INTRODUCTION

Both animal and human studies demonstrate marked individual differences in the propensity to develop alcohol (and other drug) taking behaviour (Cloninger et al., 1988; Piazza et al., 1989; Bisaga and Kostowski, 1993). Thus, it would be of particular importance to define biological markers (predictors) of the vulnerability towards alcohol/drug self-administration. One approach involves searching for individual behavioural features that are correlated with excessive alcohol/drug taking. For example, several authors have reported that individuals with a high preference for sweet substances consume more ethanol and acquire amphetamine self-administration more quickly than those with a low preference for these substances (Piazza et al., 1989; Gosnell and Krahn, 1992; Kampov-Polevoy et al., 1996). Similarly, it has been shown that rats with higher locomotor response to novelty (high responders, HR) acquire amphetamine self-administration more readily than low responders (LR) (Piazza et al., 1990). In contrast, no consistent relationship between locomotor activity and ethanol intake has been found in genetically selected alcohol-preferring and -non-preferring rats (Badishtov et al., 1995) or in outbred Wistar rats (Bisaga and Kostowski, 1993; Samson and Chapelle, 1995).

We have recently shown that parameters of saccharin drinking behaviour were highly correlated with the initial acceptance of low ethanol concentrations in Wistar rats. However, this relationship disappeared during further weeks of ethanol presentation. In contrast, locomotor activity in an open field area did not predict any subsequent ethanol consumption (Koros et al., 1998). These findings suggest that certain behavioural features may predict only initial alcohol drinking behaviour. It could also be speculated that other behavioural...
parameters correlate with ethanol intake in later stages of alcohol self-administration (maintenance phase). Thus, one aim of the present study was to assess possible relationships between long-term ethanol self-administration and several behavioural parameters derived from the open field and the saccharin drinking tests.

The alcohol deprivation effect (ADE) was first described by Sinclair and Senter (1968) as a transient increase in ethanol consumption/preference after a period of forced abstinence. In rats, the ADE was reported to occur after 18 or 21, but not 1 or 7, days of access to alcohol (Sinclair and Senter, 1968; Sinclair, 1972). More recently, Spanagel et al. (1996) have described a model of long-term free-choice alcohol self-administration with repeated deprivation episodes. In this latter study, rats showed the ADE after 2 months of access to water and three ethanol solutions (5, 10 and 20%, v/v). In contrast, we did not observe any changes in ethanol drinking after the deprivation episode, which followed a 50-day period of alcohol drinking (Koros et al., 1998). Thus, another aim of the present study was to examine the pattern of ethanol consumption after repeated episodes of deprivation in the rats used by Koros et al. (1998).

**MATERIALS AND METHODS**

**Subjects**

Twenty-four male Wistar rats (275–350 g at the beginning of the study) served as subjects. The rats were obtained from a licensed breeder (HZL, Warsaw, Poland) 2 weeks before the start of the investigation. During this time, the animals were housed six to a cage, weighed, and handled several times. The rats were kept under standard laboratory conditions [at 22 ± 1°C, 60% relative humidity, and a 12 h light/12 h dark cycle (lights on at 07:00)]. After 2 weeks of acclimatization, the subjects were transferred to individual wire cages (20 × 25 × 28 cm, L × W × H) with two graduated drinking tubes mounted at the front. During the entire investigation, the subjects had free access to standard lab chow (Bacutil, Poland) and tap water. Ethanol solutions (see below) were prepared from 95% stock ethanol and tap water. All solutions were replaced completely twice a week. Fluid intake was measured daily at 10:00 and body weights were recorded once a week throughout the study.

**Study design**

Table 1 presents a general design of the study. One week after the completion of the initial

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Fluids</th>
<th>Availability (days)</th>
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<tbody>
<tr>
<td>Initial saccharin drinking and open field test</td>
<td>Water</td>
<td>7</td>
</tr>
<tr>
<td>Washout</td>
<td></td>
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<tr>
<td>Two-bottle choice test</td>
<td>2–8% ethanol vs water</td>
<td>22</td>
</tr>
<tr>
<td>Three-bottle choice test</td>
<td>8% vs. 16% ethanol vs water</td>
<td>28</td>
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<tr>
<td>Ethanol deprivation</td>
<td>Water</td>
<td>5</td>
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<tr>
<td>Three-bottle choice test</td>
<td>8% vs. 16% ethanol vs water</td>
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<td>Ethanol deprivation</td>
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<td>Ethanol deprivation</td>
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<tr>
<td>Three-bottle choice test</td>
<td>8% vs. 16% ethanol vs water</td>
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<td>Washout</td>
<td>Water</td>
<td>7</td>
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*Parameters already reported and analysed by Koros et al. (1998).*

*For details, see Materials and methods section.*
saccharin drinking and the open field tests, the ethanol drinking tests began. For the first 22 days, the animals were exposed to increasing concentrations of ethanol (2–8%, v/v) and tap water in a two-bottle choice situation. Then, for the next 28 days, the animals were presented with two different ethanol solutions (8 and 16%, v/v) and tap water (a three-bottle choice test). The drinking tubes were rotated daily to prevent position preference. After 50 days of continuous access to alcohol and water the rats were deprived of alcohol for 5 days. During the deprivation period water was available in all three bottles. After the 5-day deprivation period, both alcohol solutions were presented again along with water for the next 28 days. [The correlations between the initial behavioural tests, ethanol self-administration during the first 50 days and the ADE after the first ethanol deprivation were described in detail by Koros et al. (1998).] After the completion of six three-bottle ethanol drinking/deprivation cycles, saccharin drinking and the open field tests were performed again (Table 1).

To assess the ADEs, i.e., changes in the ethanol intake after the deprivation episodes, ethanol drinking was measured for 3 days before and 3 days after each deprivation (Koros et al., 1998). The ADE was separately calculated using the ethanol consumption from the first 24 (ADE$_{24}$h) or 72 h (ADE$_{72}$h) after each deprivation. Positive and negative values of the ADE reflected increases or decreases in ethanol consumption after deprivation, respectively.

**Saccharin drinking test**

During the 48-h saccharin drinking test, the animals were given the choice between a saccharin solution (0.1% w/v; saccharin sodium salt, Aldrich, Gillingham, UK) and tap water. The position of bottles was changed after 24 h to prevent place preference. For each fluid, an intake measure was obtained, averaged across 2 days of choice and corrected for body weight of the subject (ml/kg/24 h).

It has been reported that the increase in total fluid intake (TFI) when saccharin is offered is a better predictor of subsequent ethanol intake than absolute saccharin consumption (Kampov-Polevoy et al., 1995). Thus, the increase in the TFI in the presence of saccharin was calculated as the percentage difference between the TFI when saccharin was available and a control TFI when only water was available (Kampov-Polevoy et al., 1995). The mean water intake (ml/kg/24 h) during the 2 days preceding the saccharin drinking test was treated as the control TFI.

**Open field test**

One week after the completion of the saccharin drinking test, the rats were used in the open field test. The open field apparatus consisted of four identical, computer-controlled cages (60 × 60 × 40 cm, L × W × H; COTM, Bialystok, Poland). Each cage was transected by two perpendicular, co-planar arrays of 16 infrared photocells which were intended to measure forward locomotion by determining the rat’s position every 0.1 s (Bienkowski et al., 1997a; Koros et al., 1998). The forward locomotion was defined as the distance (in in.) travelled by the rat during the 20-min test session. Another set of photocells located 15 cm above the cage floor measured the number of rearings. After the initial habituation of 20 min to the test room, each rat was introduced to the test cage for another 20 min. The cages were cleaned carefully between the recordings. The test sessions were conducted between 10:00 and 14:00 to avoid errors attributable to the variation in motor activity of the day activity cycle (File and Day, 1972).

**Statistical analysis**

Absolute changes (increases or decreases) in ethanol consumption (Δ g/kg) after repeated deprivation episodes were compared by a one-way ANOVA. In addition, the average ethanol drinking from pre- and post-deprivation days was analysed with a two-way ANOVA (ADE × episode). The Newman–Keuls test was used for post-hoc comparison. Multiple regression analysis was employed for testing the relationship between behavioural variables and ethanol intake. Due to the risk of a Type I error a probability below 0.01 was considered significant.

**RESULTS**

**Ethanol drinking behaviour**

Figure 1 presents the total and the 16% ethanol intake in the six 28-day access periods. One-way ANOVA showed that total ethanol intake remained stable over time [F(5,138) = 2.05, P = 0.11]. Similarly, ANOVA did not reveal any significant
changes in the 16% ethanol consumption \( [F(5,138) = 1.51, P = 0.19] \).

The initial acceptance of low ethanol concentrations (2–6%, v/v; see Koros et al., 1998) did not correlate with ethanol intake in the three-bottle choice situation and the subsequent ADE values. The baseline ethanol consumption did not predict the magnitude of the ADE (data not shown). The only exception was the ADE after the first deprivation episode, which was negatively correlated with the baseline ethanol consumption (Koros et al., 1998).

**The ADE calculated for changes in total ethanol intake**

Figures 2A and B show the ADE expressed as the change in the total ethanol intake. One-way ANOVA indicated a significant difference between the subsequent ADEs \( \text{ADE}_{24\text{ h}} \), \( F(5,23) = 3.52, \)

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**Fig. 1.** Voluntary ethanol consumption in the three-bottle choice test.

Mean (± SEM) consumption in g/kg/24 h in the three-bottle choice test (8% ethanol vs 16% ethanol vs water) \( (n = 24 \text{ rats}) \). ■, Total ethanol intake in subsequent 28-day periods of ethanol availability, □, intake of 16% ethanol in subsequent 28-day periods of ethanol availability.

**Fig. 2.** Mean (± SEM) increase in total (A,B) or 16% (C,D) ethanol consumption after subsequent periods of ethanol deprivation (alcohol deprivation effect, ADE).

The ADE was calculated for the first 24 h \( \text{ADE}_{24\text{ h}} \) (left panel) or 72 h \( \text{ADE}_{72\text{ h}} \) (right panel) of ethanol access after deprivation. The data points marked with different letters differ significantly with \( P \) less than 0.01. For other details, see Fig. 1.
$P < 0.01$; $\text{ADE}_{72\ h}$, $F(5,23) = 3.37$, $P < 0.01$; see Figs 2A and B, respectively]. Visual inspection of the data and the post-hoc analysis revealed that both the $\text{ADE}_{24\ h}$ and the $\text{ADE}_{72\ h}$ values increased with repeated deprivation episodes. The above results were partially confirmed by two-way ANOVA, which revealed a trend towards significant $\text{ADE} \times$ episode interaction for the $\text{ADE}_{72\ h}$ [$F(5,230) = 2.78$; $0.01 < P < 0.05$].

The ADE calculated for changes in 16% ethanol intake

One-way ANOVA for the ADEs did not reveal any significant differences ($F < 1.46$, $P > 0.2$) ($\text{ADE}_{24\ h}$, Fig. 2C; $\text{ADE}_{72\ h}$, Fig. 2D). Two-way ANOVA did not indicate any significant effects. It is clear, however, that the ADE calculated for the total ethanol intake was mainly the result of the increase in the 16% ethanol intake.

Correlational analysis

The mean ($\pm$ SEM) weight of the rats after the completion of all ethanol self-administration procedures was $559 \pm 16$ g. The results of the saccharin drinking and the open field tests are summarized in Table 2. Parameters of saccharin drinking did not correlate with the open field behaviours ($r$ values ranged from $-0.18$ to $+0.10$; $P > 0.35$).

As shown in Table 3, total ethanol intake correlated neither with the saccharin drinking (left) nor with the increase in the TFI in the presence of saccharin (right). Similarly, there were no significant correlations between total ethanol intake and the open field parameters (Table 4).
As shown in Tables 5 and 6, the ADE\textsubscript{24 h} values did not correlate with the parameters derived from the behavioural tests. In line with the above, the ADE\textsubscript{72 h} values were correlated neither with saccharin drinking nor with open field test parameters (data not shown).

In addition, the analysis of the relationships between the parameters from the initial behavioural tests (reported by Koros et al., 1998) and ethanol drinking in the subsequent access/deprivation cycles did not reveal any significant correlations (data not shown).
DISCUSSION

One aim of the present experiments was to evaluate the pattern of long-term ethanol self-administration with repeated deprivation periods. The total ethanol intake in the three-bottle choice situation typically ranged between 3 and 4 g/kg/24 h and remained stable across several months of drinking. Notably, the mean ethanol consumption in the first (4.0 ± 0.41 g/kg/24 h) and the sixth (3.6 ± 0.39 g/kg/24 h) 28-day access did not differ significantly. The consumption of 16% ethanol did not substantially change over time. Similar values for total ethanol intake have been found by Spanagel et al. (1996) in the four-bottle model of long-term ethanol self-administration. Generally, in agreement with previous reports (Sinclair, 1972, 1979), baseline ethanol consumption did not predict the ADE in our rats.

The ADE has been repeatedly shown after relatively short ethanol presentations in different rat strains (Sinclair and Senter, 1968; Sinclair, 1972; Sinclair and Tiinhonen, 1988; Sinclair and Li, 1989). The initial ADEs in the present study were relatively weak in comparison with the results cited above. Sinclair and Senter (1968), using Long-Evans rats, have shown that 1 and 7 days of ethanol (7%, v/v) access did not result in any significant ADE, whereas that for 21 days did. Similarly, Spanagel et al. (1996), using Wistar rats, have reported a significant ADE after a 3-day deprivation episode which followed 2 months of continuous access to water and three ethanol concentrations. In a more recent study from the same laboratory, Höltner et al. (1998), using the drinkometer system, did not find the ADE in Wistar rats pre-exposed to ethanol for 24 weeks. However, the baseline drinking in this latter study was relatively high (6.79 g/kg/24 h) and a 'ceiling effect' might have occurred.

When the ADEs after repeated deprivation episodes were compared, a clear-cut difference between the initial and the last ADE was found. The last ADE_{24 h} exceeded 1.5 g/kg and was fully comparable with the results reported previously by others (Sinclair, 1979; Spanagel et al., 1996). Based on the above findings, one could hypothesize that the ADE in the present study increased with repeated deprivation episodes. However, at least two other possible explanations should be mentioned. First, the duration of ethanol exposure rather than the deprivation episodes might have been critical for the increase in the ADE magnitude. Second, the possibility exists that aged rats are more prone to increased ethanol drinking after deprivation. This latter hypothesis seems to be less likely, as Sinclair (1972) reported that young and old Wistar rats (aged 3 and > 6 months, respectively) showed a similar ADE after 18 days of access to water and ethanol solution (5%, v/v). Obviously, future studies with additional control groups are needed to clarify the above hypotheses.

The second purpose of the present study was to evaluate correlations between long-term ethanol intake and parameters from the open field and saccharin drinking tests. Neither saccharin drinking nor the increase in the TFI in the presence of saccharin correlated with ethanol consumption or the ADE. This finding indicates that saccharin drinking is not a good predictor of ethanol intake in the maintenance phase of ethanol self-administration. Previously, Koros et al. (1998), using the same rats, have shown that the association between saccharin drinking and ethanol intake was limited to initial acceptance of low ethanol concentrations (2–6%, v/v). The correlation between saccharin and ethanol intake was also strongest for initial ethanol drinking in the study of Kampov-Polevoy et al. (1996). In our previous report (Koros et al., 1998), we used parameters of the saccharin drinking test performed before the start of the ethanol presentation (not shown here). In the present paper, we used these parameters again but, as expected, the results of the correlational analysis were negative. Thus, the parameters of the saccharin drinking test performed either before or after the period of ethanol presentation did not predict ethanol self-administration behaviour.

The obvious limitation of the present study is that only one strain of rats (Wistar) was used. It is possible that this rat strain does not possess the genetic diversity connecting long-term ethanol drinking and saccharin intake. As mentioned above, robust correlations between initial ethanol acceptance and saccharin intake have been found in the first part of the study (Koros et al., 1998). This finding was in agreement with the results of other short-term ethanol drinking experiments, including those done on genetically selected rats. Thus, it remains to be established if saccharin drinking might also predict long-term ethanol drinking behaviour in other rat strains.
In a recent clinical study, Kampov-Polevoy et al. (1997) have shown that a majority (80%) of their alcoholic patients were sweet likers, i.e. preferring high sucrose concentrations. In contrast, only 41% of the control group of non-alcoholic subjects preferred highly-concentrated sucrose solutions. Thus, it should be noted that our paradigm differs substantially from the clinical study mentioned above, as we have used only one concentration of saccharin. Interestingly, Sinclair et al. (1992) have reported that alcohol-prefering lines of rats accepted much higher sweet concentrations than their alcohol-non-prefering partners. The concentration of saccharin used by us (0.1%, w/v) is much preferred by naive rats (Kampov-Polevoy et al., 1996;Bienkowski et al., 1997b). However, the study of Kampov-Polevoy et al. (1997) suggests that, in future preclinical research, even higher concentrations of saccharin should be used. Possibly, the positive association between saccharin and ethanol consumption exists only when drinking relatively high (possibly even aversive for some rats) concentrations of the sweetener is considered.

In line with many previous papers (Bisaga and Kostowski, 1993; Badishtov et al., 1995; Fahlke et al., 1995; Samson and Chappelle, 1995; Nadal et al., 1996), spontaneous locomotor activity during a single exposure to a novel environment failed to predict the ethanol intake. Interestingly, Gingras and Cools (1995) have even shown that their HR rats drank significantly less alcohol than LR rats. Moreover, no major differences in locomotor stimulant effects of dexamphetamine in the HR and the LR rats have been reported by the same authors (Gingras and Cools, 1997). It is noteworthy that, in the study of Goeders and Guerin (1996), no association between the locomotor response to a novel environment and self-administration of a 0.125 mg dose of cocaine has been found.

In conclusion, the results of the present study indicate that neither saccharin consumption nor locomotor activity predicts long-term ethanol self-administration behaviour in Wistar rats. In addition, our findings suggest that the magnitude of the ADE may increase with repeated deprivation episodes.

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REFERENCES


