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Pharmacology Biochemistry and Behavior, Vol. 53, No. 1, pp. 11–25, 1996

0091-3057/96 $15.00 + .00

0091-3057(95)00193-X

The Development of Opiate Addiction in the Rat

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Received 2 December 1994; Accepted 4 May 1995

HEYNE, A. *The development of opiate addiction in the rat.* PHARMACOL BIOCHEM BEHAV **53**(1) 11–25, 1996.– Opiate intake was studied in rats, which were given free choice between water and etonitazene (ETZ) solutions (2, 4, and 8 mg/l) for 30 weeks. After an abstinence of 19 weeks, the opiate was reoffered. The long-term course of intake could be subdivided in three phases: a period of controlled intake (25 weeks), a period of increasing consumption (week 25–30), and the stage of drug addiction (retest). During controlled intake, environmental and individual variables reversibly influenced ETZ intake (high intake in socially deprived and in subordinate rats, low intake in group-housed and in dominant rats). After 25 weeks of situation-specific intake, the rats spontaneously increased ETZ consumption. In the retest after long-term ETZ-abstinence, their intake was strongly increased compared to both their own intake before and to that of drug-naive controls. ETZ intake could no longer be influenced by environmental, gustatory, or individual factors ("loss of control") indicating opiate addiction. In contrast, rats that have formerly had forced administration by means of a 2 mg/l ETZ solution did not become addicted. Signs of opiate withdrawal, however, occured in both series of forced and voluntary intake. Principles of the temporal development of opiate addiction are compared with those described previously for ethanol addiction.

Addiction Dependence Loss of control Animal model Rat Etonitazene Opiate Ethanol Social behavior Social isolation Dominance rank

ABUSE OF psychotropic drugs and drug addiction typically occur in humans. Two distinct stages can be differentiated, according to an altered attitude towards the drug. During the first period, the consumer is able to control his drug consumption [i.e., he takes the drug contingent upon his external, environmental situation and his internal, individual state (21)]. An example is given by "social drinking" [i.e., a specific alcohol intake pattern depending on the social environment (27)]. With time, drug taking becomes less and less modifiable by external and internal parameters, and the motivation for drug seeking and drug taking rules behavior (3,25). The corresponding subjective condition of the consumer can be described as "craving for the drug" (22,33). This state is considered as drug addiction (10,33). According to the diagnostic manual of the American Psychiatric Association (3,25) and to the WHO (18), the two major features of a person said to be addicted are:

1. Compulsive drug seeking and drug taking which can hardly be influenced ["loss of control," (3)],
2. A high risk to relapse even after several years of drug abstinence (10,22), which can be interpreted as "loss of reversibility."

Consequently, it appears appropriate to differentiate between the stages of "controlled drug intake" and "drug addiction" (10,15,48).

To understand the mechanisms underlying the development of drug addiction (i.e., the transition from controlled drug intake to drug addiction), it is necessary to analyse the factors determining drug consumption. Consequently, descriptive studies in humans have to be supplemented by experimental research. Because nontherapeutical experimental interventions are ethically not justifiable, adequate animal models must be used. Our interest is focussed on the long-term temporal development of drug taking behavior leading to drug addiction and on the influence of social determinants. Several studies reveal that drug taking behavior is influenced by the social environment and the social role of individuals within a group. Short- and long-term isolation have been shown to enhance the intake of ethanol or morphine in rats (2,47), mice (14) or monkeys (26). Dominant rats consumed less ethanol or benzodiazepine than subordinate ones (8,19,50).

For these purposes, we have recently proposed an animal model for the development of alcohol addiction in rats (48, 50). The model bases on long-term free access to alcohol in addition to water as drinking fluids. The ethanol taking behavior was analyzed in a rat's life span (48,50). Over a period of 9 months, the animals could choose continuously between water and different concentrations of ethanol. The alcohol-solutions were then withdrawn for 9 months. Afterwards, the rats were given again the choice between water and ethanol-solutions. By means of this retest, long-lasting drug preference

could be studied. To analyse how long-term drug taking was affected by social determinants, the relationship of ethanol choice to both social rank (49) and social housing conditions, were studied. For several months, the rats showed an individually stable pattern of alcohol intake, which depended on both social housing conditions and dominance rank. Isolated rats consumed more ethanol than group-housed ones. Under stable social conditions, dominant rats took smaller doses than subordinate animals. After 6 months of alcohol access, the rats began to increase ethanol intake. Following a subsequent period of forced abstinence, ethanol in addition to water was reoffered. The animals took extraordinarily high doses of alcohol even when all alcohol-solutions (but not water) were adulterated with quinine, which has an aversive taste in rats (4). Age-matched, alcohol-naive controls took in the retest only small doses of ethanol, and nearly ceased drug intake when quinine was added. In contrast to the latter, ethanol intake of alcohol-experienced rats was no longer modified by external or internal factors. This reduced adaptability has been interpreted as "loss of control" (48). The persisting and even enhanced drug preference can be seen as equivalent to the high risk of relapse; it has been described as "loss of reversibility" (48). Briefly, free choice intake of ethanol by rats revealed a temporal development running through several stages, the last of which was considered to be equivalent to alcohol addiction in humans (48).

The aim of the present article is to determine whether this temporal development is characteristic only for alcohol, or whether some general principles in long-term drug taking might exist that are independent of the specific drug. We have studied the long-term oral self-administration of the opiate etonitazene (ETZ) in the rat. ETZ is a highly potent (44) μ-agonist (31), which is soluble in water. In a former study, the preference for ETZ in rats, which had previously been made physically dependent on morphine has been measured (43). In contrast to the present experiments, the preference for ETZ has been tested in this study only on 8 single days over a period of 1 year (in the meantime the rats received water). Consequently, no continuous drug taking behavior could be observed. Because we were interested in the latter, and in particular in the development of addiction, we used a very similar experimental design to that employed in a former study on the development of alcohol addiction (50). Continuous free access to ETZ solutions and water for several months, a long ETZ-free period, and a retest followed each other. During ETZ withdrawal, behavioral measurements were performed to estimate the degree of physical dependence. Over the whole period of ETZ intake, the influence of the social environment and of individual factors on drug choice were studied. Rats that remained ETZ-naive until the retest and rats that were forced to take ETZ served as controls. Further, all rats were submitted to a free-choice test with ethanol introduced in the middle of the ETZ-abstinence period. The comparison of alcohol intake in ETZ-experienced and ETZ-naive animals enables an assessment of interdrug relationships.

METHODS

Animals and Housing

All experiments were performed with male Wistar rats (breeder: Lippische Versuchstierzucht, Extertal, Germany). At the beginning of the experiments (tetradic encounters) the body weights ranged between 300 g and 350 g. When the opiate was offered the rats weighed between 450 g and 550 g. All experiments took place in air-conditioned rooms with a temperature of 21° ± 2°C, a humidity of 40%–60%, and a light/dark cycle of 12h/12h (dark phase from 1700 to 0500 h).

The housing condition of the animals depended on the experimental situation (Tables 1 and 2). Three different conditions were used: (1) group caging of four rats per cage (60 * 38 * 20 cm); (2) single caging (43 * 26 * 15 cm); (3) contact caging of four single-housed rats, whose cages were arranged in a square pattern. This enabled limited contact to the conspecifics through a grid of metal bars in the center of the arrangement (46). Group-housed rats were isolated once every week in single cages for 24 h (short-term isolation). All rats received standard diet (Altromin 1324) ad lib. The kind of drinking fluid depended on the actual experimental condition (Tables 1 and 2). All drinking fluids were offered in glass bottles. The rats took the fluids by licking on glass tubes with a terminal hole (diameter = 1 mm). At maximum, 0.1 ml got lost when the bottles were manipulated (47). All drinking fluids were completely replaced every 2 weeks. At least four times a week, body weights, food, and fluid consumption out of each bottle were measured. The position of the bottles was changed from one registration to the next.

Tetradic Encounters

Prior to their first access to the drug, all animals were individually characterized by their social behavior in tetradic encounters (49). Four rats living in the same housing condition, familiar with each other and with the test situation due to two previous training sessions, were put together in an open field (1 * 1 m). Their behavior was recorded on videotape by means of a camera placed 2 m above the test arena. Each encounter lasted for 15 min. This test was repeated 3 days later as a check on the stability of behavior. To enable an individual discrimination, the rats were labelled by black dots on their back. All encounter sessions were performed 1 h after the dark phase started (i.e., when the rats' activity was high). The open field was diffusely illuminated by low intensity white light (2 ± 0.5 lx).

Main Test Series: Voluntary Opiate Intake (VI1, VI2, VI3)

The purpose of this series (n = 36) was to study the long-term oral intake of the opiate etonitazene (ETZ, etonitazene hydrochloride; Ciba Geigy, Basel, Switzerland) in a free choice paradigm (Table 1). Over 30 weeks, the rats were given the continuous choice between tap water and 2, 4, and 8 mg/l ETZ-solutions. The pure substance was diluted with tap water and acetic acid (0.25, 0.5, and 1 ml/l, respectively, resulting in pH-values of 4.5, 4.2, and 3.9, respectively). The acid was added to facilitate solubility of ETZ. Further, its specific odor and taste enabled the animal to discriminate between different ETZ-concentrations and acid-free water. The influence of acetic acid on fluid choice was tested in an additional series (see below).

To study the influence of environmental factors on drug taking behavior, the rats were kept in different social housing conditions: group housing that included weekly short-term isolation (G; VI1 rats), contact housing (C; VI2 rats), and single housing (I; VI3 rats) (Table 1). These housing conditions were kept stable during the first 10 weeks. All housing conditions were then changed according to a given sequence (group → contact, contact → isolation, isolation → group). The same procedure was repeated after another 10 weeks (Ta-

TABLE 1

TIME SCHEDULE OF THE *MAIN SERIES*

Weeks	VI 1	VI 2	VI 3	
1-10	G ETZ	C ETZ	I ETZ	ETZ choice
11-20	C ETZ	I ETZ	G ETZ	ETZ choice after first change of housing
21-30	I ETZ	G ETZ	C ETZ	ETZ choice after second change of housing
31-36	I water	G water	C water	drug abstinence*
37-43	I ETOH	G ETOH	C ETOH	ETOH choice
44-49	I water	G water	C water	drug abstinence
50-53	I ETZ	G ETZ	C ETZ	ETZ choice (retest)
54-55	I ETZ + quinine	G ETZ + quinine	C ETZ + quinine	(ETZ adulterated with quinine)
56-68	G water	G water	G water	drug abstinence
69-72	G ETZ	G ETZ	G ETZ	ETZ choice (retest)

G = group housing; C = contact housing; I = individual housing; ETZ = choice between water and 2, 4, and 8 mg/l ETZ solutions; ETOH = choice between water and 5, 10, and 20 Vol% ETOH solutions; VI = voluntary intake.

*Periwithdrawal tests.

ble 1). Subsequently, the housing conditions remained unchanged until week 56 of the experiment.

After 30 weeks of ETZ access, the opiate was withdrawn (behavioral tests during withdrawal, see below) for 19 weeks. Following this ETZ-free period, the preference for ETZ was tested again. As before, the rats were given in this retest the choice between tap water and 2, 4, and 8 mg/l ETZ-solutions. During the last 2 weeks of the retest, all ETZ-solutions (but not water) were adulterated with 0.1 g/l quinine hydrochloride (Buchler, Merrel Dow Pharma GmbH) (Table 1). Quinine is known to have an aversive taste in rats (4). Nonaddicted, "normal" rats were, therefore, expected to avoid the adulterated ETZ-solutions. A high opiate intake in spite of the aversive stimulus can be taken as indication for a "loss of control" (48) and, therefore, for drug addiction. With ethanol, addicted rats maintained the preference for alcohol-containing solutions when these solutions were adulterated (48).

The purpose of the next experimental period was also to test to what extent voluntary opiate intake was modifiable. All animals were kept in group cages for 6 days a week. On the seventh day they were isolated in single cages (short-term isolation). This test took place after a second ETZ-free period of 13 weeks, during which the rats adapted to the new social situation. Then, another free choice-test with ETZ was performed for 4 weeks (Table 1).

To study whether or not the preference for the opiate was drug-specific, an ethanol (ETOH) choice test was performed. For 7 weeks in the middle of the first ETZ-free period (i.e., after 30 weeks of experience with the opiate and 6 weeks of abstinence, Table 1), 5, 10, and 20 Vol% ETOH (pure ethyl alcohol diluted with tap water) were offered in addition to tap water.

Additional Test Series

Forced opiate intake (FI) The only difference to the main series concerned opiate access during the first 30 weeks of the experiment. Rats of this series (n = 9) received the low concentrated ETZ-solution (2 mg/l ETZ, 0.25 ml/l acetic acid) as the only drinking fluid. They were, therefore, forced to take the opiate (Table 2). During the first 10 weeks, the rats were housed in single cages. Subsequently, housing conditions were changed according to the same schedule as in the main series (Tables 1 and 2).

Drug-naive controls (CO1, CO2, CO3, CO4) All rats of these control series did not receive any drugs during the first 30 weeks. CO1, CO2, and CO3 rats (n = 12) underwent first the ETOH-choice test and thus served in both this test and in the following ETZ-retest as opiate-naive controls (Table 2). At the beginning of the experiment they were housed either in group cages (CO1), or in contact cages (CO2) or in single cages (CO3) (Table 2). The regular changes of housing conditions during the course of the experiment, were the same as in the main series. To test the influence of the addition of acetic acid on fluid choice, these animals underwent an acetic acid-choice test during the first 10 weeks of the experiment. In addition to tap water, the same concentrations of the acid as contained in ETZ solutions (0.25, 0.5, and 1 ml/l) were offered.

TABLE 2

TIME SCHEDULE OF *ADDITIONAL SERIES*

Weeks	FI	CO1	CO2	CO3	CO4
1-10	I ETZ-forced	G water†	C water†	I water†	I water
11-20	G ETZ-forced	C water	I water	G water	I water
21-30	C ETZ-forced	I water	G water	C water	I water
31-36	C water*	I water*	G water*	C water*	I water
37-43	C ETOH	I ETOH	G ETOH	C ETOH	I water
44-49	C water	I water	G water	C water	I water
50-53	C ETZ	I ETZ	G ETZ	C ETZ	I ETZ
54-55	C ETZ + quinine	I ETZ + quinine	G ETZ + quinine	C ETZ + quinine	I ETZ + quinine
56-68	G water	G water	G water	G water	G water
69-72	G ETZ	G ETZ	G ETZ	G ETZ	G ETZ

G = group housing; C = contact housing; I = individual housing; ETZ forced = one ETZ solution (2 mg/l); ETZ = choice between water and 2, 4, and 8 mg/l ETZ solutions; ETOH = choice between water and 5, 10, and 20 Vol% ETOH solutions; FI = forced intake; CO = drug-naive controls.

*Periwithdrawal tests; †choice between water and acetic acid solutions.

During the first 49 weeks of the experiment, CO4 rats (n = 8) obtained nothing but tap water and were kept in single cages (Table 3). These rats did not have any experience with ETOH or with ETZ before the ETZ-retest and thus served as ETOH- and ETZ-naive controls.

Periwithdrawal Tests

After 30 weeks of opiate intake (free choice or forced), the ETZ solutions were removed and all animals received tap water. Daily measurements took place from 4 days before to 4 days after cessation of ETZ access and again on the eighth day of withdrawal. The following tests were performed: registration of body weight (0700-0900 h), measurement of the pain threshold (1100-1300 h), and analysis of locomotor and social behavior in tetradic encounters (46,49) (1800-2100 h). The results of the latter are not shown in this article. On test days, all drinking fluids were removed at 0700 h and replaced at 2100 h. To measure the pain threshold a wooden black box (20 * 20 * 25 cm) with a metal grid (15 bars) on the ground was used. The grid was connected with a foot shock generator (Getaa BN 2002). The animal was repeatedly stimulated by an electric current, which was increased stepwise (from 0.5 mA in steps of 0.1 mA, scramble 2 Hz), until it responded by a characteristic twitch of the body. Provided the reaction could be reproduced, the minimum value of the amperage was taken as the threshold value.

Evaluation of Housing Data and Withdrawal Tests

The time courses of the values per week of body weight, food consumption, total fluid intake, drug intake [dose in μg/kg body weight (ETZ) and g/kg body weight (ETOH)], and the percentages of the doses taken from differently concentrated drug solutions were calculated for each individual. Based on the resulting time series, interindividual means and standard errors of the means (SEM) were calculated. In group-housed rats, a maximum limit of SEM was estimated by assuming that one out of the four rats was the only consumer.

The statistical analyses concerned two different purposes: (a) the comparison among independent samples and (b) the comparison of repeated measures. Tests for (a) used temporal averages or certain temporal cross sections. Depending on the homogenity of the variances (checked by Bartlett's test), ANOVA or nonparametric procedures (H-test, U-test) were used. Linear relationships between two parameters (e.g., drug intake and total fluid intake or drug intake in 2 different weeks ("interweek-correlations"), respectively) were studied by linear regression and correlation analyses. For a nonparametric comparison of two independent distributions the Kolmogorov-Smirnov test was used. Time courses (b) were analyzed by means of ANOVA for repeated measurements and by paired Student's t-test in the case of a single repetition. Friedman's test and Wilcoxon's test were used for the respective nonparametric analyses.

TABLE 3

BODY WEIGHT, FOOD CONSUMPTION, AND TOTAL FLUID INTAKE DURING THE COURSE OF THE EXPERIMENT

	Body weight (g)			Food consumption (g/day)			Total fluid intake (ml/day)		
	VI	FI	CO	VI	FI	CO	VI	FI	CO
Weeks 1-10	522	453	527	25.2	23.2	26.2	35.4	39.9	37.3
(ETZ choice)	(29)	(13)	(33)	(1.4)	(0.5)	(1.6)	(3.2)	(5.1)	(3.1)
Weeks 21-30	527	459	529	23.9	25.3	24.6	34.8	51.3	33.8
(ETZ choice)	(28)	(12)	(36)	(1.1)	(0.9)	(1.6)	(3.7)	(2.7)	(3.8)
Weeks 37-43	511	487	528	24.0	25.1	24.5	35.9	35.9	32.2
(ETOH choice)	(25)	(16)	(32)	(1.4)	(1.0)	(1.8)	(2.0)	(2.4)	(3.2)
Weeks 50-53	506	483	536	23.5	23.8	25.0	36.1	33.3	32.1
(ETZ retest)	(30)	(15)	(20)	(1.6)	(0.9)	(1.0)	(2.8)	(2.0)	(2.0)
Weeks 54-55	498	480	534	23.5	24.1	23.9	36.0	33.0	31.6
(+quinine)	(29)	(15)	(18)	(1.3)	(1.0)	(1.2)	(2.2)	(2.1)	(2.5)
Weeks 69-72	493	483	535	22.5	22.4	23.8	35.9	35.0	32.4
(ETZ retest)	(28)	(10)	(16)	(1.6)	(0.8)	(1.8)	(2.8)	(1.9)	(2.7)

See also Tables 1 and 2. Mean values (SEM in parantheses).
VI = voluntary ETZ intake (VI 1-3); FI = forced ETZ intake; CO = ETZ naive controls (CO 1-3); ETZ = etonitazene.

To assess changes in body weight during withdrawal, the individual increases or decreases from one day to the next were calculated. The values of the pain threshold were individually standardized according to ANOVA for repeated measurements. The remaining variances, standard deviations, and SEM only represent individual variation from day to day. Mean values remained unchanged by this procedure.

Evaluation of the Tetradic Encounters

It has previously been shown that tetradic encounters are useful for the identification of the dominance rank of a male rat (49). Dominant rats are more aggressive, and receive more social interactions from their encounter partners than subordinate ones (49). For the present simplified purposes, the number of aggressive acts [mounting and aggressive posture, (20)] during one encounter session was counted. By a median split the most and second most aggressive animal of an encounter group was classified as "dominant," the two others as "subordinate." The individual dominance rank determined by this procedure, seems to represent a very stable individual feature. The correlations, not only between two subsequent encounters at the beginning of the experiment, but also between the initial encounters and those performed nearly 1 year later during withdrawal, were significantly positive ($r(34) = +0.81$, $p < 0.001$ and $r(32) = +0.72$, $p < 0.001$, respectively).

RESULTS

First Experimental Period (Weeks 1-10)

Control rats (CO1-CO3), which were kept in three different housing conditions (Table 2), were given the choice between water and different acetic acid concentrations. All control rats avoided the acid. None of these rats revealed an individually stable preference for any of the acid solutions. Rats of the main series (VI1-VI3) were given the choice between acid-free water and three acid-containing ETZ solutions. The distributions of all volumes taken from the different ETZ solutions during the first 10 weeks of the experiment, differed significantly from those of the respective acid solutions in control rats. In total, volumes taken from acid-containing ETZ solutions were significantly higher [Kolmogorov-Smirnov test with $n_1 = 296$, $n_2 = 296$, each: $l = 0.106$, $p < 0.05$ (2 mg/l); $l = 0.118$, $p < 0.01$ (4 mg/l); $l = 0.117$, $p < 0.01$ (8 mg/l)] than those taken from the respective pure acid-solutions. Furthermore, rats of the main series (in contrast to control rats) developed individual preferences for a given ETZ concentration. It was concluded that the ETZ intake resulted from an active choice behavior. Not more than 4% of the amounts taken from the ETZ solutions, could be attributed to a leak of the bottles.

The mean daily ETZ doses taken voluntarily by rats of the main series depended on the housing condition (Fig. 1). Both kinds of socially deprived animals [contact caged (C) and isolated (I)] consumed significantly more opiate than group-housed (G) rats [$H(2) = 16.34$, $p < 0.001$]. Differences between C and I were not significant. Apart from the doses, the preferences for different ETZ concentrations depended also on housing. C and I took 55% ± 3% and 54% ± 3%, respectively, of their daily opiate doses from the 8 mg/l solution, whereas G rats took only 33% ± 5% from the highest concentrated solution (Fig. 1). The temporal pattern of voluntary ETZ intake was similar among all rats. During the first week, the mean daily doses were more than twice as high as during the following weeks (Fig. 1). Subsequently, drug intake decreased exponentially reaching a constant level. To analyse long-term individual stability in ETZ intake, individual drug doses taken in a given week were correlated with those of the following weeks. During the first 4 weeks no significant "interweek correlations" were detected, indicating that individual ETZ consumption was nearly unpredictable. With the beginning of the fifth week, the individuals established a stable pattern of opiate intake. The "interweek correlations" of opiate intake were significantly positive [e.g., week 5 vs. 6:

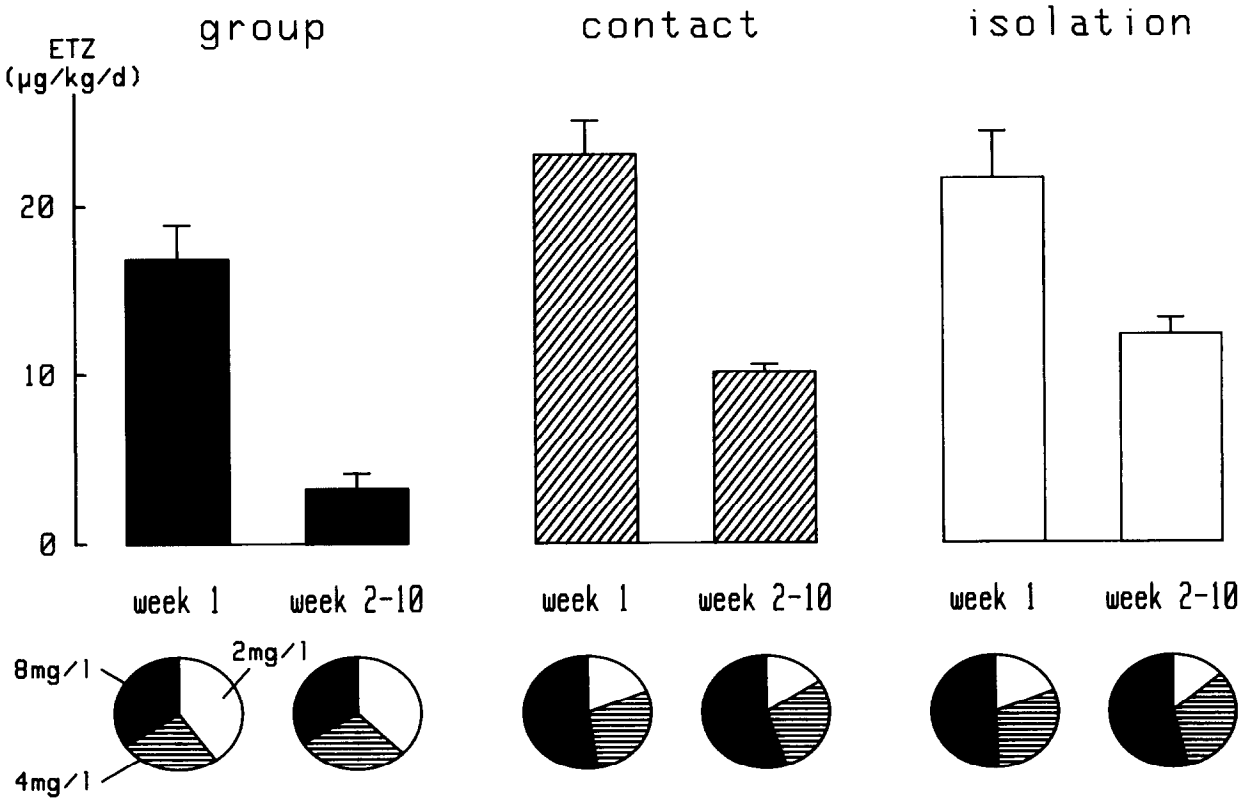

FIG. 1. Influence of social housing conditions [group (VI1), contact (VI2), and single (VI3) caging] on voluntary etonitazene (ETZ) intake during week 1 and weeks 2-10. Above: mean values (± SEM). Below: partial doses of ETZ taken from the 2, 4, and 8 mg/l ETZ solutions.

$r(14) = 0.83$, $p < 0.001$; week 6 vs. 7: $r(14) = +0.73$, $p < 0.01$; week 7 vs. 8: $r(14) = +0.81$, $p < 0.001$]. The preference for different ETZ solutions revealed a similar pattern.

No significant differences in body weight, food consumption or total fluid intake (TFI) were found among the differently housed groups of the main series (VI1-VI3) not even between main series and controls (CO1-CO3) (Table 3). These parameters did not exhibit significant temporal changes in any of the experimental series. Thus, the differences in ETZ intake between socially deprived and group-housed rats did not result from differences in TFI (G: 40.4 ± 1.8 ml/day; C: 31.1 ± 2.7 ml/day; I: 34.8 ± 3.0 ml/day). There were no significant correlations between individual ETZ choice and TFI [e.g., week 1: $r(14) = +0.01$, NS; week 5: $r(14) = +0.20$, NS; week 10: $r(14) = +0.17$, NS).

The time course of forced ETZ intake in FI-rats was different. These rats, which had one ETZ solution as the only drinking fluid, increased their fluid intake and, consequently, the intake of ETZ between week 2 and 8 from 136 ± 14 µg/kg/day to 211 ± 30 µg/kg/day. The corresponding TFI was significantly higher than in controls (FI: 46.5 ± 5.0 ml/day, CO1-CO3: 35.4 ± 1.6 ml/day; $F(1,19) = 4.38$ $p < 0.05$). Neither body weight (FI rats started with least weight) nor food consumption were affected by forced ETZ intake (Table 3).

In VI2 and VI3 rats of the main series, ETZ choice depended on the individual dominance rank. Beginning with the fifth week of ETZ choice, stable interindividual differences appeared. Dominant rats that reduced ETZ intake, consumed significantly less ETZ than subordinate ones [$t(14) = 2.99$, $p < 0.01$, Fig. 2a]. Differences between VI2 and VI3 were not significant. Body weights (521 ± 19 g and 523 ± 19 g, respectively), food consumption (25.9 ± 0.8 g/day and 25.1 ± 0.7 g/day, respectively), and TFI (36.0 ± 2.9ml/day and 35.0 ± 3.2 ml/day, respectively) were similar in dominant and subordinate rats. During intermittent short-term isolation, the animals consumed seven times more ETZ than before and afterwards when housed in groups [$t(7) = 16.89$, $p < 0.001$), whereas TFI was slightly reduced (Fig. 2b). The effect did not habituate over 10 weeks. Dominant and subordinate G rats took similar ETZ doses during the day of isolation (24.5 ± 2.2 µg/kg/day and 24.0 ± 1.0 µg/kg/day, respectively).

ETZ Intake after Change of Housing Conditions (Weeks 11-20 and weeks 21-30)

After 10 weeks and again after 20 weeks, the housing conditions were changed in all experimental groups (Tables 1 and 2). In all cases, ETZ intake was adjusted to the actual housing condition. The rats consumed significantly more ETZ when being socially deprived, than during group housing [weeks 11-20: $F(2, 21) = 37.51$, $p < 0.001$; weeks 21-30: $F(2, 20) = 30.61$, $p < 0.001$, Fig. 3). Differences between C and I were not significant. With the exception of changes into the group

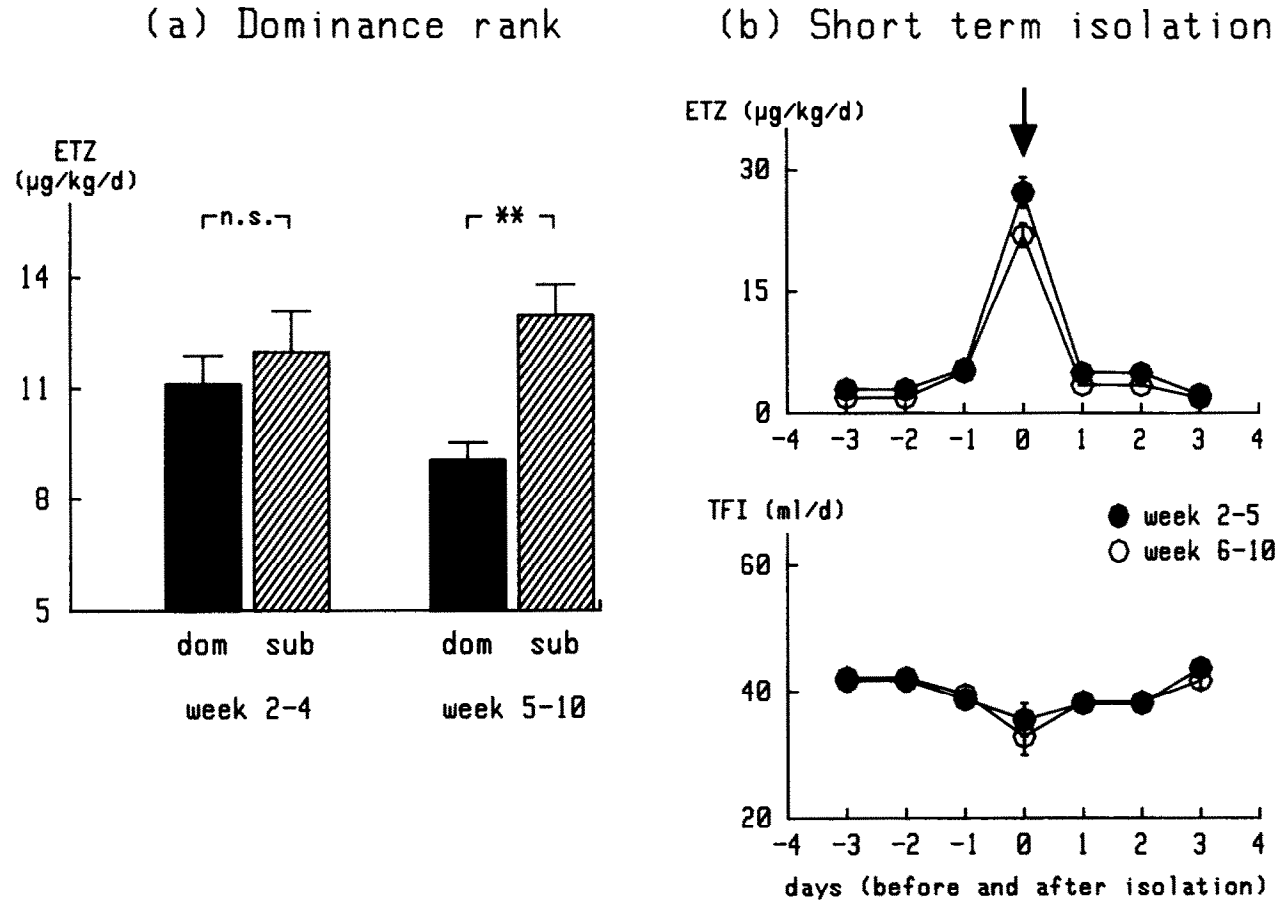

FIG. 2. (a) Mean etonitazene (ETZ) intake (± SEM) of dominant (dom) and subordinate (sub) rats kept in single and contact cages (VI2 and VI3) during weeks 2-4 and weeks 5-10; $^{**}p < 0.01$. (b) ETZ intake (above) and total fluid intake (TFI; below) before, during (arrow), and after 24-h isolation of group-housed rats (VI1); isolation was repeated once every week (mean values ± SEM).

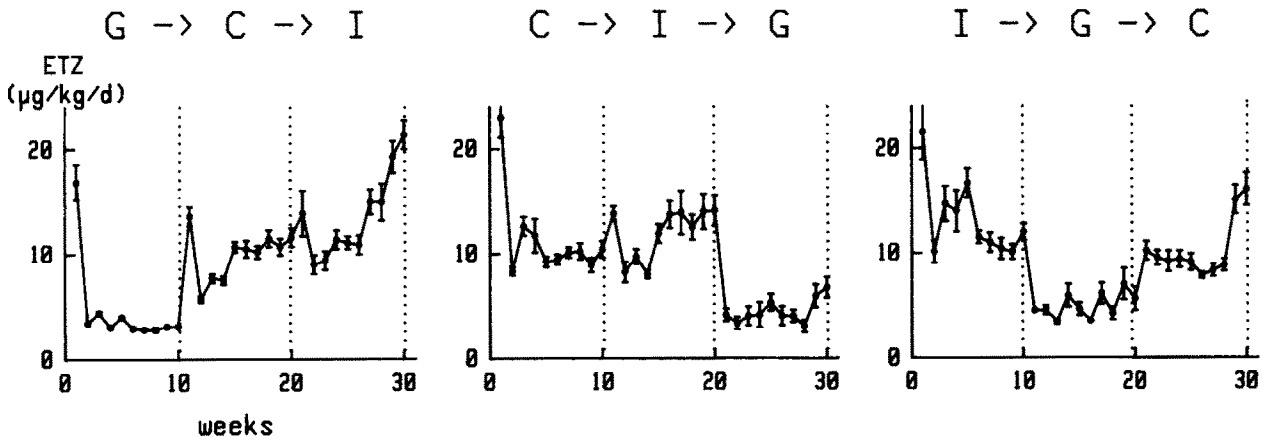

FIG. 3. Time courses of voluntary etonitazene (ETZ) intake (mean values ± SEM). After 10 and again after 20 weeks, the housing conditions were changed (Table 1). Left: VI1; middle: VI2; right: VI3. G = group housing, C = contact housing, I = single housing.

housing condition, the time courses of ETZ intake revealed a transient increase of ETZ intake during the first week in the new environment (Fig. 3). No correlations between individual ETZ doses taken in different housing conditions were found. Within any period of unchanged housing, however, the rats revealed an individually stable pattern of drug intake. Similar to the group-housed rats during the first 10 weeks, the respective group-housed rats after changes of housing, always significantly increased ETZ intake during the day of short-term isolation (VI2 rats: 360%, $t(7) = 4.60$, $p < 0.01$; VI3 rats: 670%, $t(7) = 7.61$, $p < 0.001$). Differences between the ETZ intake of the respective dominant and subordinate rats were not significant.

The relationship between the dominance rank of a rat and its ETZ intake (Fig. 2a), changed when the social environment was changed. Dominant rats, which previously had consumed less ETZ than subordinate ones [$t(13) = 4.10$, $p < 0.01$], now increased their intake. Subordinate rats did not substantially change their behavior (Fig. 4). Subsequently, dominant rats consumed more ETZ than subordinate ones [$t(13) = 2.52$, $p < 0.05$).

Forced ETZ intake neither depended on the housing condition, nor changed with time. The mean daily doses of FI rats in weeks 11-20 (227 ± 19 μg/kg) and in weeks 21-30 (211 ± 27 &mgrg/kg) were similar to those in weeks 9-10 (207 ± 29 μg/kg).

During the last 5 weeks of opiate access, all socially deprived rats (VI1 and VI3) revealed a spontaneous increase of voluntary ETZ intake (Fig. 5). On an average, their mean ETZ intake was significantly higher in weeks 26-30 than in weeks 21-25 [$t(28) = 3.08$, $p < 0.01$]. During the same time period, interindividual differences in ETZ choice between dominant and subordinate rats as seen in weeks 21-25 (dominant: 11.9 ± 0.7 μg/kg/day; subordinate: 8.2 ± 0.2 μg/kg/day) disappeared in weeks 26-30 (dominant: 14.2 ± 1.1 μg/kg/day; subordinate: 13.0 ± 1.6 μg/kg/day). The mean ETZ intake of group-housed rats (VI2) remained at a low level (weeks 21-25: 4.2 ± 0.7 μg/kg/day; weeks 26-30: 4.7 ± 0.8 μg/kg/day).

ETZ Withdrawal

After 30 weeks of ETZ access all opiate solutions were removed, and the rats obtained nothing but tap water. Contact-housed and single-housed rats with previous voluntary ETZ intake (VI3 and VI1) and rats with previous forced ETZ administration (FI) revealed signs of withdrawal. In group-housed rats with previous voluntary intake (VI2), no significant changes were observed. In VI3, VI2, and FI, the time courses of the pain threshold from day to day before and during withdrawal were similar. The threshold was significantly lower during the first 3 days of withdrawal, than during the last 4 days of ETZ access (Fig. 6). This supersensitivity disappeared at the end of the first week of opiate withdrawal [no more differences to controls, $H(4) = 1.96$, NS. High threshold values in FI rats during the last days with ETZ access might be due to a residual analgesic effect of the opiate. During the first 3 days of withdrawal, FI rats significantly lost body weight (Table 4) without any reduction of their food consumption (days −4 to −1: 23.9 ± 0.9 g/day; day +1: 25.3 ± 0.8 g/day; day +2: 25.6 ± 1.0 g/day). Therafter, these animals gained weight and finally reached the same body weight as before. The changes in pain threshold (days +1 and +2) and body weight (day +1) in FI rats were positively correlated [$r(7) = +0.69$, $p < 0.05$ and $r(7) = +0.68$, $p < 0.05$, respectively), indicating the existence of a withdrawal syndrome.

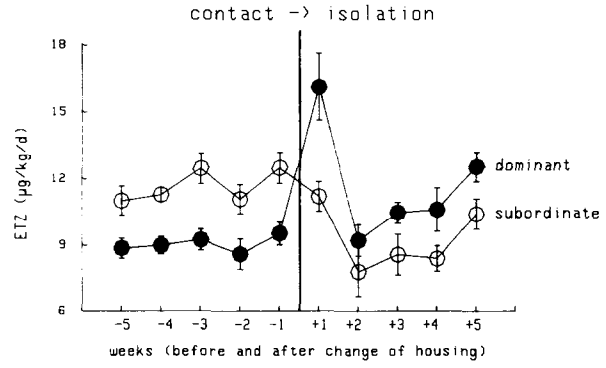

FIG. 4. Voluntary etonitazene (ETZ) intake of individually classified rats before (weeks −5 to −1) and after (weeks +1 to +5) the move from contact cages to single cages. Mean time courses (± SEM) of dominant and subordinate rats. (VI2: weeks 6-15, VI1: weeks 16-25, Table 1).

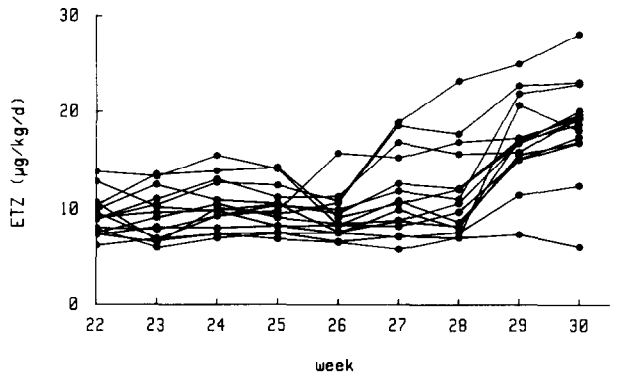

FIG. 5. Individual time courses of voluntary etonitazene (ETZ) intake of socially deprived rats during weeks 23-30 (VI1 and VI3).

The total fluid intake of FI rats abruptly decreased when water was offered and reached the same level as at the beginning of the experiment (days −4 to −1: 50.5 ± 6.3 ml/day; day +1: 33.6 ± 2.3 ml/day; day +2: 35.0 ± 2.1 ml/day). No changes in TFI were observed in the other experimental groups.

Free Choice Intake of ETOH (Weeks 37-43)

After 6 weeks of ETZ abstinence, rats with previous voluntary ETZ intake (VI1-VI3), previous forced ETZ intake (FI), and no ETZ experience (CO1-CO3) obtained ETOH solutions in addition to water. The mean daily doses of ETOH intake of all experimental series ranged from 0.5-1.4 g/kg. ETOH taking was significantly affected by former experience with the opiate. Both VI and FI rats consumed more ETOH than ETZ-naive animals [$H(2) = 13.04$, $p < 0.01$, Fig. 7a]. The individual values of ETZ intake and subsequent ETOH intake did not correlate with each other (voluntary ETZ intake in weeks 26-30 vs. ETOH intake: $r(13) = +0.19$, NS; forced ETZ intake in weeks 26-30 vs. ETOH intake: $r(6) = +0.14$, NS). Previous ETZ experience tended to enhance the preference for ETOH, however, ETZ-preferring rats did not reveal the highest consumption of ETOH.

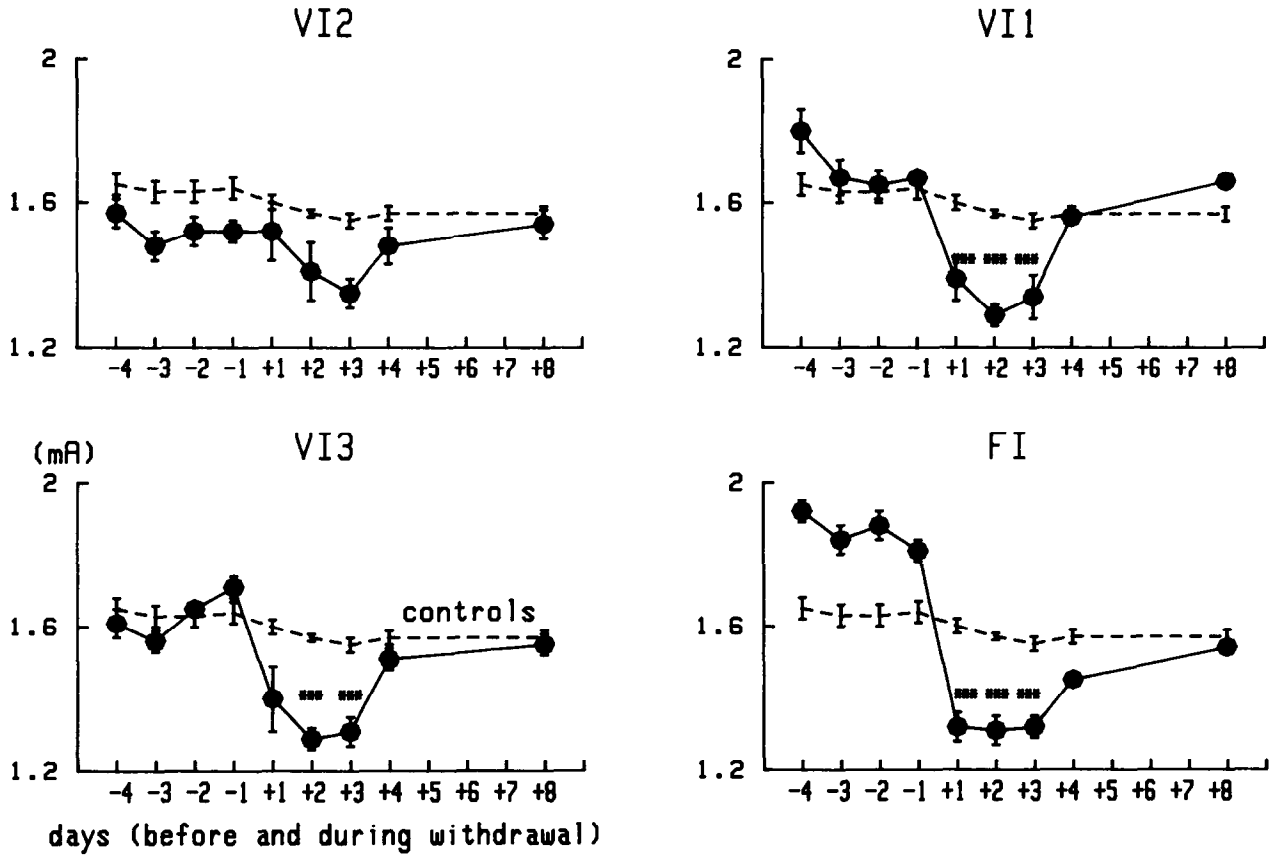

FIG. 6. Mean values (± SEM) of the pain threshold before (days −4, −3, −2, and −1) and during (days +1, +2, +3, +4, and +8) ETZ withdrawal. ETZ = etonitazene; VI = previous voluntary ETZ intake of group-housed (VI2), contact-housed (VI3), and single-housed (VI1) rats; FI = previous forced ETZ intake; CO = ETZ-naive controls (CO1-CO3). ***$p < 0.001$ (compared to days before withdrawal).

In both rats with previous voluntary ETZ intake and ETZ-naive controls, the influence of housing conditions on voluntary ETOH intake was similar. Isolated rats (VI1 and CO1, respectively) took higher ETOH doses as compared to the mean daily doses of contact caged (VI3 and CO3, respectively) and group-housed rats (VI2 and CO2, respectively) that were significantly lower ($F(2, 14) = 4.54$, $p < 0.05$ and $F(1, 4) = 12.39$, $p < 0.05$, respectively, Fig. 7b). During short-term isolation, VI2 rats and CO2 rats consumed significantly more ETOH than before and afterwards in the group-housing condition [1.9 ± 0.3g/kg/day, $U(6, 6) = 6, p < 0.05$ and 1.9 ± 0.4g/kg/day, $U(4, 4) = 0, p < 0.01$, respectively].

TABLE 4

ETZ WITHDRAWAL: DAILY CHANGES IN BODY WEIGHTS

	VI 2	VI 3	VI 1	FI	CO 1-3
Days −4 to −1	+0.5 (0.4)	+1.3 (0.6)	+0.1 (0.5)	+2.2 (0.5)	−0.1 (0.7)
Day +1	−0.3 (2.1)	−2.0 (2.5)	−1.3 (1.6)	−12.1 (2.0)†	+0.3 (0.9)
Day +2	+0.1 (1.7)	−1.5 (2.3)	−1.3 (2.6)	−1.3 (1.3)	+1.3 (1.4)
Day +3	−0.1 (1.1)	−6.1 (3.0)	+2.4 (1.2)	+3.6 (1.7)	−1.6 (1.2)
Day +4	+1.6 (1.3)	+1.5 (1.9)	+0.7 (1.6)	+3.6 (0.8)	+0.7 (1.3)
Day +8	−1.0 (1.0)	−5.8 (3.1)	−3.4 (2.2)	+8.2 (1.8)*	−0.7 (1.4)

Average changes of body weights (g) from day to day (SEM in parantheses) before (days −4 to −1) and during (days +1, +2, +3, +4, +8) etonitazene (ETZ) withdrawal.

VI = previous voluntary intake of group housed (VI2), contact housed (VI3), and individually housed (VI1) rats; FI = previous forced ETZ intake; CO = ETZ naive controls (CO1-3).

*$p < 0.05$; †$p < 0.001$ (compared to days before withdrawal).

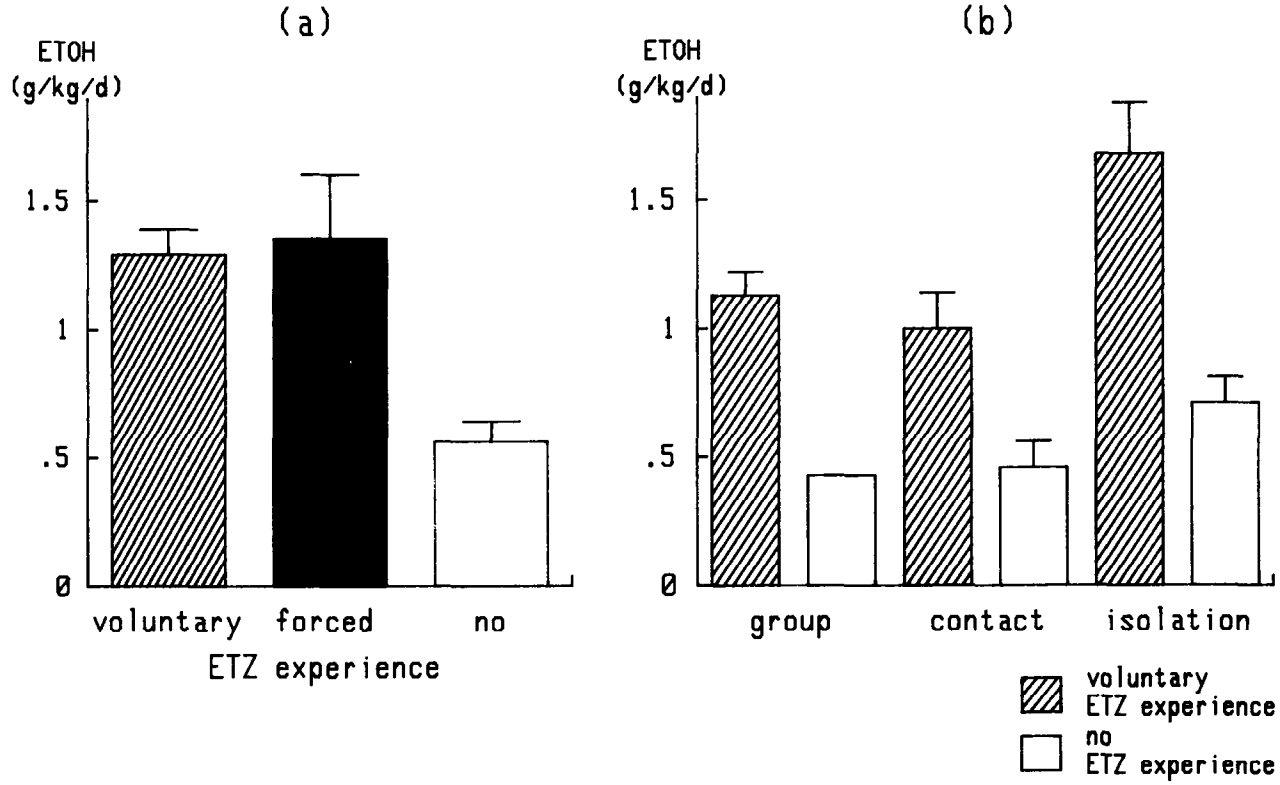

FIG. 7. Mean voluntary intake of ethanol (± SEM). (a) Influence of previous etonitazene (ETZ) intake on ethanol (ETOH) choice. (b) Influence of social housing conditions (group, contact, single housing) on ETOH choice in VI and CO rats. VI1–VI3 = previous voluntary ETZ intake; FI = previous forced ETZ intake; CO1–CO3 = no ETZ experience.

ETZ Retest (Weeks 50–55 and Weeks 69–72)

After 19 weeks without ETZ, all rats were given the choice between ETZ solutions and water. By means of this retest, persisting effects of previous ETZ experience on long-term opiate intake should be detected. Both rats with previous forced ETZ administration (FI) and ETZ-naive rats (CO), revealed a similar temporal pattern of ETZ choice in the retest, as rats of the main series (VI) did at the beginning of the experiment. An initial high intake was followed by a reduction of the daily ETZ doses in the following weeks (Fig. 8). In contrast, socially deprived rats with previous voluntary ETZ intake (VI3 and VI1), maintained and even increased their high drug intake (Fig. 8). During the first 4 weeks of the retest, these rats nearly took twice as much opiate (65.9 ± 10.3 μg/kg/day), than aged-matched CO rats (22.7 ± 2.3 μg/kg/day) and FI rats (36.1 ± 6.1 μg/kg/day) [$H(2) = 14.53$, $p < 0.001$, Fig. 8]. Neither the difference between FI and CO, nor differences between contact caged (VI3) and single-housed (VI1) rats of the main series were significant. Opiate intake of the respective group-housed rats (VI2) could not be studied because six out of eight rats had died. The last two VI2 rats revealed only a small preference for the opiate (10.6 μg/kg/day and 8.1 μg/kg/day, respectively). At no time of the retest, differences between the opiate intake of ETZ-naive controls having previous experiences with ETOH (CO1–CO3), and of drug-naive controls having no experience with any drug (CO4), were significant (mean daily ETZ doses: 22.7 ± 4.3 μg/kg and 22.7 ± 2.5 μg/kg, respectively).

When ETZ solutions (but not water) were adulterated with quinine during the last 2 weeks of the retest, both CO and FI rats reduced opiate intake (CO: 9.9 ± 0.7 μg/kg/day; FI: 8.9 ± 0.4 μg/kg/day). VI-rats, however, maintained their high preference for the opiate (VI: 83.0 ± 11.4 μg/kg/day) despite the addition of quinine (Fig. 8). During adulteration, these rats preferred the highest concentrated of the ETZ solutions. 89.2 ± 4.9% of the total daily ETZ dose was taken from this concentration, which was significantly [$t(11) = 4.28$, $p < 0.01$] more than before quinine was added (45.8 ± 4.8%). This way, the rats maintained a high intake of ETZ (Fig. 8) simultaneously reducing the amount of quinine intake.

The influence of individual factors on ETZ intake of VI rats disappeared. No more differences were found between ETZ intake of dominant and subordinate rats (Fig. 9). In contrast, dominant CO and FI animals consumed significantly less opiate than subordinate ones [$t(13) = 3.82$, $p < 0.01$, Fig.9), in the same way as VI rats had done before (Fig. 2a).

To test the influence of short term isolation on ETZ intake, all animals were moved in group cages. When the opiate was offered again, VI rats maintained their high preference for ETZ. On the average, they consumed 10 to 20 times more ETZ (104.3 ± 6.1 μg/kg/day) than CO and FI rats (CO: 3.1 ± 0.2 μg/kg; FI: 8.8 ± 0.3 μg/kg). During short-term isolation, ETZ intake of VI rats was not significantly altered whereas both CO and FI rats increased their opiate intake [CO: $t(10) = 8.63$, $p < 0.001$; FI: $t(6) = 4.39$, $p < 0.01$; Fig. 9]. The extent of this increase (CO: +1125%; FI: +665%) was similar to that of VI rats during their first weeks

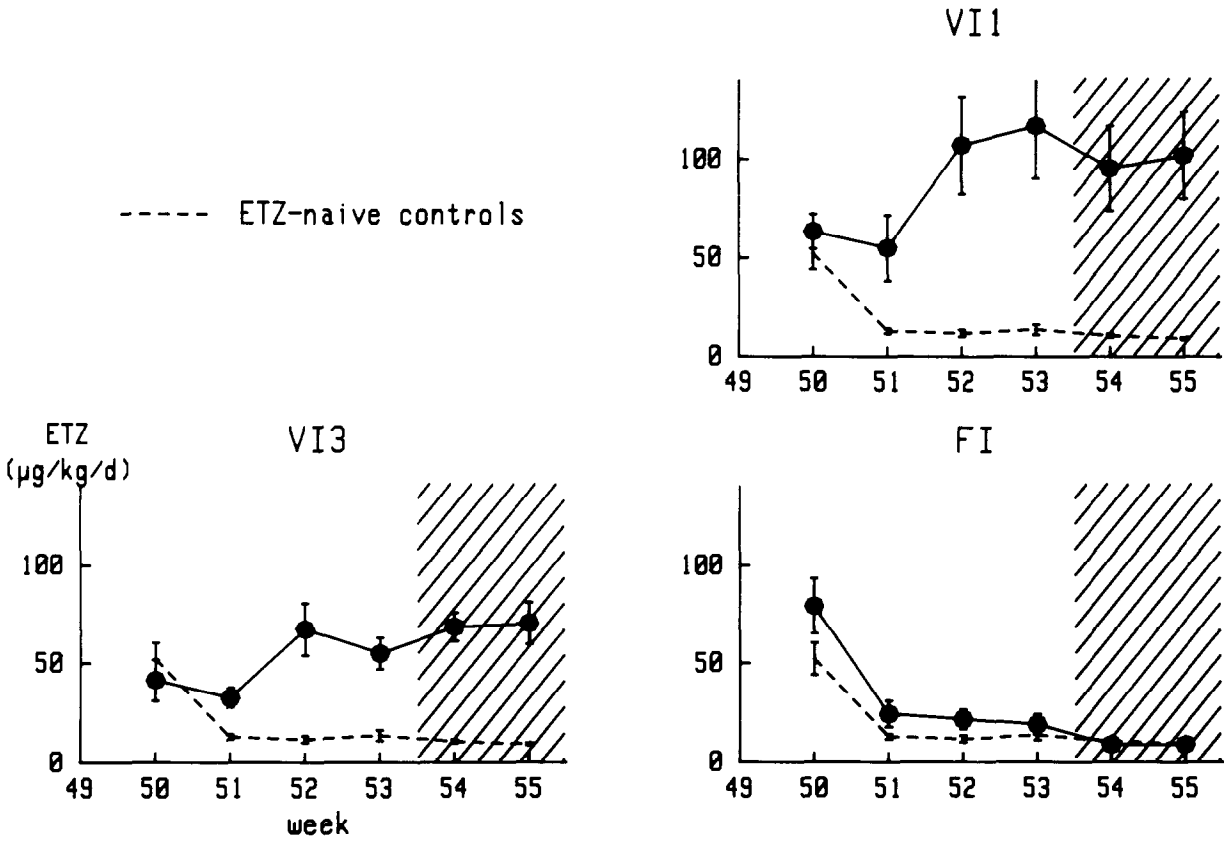

FIG. 8. Voluntary etonitazene (ETZ) intake during the retest after 19 weeks of ETZ abstinence. Mean time courses (± SEM). Hatched area = ETZ solutions, but not water, were adulterated with quinine; VI = contact-housed (VI3) and single-housed (VI1) rats with voluntary ETZ intake before abstinence. FI = forced ETZ intake before abstinence; CO = no ETZ experience before (CO1,CO3,CO4).

of ETZ access (Fig. 2b). All rats slightly reduced total fluid intake during short-term isolation.

As during the previous experimental phases, neither body weight nor food consumption or total fluid intake were systematically influenced by housing conditions or drug intake (Table 3). At no time of the experiment, were significant positive correlations between total fluid intake and voluntary ETZ or ETOH intake detected.

DISCUSSION

Temporal Development of Opiate Intake Behavior

To study underlying biological mechanisms of drug addiction, adequate animal models are necessary. Operant self-administration of drugs is a classical method in animal research on drug taking behavior (39,40,52). The increase of an animal's response rate to obtain the drug is considered as a direct measurement of reinforcing properties of a drug. The latter have been suspected to be closely related to the drug's potential of causing addiction (39,40,52). However, the observation that an animal is self-administering a drug is not a proof for drug addiction, unless the criteria of "loss of control" and "loss of reversibility" (3,18,22,25). Furthermore, the animal does not have continuous free acces to the drug for a long time (as in the human case). Therefore, we have used an experimental design, which allows the animal to have continuous free access to opiate solutions and to water for a long time (48,50). Such a paradigm seems to be closely related to the situation of a human consumer. By means of a retest (i.e., again free choice) after a long-term forced abstinence, a long-lasting drug preference could be measured ("loss of reversibility"). The analyses of the influence of aversive stimuli (adulteration of the opiate solutions with quinine) and of social variables on drug choice enables the detection of a "loss of control."

First of all, it has to be ensured that drug taking based on a veritable choice for the drug because of its pharmacological effects. There are several indications that this was the case. The taste of ETZ-containing solutions did not enhance ETZ intake because it appeared to be aversive due to the addition of acetic acid. The rats took higher volumes from the ETZ-containing acid solutions than control rats did from the ETZ-free acid solutions. If only taste would account for the opiate choice, one would not expect any relationship between ETZ intake and the social environment. Actually, ETZ intake highly depended on the social housing conditions (Fig. 3), whereas acid intake was independent from the social environment. An individually stable pattern of ETZ choice concerning both the dose and the preference for a certain ETZ concentration developed with time indicating that the rats did not drink by chance from the bottles. These results provide evidence for a choice that was based on the reinforcing properties

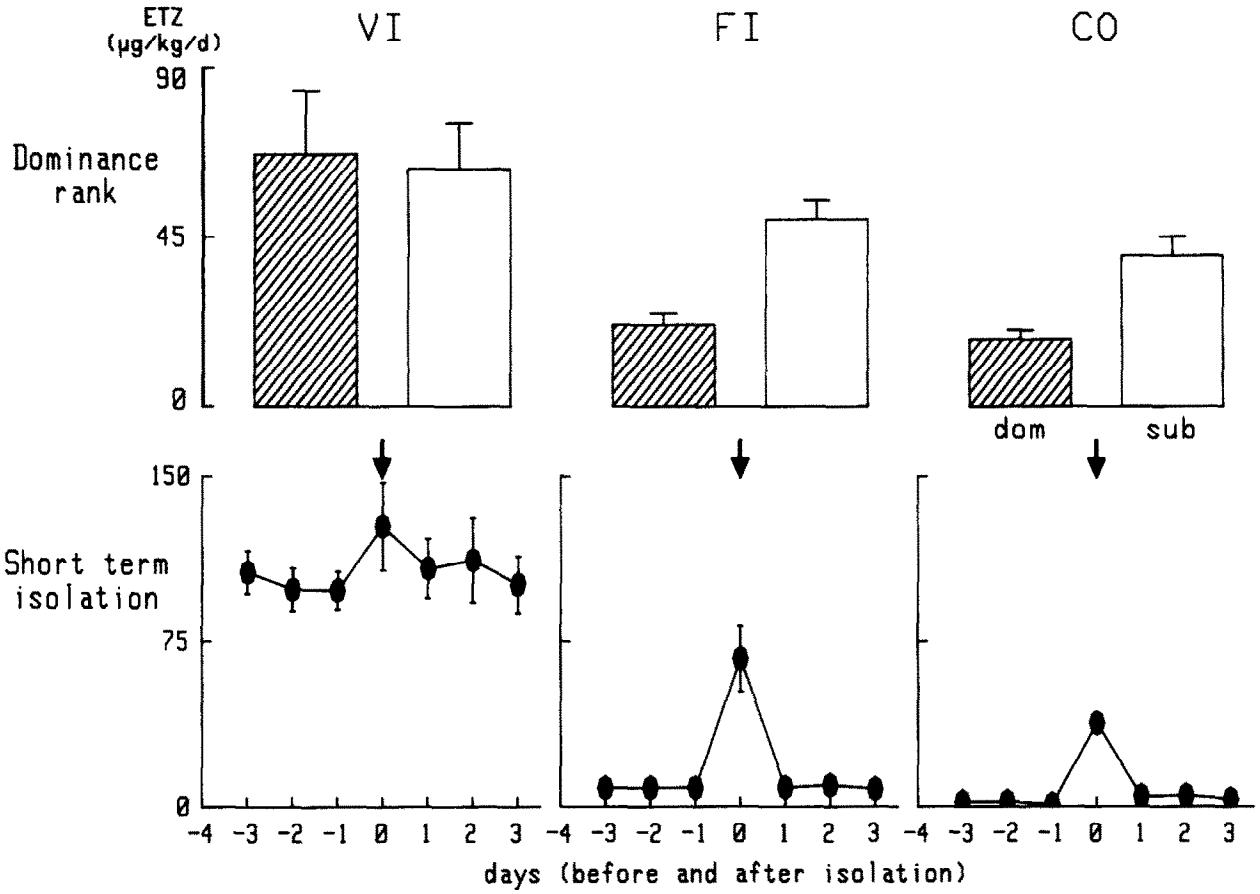

FIG. 9. Modifiability of voluntary etonitazene (ETZ) intake during the retests (mean values ± SEM). Above: ETZ intake of dominant (dom) and subordinate (sub) rats during weeks 50–53. Below: ETZ intake before, during (arrows), and after 24-h isolation of group-housed rats during weeks 69–72; isolation was repeated once every week. VI, FI, and CO as in Fig. 8.

of the opiate. This assessment is consistent with earlier findings (38). Rats that were either made physically dependent on morphine or remained drug-naive, were given the choice between water and a morphine solution. Despite of the aversive taste of the morphine solution, both physically dependent and nondependent rats developed a preference for the opiate (38). It has been concluded that positive reinforcement by the drug's effects (rather than negative reinforcement by a relief of withdrawal) accounted for the opiate preference in the choice test (38).

The intake of relatively small volumes from ETZ solutions in the present study [cf. ETZ is 1000 times more potent than morphine (44)] led to daily doses of 5–20 µg/kg. This dose range has been shown to elicit behavioral responses (41). Preliminary studies with rats in our laboratory demonstrated that orally administered ETZ significantly enhanced locomotor activity and reduced vertical exploration by doses ranging between 5 and 30 µg/kg. Direct measurements of the reinforcing properties of ETZ in an operant self-administration procedure have demonstrated that these doses of ETZ are effective as a reinforcer (12,29).

The temporal development of ETZ intake can be subdivided into distinct phases:

First Experiences (First Days to Week 5). On the very first days of opiate access, the rats consumed extremely high doses. Afterwards, mean intake decreased but still remained unpredictable, that is, days with individual high intake were followed by days with low intake and the rat repeatedly switched its preference for different ETZ-concentrations. This behavior might be interpreted as a progressive learning process involving both classical and operant conditioning. An association between external stimuli like odor or taste of the solution with a respective content (i.e., the classical component), happens very quickly (11). The operant component may consist of an association between the animal's own actions (intake of a given amount from a certain ETZ concentration) and subsequent psychotropic effects. This way the animal gathers experiences with the spectrum of drug actions, and in particular learns how desired effects can be induced by taking a particular fluid. In contrast to the classical component ("exterior cues"), the development of long-term stable preferences (or aversions) via CNS effects ["interior cues," (13)] takes several days (11).

Controlled Opiate Intake (Weeks 5 to 25). After about 5 weeks, the animals developed a stable individual pattern of opiate intake. Over several months, drug intake of the rats can be described as "controlled," because it is modulated by environmental and individual factors. When the housing conditions were changed, opiate intake completely depended on the situation and did not show residual effects from former

social experiences. The individual social rank influenced ETZ choice in a predictable manner: in stable social conditions, subordinate rats took more opiate than dominant ones. Similar effects have been described for the intake of alcohol in rats (8,19). The enhanced drug intake of subordinate animals was sometimes interpreted as a result of social stress (8,19). Both the present results and former experiments with ethanol (50), argue against this interpretation. The dominance rank was determined prior to drug choice, whereas correlations to ETZ intake appeared much later when the rat had no opportunity to "express" his dominance. The dominance rank seemed to be a very constant feature (7) over long periods, because the rats maintained their "rank" when observed 1 year later, in spite of drug experiences and repeated changes of the social environment. The relationship between individual features and drug choice might be interpreted as an expression of an individual predisposition to drug taking (6,17). Inbred strains of drug-preferring rats (28) may in part be a result of such predispositions. In these rats, which always consume more from the drug than the nonpreferring line, however, a similar interindividual variability in drug intake as in outbred strains was observed (32). Thus, it is not clear, to what extent the predispositional behavioral factors result from genetic differences.

Increasing Drug Intake (Beginning With Week 25). After 25 weeks of ETZ free choice, socially deprived rats started to raise ETZ intake continuously, although at this time no experimental conditions were changed. Interindividual differences in opiate intake disappeared. Because the exact beginning of increase differed among the rats, it is not likely that an unknown artificial stimulus could synchronize the animals. It might be assumed instead that either the "general" desire for the drug starts to raise (motivational changes), or that the effects of the drug become less effective [tolerance (16)]. Tolerance towards the effects of opiates is expected to occur quickly after some days of drug intake (16), and is stronger the higher the dose (16). In the present experiment, however, socially deprived animals raised ETZ intake not earlier than after 24 weeks of continuous drug supply. Further, group-housed rats, which until this time had taken in total even higher amounts of ETZ than socially deprived rats (Fig. 3), did not increase opiate intake. It is, therefore, more likely that the raised intake was due to motivational changes rather than to pharmacological tolerance.

Opiate Addiction. After more than 4 months of opiate abstinence, rats that had been socially deprived and had undergone the free choice paradigm, revealed a different pattern of ETZ intake as compared to both their own intake before abstinence and to that of ETZ-naive controls. The former had enhanced their preference for the drug and maintained it for the rest of their lives. Opiate intake could no longer be modified by external stimuli like short-term isolation, adulteration of the drug solutions or by individual parameters. These drastic changes are interpreted as:

(1) A loss of reversibility in drug preference
(2) A loss of control of drug taking. The animals loose their ability to consider carefully alternative behaviors (e.g., the desire for the psychotropic effects of ETZ vs. the aversive taste of quinine).

Because these features characterize drug addiction (3,18,22), it is suggested that under the given conditions the rats became addicted to the opiate.

As opposed to the addicted rats, both aged-matched ETZ-naive controls and rats with previous forced opiate supply, revealed a "controlled" pattern of intake in the retest. The intake behavior of addicted rats, therefore, could not be a result of age-dependent effects. It also cannot be regarded as "accidental," because the reduced adaptability of drug taking in addicted rats concerned at least three independent aspects.

Rats with forced opiate supply did not became addicted, although their withdrawal syndrome was most expressed among all experimental series. Consequently, physical dependence on the opiate did not necessarily lead to opiate addiction. According to recent findings, physical dependence has to be regarded, therefore, as independent from drug addiction. Addiction seems to be due to positive reinforcement (drug-induced reward) rather than to negative reinforcement (prevention of withdrawal symptoms) (33,34,40,42).

Addiction to the opiate developed only in rats that had voluntary ETZ intake and have been socially deprived during the last weeks of ETZ choice. The main discriminating features among all experimental groups, concerned the present social housing condition, previous social experiences, the present amount of ETZ intake, the total amount of ETZ taken previously, the kind of drug access (free choice or forced), and the time of drug experience. Voluntary drug intake appears to be much more important, and perhaps necessary, for the development of addiction than continuous high drug intake by forced administration. Since ETZ intake in social deprivation was always higher than in group caging, the present amount of ETZ intake during the last weeks of ETZ choice, due to social deprivation, could not explain the development of addiction. It can be assumed that after a certain time of voluntary drug intake, an endogenous rise in the motivation for drug taking develops (51), which might be facilitated by certain environmental factors. Recently, we could show that the preference for ethanol in a free choice pradigm can "skip" periods of drug abstinence of up to 4 weeks (51).

Opiate-Specific and General Principles in the Development of Drug Addiction

The experimental design of the present study was nearly the same as that of a previously described animal model of long-term ETOH intake (50). A comparison between opiate and alcohol intake behavior may be useful to identify superordinate principles in the development of drug addiction. The main similarity concerned the succession of the stage of controlled drug intake by the state of drug addiction (48,50).

During controlled drug choice, both ETOH and ETZ intake were modified by environmental and individual factors. The influences were in principle similar, that is, social isolation (long- or short-term) increased drug intake, and dominant rats took less drugs in a stable condition, but were more sensitive to social changes (50). In general, ETZ choice was influenced to a higher extent by environmental factors, whereas the intake of ETOH was more influenced by individual factors (50). The long-term qualitative changes in drug intake as characterized by loss of reversibility and loss of control in drug choice, were the same for ETZ and for ETOH. In the retest after long-term abstinence, neither aversive gustatory stimuli nor individual or environmental parameters substantially modulated the high preference for the respective drug (50).

In spite of this general similarity, the addictive drug could not be substituted by another one. Previous experience with the opiate enhanced the preference for ETOH, but these rats were not addicted to ETOH. Both the low ETOH intake (1.3 g/kg/day compared to that of ETOH addicted rats [3.7 g/kg/day (50)] and its modifiability by social parameters indicated

a controlled consumption of alcohol. On the other hand, ETOH-addicted rats revealed a similar pattern of voluntary intake of diazepam as that of ETOH-naive animals (50). Previous experience with a certain drug obviously can but does not necessarily affect the preference for another one. Drug discrimination studies showed that the influence of previous experience with one drug on the preference for another one depends on the degree of pharmacological similarity (39). To what extent a generalization between the effects of two drugs is maintained when the state of addiction is reached remains to be clarified. Based on the present findings, an established addiction appeares to be rather substance-specific. On the other hand, the principles of the temporal development of an addiction seem to be more general.

Due to the high degree of qualitative similarities in long-term alcohol and opiate intake in the present animal model, a common biological mechanism for the development of drug addiction is suggested. Many investigations demonstrated that the mesolimbic reward system of the mammalian brain plays a substantial role in mediating drug induced reward processes (9,24,45). Very good correlations exist between positive reinforcement and the release of dopamine in the nucleus accumbens septi from terminals of the ventral tegmentum area (5,24,37). Therefore, it has been proposed that drug-induced release of dopamine is rated by the animal as a "reward." The resulting motivational changes act as a superordinate control of drug seeking and drug taking (23,35,36). The predictions of this model have been confirmed for opiates, amphetamines, and cocaine (24,45). Other drugs like alcohol, benzodiazepines, barbiturates, or LSD less directly linked with dopaminergic transmission, however, failed to exhibit this clear relationship (33,36). Nevertheless, these drugs act as positive reinforcers and have a high abuse and dependence potential (22). One should consequently expect the existence of rather drug-specific mechanisms for controlled intake. The apparent similarities of principles of controlled opiate and ethanol intake require a hypothesis against such an assumption. A contribution of the reward system to the control of drug intake seems to be certain, but different drugs might affect different limbic structures (amygdala, frontal cortex, or hippocampus), which influence drug reward via modulation of dopaminergic activity in the nucleus accumbens (24,30). Dopamine release, consequently, may not be related to the reward itself (45) but to accompaning features of the motivational control.

As for controlled drug intake, one might assume a common mechanism for drug addiction, too. Robinson and Berridge proposed recently a common "neuroadaptationist model" (33). The authors assume an irreversible sensitization (i.e., an increased responsiveness) of the neural functions, probably in the mesolimbic reward system, controlling drug intake behavior (33). This might be the neural basis for an irreversible drug preference in the state of drug addiction (33). Consequently, sensitization of dopamine release after repeated drug intake increases the rewarding effects and, thus, the addictive properties (1,33). Sensitization, however, has not been proved to be consistent for different classes of addictive drugs like alcohol, benzodiazepines, barbiturates, or LSD (33). The similarities in the development of opiate and alcohol addiction in the rat may not sufficiently be explained by the recent concepts. Until now, it seems that altered functions of the reward system during the state of drug addiction still lack a conclusive demonstration. This might be due to the fact, that in most experiments, the animals have not been addicted, that is, they did not meet the criteria of loss of control and loss of reversibility in long-term drug taking behavior.

ACKNOWLEDGEMENTS

This study was supported by a grant from the Bundesgesundheitsamt, Institut für Arzneimittel, Berlin (Fo 2.1-1326-001). Etonitazene was a generous gift from CIBA GEIGY, Basel, Switzerland.

I am very grateful to Dr. J. Wolffgramm for many discussions and helpful advice. I also wish to thank Prof. H. Coper for helpful comments on the manuscript and David Kirya for the grammatical and stylistic revision of the manuscript. Special thanks to G. Slodkovska who collected the housing data and carefully looked after our animals.

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