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European Journal of Pharmacology 401 (2000) 365–373

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Ethanol modulates evoked dopamine release in mouse nucleus accumbens: dependence on social stress and dose

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Received 6 March 2000; received in revised form 15 June 2000; accepted 23 June 2000

Abstract

Ethanol may modulate the activity of presynaptic terminals to increase extracellular dopamine release in the nucleus accumbens though conflicting results have been published. It has been suggested that the stress of social defeat might be a factor influencing the effects of ethanol. We investigated the effects of ethanol on the evoked dopamine overflow in the nucleus accumbens in anaesthetised mice by in vivo voltammetry. Dominant animals, subordinates which had been defeated following eight intruder–resident encounters, and subordinate nondefeated mice were used. The overflow was evoked by electrical stimulation of the median forebrain bundle (100 pulses) at low (20 Hz) and high (50 Hz) frequencies of stimulation. Ethanol at 0.1 and 2 g/kg had no effects on evoked dopamine overflow in aggressive and nondefeated mice. Ethanol increased dopamine release at 0.1 g/kg and decreased release at 2 g/kg following high frequency stimulation in defeated mice. These data suggest that the stress of social defeat may have sensitised the machinery involved in dopamine release to ethanol, a process that may increase the reinforcing properties of this compound. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Ethanol; Dopamine release; Stress of social defeat; Voltammetry in vivo; Nucleus accumbens; (Mouse)

1. Introduction

The effects of ethanol on dopamine neurotransmission within the mesolimbic system have been under experimental investigation for decades, since it is suggested that the reinforcing properties of drugs of abuse are mediated via the dopaminergic system (Wise, 1978; Wise and Rompre, 1989; Koob and Nestler, 1997). It is now well documented that ethanol can elevate the extracellular concentrations of dopamine in the rat nucleus accumbens of freely moving animals (Imperato and Di Chiara, 1986; Yoshimoto et al., 1991; Blanchard et al., 1993a; Rossetti et al., 1993; Weiss et al., 1993; Kiianmaa et al., 1995; Samson et al., 1997; Yim et al., 1998). It has been reported that ethanol increases the firing rate of dopamine neurones in the ventral tegmental area after its application at concentrations of 20–120 mM (Gessa et al., 1985; Brodie et al., 1990). This might be the reason for elevations in the extracellular dopamine concentrations in the terminal fields of the ventral striatum following ethanol administration. Another possible mechanism through which ethanol could modulate dopamine increase is at the level of presynaptic terminals. However, data concerning this effect are somewhat conflicting. Local perfusion of toxic concentrations of ethanol (510 mM and higher) through a dialysis capillary situated in the nucleus accumbens increased basal extracellular concentrations of dopamine, though ethanol at 170 mM had no effects on dopamine release (Yim et al., 1998). In other reports, utilising the same methodological approach, ethanol at 100 mM (Wozniak et al., 1991; Yoshimoto et al., 1991) and 50 mM (Yoshimoto et al., 1991) increased the concentrations of extracellular dopamine. Local application of ethanol on striatal or hypothalamic slices either increased (Russel et al., 1988; Widdowson and Holman, 1992) or had no effect (Hyatt and Tyce, 1985; Murphy et al., 1985) on the basal dopamine level. Reports on the modulation of K^+ or electrically stimulated dopamine release are also contradictory; ethanol either decreased (Carmichael and Israel, 1975; Lynch et al., 1985), in-

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PII: S0014-2999(00)00456-8

creased (Hyatt and Tyce, 1985; Snape and Engel, 1988) or did not change (Russell et al., 1988; Widdowson and Holman, 1992) stimulated dopamine release in slices. Local application of ethanol also did not change stimulated dopamine release in anaesthetised and freely moving rats (Samson et al., 1997). Therefore, it is still unclear whether ethanol at non-anaesthetic concentrations can modulate dopamine overflow at the level of presynaptic terminals.

These inconsistencies in the literature may be due to some factors that have not been taken into account in previous experiments. Experimental animals, usually mice and rats, are routinely housed separately or in small groups. The level of stress induced by unavoidable social interactions are very high, and differ between the animals within the group, depending on their social status (Blanchard et al., 1993b; Koolhaas et al., 1997). Separate housing is also stressful due to the social isolation. The effects of social stress on the dopaminergic system are well known. Social defeat has been shown to increase extracellular dopamine concentration in the nucleus accumbens (Tidey and Miczek, 1996, 1997) and ethanol consumption (Adams, 1995; Bowers et al., 1997; Nunez et al., 1999). The stress of social defeat sensitised animals to the effects of amphetamine and cocaine (Miczek and Tornatzky, 1996; Miczek et al., 1999). Social rank (i.e. aggressiveness) also influences the effects of dopaminergic drugs (Miczek and Gold, 1983; Martin et al., 1990; Benus et al., 1991).

We investigated the possible role of social stress on the effects of ethanol on dopamine overflow at the level of presynaptic terminals. The terminals in the mouse nucleus accumbens core were chosen so as to be able to compare the results with previously published data on the effects of ethanol on dopamine release. In vivo voltammetry was used to measure extracellular concentrations of dopamine induced by electrical stimulations of the median forebrain bundle. Monitoring of stimulated dopamine overflow by in vivo voltammetry permitted the investigation of the effects of ethanol at the level of presynaptic terminals without interfering with its effects on the firing rate of dopamine neurones. This approach also allowed the measurement of dopamine overflow with minimal contribution of dopamine metabolites and other electrochemically active substances. We present evidence that (social) stress can sensitise the presynaptic terminals to ethanol.

2. Methods

2.1. Behavioural tests

The experiments were carried out on male BALB/C mice (26–32 g), bred in the National Animal Centre, Kuopio, Finland. The mice were housed in groups of eight in stainless steel cages ($30 \times 40 \times 20$ cm) in a temperature (20°C), humidity (50–60%) and light period (lights on 07:00–21:00) controlled environment. The animals had free access to food and water. One week after delivery, mice were regrouped and placed in smaller cages ($15 \times 25 \times 12$ cm) in groups of three for 1 h. During this period of establishing of social hierarchy, patterns of aggressive behaviour (threats, pursuits, attacks) were monitored in these small groups. Mice with aggressive behavioural patterns (dominants) were marked and animals were immediately regrouped for a second time. Dominant mice were housed in pairs with females of the same strain to eliminate possible effects of social isolation. These mice were used as residents in the following behavioural test. The remaining mice (subordinants) were regrouped in the larger cages of eight mice per cage. Even though the most aggressive animals were already removed, new dominant–subordinant relationships were formed and new dominants appeared. Only those groups were chosen in which new dominants were not so aggressive as to leave any signs of bite marks on the subordinants during the first week after forming the group. Mice from these groups were used as intruders in the following behavioural test and as controls for in vivo voltammetry experiments.

The tests of resident–intruder confrontations were started 1 week after the last regrouping. The test was repeated twice a week with the same intruders in pairs for 4 weeks. The dominant mouse was placed in a neutral acrylic cage (14×14 cm), and 60 min later an intruder from the group-housed mice was placed into the same cage for 4 min. To verify the intensity and stability of social stress over the experimental period, occurrences of behavioural elements were recorded on every second test on the keyboard of a notebook computer with a custom-written program for the registration and analysis of ethological data. Since we could not simultaneously record the behaviour of both animals in the pair, and because the intruder's defensive behaviour patterns were matched to the behaviour of aggressive resident, only the ethogram of the resident mouse was documented in these experiments. Sequences and durations of aggressive, defensive, non-agonistic (social) and individual acts and postures (an ethogram which included 20 species-specific behavioural elements) were recorded and stored for further statistical analysis. The most frequent behavioural elements were combined into three categories: aggression (pursuit, threat, attack bites, tail rattling and circulation), non-agonistic contacts (nasal and anogenital contacts) and individual behaviour (sitting, self-grooming, rearing, locomotion, digging). The occurrence of other behavioural elements was very infrequent in the resident mice.

2.2. Preparation of animals

Residents, group-housed intruders or intact group-housed mice were used for the determination of evoked dopamine overflow in the nucleus accumbens. Mice were

anaesthetized with chloral hydrate (450 mg/kg i.p.) and fixed in a stereotaxic frame. Anaesthesia was maintained at a level sufficient to prevent corneal reflexes by repeated injections of the anaesthetic at 100 mg/kg every 30–40 min. Rectal temperature was kept at 37 ± 0.5°C. A working electrode (detector) was placed in the nucleus accumbens core (AP: 1.2 mm, L: 1.0 mm, V: −4.3 mm vs. bregma and cortical surface) according to coordinates from the mouse brain atlas (Slotnick and Leonard, 1975). The position of the working electrode was histologically verified in all mice after electrolytic marking through the electrode. A miniature Ag/AgCl reference electrode (DRIREF-2SH, WPI, Sarasota) was positioned on the contralateral side of the skull. The auxiliary electrode (a stainless steel screw) was embedded in the occipital bones.

The experiments were carried out with the approval of the local committee on animal welfare.

2.3. Electrochemical technique

The dopamine overflow after stimulation of the median forebrain bundle was measured by constant potential amperometry (Dugast et al., 1994). Short stimulation of the median forebrain bundle induces immediate and prompt dopamine release in mouse striatum. As long as stimulation lasts for only a few seconds, the contribution of dopamine metabolites in the peak dopamine overflow is negligible. The working electrode (detector for dopamine) was a single untreated carbon fibre, 12 μm in diameter, within glass insulation. The end of the fibre, protruding beyond the glass insulation, was trimmed to a length of 350 ± 50 μm. The custom-built three-electrode potentiostat, combined with an IBM computer with digital–analogue converter for the recording and storage of data, was used with this carbon fibre electrode. The catechol oxidation current was monitored at +0.5 V vs. Ag/AgCl electrode every 0.25 s. Electrodes were conditioned for 60 s every 20 min, without removal from the brain, with triangular pulses of 0.1–1.5 V at 60 Hz after each measurement of dopamine overflow following the 50 Hz stimulation. This allowed for the maintenance of the sensitivity of working electrode throughout the experiment, and did not change its parameters.

2.4. Electrical stimulation

A bipolar stimulating electrode, with active tips 0.2-mm thick and 0.3-mm long, was implanted in the median forebrain bundle (AP: −2.0 mm, L: 1.2 mm, V: −5.2–5.4 mm vs. bregma and cortical surface). The electrode was lowered to 0.2 mm above the region of interest and stimulation began while the electrode was advanced (0.2 mm at a time). The stimulating electrode was fixed at the depth where the oxidation current at the working electrode was maximal. A Master-8 stimulator (A.M.P.I., Jerusalem) and battery-operated Stimulus Isolator (A 365, WPI, Sarasota) were used for electrical stimulation, which consisted of 100 constant-current bipolar pulses of 200 μA peak amplitude and 2 ms in length. In attempt to separate, at least qualitatively (see Section 4 for more details), the effects of ethanol on the processes of release and re-uptake we used two stimulation frequencies. The stimulation was electronically switched on for a 2-s period at 50 Hz (high frequency stimulation) or for 5 s at 20 Hz (low frequency stimulation). Two consecutive stimulations at 20 and 50 Hz were repeated every 20 min with 5-min intervals between stimulations.

2.5. Experimental procedures, data presentation and statistics

Ethanol or saline (10 ml/kg, i.p.) was administered immediately after the third stimulation of the median forebrain bundle that produced a stable dopamine release. The time-courses of the effects of either ethanol or saline were established for at least 2 h after injection at 20 min intervals. The actions of ethanol on the peak amplitude of the evoked dopamine overflow were expressed as a percentage of the peak effect from the last stimulation recorded before drug injection.

Statistical analysis of the effects of ethanol was performed by using the multivariate analysis of variance (MANOVA) for repeated measures with different treatments (two doses of ethanol and saline) as between-subjects factors and the effects of ethanol at different times after the treatment as the within-subjects factor. The stability of dopamine overflow after repeated stimulation was analyzed by using a multivariate test of significance. The effects of ethanol at different times after treatment were analyzed by using MANOVA's multiple comparisons with Bonferroni corrections. Only those mice which had the detector electrode within the nucleus accumbens were included in the final statistical analysis.

Since the resident–intruder encounter lasted only 4 min for each pair, nine animals in a group were tested in 1 day, and only one mouse per day from this group could be analyzed by in vivo voltammetry. To decrease possible variations in response connected to this time gap, the in vivo voltammetry experiments were started 10 days after the last behavioural test. Accordingly, mice were tested in the in vivo voltammetry experiments 10–18 days after the behavioural test. Analysis of time-course of the effects of ethanol was beyond the scope of the present work. However, when the results were obtained it became possible to perform a preliminary analysis of the stability of the effects of ethanol with time from the last behavioural test. For this purpose we compared the average effects of ethanol 20, 40 and 60 min after treatment in six mice which had been used in in vivo voltammetry experiments during the first 3 and last 3 days of the overall period of measuring. The data were compared using two-tailed unpaired t-test.

Behavioural data were presented as combined categories. However, before being combined, each behavioural element was calculated in terms of its relative duration (the ratio of a duration of particular element to the duration of all registered elements), probability (the ratio of the number of the particular element to the number of all registered elements) and duration of each behavioural element (in seconds). Statistical analysis of behavioural data (these data were obtained for every second resident–intruder encounter, i.e. for four encounters) was performed by using MANOVA for repeated measures, with days of testing (four levels) and behavioural categories (three levels) as within-subjects factors. Pairwise comparisons of the results of individual tests (days) were made by multiple comparisons with Bonferroni corrections. All average data are presented as arithmetical mean ± standard error of mean (S.E.M.).

3. Results

During the eight episodes of resident–intruder confrontations, the aggressive behaviour of residents became significantly elevated (Fig. 1). Residents demonstrated a progressive increase in relative duration (the effects of day of testing; $F_{(3,15)} = 4.206$, $P = 0.024$), probability ($F_{(3,15)} = 3.336$, $P = 0.048$) and duration ($F_{(3,15)} = 3.623$, $P = 0.038$) of aggressive behavioural elements. The relative duration ($F_{(3,15)} = 5.780$, $P = 0.008$), and duration ($F_{(3,15)} = 5.756$, $P = 0.008$) of the elements of social behaviour decreased over time. However, this decrease happened after the very first test and all parameters of non-agonistic contacts were stable in the following days. The patterns of individual behaviour were generally stable from test to test. There were no significant interactions between behavioural categories (aggressive, social and individual behaviour) and the days of the test. Thus, the general structure of the resident–intruder confrontations did not change after the tests. In absolute values (the mean results of all tests, $n = 72$), following 4 min of resident–intruder confrontation, residents demonstrated aggressive behavioural elements 18.3 ± 2.5 times and each incident lasting 0.95 ± 0.07 s.

Saline injections had no effects on the dopamine overflow in the mouse nucleus accumbens core following repeated over 3 h electrical stimulation of the median

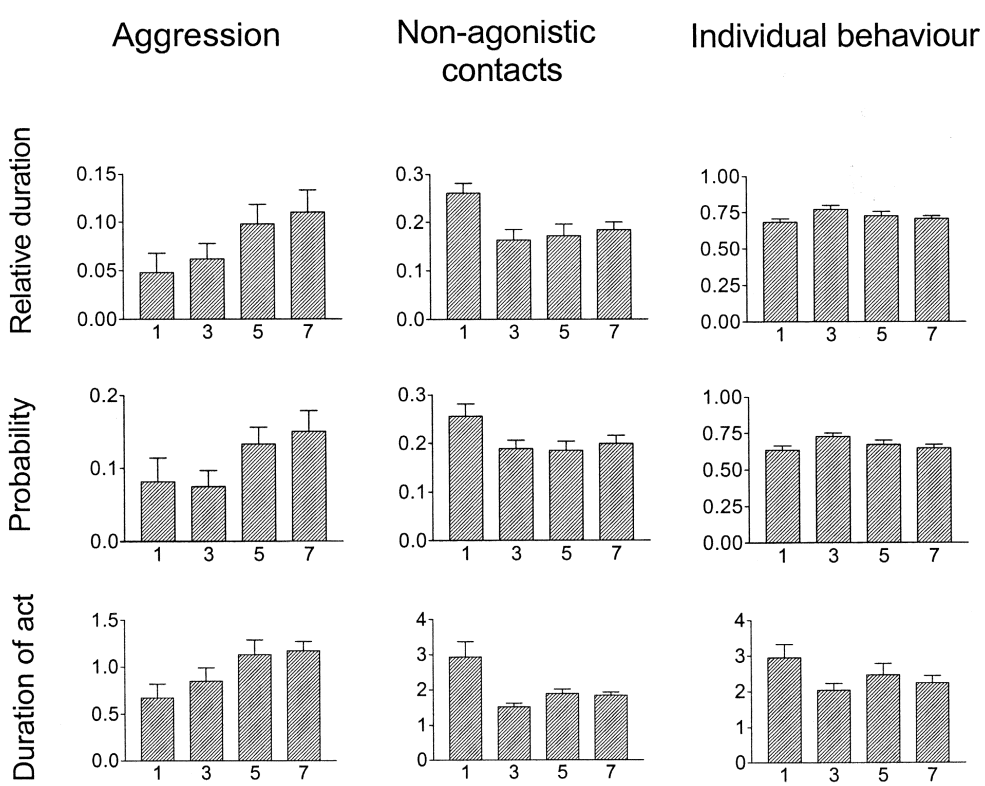

Fig. 1. Behavioural profile of aggressive resident mice during resident–intruder encounters. Behavioural elements were combined in three categories: aggression (pursuit, threat, attack bites, tail rattling and circulation), non-agonistic contacts (nasal and anogenital contacts), individual behaviour (sitting, self-grooming, rearing, locomotion, digging). *Y*-axes: relative duration (the ratio of the duration of particular element to the duration of all registered elements), probability (the ratio of the number of the particular element to the number of all registered elements) and duration of each behavioural element (in seconds). The test of resident–intruder encounter was repeated twice a week with the same intruders in pairs for 4 weeks. Data are presented as arithmetical mean ± S.E.M. for every second encounter.

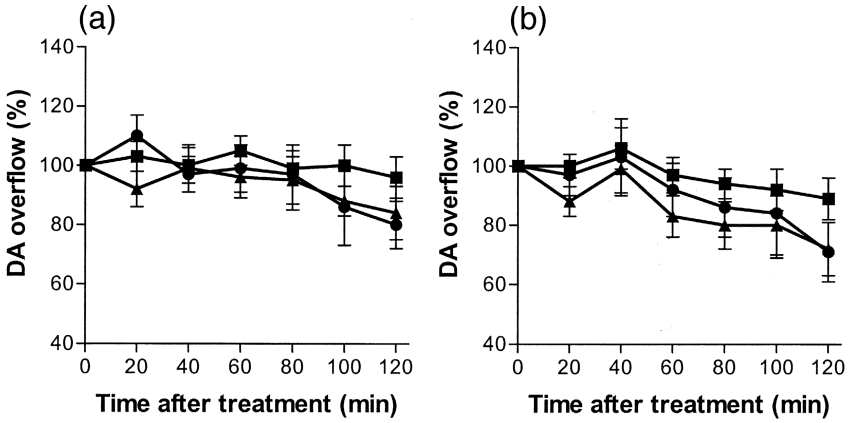

Fig. 2. Time-course of the effects of ethanol on the evoked dopamine overflow in the nucleus accumbens of aggressive resident mice. Each data point represents the peak dopamine overflow (a percentage of the predrug level, mean ± S.E.M.) evoked by electrical stimulation of the median forebrain bundle at low ((a) 20 Hz-5 s) and high ((b) 50 Hz-2 s) frequencies. Saline (■, $n = 8$), ethanol 0.1 g/kg (▼, $n = 9$) and ethanol 2.0 g/kg (●, $n = 8$) were injected immediately after the stimulation at time point 0.

forebrain bundle (the effects at different time after treatment; 20 Hz stimulation: $F_{(5,3)} = 2.513$, $P = 0.239$; 50 Hz stimulation: $F_{(5,3)} = 6.595$, $P = 0.076$).

Ethanol at 0.1 and 2 g/kg in aggressive residents induced some trend to decrease the dopamine overflow evoked by low frequency stimulation at the end of the observation period (Fig. 2a, the effects at different time after treatment: $F_{(5,18)} = 11.119$, $P = 0.001$). However, no differences were seen between the effects of saline and ethanol. Ethanol at 0.1 and 2 g/kg induced the same trend towards decreased dopamine overflow over time when release was evoked by high frequency stimulation in aggressive residents (Fig. 2b, $F_{(5,18)} = 12.082$, $P = 0.001$). This was the only significant effect of ethanol, and no differences were found between the effects of saline and ethanol at any time after the treatment (Fig. 2b, the effects of treatment × time after treatment: $F_{(10,38)} = 0.709$, $P = 0.710$).

Evoked dopamine overflow in defeated intruder mice also showed a general tendency to decrease over time following ethanol administration at 0.1 and 2 g/kg (Fig. 3a and b, the effects at different time after treatment; low frequency stimulation: $F_{(5,13)} = 6.038$, $P = 0.004$; high frequency stimulation: $F_{(5,13)} = 9.978$, $P = 0.001$). Apart from this general trend, ethanol had virtually no effects on dopamine overflow evoked by low frequency stimulation at either dose in defeated intruders in comparison with saline treated mice ($F_{(10,28)} = 1.524$, $P = 0.183$). Significant differences between the saline and ethanol treated groups (between-subject effects of treatment, $F_{(2)} = 9.708$, $P = 0.002$) were observed in the defeated intruder mice following high frequency stimulation. Ethanol at 0.1 g/kg

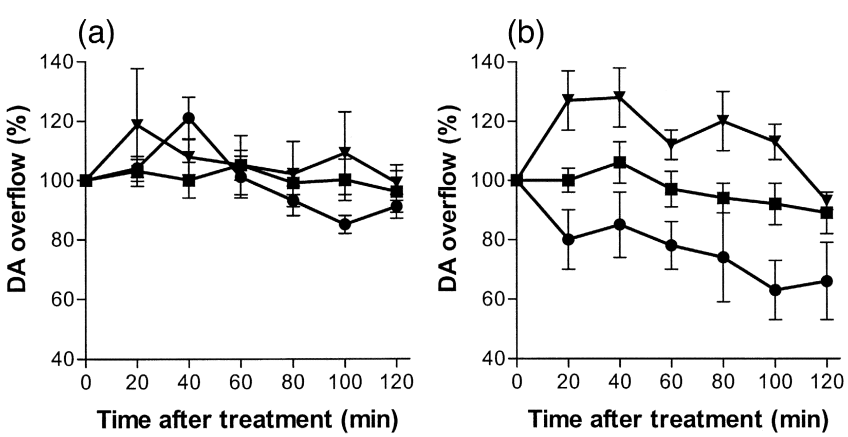

Fig. 3. The effects of ethanol on the evoked dopamine overflow in the nucleus accumbens of defeated intruder mice. Each data point represents the peak dopamine overflow (a percentage of the predrug level, mean ± S.E.M.) evoked by electrical stimulation of the median forebrain bundle at low ((a) 20 Hz-5 s) and high ((b) 50 Hz-2 s) frequencies. Saline (■, $n = 8$), ethanol 0.1 g/kg (▼, $n = 6$) and ethanol 2.0 g/kg (●, $n = 6$) were injected immediately after the stimulation at time point 0.

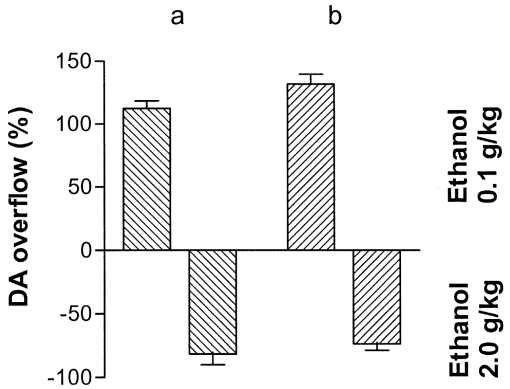

Fig. 4. Dopamine overflow following ethanol treatment in defeated mice at different times after the last resident–intruder encounter. The columns represent average effects (mean ± S.E.M.) of ethanol in six mice for each dose. These mice were used in in vivo voltammetry experiments during the three first (a) and last 3 (b) days of the overall period of measurements (10–18 days after last behavioural test). Upper panel: the effects of ethanol at 0.1 g/kg. Lower panel: the effects of ethanol at 2.0 g/kg.

increased dopamine overflow by 27 ± 10% ($P = 0.013$) already 20 min after injection (Fig. 3b). Ethanol induced the opposite effects at 2 g/kg. The tendency ($P = 0.065$) of decreased dopamine overflow following ethanol administration at this dose was already seen 20 min after treatment. Ethanol induced a 26 ± 12% ($P = 0.048$) decrease in the evoked dopamine overflow at 40 min after injection. The effects lasted at least 2 h after the intraperitoneal administration of ethanol.

Experiments with ethanol at a concentration of 0.1 g/kg were repeated with group-housed mice, which had no experience of submissive behaviour with aggressive residents. The effects of ethanol on dopamine overflow did not differ from the effects of saline treated mice and aggressive residents (data not shown).

Mice were tested for the effects of ethanol 10–18 days after the last resident–intruder confrontation (one mouse per day). The effects of both doses of ethanol were compared in defeated mice in the first and the last 3 days of this period. This approach was used to obtain preliminary data on the time-course (stability) of the effects of ethanol over the period of measurements. There were no significant differences between these groups for both doses of ethanol (Fig. 4, upper panel: ethanol at 0.1 g/kg, $t = 1.913$, $df = 16$, $P = 0.08$; lower panel: ethanol at 2.0 g/kg, $t = 0.081$, $df = 16$, $P = 0.431$).

4. Discussion

These results suggest that social stress may sensitise presynaptic dopamine terminals in the mouse nucleus accumbens to ethanol. Ethanol modulated dopamine overflow in subordinant mice that had been defeated by aggressive residents. Aggression itself did not sensitise mice to the effects of ethanol, as ethanol had no effects on the evoked dopamine overflow in aggressive mice. The effects of ethanol in subordinate intruder mice were dependent on dose. At a small dose (0.1 g/kg) ethanol increased the evoked dopamine overflow in the nucleus accumbens and at 2.0 g/kg this effect was decreased.

In all experimental series, ethanol induced a slight decrease in evoked dopamine overflow 1–2 h after administration in resident and intruder mice at low and high frequency stimulation and at low and high doses. This trend reached significance when the overall effects of the group/doses were considered. At the same time, and at all time points after administration, there were no detectable differences between the saline and ethanol treated groups. Two factors may explain these results; attenuation of the detector's sensitivity over time, and/or general anaesthetic properties of ethanol. With respect to detector sensitivity, regular conditioning of the working electrodes allowed for more stable results with saline than in previous work (Yavich et al., 1997). Anaesthesia itself has only minimal effects on dopamine re-uptake, but inhibits electrically evoked dopamine release (Garris et al., 1997), and therefore we cannot completely rule out the possibility of some depressing effects from the combination of chloral hydrate and ethanol, superimposed on existing detector sensitivity. This trend might be considered as nonspecific effects of ethanol. It seems clear that bidirectional modulation of dopamine overflow that also depended on a previous history of social stress cannot be associated with the interaction with anaesthetics.

Ethanol produced significant dose-dependent bidirectional effects in only subordinates that had been defeated by aggressive residents; i.e. the stress of social defeat had sensitised these animals to ethanol. Indeed, the stress of subordination is a high-level stressor that is associated with high mortality, oscillations in blood pressure, heart rate, core temperature, elevation of plasma corticosterone level and major changes in other physiological parameters (for a review see Blanchard et al., 1993b; Miczek and Tornatzky, 1996). In the present study, intruders were confronted by aggressive residents for 8 min/week, during which time direct assaults (that included also ambivalent behaviour: circulation and tail rattling) lasted for about 36 s. Residents demonstrated a progressive increase in the number and time of aggressive behavioural patterns, but this should not significantly affect the intensity of stress. It has been clearly documented by measured autonomic reactions, stress hormone levels in plasma and extracellular dopamine concentrations in the nucleus accumbens of defeated rats that such stressful situation lasted throughout the entire time of the encounter (Miczek and Tornatzky, 1996; Tidey and Miczek, 1996). However, the minimal intensity and length of stress-inducing stimuli that are required to produce this sensitisation to the effects of ethanol remain to be clarified. Possibly even a single defeat could be sufficient to sensitise intruders (see, for example, Koolhaas et al., 1997; Kulling et al., 1988;

Miczek and Tornatzky, 1996; Miczek et al., 1991, 1999). Whether or not other situations related to housing, handling or brief restraint may induce sensitisation to the effects of ethanol on dopamine release is not yet clear. It is also unknown how long this effect lasts. For example, sensitisation to amphetamine and cocaine in mice develops slowly, with the peak time around 1 week after the stress of social defeat (Miczek and Tornatzky, 1996; Miczek et al., 1999). Defeated rats demonstrate tolerance to the analgesic effects of morphine at least 30 days after a single aggressive encounter (Miczek et al., 1991). The measurements of the effects of ethanol on dopamine overflow were started 10–18 days after the last behavioural encounter. We did not find any differences between the effects of ethanol in mice which were used in in vivo voltammetry experiments during the first and the last 3 days of testing. The results indicate that sensitisation to ethanol is maintained at a stable level during this period.

Dopamine overflow in the terminal fields induced by brief electrical stimulation of ascending pathways merely reflects the processes of release and re-uptake. The firing rate of dopamine cells has no impact on the evoked dopamine overflow, as externally applied stimulation overwhelms neuronal tone, and the sensitivity of working electrodes (about 100 nM) is less than the basal extracellular dopamine levels. Thus, we conclude that the stress of subordination can modify the effects of ethanol on the release and/or re-uptake of dopamine at the level of presynaptic terminals. The dopamine overflow registered by a working electrode is the sum of these two processes, and it follows Michaelis–Menten kinetics (May et al., 1988; Wightman et al., 1988). Accordingly, drugs affecting merely re-uptake produce larger effects on dopamine overflow than is induced by low frequency stimulation, since the dopamine concentrations that are evoked by low frequency stimulation are comparable with the K_m of re-uptake. Drugs that elevate release per pulse of stimulation have larger effects at higher stimulation frequencies which evoke larger extracellular dopamine concentrations. In the present experiment, we used stimulation at low and high frequencies as a tool to qualitatively separate release and re-uptake. Ethanol modulated dopamine overflow only at the high frequency stimulation. We suggest that the effects of ethanol are primarily due to the modulation of dopamine release. These results do not support previous proposals that ethanol should inhibit (Lin and Chai, 1955) or facilitate (Wang et al., 1997) dopamine re-uptake. In the first case, a local ethanol infusion (200 mM) prolonged the voltammetric signal of dopamine evoked by locally applied *N*-methyl-D-aspartate (NMDA) or exogenously applied dopamine. It was concluded that ethanol inhibited dopamine re-uptake. In the second case local ethanol infusion (100mM) diminished the voltammetric signal from locally applied dopamine, which was regarded as activation of re-uptake. In both experiments, these high concentration local ethanol infusions decreased levels of evoked dopamine that coincided with our results on the effects of ethanol at 2 g/kg. The fact that ethanol did modulate dopamine re-uptake in the above mentioned experiments might be due to the methodology used to evoke dopamine overflow, particularly as a factor of the time that dopamine persisted (and was subjected to re-uptake) in the extracellular space after local infusion of K^+ or NMDA. This time (from 40 to 200 s) was about 100 times longer than might be expected to occur in a real situation of neuronal firing in bursts (Grace and Bunney, 1984). Following a brief electrical stimulation, one that more closely resembles physiological conditions, ethanol at small and large doses did not change the re-uptake of dopamine.

The present experiments were started with two extreme and behaviourally relevant doses of ethanol (0.1 and 2.0 g/kg) without examining any intermediate doses. Nevertheless, the effects of ethanol in defeated mice were only about 30–40% of control and the variation in these results was much higher than in the experiments with resident and group-housed mice. We propose that the way to obtain more data points on the dose-response curve for the effects of ethanol in defeated mice may require standardisation of the levels of aggression in the resident mice.

The stress of subordination affects opioids, γ-aminobutyric acid (GABA), excitatory amino acids and the dopaminergic system itself (for a review see Miczek and Tornatzky, 1996) which, in turn, may modulate evoked dopamine overflow following ethanol administration. Ethanol decreased evoked dopamine overflow at 2.0 g/kg and elevated it at 0.1g/kg in defeated mice. It seems unlikely that ethanol has any direct actions on the neuronal membranes, because these would become apparent only at higher concentrations. Ethanol enhances synthesis of dopamine in the striatum (Carlsson et al., 1974; Blomqvist et al., 1993). This process is regulated by dopaminergic receptors (Carlsson et al., 1974; Rubinstein et al., 1997). Stress-induced modifications of the receptor sensitivity could have some impact on the effects of ethanol, particularly dopamine synthesis and release. Ethanol can interact with opioids in their effects on dopamine release (for a review see Herz, 1997). It could be suggested that opioid potentiation and tolerance induced by the stress of social defeat could modulate the effects of ethanol on dopamine release. $GABA_A$ and NMDA receptors also regulate dopamine release presynaptically (Chéramy et al., 1998) and ethanol can interact with these receptors (Faingold et al., 1998). This might represent another site of action for ethanol in defeated mice. It seems that the dose-dependent bidirectional effects of ethanol are due to its multiple actions on different neurochemical systems, all with diverse sensitivity to ethanol after stress. However, any suggestions about precise mechanisms underlying the opposing effects of ethanol would be prematurely speculative.

The consumption of ethanol is elevated after social stress (Adams, 1995; Bowers et al., 1997; Nunez et al.,

1999). Recent experiments showed that the reinforcing properties of ethanol can be potentiated by conditioned fear stress (Matsuzawa et al., 1998, 1999). Generally, the reinforcing effects of drugs are associated with activation of the mesolimbic dopaminergic system. Sensitisation of presynaptic dopaminergic terminals to ethanol following social stress may play some role in the increase in its reinforcing properties. We propose that the sensitisation to the effects of ethanol on dopamine release in the nucleus accumbens is a part of the neurochemical mechanisms leading to elevated consumption of ethanol in stressed animals.

References

Adams, N., 1995. Sex differences and the effects of tail pinch on ethanol drinking in Maudsley rats. Alcohol 12, 463–468.

Benus, R.F., Bohus, B., Koolhaas, J.M., van Oortmerssen, G.A., 1991. Behavioural differences between artificially selected aggressive and non-aggressive mice, response to apomorphine. Behav. Brain Res. 43, 203–208.

Blanchard, B.A., Steindorf, S., Wang, S., Glick, S.D., 1993a. Sex differences in ethanol-induced dopamine release in nucleus accumbens and in ethanol consumption in rats. Alcohol.: Clin. Exp. Res. 17, 968–973.

Blanchard, R.J., Yudko, E., Blanchard, K., 1993b. Alcohol, aggression and stress of subordination. J. Stud. Alcohol 11, 146–155.

Blomqvist, O., Engel, J.A., Nissbrandt, H., Soderpalm, B., 1993. The mesolimbic dopamine-activating properties of ethanol are antagonized by mecamylamine. Eur. J. Pharmacol. 249, 207–213.

Bowers, W.J., Sabongui, A.G., Amit, Z., 1997. The role of ethanol availability on stress-induced increases in ethanol consumption. Alcohol 14, 551–556.

Brodie, M.S., Shefner, S.A., Dunwiddie, T.V., 1990. Ethanol increases the firing rate of dopamine neurons of the rat ventral tegmental area in vitro. Brain Res. 508, 65–69.

Carlsson, A., Engel, J., Strombom, U., Svensson, T.H., Waldeck, B., 1974. Suppression by dopamine-agonists of the ethanol-induced stimulation of locomotor activity and brain dopamine synthesis. Naunyn-Schmiedeberg's Arch. Pharmakol. 283, 117–128.

Carmichael, F.J., Israel, Y., 1975. Effects of ethanol on neurotransmitter release by rat brain cortical slices. J. Pharmacol. Exp. Ther. 193, 824–834.

Chéramy, A., L'hirondel, M., Godeheu, G., Artaud, F., Glowinski, J., 1998. Direct and indirect presynaptic control of dopamine release by excitatory amino acids. Amino Acids 14, 63–68.

Dugast, C., Suaud-Chagny, M.F., Gonon, F., 1994. Continuous in vivo monitoring of evoked dopamine release in the rat nucleus accumbens by amperometry. Neuroscience 62, 647–654.

Faingold, C.L., N'Gouemo, P., Riaz, A., 1998. Ethanol and neurotransmitter interactions from molecular to integrative effects. Prog. Neurobiol. 55, 509–535.

Garris, P.A., Christensen, J.R., Rebec, G.V., Wightman, R.M., 1997. Real-time measurement of electrically evoked extracellular dopamine in the striatum of freely moving rats. J. Neurochem. 68, 152–161.

Gessa, G.L., Muntoni, F., Collu, M., Vargiu, L., Mereu, G., 1985. Low doses of ethanol activate dopaminergic neurons in the ventral tegmental area. Brain Res. 348, 201–203.

Grace, A.A., Bunney, B.S., 1984. The control of firing pattern in nigral dopamine neurons, burst firing. J. Neurosci. 4, 2877–2890.

Herz, A., 1997. Endogenous opioid systems and alcohol addiction. Psychopharmacology (Berlin) 129, 99–111.

Hyatt, M.C., Tyce, G.M., 1985. The effects of ethanol on the efflux and release of norepinephrine and 5-hydroxytryptamine from slices of rat hypothalamus. Brain Res. 337, 255–262.

Imperato, A., Di Chiara, G., 1986. Preferential stimulation of dopamine release in the nucleus accumbens of freely moving rats by ethanol. J. Pharmacol. Exp. Ther. 239, 219–228.

Kiianmaa, K., Nurmi, M., Nykanen, I., Sinclair, J.D., 1995. Effect of ethanol on extracellular dopamine in the nucleus accumbens of alcohol-preferring AA and alcohol-avoiding ANA rats. Pharmacol., Biochem. Behav. 52, 29–34.

Koob, G.F., Nestler, E.J., 1997. The neurobiology of drug addiction. J. Neuropsychiatry Clin. Neurosci. 9, 482–497.

Koolhaas, J.M., De, B.S.F., De, R.A.J., Meerlo, P., Sgoifo, A., 1997. Social stress in rats and mice. Acta Physiol. Scand., Suppl. 640, 69–72.

Kulling, P., Frischknecht, H.R., Pasi, A., Waser, P.G., Siegfried, B., 1988. Social conflict-induced changes in nociception and beta-endorphin-like immunoreactivity in pituitary and discrete brain areas of C57BL/6 and DBA/2 mice. Brain Res. 450, 237–246.

Lin, A.M., Chai, C.Y., 1955. Dynamic analysis of ethanol effects on NMDA-evoked dopamine overflow in rat striatum. Brain Res. 696, 15–20.

Lynch, M.A., Samuel, D., Littleton, J.M., 1985. Altered characteristics of [^{3}H]dopamine release from superfused slices of corpus striatum obtained from rats receiving ethanol in vivo. Neuropharmacology 24, 479–485.

Martin, S.P., Smith, E.O., Byrd, L.D., 1990. Effects of dominance rank on D-amphetamine-induced increases in aggression. Pharmacol., Biochem. Behav. 37, 493–496.

Matsuzawa, S., Suzuki, T., Misawa, M., 1998. Conditioned fear stress induces ethanol-associated place preference in rats. Eur. J. Pharmacol. 341, 127–130.

Matsuzawa, S., Suzuki, T., Misawa, M., Nagase, H., 1999. Involvement of dopamine D(1) and D(2) receptors in the ethanol-associated place preference in rats exposed to conditioned fear stress. Brain Res. 835, 298–305.

May, L.J., Kuhr, W.G., Wightman, R.M., 1988. Differentiation of dopamine overflow and uptake processes in the extracellular fluid of the rat caudate nucleus with fast-scan in vivo voltammetry. J. Neurochem. 51, 1060–1069.

Miczek, K.A., Gold, L.H., 1983. D-Amphetamine in squirrel monkeys of different social status, effects on social and agonistic behavior, locomotion, and stereotypes. Psychopharmacology (Berlin) 81, 183–190.

Miczek, K., Tornatzky, W., 1996. Ethopharmacology of aggression, impact on autonomic and mesocorticolimbic activity. Ann. N. Y. Acad. Sci. 794, 60–77.

Miczek, K., Thompson, M.L., Tornatzky, W., 1991. Subordinate animals, behavioral and physiological adaptations and opioid tolerance. In: Brown, M.R., Koob, G.F., Rivier, C. (Eds.), Stress, Neurobiology and Neuroendocrinology. Marcel Dekker, New York, pp. 323–357.

Miczek, K.A., Nikulina, E., Kream, R.M., Carter, G., Espejo, E.F., 1999. Behavioral sensitization to cocaine after a brief social defeat stress, c-fos expression in the PAG. Psychopharmacology (Berlin) 141, 225–234.

Murphy, J.M., Cunningham, S.D., McBride, W.J., 1985. Effects of 250 mg% ethanol on monoamine and amino acid release from rat striatal slices. Brain Res. Bull. 14, 439–442.

Nunez, M.J., Riveiro, P., Becerra, M.A., De, M.S., Quintans, M.R., Nunez, L.A., Legazpi, M.P., Mayan, J.M., Rey-Mendez, M., Varela, M., Freire-Garabal, M., 1999. Effects of alprazolam on the free-choice ethanol consumption induced by isolation stress in aged rats. Life Sci. 64, PL213–PL217.

Rossetti, Z.L., Hmaidan, Y., Diana, M., Gessa, G.L., 1993. Lack of tolerance to ethanol-induced dopamine release in the rat ventral striatum. Eur. J. Pharmacol. 231, 203–207.

Rubinstein, M., Phillips, T.J., Bunzow, J.R., Falzone, T.L., Dziewczapolski, G., Zhang, G., Fang, Y., Larson, J.L., McDougall, J.A., Chester, J.A., Saez, C., Pugsley, T.A., Gershanik, O., Low, M.J., Grandy,

D.K., 1997. Mice lacking dopamine D4 receptors are supersensitive to ethanol, cocaine, and methamphetamine. Cell 90, 991–1001.

Russell, V.A., Lamm, M.C., Taljaard, J.J., 1988. Effect of ethanol on [^{3}H]dopamine release in rat nucleus accumbens and striatal slices. Neurochem. Res. 13, 487–492.

Samson, H.H., Hodge, C.W., Erickson, H.L., Niehus, J.S., Gerhardt, G.A., Kalivas, P.W., Floyd, E.A., 1997. The effects of local application of ethanol in the n. accumbens on dopamine overflow and clearance. Alcohol 14, 485–492.

Slotnick, B.M., Leonard, C.M., 1975. A Stereotaxic Atlas of the Albino Mouse Forebrain. U.S. Department of Health, Education, and Welfare. Public Health Service, Rockville, MD.

Snape, B.M., Engel, J.A., 1988. Ethanol enhances the calcium-dependent stimulus-induced release of endogenous dopamine from slices of rat striatum and nucleus accumbens in vitro. Neuropharmacology 27, 1097–1101.

Tidey, J.W., Miczek, K., 1996. Social defeat stress selectively alters mesocorticolimbic dopamine release, an in vivo microdialysis study. Brain Res. 721, 140–149.

Tidey, J.W., Miczek, K.A., 1997. Acquisition of cocaine self-administration after social stress, role of accumbens dopamine. Psychopharmacology (Berlin) 130, 203–212.

Wang, Y., Palmer, M.R., Cline, E.J., Gerhardt, G.A., 1997. Effects of ethanol on striatal dopamine overflow and clearance, an in vivo electrochemical study. Alcohol 14, 593–601.

Weiss, F., Lorang, M.T., Bloom, F.E., Koob, G.F., 1993. Oral alcohol self-administration stimulates dopamine release in the rat nucleus accumbens, genetic and motivational determinants. J. Pharmacol. Exp. Ther. 267, 250–258.

Widdowson, P.S., Holman, R.B., 1992. Ethanol-induced increase in endogenous dopamine release may involve endogenous opiates. J. Neurochem. 59, 157–163.

Wightman, R.M., Amatore, C., Engstrom, R.C., Hale, P.D., Kristensen, E.W., Kuhr, W.G., May, L.J., 1988. Real-time characterization of dopamine overflow and uptake in the rat striatum. Neuroscience 25, 513–523.

Wise, R.A., 1978. Catecholamine theories of reward, a critical review. Brain Res. 152, 215–247.

Wise, R.A., Rompre, P.-P., 1989. Brain dopamine and reward. Annu. Rev. Psychol. 40, 191–225.

Wozniak, K.M., Pert, A., Mele, A., Linnoila, M., 1991. Focal application of alcohols elevates extracellular dopamine in rat brain, a microdialysis study. Brain Res. 540, 31–40.

Yavich, L., Lappalainen, R., Sirvio, J., Haapalinna, A., MacDonald, E., 1997. Alpha-2 adrenergic control of dopamine overflow and metabolism in mouse striatum. Eur. J. Pharmacol. 339, 113–119.

Yim, H.J., Schallert, T., Randall, P.K., Gonzales, R., 1998. Comparison of local and systemic ethanol effects on extracellular dopamine concentration in rat nucleus accumbens by microdialysis. Alcohol. Clin. Exp. Res. 22, 367–374.

Yoshimoto, K., McBride, W.J., Lumeng, L., Li, T.-K., 1991. Alcohol stimulates the release of dopamine and serotonin in the nucleus accumbens. Alcohol 9, 17–22.