differences that do seem to exist in the abovementioned studies as evidenced by rather large S.D.s or S.E.M.s in the group means, by sometimes citing that not all animals could be trained to self-administer nicotine or by indicating that animals showed quite different SA patterns (Brower et al., 2002; Corrigall and Coen, 1989; Donny et al., 1998; Glick et al., 1996; Kalman, 2002; Rosecrans, 1995).

However, these individualized responses to nicotine and the individual variations in avoidance and/or preference among animals are of special interest because they resemble

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the human situation where humans are known to vary greatly in the avoidance and use or abuse of this substance (DiFranza et al., 2000; Eisenberg and Balster, 2000; Griffin et al., 1999; Koplan, 2002; Satcher, 2002; Unger et al., 1987, 2002) as well as in their success of stopping this addiction (Fiore et al., 2000; Fagerstrom et al., 1993; Foulds et al., 1992). To fill this gap we investigated the oral SA of nicotine in a large number of both male and female rats at a young age under a variety of experimental conditions. While group means were obtained, particular attention was paid to the individual responses of each rat and the stability of this particular response to nicotine over the entire experiment.

The method used was the two-bottle free-choice method, where animals could choose freely between water and a nicotine solution. This allows the simultaneous observation of a large number of male and female animals. The strain chosen was the strain of N:NIH rats, which was derived from cross-breeding eight different rat strains. The original purpose was to obtain a "true" outbred strain, and breeding was not directed towards a specific biochemical or behavioral parameter. Such an outbred strain is expected to show marked individual differences among its members. This was the reason why this strain has been chosen. Like the human situation where most smokers begin to smoke at puberty, the experiment was started with young male and female rats during sexual maturation and then continued for 42 days.

The different experimental conditions used were, in succession, exposure of the animals to (1) water and three successively increasing concentrations of nicotine for 6 days each to see if a gradual increase in the nicotine concentration would lead to an increase in the SA of this substance, (2) a period of nicotine withdrawal and water only followed again by a voluntary water and nicotine choice to see how a nicotine-free period would affect a subsequent voluntary consumption of nicotine and (3) a period of nicotine forcing (with no choice) followed again by a choice between water and nicotine to see how a forced exposure to a high amount of nicotine would affect a subsequent free choice of this substance.

2. Method

2.1. Chemicals

Nicotine as the nicotine (−) tartrate salt was obtained from Sigma, St. Louis, MO. The salt was dissolved in tap water to obtain the neutral different concentrations (wt/vol).

2.2. Animals

The male and female rats were obtained through the courtesy of Dr. C. Hansen from the National Institutes of Health (NIH), Bethesda, MD.

The animals were housed individually in standard cages in rooms controlled for climate (temperature about 22 °C and humidity about 55%) and a light/dark cycle (light from 0800 to 2000 h). Animals always had free access to standard rat chow. Male and female rats were about 40 to 45 days of age when the experiment started. The institutional committee approved the use of the animals and experiments performed and all animals were kept in the general quarters of the University according to NIH principles of animal care.

2.3. Procedure

During the experiment, the animals were always exposed to two bottles, which were alternated each day. The experimental design was as follows:

P1: Days 1–6	Choice between water and 0.003% nicotine
P2: Days 7–12	Choice between water and 0.006% nicotine
P3: Days 13–18	Choice between water and 0.012% nicotine
P4: Days 19–24	Water and water
P5: Days 25–30	Choice between water and 0.006% nicotine
P6: Days 31–36	Only 0.006% nicotine and 0.006% nicotine
P7: Days 37–42	Choice between water and 0.006% nicotine

The time for each period of 6 days and the doses used had been found to be optimal from previous experiments (Maehler, 1999; Todte et al., 2001). For body weight determinations and the consumption of water and nicotine solutions, rats and bottles were weighed each morning starting between 8:00 and 9:00. At this time solutions were also reconstituted or changed as indicated in the schedule.

2.4. Statistical analyses

Data were analyzed statistically using a repeated, multifactorial analysis of variance with the concentration of nicotine offered and the gender as independent variables and the amount of nicotine consumed as the outcome variable followed by Neuman–Keuls post hoc tests; correlations were determined using the Spearman rank correlation test (Prostat program).

3. Results

3.1. Voluntary nicotine consumption of different nicotine concentrations

The purpose of this part of the experiment was to investigate if increasing the nicotine concentrations offered would affect the voluntary consumption of this substance. Three different nicotine solutions were offered successively, each for 6 days, with the rats having a choice between water and a particular nicotine concentration.

The data from Table 1 show the voluntary consumption of nicotine at the three nicotine concentrations by 20 male rats. The rats weighed about 125 ± 13 g at the start of the experiment and had a total fluid intake of 158 ± 14 ml/kg/day. The weight increased and total fluid intake declined slightly as the animals aged but both were not influenced by the nicotine concentration or were consistent among animals. Group values for nicotine intake were similar for the 0.003% and 0.012% concentrations but were slightly higher for the 0.006% concentration, indicating a possible preference for this nicotine concentration.

Of particular importance, however, are the differences in the voluntary consumption of nicotine observed in individual animals. Intakes of nicotine as high as 7.59 and as low as 0.43 mg/kg/day were found. Based on the nicotine consumption over the three periods, animals could be divided readily into high consumers (e.g., nos. 4, 19, 20), low consumers (e.g., nos. 6, 7, 15) and intermediate consumers (e.g., nos. 9, 13, 14). Each animal showed its characteristic pattern over all three periods as evidenced by the significant correlations obtained between the three periods; that is, the rank of an individual animal in the group remained largely the same in each period. Thus, the individual nicotine intake was mostly determined by a particular animal, was relatively consistent for this animal and generally independent of the nicotine concentration offered.

Table 1
Self-administration of different nicotine concentrations by male rats

Male rats	P1 nicotine intake (0.003%) [mg/kg/day]	P2 nicotine intake (0.006%) [mg/kg/day]	P3 nicotine intake (0.012%) [mg/kg/day]
1	1.05 ± 0.62	3.12 ± 0.59	0.69 ± 0.44
2	2.14 ± 0.29	3.79 ± 2.66	0.64 ± 0.25
3	0.75 ± 0.73	1.62 ± 1.29	0.75 ± 0.21
4	2.32 ± 0.89	4.80 ± 2.00	2.38 ± 1.59
5	1.24 ± 1.01	2.24 ± 2.09	0.87 ± 0.16
6	0.82 ± 0.38	0.59 ± 0.64	0.60 ± 0.14
7	0.43 ± 0.29	0.73 ± 0.38	0.53 ± 0.20
8	0.90 ± 0.26	2.34 ± 0.77	0.81 ± 0.22
9	1.15 ± 1.06	2.65 ± 1.22	1.05 ± 0.34
10	1.27 ± 1.70	1.87 ± 1.98	0.77 ± 0.43
11	1.96 ± 0.36	4.36 ± 2.31	1.42 ± 0.68
12	1.29 ± 1.38	1.57 ± 0.71	0.65 ± 0.37
13	1.26 ± 0.63	2.15 ± 1.17	1.03 ± 0.68
14	1.41 ± 0.60	2.52 ± 1.19	1.24 ± 1.19
15	0.54 ± 0.56	0.64 ± 0.34	0.91 ± 0.31
16	1.22 ± 0.67	0.87 ± 0.17	0.77 ± 0.27
17	1.94 ± 1.60	4.33 ± 2.07	1.40 ± 0.88
18	0.83 ± 0.39	1.03 ± 1.02	0.94 ± 0.11
19	2.00 ± 0.87	4.53 ± 1.37	2.06 ± 0.94
20	4.41 ± 1.45	7.59 ± 3.48	2.47 ± 0.87
Average	1.46 ± 0.88	2.67 ± 1.79	1.22 ± 0.72

Values represent means and S.D.s. The animals were about 42 days old at the beginning of the experiment. Animals had a choice between water and different nicotine concentrations in succession as indicated given for 6 days during each of the three periods.
Group differences: $P < .05$ between Ps 1 and 2; $P < .05$ between Ps 2 and 3.
Rank correlations: $P < .05$ between Ps 1 and 2 (r=.88); $P < .05$ between Ps 2 and 3 (r=.79); $P < .05$ between Ps 1 and 3 (r=.82).

Table 2
Self-administration of different nicotine concentrations by female rats

Female rats	P1 nicotine intake (0.003%) [mg/kg/day]	P2 nicotine intake (0.006%) [mg/kg/day]	P3 nicotine intake (0.012%) [mg/kg/day]
1	1.62 ± 0.61	0.82 ± 0.46	0.97 ± 0.08
2	1.53 ± 0.65	3.07 ± 1.14	1.24 ± 0.31
3	1.91 ± 0.81	0.99 ± 0.70	1.24 ± 0.24
4	2.34 ± 1.26	3.05 ± 1.07	0.90 ± 0.12
5	0.55 ± 0.58	0.44 ± 0.10	0.84 ± 0.28
6	0.39 ± 0.27	0.57 ± 0.14	1.00 ± 0.19
7	1.58 ± 0.56	0.95 ± 0.13	1.24 ± 0.43
8	1.31 ± 0.77	1.14 ± 1.02	0.93 ± 0.31
9	0.41 ± 0.35	0.54 ± 0.04	0.75 ± 0.12
10	0.35 ± 0.22	0.38 ± 0.06	0.61 ± 0.16
11	1.08 ± 0.42	1.05 ± 0.34	0.85 ± 0.31
12	1.58 ± 1.29	3.29 ± 1.36	2.77 ± 0.56
13	0.71 ± 0.78	0.43 ± 0.06	0.97 ± 0.27
14	1.04 ± 0.89	0.61 ± 0.09	1.23 ± 0.40
15	1.19 ± 1.06	1.46 ± 0.83	1.48 ± 0.59
16	1.56 ± 0.99	1.04 ± 0.58	0.88 ± 0.38
17	0.77 ± 0.30	3.21 ± 0.74	0.97 ± 0.21
18	1.04 ± 0.58	0.88 ± 0.52	0.87 ± 0.26
19	2.81 ± 0.68	4.69 ± 1.11	1.58 ± 0.76
Average	1.25 ± 0.66	1.51 ± 1.27	1.12 ± 0.47

Values represent means and S.D.s. The animals were about 42 days old at the beginning of the experiment. Animals had a choice between water and different nicotine concentrations in succession as indicated given for 6 days during each of the three periods.
Group differences: none.
Rank correlations: $P < .05$ between Ps 1 and 2 (r=.69); $P < .05$ between Ps 2 and 3 (r=.59); $P < .05$ between Ps 1 and 3 (r=.59).

Table 2 shows the voluntary intake of different concentrations of nicotine by 19 female rats, which were tested at the same time under identical conditions. The initial weight was 109 ± 10 g at the start of the experiment and the total fluid intake was 171 ± 13 ml/kg/day. Weight increased and total fluid intake declined during the experiment, but this increase and decline were independent of the nicotine concentrations offered. Group means showed no differences in the voluntary intake of nicotine during the three periods, indicating that the concentration offered did not affect the voluntary intake of this substance.

Again, large individual differences in nicotine consumption ranging from 0.38 to 4.69 mg/kg/day were detected among the animals and high (e.g., nos. 2, 12, 19), low (e.g., nos. 5, 9, 10) and moderate (e.g., nos. 8, 11, 15) consumers could be identified. Correlation analyses over the three periods were significant, indicating that each animal consumed nicotine at its own preferred rate and the rank order remained relatively stable in spite of the different nicotine concentrations. However, the correlation coefficients were weaker than those for the male rats. Like male rats, voluntary intake of nicotine by female rats was determined by a particular animal, was quite stable over time and independent of the nicotine concentration offered.

3.2. Water interlude and nicotine forcing on the voluntary consumption of nicotine

After this part of the experiment, the male and female animals received water only for 6 days and were then again given for the next part of the experiment a choice between water and a 0.006% solution of nicotine. This was followed by a period of forced nicotine exposure and, again, followed by a choice situation between nicotine and water. The purpose of this experiment was to determine the effects of a water-only/nicotine-free period and a period with nicotine forcing on subsequent choices of nicotine.

Table 3 shows first the same male animals as shown in Table 1 but after the water-only period. The animals had now reached a weight of 235 ± 21 g and the total fluid intake had declined to 119 ± 17 ml/kg/day. It can be noticed that the group intake of the 0.006% nicotine solution (Table 1, P2 vs. Table 3, P5) has decreased ($P < .05$), whereas no decrease was apparent when the comparison was made with the 0.012% nicotine solution (Table 1, P3 vs. Table 3, P5). Forcing the rats to consume only nicotine during P6 markedly increased their involuntary nicotine intake. During this forced-nicotine intake, weight gain was slightly reduced (P5 −2.19 ± 0.77 vs. P6 −1.3 ± 1.15 g/day) and total fluid intake was also slightly less (P5—about 119 vs. P6—about 99 ml/kg/day). However, given a choice again during P7 the rats decreased their nicotine intake to the same level as they had maintained before the forced-nicotine intake (P5 vs. P7). Forced-nicotine exposure did not increase a subsequent voluntary nicotine intake.

Table 3
Self-administration of nicotine after a water-only period and forced intake of nicotine by male rats

Male rats	P5 nicotine intake (0.006%) [mg/kg/day]	P6 nicotine intake (0.006%) [mg/kg/day]	P7 nicotine intake (0.006%) [mg/kg/day]
1	0.56 ± 0.51	5.54 ± 0.85	0.27 ± 0.14
2	2.24 ± 1.11	7.98 ± 0.79	3.66 ± 1.27
3	0.78 ± 0.56	4.63 ± 1.66	0.60 ± 0.30
4	1.47 ± 0.55	5.94 ± 0.01	0.60 ± 0.22
5	0.42 ± 0.16	4.95 ± 0.04	2.43 ± 1.10
6	0.61 ± 0.31	4.76 ± 1.19	0.67 ± 0.20
7	0.20 ± 0.05	4.67 ± 1.02	0.16 ± 0.07
8	0.39 ± 0.09	5.07 ± 0.20	1.38 ± 0.73
9	0.36 ± 0.08	4.56 ± 0.78	0.19 ± 0.07
10	0.90 ± 0.27	4.48 ± 0.68	0.29 ± 0.11
11	2.00 ± 1.13	5.56 ± 0.73	1.49 ± 0.44
12	0.93 ± 0.87	5.46 ± 0.13	0.54 ± 0.12
13	0.60 ± 0.26	7.88 ± 1.24	0.49 ± 0.31
14	0.33 ± 0.13	5.33 ± 0.78	0.48 ± 0.19
15	0.38 ± 0.11	5.61 ± 0.02	2.00 ± 0.59
16	0.53 ± 0.16	5.01 ± 0.64	1.14 ± 0.43
17	0.25 ± 0.08	4.99 ± 0.39	0.22 ± 0.08
18	0.53 ± 0.10	5.64 ± 0.71	1.98 ± 0.65
19	0.73 ± 0.36	7.12 ± 0.36	0.36 ± 0.10
20	1.40 ± 0.55	9.28 ± 1.91	3.28 ± 1.41
Average	0.78 ± 0.57	5.72 ± 1.32	1.11 ± 1.05

Values represent means and S.D.s. This table shows the same animals as were shown in Table 1 after they had been on water only for 6 days. Animals had a choice between water and nicotine in P5 (6 days), were forced to consume only nicotine in P6 (6 days) and again had a choice between water and nicotine in P7 (6 days). The nicotine concentration in all cases was 0.006%.
Group differences: none.
Rank correlations: none.

However, a survey of individual animals shows large individual differences in the voluntary consumption of nicotine. Responses to the water interlude had different effects on these animals in that some low consumers remained low (e.g., nos. 6, 15, 16), some high consumers remained high (e.g., nos. 2, 4, 11), some high consumers decreased (e.g., 9, 13, 14) and none of the rats increased their intake of nicotine. There was no significant correlation ($r = .41$; $P > .05$) between the nicotine consumption before (Table 1, P2 = 0.006%) and after (Table 3, P5 = 0.006%) the water interlude. Forcing the animals to only consume nicotine caused a marked increase in nicotine consumption with no differentiation if the rat showed previously a low, moderate or high preference for nicotine before the forced exposure. After forcing, some animals increased (e.g., nos. 5, 15, 20), some animals decreased (e.g., nos. 4, 10, 19) and some animals remained the same (e.g., nos. 3, 6, 7) in their voluntary intake when comparing Ps 5 and 7 of this table. No significant correlation ($r = .42$; $P > .05$) was detected in the nicotine intake before and after forcing. Thus, a nicotine-free period and a forced exposure to nicotine affected rats quite differently in the choice of this substance.

Table 4 shows the same female animals as shown in Table 2, which were exposed to the same schedule at the same time. At the start of this part of the experiment, their weights had increased to 205 ± 14 g and their total fluid intake had decreased to 135 ± 12 ml/kg/day.

The average nicotine intake after the water interlude was significantly lower than the intake before (Table 2, P2; 0.006% vs. Table 4, P5; 0.006% = $P < .01$). Forced intake of nicotine increased the consumption of nicotine without affecting weight gain (P5: 1.3 ± 0.6 vs. P6: 1.1 ± 05 g/day) or total fluid intake (P5: about 135 ± 12 vs. P6: about 116 ± 13 ml/kg/day). After the period of forcing, animals again had a choice between water and nicotine and, this time, the mean nicotine intake of the group increased significantly.

A survey of individual animals shows that, after the water interlude, some animals increased (e.g., nos. 1, 9), some animals decreased (e.g., nos. 2, 4, 12, 17, 19) and some animals stayed the same (e.g., nos. 5, 14, 18). During the forced intake period, animals consumed markedly more nicotine but no correlation was apparent between rats with a previously high or low nicotine intake before the forcing ($r = .41$; $P > .05$). After the forced nicotine intake (comparing P5 vs. P7), some animals increased (e.g., nos. 3, 4, 6, 9, 18), some animals decreased (e.g., nos. 7, 8, 15) and some animals remained the same (e.g., nos. 1, 2). No significant correlation between the nicotine intake between Ps 5 and 7 of this table was found ($r = .39$; $P > .05$). Again, animals

Table 4
Self-administration of nicotine after forced intake of nicotine by female rats

Female rats	P5 nicotine intake (0.006%) [mg/kg/day]	P6 nicotine intake (0.006%) [mg/kg/day]	P7 nicotine intake (0.006%) [mg/kg/day]
1	1.42 ± 0.57	6.31 ± 0.62	1.27 ± 2.02
2	0.60 ± 0.32	7.87 ± 1.17	0.62 ± 0.37
3	0.41 ± 0.05	7.42 ± 0.62	4.02 ± 1.96
4	0.38 ± 0.11	8.56 ± 0.17	2.12 ± 1.41
5	0.43 ± 0.04	7.82 ± 3.00	0.27 ± 0.15
6	0.37 ± 0.08	7.40 ± 0.25	6.08 ± 1.70
7	0.73 ± 0.33	6.14 ± 0.05	0.49 ± 0.10
8	0.43 ± 0.09	5.57 ± 1.53	0.27 ± 0.09
9	1.10 ± 1.29	6.57 ± 0.95	5.86 ± 4.34
10	0.65 ± 0.31	5.94 ± 1.37	0.47 ± 0.20
11	0.43 ± 0.18	5.53 ± 0.34	0.25 ± 0.08
12	0.73 ± 0.43	6.36 ± 0.13	1.43 ± 0.87
13	0.41 ± 0.04	8.28 ± 0.96	2.06 ± 1.42
14	0.53 ± 0.08	7.86 ± 0.18	2.01 ± 2.33
15	0.63 ± 0.22	7.75 ± 1.09	0.36 ± 0.09
16	0.64 ± 0.33	7.90 ± 0.31	1.76 ± 2.43
17	0.53 ± 0.39	5.99 ± 0.75	0.35 ± 0.15
18	0.79 ± 0.34	9.54 ± 0.01	4.69 ± 2.55
19	0.45 ± 0.19	8.35 ± 0.81	1.64 ± 1.05
Average	0.61 ± 0.26	7.22 ± 1.14	1.90 ± 1.90

The values represent means and S.D.s. This table shows the same animals as were shown in Table 2 after they had been on water only for 6 days. Animals had a choice between water and nicotine in P5 (8 days), were forced to consume only nicotine in P6 (6 days) and again had a choice between water and nicotine in P7 (6 days). The nicotine concentration in all cases was 0.006%.
Group differences: $P < .05$ between Ps 1 and 3.
Rank correlations: none.

responded in an individual matter in the voluntary nicotine intake to the water interlude and to the forced-nicotine challenge.

3.3. Gender differences in the voluntary nicotine consumption

We also compared the nicotine consumption between the genders. No significant differences were noted except that the male rats showed a preference for the 0.006% nicotine solution during the first part of the experiment and that female rats were more sensitive to a period of nicotine forcing because the group mean increased after the forced nicotine intake period. A comparison of all concentrations (Tables 1 and 2) showed an inverted U-shaped pattern for nicotine intake for the males that was absent in our female animals. We also checked for a possible influence of the estrous cycle in females on nicotine consumption but were unable to find a correlation.

4. Conclusion

Studies on the SA of nicotine by animals are usually performed by operant methodology where the animal is trained to work for the reward of a psychoactive substance (Brower et al., 2002; Cabeza de Vaca and Carr, 1998; Corrigall and Coen, 1989; Donny et al., 1998, 1999, 2000; Glick et al., 1996; Horan et al., 1997; LeSage et al., 2002; Maehler et al., 2000; Todte et al., 2001; Valentine et al., 1997). Although the advantages of this method are obvious, drawbacks are that only a relatively small number of older animals can be used, that testing occurs usually only during a restricted time of the day for a few days, that weight/food/water restrictions have to be used, that different operant schedules produce different results and that the SA requires a learning process, all of which can potentially affect the SA of the compound under investigation (Brower et al., 2002; Cabeza de Vaca and Carr, 1998; Donny et al., 1998, 2000; Gauvin et al., 1993; Kalman, 2002; McMillan and Katz, 2002). The oral method using the two-bottle free-choice method does not involve food/water restrictions or weight reductions, can be applied to a large number of animals without learning or shaping of the animal even at an early age and measures the voluntary intake of the psychoactive substance over a 24-h period. The drawbacks of the method are the caloric content of the substance to be studied (which does not apply to nicotine) and its taste. In the case of nicotine, we and other investigators found that taste does not seem to play a role in the voluntary selection of nicotine (De la Garcia and Liu, 2002; Glick et al., 1996; Maehler, 1999; Parker, 1991; Wilmpouth and Spear, 2002). Furthermore, the daily nicotine intake found in this study of about 1–2 mg/kg/day is similar to the intake of about 1 mg/kg/day reported in studies using the operant procedure (e.g., Donny et al., 1999; LeSage et al., 2002) or in humans where the daily inhaled amounts of nicotine have been found to be about 1 mg/kg/day (Benowitz and Jacob, 1984). Thus, the two-bottle, free-choice method seems a valid procedure to investigate the SA of nicotine.

In our case, the oral method was chosen because it allowed the simultaneous observation of a large number of individual rats over a longer period. The animals chosen were the outbred N:NIH rats expected to show individual differences. They were tested at an early age because most humans do start to experiment with smoking during puberty (DiFranza et al., 2000; Eisenberg and Balster, 2000; Griffin et al., 1999; Unger et al., 2002). We used both male and female rats because gender could play a role in nicotine preference (Donny et al., 2000; Eisenberg and Balster, 2000; Koplan, 2002; Unger et al., 2002). During the study we obtained and compared mean values but paid special attention to the particular behavior of an individual rat.

The total fluid intake was somewhat different in the male and female rats and declined slightly over the course of the experiment. Total fluid intake varied slightly among animals but was not consistent for a particular rat in that animals increased and decreased their intake slightly from day to day. Intake was independent of the nicotine concentration offered. Thus, total fluid intake did not influence the choice

of nicotine, which was very different, but consistent, among animals.

A comparison of the average intakes of nicotine over the three successively increasing nicotine concentrations (Tables 1 and 2) shows that male and female N:NIH rats are willing to voluntarily consume different nicotine concentrations with the male rats preferring the 0.006% nicotine solution, whereas no such preference was seen with the female rats. In most cases, however, mean intake of nicotine was independent of the nicotine concentration offered. This was achieved in that the animals kept their total fluid intake the same but changed the amounts of water and of the nicotine solution. For instance, to keep the nicotine intake the same for the 0.003% and 0.012% nicotine concentration, they reduced the consumption of the 0.012% nicotine solution by correspondingly increasing the amount of water consumed so that total fluid intake remained quite similar. This resembles the behavior of smokers being offered low and high nicotine containing cigarettes. As the nicotine content of the cigarettes decreased, individuals smoked more aggressively, increased their puff volumes, puffed faster and increased puff duration, keeping nicotine delivery to the body relatively constant (Armitage et al., 1988; Collins et al., 1996; De Grandpre et al., 1992; Pickworth et al., 2002).

After the water interlude (Tables 3 and 4), nicotine consumptions went down significantly ($P < .05$) compared to the 0.006% solution, but not when compared to the 0.012% solution; the latter was the last nicotine choice before the water interlude. At the same time animals also grew older, which could have reduced their nicotine consumption (Maehler, 1999; Maehler et al., 2000). Nevertheless, if the effect is not due to aging but due to the water interlude, it is relatively small. This is similar to data reported on a water interlude using an operant procedure where animals resumed previous nicotine intake pattern (Wilmpouth and Spear, 2002).

Forcing rats with nicotine increased nicotine intake greatly without reducing total fluid intake markedly. This indicates that rats, when forced, are willing to consume a higher amount of nicotine as they would have consumed voluntarily—if the taste of nicotine would be aversive, a marked drop in total fluid consumption should have occurred at least on the first day; but this was not seen. The willingness to consume large amounts of nicotine then suggests that nicotine does not have an aversive taste per se. However, rats may have experienced more unpleasant effects, which were probably compensated for and overridden by their desire to keep total water intake constant. After forcing, male animals went back to their preforcing choice, whereas female rats increased their voluntary nicotine consumption slightly.

A comparison of individual rats, however, is much more informative and does not support some of the generalizations based on group means mentioned earlier. Male and female rats selected the amounts of nicotine on a very individual basis and varied greatly in their nicotine intakes (Tables 1 and 2). Large individual differences were detected, which remained relatively stable for an animal over time. High or low consumers remained high or low consumers regardless of the nicotine concentration offered. Interestingly, on Day 1 or first exposure to nicotine all animals selected an amount of nicotine that was always slightly higher than the amounts selected on the following days. However, amounts selected on Day 2 were quite predictive of the amounts consumed over the next days—or Day 2 would predict quite reliably if the animal would be a high, moderate or low consumer of nicotine. As nicotine concentrations changed, most animals continued in their relative high or low intake pattern. Only a few animals showed changes in nicotine intake. For instance, male animals 1, 2 and 12 and female animals 1, 4 and 16 reduced markedly their intake in the third period or during Days 13–18. In contrast, no male animal but female animals 6, 10 and 12 increased their nicotine consumption during Days 13–18. This shows that most animals respond at an early age with a particular pattern of nicotine consumption, which they continue for at least the period studied here. A few animals, however, changed their nicotine preference over time. This is similar to the human situation, where most humans experiment with cigarettes during puberty and where early smoking experiences already predict future attitudes towards smoking in most but not all individuals (Eisenberg and Balster, 2000; Griffin et al., 1999; Satcher, 2002; Unger et al., 1987, 2002). Although it has been claimed that humans slowly increase the amount of nicotine inhaled over time, more recent studies have shown that a smoking pattern is already established within weeks after the first cigarette and that even the experience of the "first puff" will indicate future use of tobacco (Eisenberg and Balster, 2000).

Similarly, an interlude of water only or nicotine "deprivation" affected our rats quite differently. Most male rats decreased their voluntary nicotine consumption after the water-only period (Table 1, P2 = 0.006% vs. Table 3, P5 = 0.006%; $P < .05$). However, rats 2, 4, 11 and 20 remained high consumers. Other rats were very stable in their particular intake and continued their previous consumption after the water interlude such as animals 6, 15 and 16. The female rats behaved somewhat differently in that most animals decreased their nicotine intake (Table 2, P2 vs. Table 4, Ps 5 or 6) such as rats 2, 4, 8, 11, 12, 14, 15, 17, 19, but two animals, 1 and 9, actually increased their intake. This is again similar to the human situation of smoking cessation, although our rats had a shorter period of nicotine exposure than most human smokers and the water interlude was relatively short. It is well known that smoke-free periods can prove helpful for some but not all smokers who would like to quit (Fiore et al., 2000; Fagerstrom et al., 1993; Foulds et al., 1992). Of interest are male rats 4, 17 and 19, which reduced their intakes (Table 1, P2 to Table 3, P5 in mg/kg/day) from 3.1 to 0.6, from 4.3 to 0.3 and from 4.5 to 0.7 and female rats 4, 17 and 19, which decreased their

intake from 3.1 to 0.6, from 3.2 to 0.5 and from 4.7 to 0.5. These rats would represent the smokers who are successful in their effort to reduce the use of tobacco after a smoking-free period.

A forced nicotine drinking period was used to see if forcing high amounts of nicotine on an animal would increase a subsequent voluntary consumption of this substance. Most male rats were unaffected by this exposure and resumed their previous voluntary choices (Table 3, P5 vs. Table 3, P7). This has also been reported previously by us (Maehler, 1999; Maehler et al., 2000; Todte et al., 2001) and recently in an abstract (Wilmpouth and Spear, 2002). However, some animals decreased their intake (animals 4 and 10), whereas some animals increased their intake such as animals 2, 5, 8, 15, 16, 18 and 20. In contrast, more female rats increased their nicotine intake after forcing (animals 3, 4, 6, 9, 13, 14, 16, 18 and 19), whereas only one decreased (animal 15) and the rest was unaffected. Female rats might be more sensitive to the effects of nicotine as has been shown in some studies with female smokers being at higher risk (Koplan, 2002; Leshner, 2001; Lueders et al., 2002; Unger et al., 1987, 2002). Thus, exposure to nicotine can have a differential effect on the consumer in that it is of no influence or that it can increase or decrease the desire for this substance. Again, this is quite similar to the human situation where exposure to tobacco and nicotine causes some but not all individuals to begin to smoke and only some of these to progress to the abuse of tobacco.

These data with the emphasis on the individual responses of the N:NIH rats offer a new insight into the interaction between nicotine and an animal. This interaction seems to be highly individualized and seems to be determined by certain characteristics of the animal. The different, but consistent, voluntary consumption of nicotine by individual animals suggests that not nicotine exposure per se but the response of a particular rat to nicotine is going to determine the SA pattern of this substance. This supports the common observation in humans where many young individuals experiment with tobacco but only a few will progress to heavy smoking.

Our data showing that individual differences strongly determine the choice of nicotine by an animal offer a new tool in finding the genetic and biological basis of this response to nicotine (and other psychoactive substances as well). Instead of measuring biological parameters in groups of rats resulting usually in large variations among animals and masking individual characteristics, rats of this strain can now first be screened for their nicotine avoidance or preferences and their responses to a nicotine-free period or a period of forced nicotine. After rats have been identified to fall in distinct categories, they can then be secondly examined as to their genetic and biological differences. This has already been done with cocaine where only the use of high- and low-cocaine self-administering rats allowed us to identify the extent of the dopamine release in the shell of the nucleus accumbens as a marker for cocaine avoidance or preference (Ferraro et al., 2000). Animals can also be more effectively tested pharmacologically in that only high self-administering rats should be used to test for drugs, which might reduce nicotine craving without fear that low self-administering rats will dilute any true effect of the test drug.

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