INTRODUCTION

In humans, experimental and anecdotal evidence points to a strong correlation between exposure to chronic stress and increased alcohol consumption (Sinha, 2001); an effect attributed to alcohol's ability to alleviate tension. Unlike in humans, the experimental evidence linking stress to increased alcohol consumption in laboratory animals, particularly rats, is equivocal. In animal models, chronic stress has been reported to increase (Anisman and Waller, 1974; Volpicelli et al., 1990), decrease (van Erp and Miczek, 2001) or not change alcohol intake (Fidler and LoLordo, 1996; Myers and Holman, 1967). Interestingly, and in contrast to the divergent findings on stress-induced alcohol consumption in laboratory animals, it has been repeatedly shown that exposure to acute stress evokes reinstatement of alcohol-seeking (Le and Shaham, 2002; Martin-Fardon et al., 2000). The reasons for this discrepancy are not readily apparent; however, several factors may contribute to the lack of correlation between chronic stress and increased alcohol consumption in rats. For example, stress may have different effects on alcohol consumption in non-dependent rats compared to dependent rats, or in animals that have had a prior history of dependence. In addition, stressors of a different nature may have a different impact upon alcohol consumption. Moreover, to our knowledge, the effects of different types of stress on the alcohol deprivation effect (ADE), the temporary increase in alcohol consumption seen after periods of abstinence, has not been previously examined.

Footshock has been the most commonly used stressor in the animal literature to model stress-induced changes in alcohol consumption in humans (Anisman and Waller, 1974; Fidler and LoLordo, 1996; Le and Shaham, 2002; Le et al., 1998; Martin-Fardon et al., 2000; Myers and Holman, 1967; Volpicelli et al., 1990). Interestingly, footshock has given inconsistent results with respect to alcohol consumption, but it has been repeatedly shown to reinstate alcohol-seeking behaviour in rats (Le and Shaham, 2002; Martin-Fardon et al., 2000). To contrast the effects of chronic footshock on alcohol consumption, animals were exposed to repeated injection of the endotoxin lipopolysaccharide (LPS), a model of chronic stress thought to use different stress-sensitive pathways to trigger activation of the hypothalamic-pituitary-adrenal (HPA) axis (Dayas et al., 2001; Li et al., 1996). Like footshock, LPS robustly activates the HPA axis culminating in peripheral glucocorticoid release and is also accompanied by changes in central monoamine levels (Besedovsky and del Rey, 1996; Grinevich et al., 2001). Glucocorticoid release in response to LPS plays an important role as an immunosuppressant, subsequently acting to limit the degree of inflammation (Grinevich et al., 2001).

In the present study, the effects of different types of chronic stress on the alcohol deprivation effect (ADE), the temporary increase in alcohol consumption seen after periods of abstinence, was assessed in rats made dependent on alcohol by a liquid diet. To address these issues, animals were subjected to a week of forced abstinence from alcohol self-administration, during which they were subjected to either no stress, seven days of chronic mild footshock or gradually increasing doses of the endotoxin LPS. Alcohol-reinforced responding was then measured daily for 3 weeks.

MATERIALS AND METHODS

Twenty-one, male, group housed (12 h light : 12 h dark cycle, lights off 18:00 hours) Wistar rats (Charles River, Kingston, NY) weighing 300 g on arrival were trained to self-administer alcohol in operant conditioning chambers (Coulborn Instruments, Allentown, PA) enclosed in sound-attenuating cubicles, equipped with two retractable levers. Food and water were available ad libitum except during the initiation of operant self-administration training. After 1 week of acclimatization in the vivarium, during which the rats were handled for 5 min...
each day, rats were trained to orally self-administer alcohol in daily 30-min sessions using a sweet solution fading procedure (Martin-Fardon et al., 2000). Briefly, water availability was limited to 2 h/day for 3 days. At this time, self-administration training sessions were initiated by extension of the right lever, and each lever press was reinforced by 0.1 ml of a 0.2% (v/v) saccharin solution. Water was then made available again ad libitum in the home cage and, on day four, alcohol self-administration was initiated by adding 5% (v/v) alcohol to the saccharin solution. Over the next 3 weeks, the concentration of alcohol was gradually increased to 10% (v/v) while concomitantly saccharin was eliminated. During this time, the response requirement was increased to a fixed ratio (FR) of 3, and each reinforced response paired with a white cue light (24 W) above the lever illuminated for 0.5 s. Self-administration of 10% alcohol under these contingencies continued in daily sessions for 7 weeks.

Dependence induction
After 50 days of alcohol self-administration (10%), rats were exposed to an alcohol liquid diet in order to induce dependence, as previously reported (Zorrilla et al., 2001). Briefly, rats Chow and water were replaced with a nutritionally complete, 10% (v/v) alcohol-containing liquid diet consisting of a chocolate-flavoured, liquid nutritional supplement (BOOST; Mead Johnson, Evansville, IN) supplemented with a vitamin and mineral salt mixture (0.3 and 0.5 g/100 ml, respectively; ICN Nutritional Biochemicals, Aurora, OH), distilled water and 95% alcohol diluted to a final concentration of 10% (v/v). Rats in the alcohol condition received ad libitum access to the alcohol liquid diet. The diet was prepared daily and administered at the onset of the reverse dark cycle for 21 days.

After withdrawal, alcohol self-administration was re-established for an additional 4 weeks using the same FR-3 schedule as described above.

Blood alcohol levels
To determine blood alcohol levels (BAL), tail blood (approx. 500 µl) was collected weekly into heparinized tubes 4–5 h after the onset of the dark cycle. Samples were centrifuged (10 min, 5000 r.p.m.) and plasma assayed for alcohol content by autoanalyser (Analox Instruments, Lunenburg, MA).

Alcohol deprivation and stressor exposure
Once stable baseline alcohol self-administration was re-established, animals were randomly assigned to three groups and underwent 1 week of abstinence from alcohol self-administration. During this week of abstinence, animals from group one (n = 7) received no stress but once daily sterile saline injections (i.p. 1 ml/kg). Animals from group two (n = 7) were exposed to chronic daily intermittent footshock (n = 7, 10 min/day, 0.5 mA, train length 0.5 s, administered via the grid floor under a variable-interval 40-s schedule, interval range: 10–70 s for 7 days). Animals from group three (n = 7) were injected daily (for 7 days) with gradually increasing doses of lipopolysaccharide (LPS, Escherichia coli serotype 0111 (Sigma, St Louis, MO) 25, 50, 75, 100, 150, 170, 250 µg/100 g dissolved in sterile saline, 1 ml/kg) to simulate an immune challenge. Incremental doses of LPS were administered as per previous studies (Grinevich et al., 2001), because it is known that repeated injection of LPS desensitizes cytokine production.

After 7 days, alcohol reinforced-responding was measured for 20 days.

Data analysis
BAL from the last week of the liquid diet were compared using one-way ANOVA to ensure that all animals were equally intoxicated. Alcohol-reinforced responding pre- and post liquid diet, the effect of stress on body weight, alcohol-reinforced responding and intake (g/kg) following stress were analysed using two-way ANOVA with repeated measures on one factor. After statistically significant effects were observed, post-hoc comparisons were conducted using Fisher’s LSD tests. Statistical significance was set at P < 0.05.

RESULTS
All rats acquired responding for alcohol (10%) and developed stable rates of alcohol-reinforced responding during the initial self-administration procedure. Moreover, the three groups showed statistically indistinguishable levels of alcohol-reinforced responding before and after the intoxication period [F(2, 18) = 1.1; P = 0.36]. Baseline levels of alcohol-reinforced responding prior to the liquid diet (average over 7 weeks) in the three groups (i.e. control, footshock and LPS) were 45.4 ± 3.5, 36.7 ± 1.8 and 42.6 ± 3.7 (mean ± SEM) respectively. After dependence induction, animals were allowed to self-administer alcohol for an additional 4 weeks. The mean (± SEM) number of alcohol-reinforced responses in the control, footshock and LPS groups were 37.3 ± 4.2; 33.9 ± 2.7 and 39.3 ± 3.8 respectively (Fig. 1).

After the first week of the alcohol liquid diet, the average BAL in the three groups were 5 ± 1, 6 ± 1 and 7 ± 1 mg% in the control no stress, footshock and LPS groups, respectively. No differences in BAL were observed after the last week of intoxication (control, 96 ± 11, footshock 84 ± 13 and LPS 81 ± 15 mg% [F(2, 18) = 0.35, P = 0.7]).

In the 3 groups (see Fig. 1 inset for comparison), 1 week of forced abstinence and stress had a significantly different effect on body weight, as reflected by a significant main effect on the repeated measure (i.e. weight) [F(8, 144) = 26.6, P < 0.001] and a significant stress × weight interaction [F(16, 144) = 26.4, P < 0.001]. In the control group (i.e. no stress) the animals demonstrated a gradual increase in body weight over the treatment period that was significantly different from baseline on days 5–8 (Fisher’s LSD tests: days 5–7, P < 0.05; day 8, P < 0.001), day 8 being the day of re-introduction to the self-administration chamber (Fig. 1A, inset). Chronic daily footshock produced a gradual decrease in body weight compared to pre-stress levels reaching significance on days 2 (P < 0.05), 4 (P < 0.05), 5 (P < 0.01) and 6 and 7 (P < 0.001) (Fig. 1B inset; maximum loss was approximately 3% on days 6 and 7). Injections of LPS induced a more dramatic decrease in body weight, significantly different from pre-stress levels on days 2–8 of treatment (Fisher’s LSD tests: P < 0.001) (Fig. 1C, inset; maximum loss was approximately 11% on day 7).

Alcohol reinforced responding, after 1 week of alcohol deprivation and either no stress, footshock or LPS was significantly different to baseline (Fig. 1A–C), as reflected by a significant main effect of the repeated factor: days of self-administration [F(38, 342) = 1.9, P < 0.01] and a significant
group × day of self-administration interaction [$F(38,342) = 1.95, P < 0.01$]. In control rats, 1 week of forced abstinence produced a typical ADE (i.e. increased alcohol-reinforced responding compared to baseline) the day of return to the alcohol self-administration chamber (Fisher’s LSD test, $P < 0.01$ vs. baseline; Fig. 1A). This increase in alcohol-reinforced responding was also noticeable on several other days (days 6–8, 12–15 and 18, $P < 0.01$ vs. baseline; Fig. 1A).

Fig. 1. Effects of stress on responding for alcohol and body weight (insets). (A) Animals exposed to no stress displayed the typical alcohol deprivation effect on day 1 and increased responding on days 6–8, 12–15 and 18. (B) Animals that underwent chronic daily footshock during the week of forced abstinence showed no significant increase of responding for alcohol. (C) On the first day after the last lipopolysaccharide (LPS) challenge, the animals did not show an alcohol deprivation effect (ADE) but on days 6, 7 and 9 the rats showed an increase of alcohol-reinforced responding compared to their baseline level. Control rats gradually increased their body weight reaching statistically different levels compared to baseline over the last four days of treatment (A, inset). Rats exposed to footshock gradually decreased their body weight returning to baseline levels by the end of treatment (B, inset). LPS-treated rats exhibited a typical decrease in body weight that persisted throughout the treatment period (C, inset). Fishers LSD tests: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, compared to pre-stress baseline. B, baseline; R-SA, day of return to self-administration; SA, self-administration; T1–7, treatment days.
Animals that underwent chronic daily footshock during the week of forced abstinence showed no significant increase of alcohol-reinforced responding (Fig. 1B). Animals exposed to gradually increasing doses of LPS displayed the typical behavioral response to an immune challenge consisting of piloerection and decreased locomotor activity 3–5 h after each injection. On the first day after the last LPS challenge, the animals did not show an ADE but on days 6, 7 and 9 the rats showed an increase of alcohol-reinforced responding compared to their baseline level (Fisher’s LSD test, $P < 0.05$ vs. baseline; Fig. 1C).

To confirm that alcohol-reinforced responding in response to stress was not a function of increased body weight in the control group over the treatment period, alcohol consumption was reanalysed in g/kg. As described above, after 1 week of alcohol deprivation and either no stress, footshock or LPS alcohol consumption in g/kg was significantly different to baseline (Fig. 2), as reflected by a significant main effect of the repeated factor: days of self-administration [$F(38,342) = 3.2$, $P < 0.001$] and a significant group $\times$ day of self-administration interaction [$F(38,342) = 2.1$, $P < 0.001$]. In control rats, an identical pattern to levels of alcohol-reinforced responding was observed, namely 1 week of forced abstinence produced a typical ADE (i.e., increased alcohol consumption compared to baseline), the day of return to the alcohol self-administration chamber (Fisher’s LSD test, $P < 0.01$ vs. baseline; Fig. 2A). This increase in alcohol intake was also noticeable on days 6–8, 12–15 and 18 (Fisher’s LSD test, $P < 0.01$ vs. baseline, Fig. 2). In the repeated footshock group, no significant change in alcohol intake (g/kg) was observed (Fig. 2B). Animals chronically treated with LPS did not show an ADE on day 1 but on days 6, 7 and 9 the rats showed an increase of alcohol intake compared to their baseline level (Fisher’s LSD test, $P < 0.05$, $P < 0.001$, $P < 0.01$ respectively, vs. baseline; Fig. 2C). In contrast to our analysis using alcohol-reinforced responses, an increase of alcohol intake was also observed on day 16 compared to baseline when expressed in g/kg (Fisher’s LSD test, $P < 0.05$ vs. baseline; Fig. 2C).

**DISCUSSION**

The present findings demonstrate that in dependent rats that were subjected to a week of forced abstinence from alcohol self-administration, but receiving no stress, a typical ADE and an increase in alcohol-reinforced responding was observed. Somewhat surprisingly, animals subjected to repeated footshock showed no ADE and no significant change in alcohol intake. Finally, animals that received repeated LPS injections showed a delayed ADE.

Studies in humans support a direct link between chronic stress and increased alcohol consumption (for review see (Sinha, 2001)), while in rats, a link between chronic stress and increased alcohol consumption has been harder to establish. In contrast, stress-induced relapse to alcohol-seeking has been reported in both humans and rats (Brown et al., 1995; Le and Shaham, 2002; Martin-Fardon et al., 2000; Sinha, 2001). The present study examined several important variables that might contribute to this dissociation; whether a history of dependence or different stressors has an effect on the alcohol deprivation effect. We examined these issues in rats with a history of dependence; BAL corresponding to levels previously reported to produce signs of alcohol withdrawal (Ciccocioppo et al., 2003).

Previous studies have shown that similar levels of alcohol-reinforced responding, as observed in the present study, produce pharmacologically relevant BAL (Ciccocioppo et al., 2002; Roberts et al., 1999). It is noteworthy that animals made dependent on the alcohol liquid diet did not appear to increase their intake in self-administration sessions. Increased alcohol consumption in dependent animals is a highly complex and variable phenomenon, often requiring repeated cycles of withdrawal (Roberts et al., 1996). In the present study our aim was to investigate the effects of stress on the ADE. Therefore we deliberately avoided repeated cycles of abstinence and withdrawal. Regardless, animals had a long history of ethanol exposure whether self-administering or in the homecage, the latter producing significant elevations in blood alcohol levels.

Physically dependent non-stressed rats that underwent 1 week of forced abstinence demonstrated an ADE that was observed on the first day following re-exposure to the self-administration chamber; a finding consistent with previous studies in both rats and humans (Spanagel and Holter, 2000). Moreover, these animals displayed an elevated level of alcohol-reinforced responding on days 6–8, 12–15 and 18. Potentially, the augmented level of alcohol intake reflects self-medication of adverse symptoms that commonly accompany alcohol withdrawal (Markou et al., 1998). While there are some reports using a progressive ratio schedule suggesting that the ADE produces an increase in the reinforcing value of alcohol (Spanagel and Holter, 2000), it cannot be ruled out that a decrease in the reinforcing effects of alcohol account for the increase in responding observed here. Further experiments are required to pursue this possibility. The exact mechanisms underlying the ADE are not well characterized; however, drugs successful in treating alcoholism in humans, such as naltrexone, acamprosate and baclofen, are also capable of suppressing the ADE in rats (Addolorato et al., 2002; Colombo et al., 2003; Spanagel and Holter, 2000), suggesting that dysregulation of opioid, glutamatergic and or gabaergic systems may underlie this phenomenon.

In contrast to animals that received no stress, animals exposed to chronic footshock during the week of forced abstinence demonstrated no ADE and no increase of alcohol intake. While somewhat unexpected, the literature concerning stress-induced increases in alcohol consumption in rats suggests that the present data fit within the spectrum of studies showing either an increase (Anisman and Waller, 1974; Volpicelli et al., 1990; Vengeliene, 2003), decrease (van Erp and Miczek, 2001) or no change following exposure to a stressor (Fidler and LoLordo, 1996; Myers and Holman, 1967); most commonly chronic footshock. van Erp and Miczek, attempting to understand these inconsistent findings have highlighted the importance of controlling for the many variations inherent in the stress/alcohol consumption field, such as animal housing, ratio of alcohol to an additional sweetener, intensity of the stressor and importantly, the timing of stressor exposure with respect to re-acquaintance with the alcohol solution. They reported a decrease in alcohol consumption when alcohol access occurred before a brief exposure to a social stressor or no change when alcohol access occurred after the stressor (van Erp and Miczek, 2001).

The present results confirm previous studies showing no change in alcohol consumption (Fidler and LoLordo, 1996;
Myers and Holman, 1967) in response to chronic footshock stress but more importantly, footshock suppressed the normal increase in alcohol intake observed in response to alcohol deprivation. The reason for the suppression of the ADE by footshock in the present study is not readily apparent, but one parsimonious explanation is that chronic footshock results in a passive rather than active (increased alcohol consumption) coping strategy (Bandler et al., 2000).

Animals that underwent repeated LPS injections displayed the typical symptoms of sickness associated with an immune response to stress.
challenge, as reflected by a decrease in body weight across the treatment period (Kent et al., 1992). These animals showed an initial suppression of alcohol-reinforced responding (Spanagel and Holter, 2000) lasting for up to five days following the cessation of the LPS injections. Despite this initial suppression, a delayed ADE was observed on days 6, 7, 9 and 16. In fact, in absolute terms, on day 6 this group displayed a level of alcohol-reinforced responding that was greater than on any other day and across any of the groups. The reasons for why animals exposed to an immune challenge would demonstrate such a robust rebound in responding 6 days after the cessation of repeated LPS is not self-evident, but arguably, this rebound reflects the negative reinforcement associated with the adverse symptoms that accompany withdrawal. Also, recent attention has been given to the similarity of the symptoms induced by an LPS-mediated immune challenge to the symptoms of depression such as suppression of appetite, sleep pattern disturbances, lack of energy and fatigue (Dantzer et al., 1999). Thus, an interesting possibility is that the increase in alcohol-reinforced responding seen in immune challenged animals reflects an attempt to self-medicate, a suggestion consistent with clinical data supporting a correlation between anxiety-related disorders and drug abuse (Markou et al., 1998).

CONCLUSIONS

Cautiously interpreted, the present data suggest that chronic footshock is not an appropriate paradigm to model the phenomenon of increased alcohol consumption in response to stress as reported in humans (Sinha, 2001). This is in distinct contrast to the many reports demonstrating that exposure to footshock facilitates self-administration of other commonly abused drugs including amphetamines (Piazza et al., 1990), morphine (Alexander et al., 1978; Shaham and Stewart, 1994) and cocaine (Covington and Miczek, 2001; Miczek and Mutschler, 1996), that acute footshock has been shown to evoke reinstatement of heroin-, cocaine-, nicotine- and alcohol-seeking (Le and Shaham, 2002) and that acute footshock used in the reinstatement paradigms does seem to correlate with reports of stress-induced relapse in humans (Brown et al., 1995). It seems highly likely then, at least in rats, that the mechanism(s) that trigger stress-induced reinstatement of drug-seeking and the facilitative effects of stress on stimulant and opioid self-administration, are footshock sensitive, whereas that triggering increased alcohol consumption is not (Sinha, 2001). Further work is required to identify paradigms suitable to study the phenomenon of stress-induced alcohol consumption in humans (Sinha, 2001).

Acknowledgements — This is publication number 16035-NP from The Scripps Research Institute. This work was supported by a NIH/NIAAA grant AA 10531 program grant (F.W.) and by a C. J. Martin Post-doctoral Fellowship (National Health and Medical Research Council of Australia) awarded to C.V.D. The authors thank Jeff Simms and Maury Cole for expert technical assistance and Mike Arends for assistance with the preparation of this manuscript.

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