BEHAVIOURAL RESPONSES TO ELEVATED PLUS-MAZE AND DEFENSIVE BURYING TESTING: EFFECTS ON SUBSEQUENT ETHANOL INTAKE AND EFFECT OF ETHANOL ON RETENTION OF THE BURYING RESPONSE

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(Received 13 March 2000; in revised form 14 July 2000; accepted 6 August 2000)

Abstract — Based upon repeated observations of a relationship between defensive burying (DB), ethanol intake and stress ulcer susceptibility, and recurring questions regarding what DB behaviour reflects, two experiments were performed. Experiment 1 showed that prod shock exposure per se reduced subsequent ethanol intake, as did access to burying material. In rats without burying material in the conditioning phase, subsequent access to ethanol resulted in reduced DB activity in the retest, pointing to some interference by ethanol on latent learning. Experiment 2 showed that there were no effects of anxiety as measured in the elevated plus-maze (EPM) on saccharin or ethanol intake. Behaviours in the EPM and DB test did not correlate. Rats entering the open arm on first entry into the EPM drank and preferred more ethanol than those choosing the closed arm. Saccharin intake was negatively related to burying latency, and positively related to initial ethanol intake. In conclusion, it is still questionable whether the DB test is measuring anxiety, but exposure to the test or performance of the DB activity appears to cause modifications, psychologically and perhaps physiologically, in rats. Ethanol intake may under certain conditions interfere with the acquisition of a defensive response. The testing of DB behaviour may be useful in studying drug effects on latent learning of prepared responses.

INTRODUCTION

Animals from outbred rat strains, which are exposed over several weeks to an ethanol and water free-choice test design, with food available ad libitum, will show large individual differences in their consumption of, and preference for, alcohol. Our research focus has been to identify individual differences in behaviour that may be employed in the prediction of ethanol preference. The tendency of drinking more, rather than the development of alcoholism as such, has been the scope of our interest.

In this paper, we present two experiments. In the first, we investigated whether any isolated aspects of the defensive burying (DB) test would affect later alcohol consumption, and in what way alcohol exposure might influence the conditioning of the DB response. In a second experiment, the aim was to consider whether the DB test is a test suited for studying anxiety or fear. Rats were initially tested for activity in the elevated plus-maze (EPM), considered a validated test for anxiety or fear. A problem concerning this interpretation is the common effect of many anxiolytics to reduce activity generally.

Alternatively, burying behaviour may be interpreted as a proactive coping strategy (Koolhaas et al., 1999) in response to a perceived threat. Rats engaged in DB show no greater corticosterone responses to a shock prod than rats merely exposed to the apparatus. Animals without bedding material, thus lacking the opportunity to bury, show corticosterone elevations of the same magnitude as seen after inescapable footshock (De Boer et al., 1990).

A problem regarding tests of anxiety (like the DB test) is the lack of validation, other than pharmacological, to ensure that they are measuring anxiety. Tests may be reliable in identifying the effects of benzodiazepines, but it is more difficult to distinguish between the sedative and anxiolytic effects of these compounds. The EPM test (Pellow et al., 1985; Pellow and File, 1986), based upon a procedure by Montgomery (1958), avoids this problem. With clinically effective anxiolytics (chlordiazepoxide and diazepam), exploration of the open arms was increased, without increasing exploration in the enclosed arms (Pellow et al., 1985), indicating that the test reflects anxiety. The major determinant of behaviour in this test is the unconditioned aversion to heights and open spaces that characterizes laboratory and wild rats (Barnett, 1975). The behavioural measure in this test is not related to amount of activity, but to the ratio between activity in the open and closed arms.

With regard to the relationship between anxiety and alcohol consumption, experimental studies are far from conclusive. Several studies indicate that alcohol-prefering rat strains are

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less anxious than alcohol-avoiding strains (Korpi et al., 1988; Badishtov et al., 1995; Viglinskaya et al., 1995; Knapp et al., 1997; Möller et al., 1997). As regards behaviour in the EPM, some studies have demonstrated that AA rats spend more time than ANA rats in the open arms (Viglinskaya et al., 1995; Knapp et al., 1997; Möller et al., 1997). The former study demonstrated that both alcohol-preferring P rats of the Indiana selected lines and rats from the FH (Fawn-hooded) strains show more open arm activity than their non-preferring counterparts, NP and FRL (Flinders resistant), respectively. On the other hand, there are reports that alcohol-preferring strains are more anxious, regarding both the AA (Fahlke et al., 1993), and P (Stewart et al., 1993) lines. As to studies on outbred strains, the literature is less extensive. Spanagel et al. (1995) discovered that anxious rats, as measured in the EPM test, drank more ethanol than non-anxious rats.

Another area of interest is the association between preference for sweet solutions (e.g. saccharin and sucrose) and alcohol in rodents (Sinclair et al., 1992; Kampov-Polevoy, 1996). Rat strains with high ethanol preference: AA, P, FH, MNRA (Maudsley NonReactive), show higher saccharin consumption than their non-preferring counterparts. Also, in outbred rats, a positive correlation has been reported between saccharin intake and ethanol drinking (Koros et al., 1998). Moreover, rats selectively bred for high saccharin consumption drink more ethanol than do rats bred for low saccharin consumption (Occidental HiS and LoS lines) (Dess et al., 1998). It has been suggested that this association is not mediated by taste reactions alone (Bice and Kiefer, 1990), but, instead, by common reinforcement mechanisms involving opioidergic release (Sinclair et al., 1992). Sweet substances increase release and breakdown of hypothalamic β-endorphins in rats (Dum et al., 1983; Dum and Herz, 1984) and in humans (Gatto et al., 1984). Thus sweets appear to stimulate the endogenous opiate system. Such stimulation may be seen in opioid-induced analgesia. In addition, the opioidergic system is one of the potential mechanisms mediating the reinforcement of ethanol (Kampov-Polevoy et al., 1996). Humans with high risk for alcoholism have lower basal levels of β-endorphins, but, like AA rats, they show an increase by drinking 0.5 g/kg ethanol (Gianoulakis et al., 1990). A clinical study showed that alcoholic patients preferred higher sucrose concentrations than a non-alcoholic control group (Kampov-Polevoy et al., 1997). The dopaminergic system is believed to be involved, as saccharin infusion increases dopamine (DA) release in the nucleus accumbens (Mark et al., 1991).

The relationship between preference for palatable fluids and behaviour in the EPM has been little studied. However, differences in sucrose consumption are predictive of behavioural reactivity of rats in the plus-maze, as open arm activity is higher in rats ingesting large amounts of sucrose than in those with a low intake (de Cabo et al., 1995; De Sousa et al., 1998). From this, one might predict that our rats exhibiting high anxiety as measured in the plus-maze should show both low saccharin and alcohol consumption. If the DB test were reflecting anxiety, one would also expect relationships between burying behaviour and saccharin consumption.

Hoping to clarify some of these issues, we performed two experiments. In Experiment 1, we wanted to investigate more closely certain elements of the DB test by systematically varying some of them, to see whether this would affect ethanol intake. At the same time, we asked whether ethanol would influence retention of conditioned burying. As the question of what is being measured in the DB test is still unresolved (anxiety or coping), Experiment 2 involved a study of the relationship of the DB and the EPM test (both purported tests of anxiety), to see whether they would correlate on any measure. In addition, animals were assessed for saccharin preference, and later alcohol intake.

EXPERIMENT 1: METHODS

Animals

Sixty-seven male HsdOla:WIST rats (Harlan UK, Bicester, UK) weighing 225–274 g at delivery, served as experimental subjects. The animals were initially group-housed (five per Makrolon-IV cage) under standard temperature 22°C (±2°C), with a 12-h light:12-h dark cycle (lights on at 06.00 and off at 18.00), with free access to food (standard laboratory chow, SDS-RM1E; PMI-Special Diets Services, Witham, Essex, UK) and tap water. One week of adaptation to the animal facilities was allowed before the experiment started. The animals were randomly assigned to one of four different experimental conditions (A–D) and then placed in single cages. Each of these four groups was further divided into two, one ethanol (E) and one water (W) group, thus totalling eight groups (see Table 1).

All procedures described in this article have been approved by the local responsible laboratory animal science specialist under the surveillance of the Norwegian Animal Research Authority (NARA) and registered by the Authority.

Condition A implied that the DB test was carried out the original way. The floor of the test chamber was covered with burying material and shock was administered when rats were in contact with the prod. Based on latency times for DB, the animals were evenly assigned to the ethanol (AE) and water (AW) groups. In condition B, the situation was the same, but there was no material available to allow burying. About one-third of the animals displayed freezing and these (plus the other two-thirds) were assigned evenly to the ethanol (BE) and water (BW) groups. In condition C, the chamber contained burying material, but the prod was not activated. Some animals showed burying behaviour and these (plus the rest of the group) were evenly assigned to the ethanol (CE) and water (CW) groups. Animals lacking burying latency times (equalling total test time) were assigned to the groups based on amount of activity throughout the test. In the last condition D, there

<table>
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<th>Table 1. Experimental conditions</th>
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<tr>
<td>Group (n)</td>
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<tr>
<td>AE (n = 9)</td>
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<tr>
<td>AW (n = 8)</td>
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<tr>
<td>BE (n = 8)</td>
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<tr>
<td>BW (n = 8)</td>
</tr>
<tr>
<td>CE (n = 9)</td>
</tr>
<tr>
<td>CW (n = 8)</td>
</tr>
<tr>
<td>DE (n = 8)</td>
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<tr>
<td>DW (n = 8)</td>
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</table>

For all abbreviations, see the Methods section in Experiment 1.
was neither burying material covering the floor, nor was the prod electrified. These animals were divided into two groups (DE and DW) based on amount of activity and grooming.

**Apparatus**

**Defensive burying chamber.** The test chamber (60 × 20 × 25 cm) was constructed of wood and had a clear acrylic roof and front wall. During the test phase, a shock probe (1 cm in diameter and 7 cm long) was positioned through the front wall ~5 cm over the floor, and connected to a shock source (Lafayette). During the habituation period (in all conditions) and in the test phases of the experiment (conditions A and C), the floor of the chamber was evenly covered with a 3 cm layer of burying material (small wooden chips) identical to that used in the animals’ home cages as bedding.

**Procedures**

**Defensive burying.** On 4 consecutive days, rats were habituated to the DB apparatus for 30 min, in the absence of the shock prod (burying material was available during the habituation sessions for all animals). On day 5, the rats were again placed in the apparatus, but on this occasion the shock prod was protruding into the chamber. The four different conditions were now imposed. For animals in condition A, the procedures for ordinary testing of DB were followed. The floor was covered with bedding material and when the rat made contact with the prod, a current up to 5 mA was passed through it. The B condition was similar, but burying material was absent on the test day. In the C condition there was burying material available but the prod was not electrified. In condition D there was neither burying material nor was the prod electrified.

The rat’s behaviour was observed and recorded on video for 10 min following the first contact with the shock prod. After this period, the height of burying material at the prod was measured. The rat was thereafter returned to its home cage. Video tapes were scored for latency to initiate burying after shock (defined as pushing bedding material with the snout or forelimbs forward in the direction of the prod), duration of burying, and number of burying ‘bouts’ (defined as periods of burying behaviour separated by a minimum of 30 s of non-burying). In addition, behaviours including vertical activity, grooming, freezing (no movements) and immobility (standing still) were scored.

**Ethanol consumption.** Two days subsequent to the DB test, ethanol-screening procedures were initiated for the animals of groups AE, BE, CE and DE. Animals of the other groups (AW, BW, CW and DW) continued to receive water only. The ethanol procedure involved presenting the rats with two drinking bottles attached to the front of the home cage. One bottle contained water, whereas the other initially contained an ethanol-in-water solution at an ethanol concentration of 3% (v/v). Bottles were filled at the start of each day to a level of 100 ml with either water or ethanol. The animals had access to ethanol for 23 h per drinking day. The 3% concentration of ethanol was presented every other day for 6 days; that is, every other day, the animals received two bottles of water, and on alternate days they received one bottle of 3% ethanol and one bottle of water. The position of the ethanol bottle (left/fright) was changed upon each presentation to eliminate the possibility of a position preference by the rats. The same alternate day presentation was continued for ethanol in increasing concentrations of 5% (6 days). At the end of the procedure, the rats received the 7% ethanol solution (plus a bottle of water) for 8 consecutive days. The animals were thus exposed to ethanol for a period lasting 20 days, including 14 days with access to ethanol.

Fluid consumption (both ethanol and water) and body weight were monitored daily at a fixed time of day. Ethanol consumption was calculated based on daily registered ethanol intake (g of ethanol/kg of rat body wt/da y). Ethanol preference was defined as quantity of alcohol consumed as a percentage of total fluid intake.

**Defensive burying retest.** In the retention test the animals were placed in the same test chamber as before. All animals were exposed to the same condition: with burying material and without shock being administered via the prod. Behaviour was observed and recorded for 10 min from the moment the rat was placed in the chamber. Scores were made for the same behaviours as described earlier.

**Statistics**

Data analyses were performed using two- and three-way analyses of variance (ANOVA) and planned comparisons.

**RESULTS**

**Ethanol intake**

Our first questions concerned the effects of access to burying material and exposure to shock in the initial DB test on subsequent ethanol intake. Consequently, these analyses only include groups exposed to alcohol. Following ANOVA, group comparisons to address our specific questions were performed using planned comparisons.

**Ethanol consumption.** Preliminary analysis of mean ethanol intake (g/kg/day) revealed heterogeneity of variances among the groups \( F_{\text{max}} (3 \text{ df}) = 20.81, P < 0.0001 \). Raw scores were transformed by inverting them (1/var), reducing \( F_{\text{max}} \) to 3.96 (not significant). A 2-way ANOVA of these transformed scores revealed a significant effect of burying material \( F(1,30) = 15.14, P < 0.0005 \), and a significant effect of shock \( F(1,30) = 5.05, P < 0.05 \). The interaction term was not significant \( F(1,30) < 1 \). As can be seen from Table 2, animals exposed to shock in the bedding-absent condition later drank less alcohol than animals not exposed to shock, and animals with access to burying material later drank less alcohol than animals without access (independent of shock condition). The animals which drank least were those exposed to shock and given the ability to bury, while those which drank

<table>
<thead>
<tr>
<th>Table 2. Ethanol consumption in the four defensive burying conditions</th>
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<tr>
<td><strong>Bedding</strong></td>
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<tr>
<td><strong>Absent</strong></td>
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<tr>
<td><strong>Present</strong></td>
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</table>

Mean (SEM) alcohol consumption (g/kg/day) under the bedding/no-bedding and shock/no-shock conditions.

<sup>b</sup>Differs from shock condition.

<sup>a</sup>Differs from bedding condition.
Table 3. Ethanol preference in the four defensive burying conditions

<table>
<thead>
<tr>
<th>Bedding</th>
<th>Shock condition</th>
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<tbody>
<tr>
<td>Absent</td>
<td>30.09 (2.95)</td>
</tr>
<tr>
<td>Present</td>
<td>22.54 (1.25)</td>
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</table>

Mean (SEM) alcohol preference (percentage of total fluid intake) under the bedding/no-bedding and shock/no-shock conditions.

Most were the ones not exposed to shock, and not given access to bedding material. Planned comparisons show that both in the shock and no-shock conditions, the presence of burying material reduced alcohol consumption \(F(1, 30) = 4.70, P < 0.05\) and \(F(1, 30) = 11.12, P < 0.01\), respectively. In the bedding-present condition, shock had no effect on later ethanol consumption \(F(1, 30) = 1.07\), but in the bedding-absent condition, shock reduced later drinking \(F(1, 30) = 4.46, P < 0.05\).

Ethanol preference. Preliminary analysis of ethanol preference (percentage of total fluid intake) also revealed heterogeneity of variances amongst the groups \(F_{\text{max}}(3 \text{ df}) = 16.94, P < 0.01\). Raw scores were then transformed by inverting them (1/var), reducing \(F_{\text{max}}\) to 3.26 (not significant). A two-way ANOVA of the transformed preference scores showed parallel effects to analysis of alcohol consumption. There were significant main effects of bedding condition \(F(1, 30) = 16.77, P < 0.0005\) and of shock condition \(F(1, 30) = 6.88, P < 0.05\). The interaction term was not significant \(F(1, 30) < 1\). As can be seen from Table 3, animals exposed to shock subsequently showed lower preference for alcohol than animals not exposed to shock (significantly so only in the bedding absent condition), and animals with access to burying material would later show significantly lower preference for alcohol than animals without access. The animals with lowest preference were those exposed to shock and given the ability to bury, whereas those which drank most were the ones not exposed to shock, and not given access to burying material. Planned comparisons show that, both in the shock and no-shock conditions, the presence of burying material reduced preference for alcohol \(F(1, 30) = 4.96, P < 0.05\) and \(F(1, 30) = 12.71, P < 0.005\), respectively. In the bedding-present condition, shock had no effect on later ethanol preference \(F(1, 30) = 1.49\), but in the bedding-absent condition shock reduced later preference for alcohol \(F(1, 30) = 6.02, P < 0.05\).

Retention of defensive burying. Our next questions concerned the effects of prior defensive burying experience and alcohol exposure on retention of defensive burying. Behaviours scored (Table 4) during the retention test were analysed as a function of: (a) presence of burying material during the conditioning phase; (b) shock experience; (c) exposure to alcohol during the period between conditioning and retention testing, using three-way ANOVA. Group comparisons to address our specific questions were performed using planned comparison tests.

Latency to initiate conditioned defensive burying. A three-way ANOVA (bedding \(\times\) shock \(\times\) alcohol) of latency times from shock to burying is commenced in the retention test yielded significant effects of bedding \(F(1, 59) = 7.37, P < 0.01\), shock \(F(1,59) = 59.10, P < 0.00001\) and alcohol \(F(1, 59) = 5.11, P < 0.05\). Additionally, the bedding \(\times\) alcohol interaction was significant \(F(1, 59) = 5.22, P < 0.05\), as was the shock \(\times\) alcohol interaction \(F(1, 59) = 4.64, P < 0.05\).

For animals that had the possibility to manifest the burying response during the conditioning phase (access to bedding material), the planned comparison analysis revealed that exposure to ethanol prior to retention did not affect DB latency (group AE vs group AW) \(F(1, 59) < 1\). Moreover, the absence of bedding material during conditioning did not affect latency times in the retention test in water controls \(F(1, 59) < 1\). However, access to alcohol in the interval between conditioning and retention in animals lacking bedding material resulted in significantly longer retest burying latencies \(\text{BE vs BW}, F(1, 59) = 16.15, P < 0.005\), Fig. 1. The no-shock water controls (CW and DW) showed little or no burying in the retention test, and so did not differ with respect to latency times \(F(1, 59) = < 1\).

Time spent burying in the retention test. Preliminary analysis of time spent burying revealed considerable heterogeneity of variances among the groups \(F_{\text{max}}(7 \text{ df}) = 148.43, P < 0.0001\). Raw data were therefore converted using a log_{10}+1 transform, reducing \(F_{\text{max}}\) to 17.77 (not significant). A three-way ANOVA (bedding \(\times\) shock \(\times\) alcohol) yielded significant effects of bedding \(F(1, 59) = 4.49, P < 0.05\), shock \(F(1, 59) = 46.06, P < 0.000001\) and a non-significant trend for alcohol \(F(1, 59) = 3.48, P < 0.067\), on duration of burying activity. The interaction terms were not significant.

Table 4. Defensive burying behaviour in each experimental condition

<table>
<thead>
<tr>
<th>Group</th>
<th>Latency (s) conditioning</th>
<th>Latency (s) retention test</th>
<th>Duration (s)</th>
<th>No. of bouts</th>
<th>Height (cm)</th>
<th>Grooming (no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AE</td>
<td>72.56 (19.69)</td>
<td>193.33 (36.11)</td>
<td>100.89 (34.13)</td>
<td>2.33 (0.35)</td>
<td>7.17 (1.09)</td>
<td>0.78 (0.24)</td>
</tr>
<tr>
<td>AW</td>
<td>62.38 (12.30)</td>
<td>165.50 (57.85)</td>
<td>88.25 (17.08)</td>
<td>2.25 (0.34)</td>
<td>7.50 (0.70)</td>
<td>1.88 (0.32)</td>
</tr>
<tr>
<td>BE</td>
<td>481.63 (83.82)</td>
<td>66.13 (45.59)</td>
<td>0.88 (0.51)</td>
<td>6.13 (1.34)</td>
<td>1.50 (0.53)</td>
<td></td>
</tr>
<tr>
<td>BW</td>
<td>192.00 (59.17)</td>
<td>99.89 (26.17)</td>
<td>2.56 (0.50)</td>
<td>7.89 (0.82)</td>
<td>1.78 (0.34)</td>
<td></td>
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<tr>
<td>CE</td>
<td>449.22 (53.60)</td>
<td>502.56 (59.30)</td>
<td>9.33 (7.64)</td>
<td>0.44 (0.26)</td>
<td>4.11 (0.43)</td>
<td>2.11 (0.28)</td>
</tr>
<tr>
<td>CW</td>
<td>515.50 (47.52)</td>
<td>532.13 (48.17)</td>
<td>8.13 (6.70)</td>
<td>0.38 (0.28)</td>
<td>4.44 (0.54)</td>
<td>1.75 (0.39)</td>
</tr>
<tr>
<td>DE</td>
<td>577.13 (24.45)</td>
<td>3.50 (3.74)</td>
<td>0.13 (0.13)</td>
<td>3.75 (0.27)</td>
<td>1.88 (0.32)</td>
<td></td>
</tr>
<tr>
<td>DW</td>
<td>536.88 (48.24)</td>
<td>8.50 (6.07)</td>
<td>0.50 (0.29)</td>
<td>4.06 (0.46)</td>
<td>2.25 (0.34)</td>
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</tr>
</tbody>
</table>

Defensive burying latencies in the initial test and defensive burying behaviours in the retention test for each group. Data are expressed as means (SEM). For all abbreviations, see the Methods section in Experiment 1.
For animals allowed to bury during the conditioning phase, exposure to ethanol prior to retention did not affect time spent burying [AE vs AW, F(1, 59) < 1, Fig. 2]. The absence of burying material during conditioning did not affect test burying times in the water-only exposed animals (AW vs BW) [F(1, 59) < 1]. However, access to alcohol during the interval between conditioning and retention in the groups with bedding absent (BE vs BW) resulted in significantly less time spent burying in the retention test [F(1, 59) = 8.06, P < 0.01, Fig. 2].

Number of burying bouts. Preliminary analysis of number of burying bouts again revealed heterogeneity of variances among the groups [F max (df, 7) = 16.22, P < 0.05]. Raw

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**Fig. 1.** Latency for the initiation of defensive burying in the defensive burying (DB) retest in each experimental condition. Data are expressed as means ± SEM (bars). Planned comparison revealed that in rats receiving shock, but with no access to burying material (BE versus BW), there was a significant difference due to ethanol (**P < 0.005). For group abbreviations, see the Methods section in Experiment 1.

**Fig. 2.** Amount of defensive burying in the defensive burying (DB) retest in each experimental condition. Data are expressed as means ± SEM (bars). Planned comparison revealed that in rats receiving shock, but with no access to burying material (BE versus BW), there was a significant difference due to ethanol (**P < 0.01). For group abbreviations, see the Methods section in Experiment 1.
data were therefore converted using a log_{10}+1 transform, reducing $F_{\text{max}}$ to 6.71 (not significant). A three-way ANOVA (bedding × shock × alcohol) yielded significant effects of shock [$F(1, 59) = 50.24, P < 0.001$] and ethanol [$F(1, 59) = 4.37, P < 0.05$], plus a non-significant trend for bedding [$F(1, 59) = 3.59, P < 0.063$]. Additionally, there was a significant bedding × alcohol interaction effect on number of burying bouts [$F(1, 59) = 5.91, P < 0.05$].

For animals provided with burying material during the conditioning phase, exposure to ethanol did not affect number of burying bouts [AE vs AW, $F(1, 59) < 1$, Fig. 3]. The absence of bedding material in the conditioning phase did not affect number of bouts in the water controls, as groups AW and BW were very similar on this measure [$F(1, 59) < 1$]. Access to alcohol in the intermediate interval in animals with bedding absent during conditioning resulted in significantly fewer burying bouts during retention testing [BE vs BW, $F(1, 59) = 12.19, P < 0.001$, Fig. 3].

**Height of bedding.** Preliminary analysis of height of pile of bedding covering the electric prod revealed heterogeneity of variances among the groups [$F_{\text{max}}$ (df, 7) = 24.96, $P < 0.001$]. Raw data were therefore converted using a log_{10}+1 transform, reducing $F_{\text{max}}$ to 7.66 (not significant). A three-way ANOVA (bedding × shock × alcohol) revealed a significant effect of shock [$F(1, 59) = 40.67, P < 0.000001$], whereas the effects of bedding and ethanol were not significant.

In animals with burying material present, ethanol drinking prior to the retention test did not affect height of bedding [AE vs AW, $F(1, 59) < 1$, Table 4]. Also, with bedding material absent during conditioning, amount of burying in water controls was not affected [AW vs BW, $F(1, 59) < 1$, Table 4]. However, access to alcohol during the intermediate interval in the bedding-absent group resulted in a significantly lower pile of bedding covering the prod in the retention test [BE vs BW, $F(1, 59) = 12.19, P < 0.05$, Table 4].

**DISCUSSION**

Animals provided with burying material in the initial DB test showed less alcohol consumption and lower preference for alcohol than animals exposed to this test without burying material. Those with burying material had the ability to express coping behaviour in the presence of a threat. It has been shown that animals lacking burying material exhibited corticosterone elevations of the same magnitude as seen after inescapable footshock (De Boer et al., 1990). Provision of sawdust in a DB retention test has been reported to give higher noradrenaline and lower adrenaline and corticosterone levels (Korte et al., 1992). Our results could be compatible with the suggestion that adrenal secretion of corticosterone modulates the intake of alcohol in rats (Fahlke et al., 1994a), based for example on the way ethanol intake is reduced by adrenalectomy (Fahlke et al., 1994a) and metyrapone-induced suppression of corticosteroid synthesis (Fahlke et al., 1994b).

We also asked whether shock would influence alcohol intake. When the animals had access to bedding material, shock did not affect ethanol drinking. We might surmise that animals that are able to exhibit coping behaviours would not show elevations of corticosterone. As coping implies a positive response outcome expectancy, it is associated with a decreased stress response (Ursin, 1988). Animals unable to express coping behaviours showed higher alcohol consumption, and it might be anticipated that shock in the bedding-absent condition was more
fear-provoking. The relative roles of corticosterone in the alcohol consumption observed here may of course be tested empirically.

With regard to the retention of DB, the rats that received shock, but had no means to bury the aversive stimulus in the conditioning phase, showed as much burying activity in the retention test as did rats that initially had access to material. Thus the SSDR (Bolles, 1970) may be expressed on re-presentation of a conditioned aversive stimulus even when the response is not possible during the acquisition phase. In the same way that performance of a response is not essential for traditional operant conditioning to take place (Mazur, 1990), the expression of an SSDR need not be necessary for its conditioning. Situations necessitating SSDRs will elicit these patterns (Breland and Breland, 1961). Consequently, this resembles a case of latent learning (Tolman and Honzik, 1930).

However, this particular kind of latent learning appears from our data to be disrupted by alcohol. Shocked animals given access to alcohol in the interval between the conditioning and test sessions showed as little defensive behaviours as did the no-shock animals. They differed on all DB measures from their control group (i.e. those receiving similar shock in the no-bedding condition, but followed by water only). Our experiment shows that the conditioning of the burying response, taking place when the shock is paired with the presence of the prod, is strong and long-lasting. It has been shown that repeated exposure to the test context (including prod) is necessary for extinction of the burying response to occur. The mere passage of time is not sufficient (Pinel et al., 1985). As we have seen, the response strength is not weakened by ethanol when animals are given the immediate possibility to exert the response during the acquisition phase. We can therefore only speculate on what mechanism is in play here. When the rat is prevented from doing what is required in the aversive situation (its SSDR), a less strong association or even a different type of association may be induced. This association is possibly more susceptible to interference by ethanol. The response may be weakened to such a degree that it might require a more threatening stimulus to trigger it. Moreover, ethanol consumption in the intermediate period may have reduced the general level of fear or anxiety (Blokland et al., 1992), with a further reduction in the likelihood and necessity for the defensive response to occur. Ethanol drinking in the intermediate period in animals with access to burying material had no effect on retest burying results. The association between the stimulus and the response remained undisturbed, and thus could be performed according to the animal’s defence repertoire.

As demonstrated, there are aspects of the DB test that influence ethanol drinking. In addition, ethanol intake itself affects DB behaviour. These findings necessitated the importance of establishing whether DB reflects anxiety. By correlating DB with behaviour in a more established test of anxiety, we hoped to unveil possible relations.

EXPERIMENT 2

In the following experiment we explored the relationship between two purported tests of anxiety (EPM and DB test), saccharin preference, and later alcohol intake. Rats were initially tested in the plus-maze, and assigned to anxiety categories using the criteria employed by Spanagel et al. (1995). The rats were then tested for saccharin preference. Thereafter, they were tested for DB and subsequently exposed to a voluntary ethanol screening procedure for 4 weeks.

METHODS

Animals

Forty male Mol:WIST rats (Møllegaard, Denmark) weighing 150–200 g on delivery served as the experimental subjects. Animals were initially group-housed (five per Makrolon-IV cage) under standard temperature 22°C (± 2°C), with a 12:12 light/dark cycle (lights on at 06.00 and off at 18.00). They had free access to food (standard laboratory chow; SDS-RM1E) and tap water. To reduce novelty stress, 2 weeks of acclimatization to the animal facilities were allowed. Afterwards, the animals were weighed and transferred to single cages.

Based on the ratios of time spent in open arms/total time in both types of arms and entries into open arms/total number of entries in the elevated plus-maze, the animals were divided into two groups: ‘anxious’ and ‘non-anxious’. To consider an animal anxious, the two parameters had to correlate. Thus animals with levels below 45% for entries and below 30% for time spent were considered anxious. In the non-anxious group, levels above 55% for entries and above 40% for time spent in the open arms were used as selection criteria. This approach was based on the procedure outlined by Spanagel et al. (1995). In addition, we categorized a group as ‘medium anxious’ for animals with levels below 45% for entries and between 30 and 40% of time spent in open arms. Animals that did not fit into any of these criteria were excluded from analyses concerning anxiety variables. Animals were thus divided into three categories: anxious, medium anxious, and non-anxious.

Apparatus

Elevated plus-maze. The plus-maze was built as described by Pellow et al. (1985). It consisted of four arms, each 50 cm long and 10 cm wide. Two of the arms were open (i.e. without side and end walls), whereas the two closed arms had 40 cm high side and end walls with an open roof. The arms were arranged such that the two arms of each type were opposite each other. All four arms were connected to a 10 × 10-cm centre square. The maze was made of wood and the floor and inner walls were painted black. The plus-maze was elevated to a height of 50 cm above the floor.

Defensive burying chamber. The test chamber was identical to the one described in Experiment 1.

Procedures

Elevated plus-maze. The rat was first placed in an empty Makrolon-IV cage (identical to their home-cages) 5–7 min prior to exposure to the maze. After this period the rat was immediately transferred to the plus-maze. The rat was placed at the centre of the plus-maze facing one of the enclosed arms. During the 5-min test period, the rat’s activity was observed and recorded on video. Video tapes were later scored for first choice of alley, number of entries into open and enclosed arms, time spent in each arm, number of transitions between the arms, rearing, duration of displacement activity (grooming, gnawing, chewing), incidents of freezing (no movements) or
immobility (standing still), and defecation. The maze was cleaned with ethanol solution between trials.

Saccharin test. This test was conducted according to the methods described by Dess (1993). It was carried out in the home cages and animals were not food-deprived. Three days subsequent to the EPM test, baseline water consumption was measured. On day 1, starting at 12.00, the 23-h water consumption was measured from two 100-ml bottles of tap water for each rat. On day 2, starting at 12.00, the water bottles were replaced with one containing 150-ml tap water, the other containing a 0.125% sodium saccharin solution, 150 ml. The saccharin bottle was placed on the left side for half of the animals and on the right for the other half. The bottles were removed 23 h later and residual fluids measured.

Measures used were saccharin intake (ml/kg), saccharin preference (% of total fluid intake), increase in daily fluid intake (DFI) due to the introduction of saccharin, and the so-called ‘Dess index’. This index indicates the amount of saccharin solution consumed compared with baseline water intake, expressed as a percentage of body weight (Dess, 1993). A Dess score of zero indicates that saccharin intake equals average daily water intake; positive and negative scores indicate that saccharin intake is respectively greater or less than average daily intake.

Defensive burying. The testing took place 3 weeks after the initial EPM test. All animals were habituated to the DB apparatus for 30 min for 4 consecutive days. The shock prod was absent and the floor was covered with bedding material. On the fifth day, the rats were again placed in the apparatus, but on this occasion the shock prod was protruding into the chamber. The procedure was the same as in Experiment 1, condition A.

Ethanol procedure. Four weeks after DB testing, ethanol-screening procedures were initiated. The procedure was similar to the one described in Experiment 1, with the exception of a longer duration. Ethanol at both 3% and 5% concentrations was alternately presented every other day for 8 days. The same alternate day presentation was continued for 7% (6 days). At the end of this procedure, the animals received 7% ethanol (plus a bottle of water) for 6 consecutive days. Thus, the animals were tested for a period lasting 28 days, including 17 days with access to ethanol.

Statistics

Statistical methods employed were one- and two-way ANOVAs, Student’s t-tests for independent samples and Pearson product-moment correlations.

RESULTS

Relationships of anxiety, as measured in the plus-maze, to saccharin sensitivity, DB and ethanol consumption

ANOVA revealed that there were no main effects of anxiety category regarding any of the dependent variables. There was no main effect of level of anxiety on the saccharin variables, except for a marginal effect on saccharin intake (ml/kg) \( F(2,31) = 2.97, P < 0.07 \), with the middle anxious category showing the largest intake. Furthermore, there were no effects of level of anxiety on latency to start burying in the DB test, duration of burying, number of burying bouts, or height of bedding covering the electric prod, all \( F \) values < 1. There was no main effect of anxiety level on ethanol consumption \( F(2,31) < 1 \), or ethanol preference \( F(2,31) < 1 \).

Analyses were also performed to see whether the animal’s first choice of alley after being inserted into the plus-maze was related to ethanol consumption. The first choice was otherwise strongly related to subsequent behaviour in the plus-maze. Rats that initially ran into an open alley showed significantly more open arm activity than those entering the closed alley (Fig. 4). Moreover, ANOVA indicated that animals initially entering an open arm drank more alcohol during the 3% condition than those entering the closed arm \( F(1, 35) = 6.39, P < 0.025 \), and the same applied to the 5% condition \( F(1, 35) = 1.73, P < 0.005 \), Fig. 5). Rats choosing the open alley also had a larger mean ethanol consumption throughout the entire alcohol period \( r^2 = 2.14, P < 0.05 \), and a higher preference for ethanol \( r^2 = 2.30, P < 0.05 \), Table 5). Variety in ethanol intake ranged from 0.3 to 3.3 g/kg/day. First choice of alley did not relate to any of the other variables measured in this experiment.

Correlation analyses showed that none of the behaviours observed, or ratios calculated, in the EPM test correlated with any of the DB behaviours. A negative relationship was revealed between the increase in daily fluid intake (DFI) due to saccharin and DB latency \( r = -0.38, n = 38, P < 0.05 \). In

![Open arm activity in groups based on first choice of alley](image-url)
Ethanol consumption in groups based on first choice of alley

![Graph showing ethanol consumption in groups](image)

**Fig. 5.** Ethanol consumption (in g/kg/day) in the two groups based on first choice of alley, throughout the different alcohol concentrations. Data are expressed as means ± SEM (bars). Analysis of variance revealed that the groups were different during the 3% and 5% conditions (*P < 0.025, **P < 0.005).

<table>
<thead>
<tr>
<th>First choice of alley</th>
<th>Mean EtOH consumption (g/kg/day)</th>
<th>EtOH preference (% of total fluid intake)</th>
<th>(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open arm</td>
<td>1.85 (0.20)</td>
<td>50.91 (4.33)</td>
<td>14</td>
</tr>
<tr>
<td>Closed arm</td>
<td>1.33 (0.15)*</td>
<td>38.20 (3.39)*</td>
<td>24</td>
</tr>
</tbody>
</table>

Table 5. Effect of first choice of plus-maze alley (open/closed) on ethanol (EtOH) consumption and preference

Data are expressed as means (SEM). Student’s t-test revealed significant group differences (*P < 0.05).

addition, saccharin intake (r = 0.33), increase in DFI (r = 0.49) and the DESS index (r = 0.36) correlated positively with ethanol consumption during the initial period of the ethanol regime (the 3% concentration; all n values = 39, P < 0.05). However, except for grooming duration in the DB test, showing a negative relationship to ethanol consumption and preference (r = −0.34 and r = −0.36, n = 36, P < 0.05), relationships between DB and ethanol intake were non-existent.

**DISCUSSION**

Data analyses revealed no significant main effects of level of anxiety, as measured by the plus-maze test, on ethanol intake. Thus we are unable to confirm or disprove the reported relationship between anxiety and ethanol intake (Spanagel et al., 1995). Previous reports have not been solely affirmative; an investigation of alcohol drinking in rats selectively bred for anxiety-related behaviours in the EPM revealed that the non-anxious line showed largest ethanol intake (Henniger et al., 1999). Furthermore, alcohol-prefering AA rats are less anxious, as measured in the EPM (Möller et al., 1997). Rats with long DB latencies drink more and develop less gastric ulceration after stress (Sandbak and Murison, 1996). Finally, alcohol-avoiding ANA rats (appearing to be more timid) show shorter DB latencies and develop more ulcerations (Sandbak et al., 1998).

An alternative way of categorizing animals was attained, a variable that probably reflects initial fear of the plus-maze device. This very simplistic measure — the choice of the first alley — was related to subsequent ethanol intake in the animals. About one-third of the rats chose the open arm of the plus-maze, and these rats would subsequently drink significantly more on all ethanol measures. Like rats in the non-anxiety category, rats first choosing the open alley showed considerably more open arm activity.

There were no correlations, positive or negative, between measures in the EPM test and the DB test. This again raises the question of whether DB is a measure of anxiety. Lesions in the posterior septum have an anti-anxiety effect (Gray, 1982), resulting in increasing open arm activity in the plus-maze and abolition of DB (Treit and Pesold, 1990; Pesold and Treit, 1992). On the other hand, the Maudsley Reactive (MR/Har) and Non Reactive (MNRA/Har) rat strains (a genetically based animal model for emotionality and/or anxiety) show no differences on any DB measure (Beardslee et al., 1989). A more fruitful approach to DB might be the differentiation between proactive and reactive coping (Koolhaas et al., 1999). Wild-type rats, which adopt a proactive strategy, show both highly aggressive behaviour and a high degree of burying behaviour (Sgoifo et al., 1996). When these rats are exposed to a social defeat test, it is followed physiologically by increases in plasma noradrenaline, adrenaline and corticosterone. Exposure to the shock prod, on the other hand, barely elevates noradrenaline and adrenaline from baseline values, and the elevation of corticosterone is much lower than during social defeat.

Saccharin (measured as increased daily fluid intake due to saccharin) correlates negatively to DB latencies. As we have seen, many reports show that ingestion of sweet substances leads to an increased release of endorphins (Dum et al., 1983; Dum and Herz, 1984; Gatto et al., 1984) and may prolong and potentiate opioid-induced analgesia (Davis et al., 1956). The opioiergic system may be involved in the mechanisms mediating the reinforcement of alcohol (Kampov-Polevoy et al., 1996). Ethanol also causes a release of endogenous opioids (Schulz et al., 1980), and the effect is larger in AA than in ANA animals (Gianoulakis et al., 1992). Opioid antagonists (naloxone, naltrexone) cause a suppression of ethanol drinking (Altshuler et al., 1980; Murphy et al., 1987; Sinclair, 1989, 1990; Hyytiä and Sinclair, 1993). Given that individuals with lower levels of β-endorphins tend to ingest substances that raise the endogenous levels of opioids, a possible explanation for our observation could involve lower pain thresholds in the animals showing low burying latencies. This is of course open to empirical investigation.

Several of the saccharin measures correlated positively with initial ethanol consumption. This merely confirms earlier
observations: the propensity to ingest sweet solutions is highly correlated to ethanol preference and consumption (Sinclair et al., 1989; Kampov-Polevoy et al., 1990; Overstreet et al., 1993; Bell et al., 1994; Stewart et al., 1994). As in previous studies (Kampov-Polevoy et al., 1990, 1996; Koros et al., 1998), the association was stronger during the initial alcohol period (the 3% concentration).

CONCLUSION

The findings of these experiments indicate that exposure to various aspects of the DB test have effects on consumption of, and preference for, alcohol. Furthermore, ethanol intake may under certain circumstances interfere with the acquisition of a defensive behaviour. Finally, defensive burying is not related in any simple way to the anxiety reflected by behaviour in an EPM. We regard DB behaviour to be useful in studying drug effects on latent learning of prepared responses, but pose the question whether the DB test reflects merely anxiety, or rather both elements of anxiety and coping. To resolve this, more empirical investigations are required.

Acknowledgement — The authors would like to thank Kjrantan B. Berge for building the elevated plus-maze.

REFERENCES


