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ORIGINAL INVESTIGATION

Increased accumbal dopamine during daily alcohol consumption and subsequent aggressive behavior in rats

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Abstract
Background Alcohol drinking may lead to increased aggression in certain individuals, and both fighting and drinking increase levels of dopamine and serotonin in mesocorticolimbic structures. Assessing the dynamic changes in these neurotransmitters during the course of drinking and fighting has remained challenging.
Objective The objective of the study was to learn about ongoing monoaminergic activity in the nucleus accumbens of rats that engaged in aggressive behavior after having consumed low doses of alcohol.
Materials and methods After male members of breeding pairs of Long–Evans rats displayed reliable aggression toward an intruder into their home cage, they were trained to consume a 10% alcohol solution, leading to blood alcohol levels of 20–80 mg/dl. Subsequently, the effect of daily alcohol self-administration on aggression was determined in biweekly confrontations with an intruder. Finally, rats were implanted with a microdialysis probe aimed at the n. accumbens for sample collection before, during, and after a 10-min alcohol drinking session followed by a 10-min aggressive confrontation.
Results Accumbal dopamine, but not serotonin, levels tended to increase in anticipation of the daily alcohol session, reaching significance immediately after the alcohol session and remaining significantly elevated (by 40%) during and after the subsequent confrontation. No such changes were seen in residents that confronted an intruder without preceding alcohol consumption. Animals that had a history of becoming more aggressive after consumption of low levels of alcohol showed similar changes in dopamine levels as did animals that had no such history.
Conclusions The rise in accumbal dopamine confirms previous findings and seems to reflect the anticipation of alcohol consumption; it persisted during the aggressive confrontation regardless of the level of aggression. The daily alcohol drinking for several months may have facilitated dopamine release and masked any further changes associated with the aggressive encounter.

Keywords Aggression · Dopamine · Alcohol · Nucleus accumbens · Serotonin

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Introduction

Surprisingly, little is known about the neurobiological mechanisms that link alcohol to many types of aggression and violence in a certain proportion of humans across cultures and history (Bushman and Cooper 1990; Krug et al. 2002; Roizen 1997; WHO 1992). Bidirectional dose-

dependent changes in aggression characterize alcohol effects in humans and animals, i.e., decreased aggressive behavior after higher doses due to the drug's sedative and intoxicating effects (e.g., Crowley et al. 1974; Krsiak and Borgesova 1973; Lagerspetz and Ekqvist 1978; Mos and Olivier 1988; Smoothy and Berry 1983) and increased aggression after lower doses during the activation phase (e.g., Blanchard et al. 1987; Chance et al. 1973; Ellman et al. 1972; Giancola and Zeichner 2001; Lister and Hilakivi 1988; Miczek et al. 1998; Miczek and Barry 1977; Miczek and O'Donnell 1980; Pettijohn 1979; Zeichner and Pihl 1979). Marked individual differences characterize the aggression-heightening effects of alcohol as a significant minority of human and non-human primates, rats, and mice become more aggressive (Higley and Bennett 1999; Mehlman et al. 1994; Miczek et al. 1992; 1994, 1998; Miczek and de Almeida 2001; van Erp and Miczek 1997).

The actions of alcohol are mediated by many neurotransmitter systems in the brain, such as dopamine (DA), serotonin 5-hydroxytryptamine (5-HT), and γ-aminobutyric acid, and alcohol consumption is influenced by drugs that act on these and other neurotransmitters, including opiates and glutamate (e.g., Koob et al. 1998; McBride and Li 1998). Of these neurotransmitters, the mesocorticolimbic DA and 5-HT systems are of interest because of their putative role in behavioral activation and impulse control—both relevant to fighting and drinking (Coccaro 1992; Di Chiara et al. 2004; Everitt et al. 1999; Koob 2000; Linnoila and Virkkunen 1992; Mann 1999; Robinson and Berridge 2000).

Alcohol activates dopaminergic systems in several terminal regions as demonstrated by increased concentrations of DA and its metabolites in the nucleus accumbens, frontal cortex, striatum, and amygdala after intraperitoneal injection of moderate doses of alcohol in rats (Heidbreder and De Witte 1993; Imperato and DiChiara 1986; Kiianmaa et al. 1995; Murphy et al. 1988; Yan 1999; Yoshimoto et al. 1992a, 2000). The increased accumbal DA release originates in the ventral tegmental area (VTA), as demonstrated by increased firing rates of VTA DA neurons (Brodie et al. 1990; Gessa et al. 1985). Accumbal DA increases in rats that self-administered alcohol but generally not sweet solutions (Fadda et al. 1989; Katner et al. 1996; Melendez et al. 2002; Nurmi et al. 1998; Weiss et al. 1993). However, it is possible to see increased accumbal dopamine after intake of a sucrose solution but only under some conditions such as after water restriction or passive administration (e.g., Hajnal et al. 2004).

Activation of the accumbal dopaminergic system characterizes a range of behaviors, which are under the control of many contingencies ranging from intensely rewarding cocaine injections to copulatory acts (Hull et al. 1993; Hurd et al. 1989; Mas et al. 1990; Pettit and Justice 1991; Pfaus et al. 1990; Wise et al. 1995) and food-related behavior (Bassareo and Di Chiara 1997; Hernandez and Hoebel 1988; Rada et al. 2005; Wilson et al. 1995). Moreover, ostensibly aversive electric shock pulses (Abercrombie et al. 1989; Imperato et al. 1992; Sorg and Kalivas 1991), and aggressive encounters, either in the aggressor or the victim (Louilot et al. 1986; Mos and Van Valkenburg 1979; Tidey and Miczek 1996; van Erp and Miczek 2000) increase DA levels, possibly reflecting vigilance to facilitate appropriate goal-directed, motivated behavior (Ikemoto and Panksepp 1999).

Supporting evidence for the role of 5-HT in modulating aggressive behavior has been provided by many animal studies in which reduced aggression was observed after administration of directly or indirectly acting serotonergic agonists (e.g., Miczek et al. 2002; Olivier et al. 1995). 5-HT modulates aggressive behavior in interaction with other neurotransmitters, of which corticolimbic DA continues to be of interest for its critical role in integrating motivational and motor functions (Robbins et al. 1989). For example, most serotonergic influence on accumbal dopaminergic neurons has been demonstrated to originate in the dorsal raphe nucleus (e.g., Ase et al. 2000). The dorsal raphe nucleus 5-HT system projecting to the mesolimbic DA system has also been implicated in the regulation of alcohol self-administration (Yoshimoto and McBride 1992). These observations prompted the current investigation into the activity of accumbal DA and 5-HT during the phases of initiation, execution, and termination of alcohol drinking and subsequent fighting using in vivo microdialysis.

In the present study, we assessed dopaminergic and serotonergic activity in a sample of rats with a history of repeated displays of aggressive behavior under the influence of low doses of self-administered alcohol. During the first phase of the experiment, rats were established as residents and evaluated for consistent aggressive behavior when confronted with an intruder (Miczek 1979). During the second phase, the resident rats were trained to voluntarily consume alcohol, using a sucrose-fading technique adapted from Samson (1986). During the third phase, alcohol self-administration was repeatedly followed by a confrontation with an intruder (van Erp and Miczek 1997). During the fourth phase, we assessed dynamic changes in DA and 5-HT in the brains of animals during ongoing alcohol self-administration followed by aggressive behavior, using in vivo microdialysis (van Erp and Miczek 2000). Our previous work focused on n. accumbens and prefrontal cortex. In this paper, we report on DA in the n. accumbens, as the changes in DA due to aggressive behavior were most pronounced in this structure, and we hypothesized that extracellular DA would undergo significant changes in this frequently studied region during drinking and fighting.

Materials and methods

Subjects Male Long–Evans rats (Charles River, Wilmington, MA), weighing 350–375 g at the start, were each housed with a female in a large stainless steel cage (70×45×45 cm) lined with sawdust bedding and fitted with a clear polycarbonate front panel. The cages were equipped with a wooden structure to provide cover and gnawing material. The female rats' fallopian tubes were ligated under ketamine (100 mg/kg) and xylazine (9 mg/kg) anesthesia to prevent changes in behavior due to the presence of pups. Food and water were available without restriction. The cages were kept in a vivarium with controlled temperature (20–21°C) and humidity (40–50%) under a reversed light cycle (lights on between 2000 and 800 hours). All procedures were reviewed and approved by the Tufts University Animal Care and Use Committee, following the principles of the NIH Guide.

Resident–intruder confrontations Three weeks after being housed with a female, the male resident rats confronted a naive male intruder rat (250–300 g) for 5 min, as described previously (Miczek 1979). In brief, the female rat was removed from the resident's cage for the duration of the confrontation. Typically, the resident displays a species-specific pattern of aggressive behavior, consisting of pursuits, threats, and attacks, resulting in defeat of the intruder, as defined by the intruder showing a supine posture for at least five consecutive seconds and emitting 20–30 kHz ultrasonic vocalizations (Miczek and de Boer 2005). Latency to the first attack and total number of attack bites were monitored. The confrontation was terminated after 20 attack bites or after 5 min, whichever came first. Typically, two to five confrontations were scheduled to establish reliable responses; animals that consistently showed more than ten bites in 5 min proceeded to the next phase.

Alcohol self-administration We adapted the principles of the sucrose-fading technique (Samson 1986) as described in detail elsewhere (van Erp and Miczek 2000). During the period of acquiring ethanol self-administration, no intruder confrontations were conducted. In brief, the female was removed, and the males were confined to a smaller (24×20×18 cm) wire cage inside the large home cage and presented for 30 min with a sucrose/alcohol solution. Sessions were conducted 7 days/week at 800 hours, at the beginning of the dark phase, and food was withheld overnight to ensure rapid alcohol uptake. Twenty-five milliliters of solution was presented in a plastic centrifuge tube fitted with a curved ball-type sipper tube (Ancare, Bellmore, NY) to reduce spillage. Bottles were weighed before and after the access period to assess the amount consumed. At first, a 10% sucrose (w/v) solution in tap water was presented for 1 week. Subsequently, alcohol was added in increasing concentrations up to 10% alcohol (v/v). After intake stabilized, the sucrose concentration was decreased slowly until the rats consumed a 10% alcohol solution without sucrose. During the next 10 days, the access period was reduced to 15 min. Typically, the period of acquiring consistent alcohol consumption lasted 5 to 6 weeks.

Alcohol self-administration and aggression Animals were maintained daily on 10% alcohol throughout the remainder of the study. Twice a week for several months, alternating alcohol and water self-administration was followed immediately by a 5-min intruder confrontation, as described previously (van Erp and Miczek 1997). The confrontations were conducted in a separate room to avoid disturbances of other animals. All confrontations were recorded on videotape and analyzed in detail for frequency and duration of all salient acts and postures of aggressive behavior. Immediately after each confrontation, a 0.1-ml blood sample was taken from the retroorbital sinus under isoflurane anesthesia. Blood was stored in trichloroacetic acid at 4°C until further analysis with a nicotinamide adenine dinucleotide–blood alcohol assay (Sigma Chemical, St. Louis, MO) and fluorescent detection. Behavior data were analyzed to determine which residents showed increased aggression after alcohol self-administration relative to fighting after water self-administration (Miczek et al. 1992; van Erp and Miczek 1997).

Behavior analysis Behavioral responses were analyzed using customized software (Tufts data acquisition program [see Miczek 1982]). The frequency and duration of social, aggressive, and other behaviors were recorded in detail (Miczek 1979; Miczek and de Boer 2005; van Erp and Miczek 1997). Duration of aggressive behavior was calculated by adding the duration for aggressive posture, sideways threat, dragging, and chasing.

Surgery and recovery Nine animals were implanted unilaterally and ten bilaterally with CMA/12 guide cannulae (CMA/Microdialysis, North Chelmsford, MA) aimed 2 mm above the n. accumbens at the coordinates AP +2.0, ML ±1.5, DV −6.0 from bregma, according to Paxinos and Watson (Paxinos and Watson 1997). Initially, animals (n=9) were implanted unilaterally; when it appeared that the experimental set-up was feasible and data were collected successfully, the next batch (n=4) was implanted bilaterally to maximize the amount of data obtained from each animal. A final batch of animals (n=6) was implanted with one probe aimed at the n. accumbens and another aimed at the prefrontal cortex (data not reported). Based on experience in our laboratory, stereotaxic adjustments were made to accommodate the size of the animals that weighed 500–650 g at this time. The head-mount was adapted for intruder

confrontations by adding metal eyelets on either side, allowing for a sturdy connection between the head-mount and the protective wire spring around the microdialysis tubing at the time of probe insertion. A panel was lowered into the large home cage to restrict access to the front half of the cage (35×45×45 cm), which had a sliding roof with a hole for the microdialysis tubing. During 1 week of recovery after surgery, daily alcohol sessions were conducted, and the resident was housed with the female. Two confrontations were conducted to confirm that the animals were aggressive towards an intruder after they consumed alcohol. Two animals failed to show aggression after surgery and were excluded from the experiments.

Microdialysis protocol The night before the microdialysis experiment, the female and any remaining food were removed, and the cage was moved to a quiet room. A CMA/12 microdialysis probe (800 μm OD, 2-mm exposed membrane) was lowered into the n. accumbens under isoflurane inhalation anesthesia. The probe was perfused with artificial cerebrospinal fluid (147 mM NaCl, 1.3 mM $CaCl_2$, 0.9 mM $MgCl_2$, 4.0 mM KCl, pH 6.5–7.0) at a rate of 0.5 μl/min, using a CMA/100 pump. A swivel arm (Med Associates, Georgia, VT), dual channel swivel (Instech, Plymouth Meeting, PA), and a 45-cm spring wire protecting the microdialysis tubing (FEP tubing, CMA) allowed free movement of the animal. The animal was housed singly overnight for approximately 16 h in its modified home cage to allow for neurotransmitters and behavior to reach a stable baseline. On the experimental day, the pump flow was doubled to 1.0 μl/min. Details of sample collection (every 10 min) and high-performance liquid chromatography (HPLC) analysis are described elsewhere (van Erp and Miczek 2000).

Starting at 800 hours, two sessions were conducted in counterbalanced order, separated by at least 1 h or when baseline values were recaptured. One session consisted of a resident–intruder confrontation only, and the other session included alcohol self-administration followed by a confrontation. Baseline samples were collected for 60 min, followed by 10-min alcohol access, 10-min rest, a 10-min confrontation, and at least 80-min recovery. The confrontation consisted of the introduction of an experimentally naive intruder rat into the resident's cage, as described above. The intruder was removed after 10 min, corresponding to one microdialysis sample collection period. Behavior was recorded on videotape during alcohol access and during the confrontation. Animals with bilateral cannulae were allowed to recover for at least 1 week before an identical experiment was performed with a probe inserted into the contralateral side of the brain.

Data analysis and statistics DA and 5-HT baseline levels were calculated individually by averaging five or six baseline samples. Neurotransmitter levels were expressed as percent baseline for each individual. Group data were analyzed by a one-way repeated measures analysis of variance (RM ANOVA) of 14 or 16 samples (5 during baseline, 2 during alcohol self-administration plus intermission (optional), 1 during the confrontation, and 8 afterwards). If a significant difference was observed, the ANOVA was followed by planned paired *t*-tests comparing the baseline to each time point during and after alcohol self-administration and the subsequent confrontation. The final number of animals in each group varied due to animals failing to drink or fight or due to technical difficulties with the probes or HPLC analysis.

Results

Alcohol consumption Rats showed reliable daily alcohol consumption, leading to blood alcohol concentrations of up to 85 mg/dl (Fig. 1, top). Because no blood samples could be obtained during microdialysis, blood alcohol concentrations were estimated from the amount of time spent drinking and the amount of fluid consumed (Fig. 1, bottom). During microdialysis, the animals drank for 30 to 335 s, with an average alcohol intake of 0.4 g/kg (Table 1) and an estimated blood alcohol level of 15–80 mg/dl. One animal that failed to drink alcohol during microdialysis was excluded from the data analysis.

Aggressive behavior Before the microdialysis experiments, all animals showed reliable levels of aggression, with an average of ten bites and 120 s of aggressive behavior during a 5-min confrontation. Seven of the fifteen animals showed alcohol-heightened aggression (AHA; Fig. 4, inset; [van Erp and Miczek 1997]). During the actual sampling for the microdialysis experiment, the group as a whole did not show heightened aggression (Table 1). Under the tethered microdialysis conditions, animals showed similar levels of aggression as before surgery, except for a lower number of bites (about five; Table 1). Due to the restrictions of the tether, aggressive behavior consisted of sideways threat, pinning the intruder down into the submissive-supine posture while assuming the aggressive posture and dragging the intruder, rather than roll-and-tumble fights that would frequently occur without the tether. Two animals that appeared lethargic during microdialysis and two others who did not show aggression were excluded from the data analysis.

Microdialysis Data were collected successfully from 17 probes. One probe was excluded due to improper placement

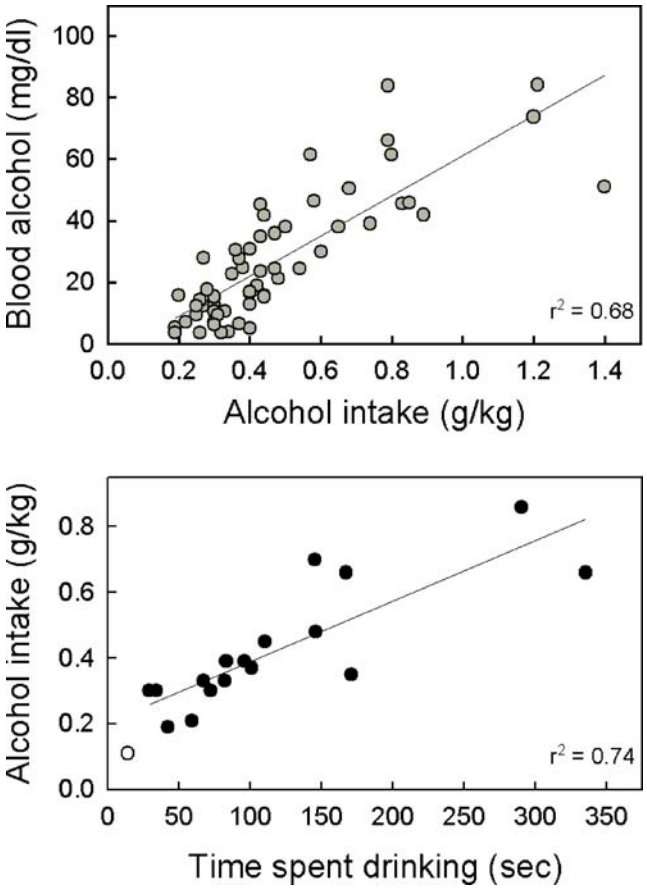

Fig. 1 Correlation between alcohol consumption and blood alcohol levels. *Top* Alcohol consumption during the training phase. Animals had access to 10% alcohol for 15 min, which was followed by a 5-min confrontation with a male intruder. Blood samples were obtained immediately after the confrontation. *Bottom* Alcohol consumption during microdialysis. Because no blood sample could be obtained, blood alcohol levels were estimated based on the amount of fluid consumed and the time spent drinking. One animal (*open symbol*) did not drink sufficient levels of alcohol and was excluded from data analysis

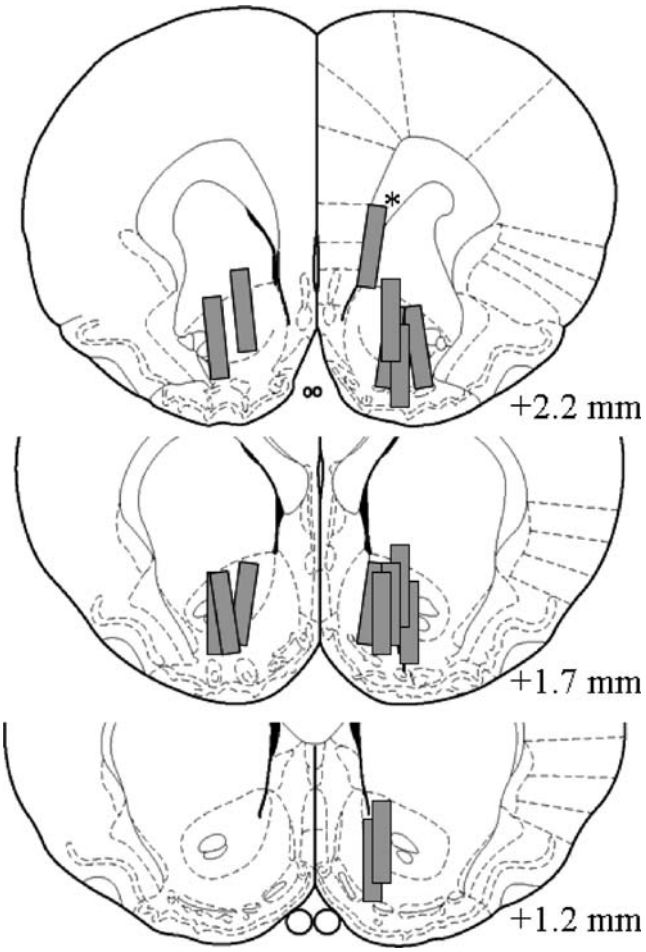

Fig. 2 Histological representation of probe placements in n. accumbens. Coronal sections were reproduced from Paxinos and Watson (1997). *Vertical bars* represent the 2-mm exposed membrane of each microdialysis probe. Probes were implanted at random in the left or right hemisphere. The *asterisk* indicates a probe placement that was excluded from data analysis

(Fig. 2). Basal probe recovery (expressed in pg/μl) was 1.89±0.43 for DA and 0.63±0.35 for 5-HT. Accumbal DA levels tended to increase in anticipation of the alcohol self-administration session, reaching significance immediately after the alcohol session and remaining significantly elevated during and after the subsequent aggressive encounter (one-way RM ANOVA: subjects df=14, samples df=15, F=2.860, p<0.001). A separate RM ANOVA performed on the five baseline samples approached significance (df=14, 4; F=2.121; p<0.10), indicating a trend for increased DA levels immediately preceding the alcohol self-administration session. 5-HT levels were not changed significantly (one-way RM ANOVA: df=13, 15; F=0.917; p>0.10; Fig. 3, bottom). Because the RM ANOVA did not reach significance, no post hoc testing was performed; although it may appear that 5-HT levels tended to increase preceding the confrontation, this could not be confirmed statistically (Fig. 3, bottom). In the absence of alcohol self-administration, neither DA nor 5-HT levels were changed significantly (one-way RM ANOVA: DA, df=13, 13; F=1.017; p>0.10; 5-HT, df=12, 13; F=1.192; p>0.10; Fig. 3,

Table 1 Behavioral responses observed during microdialysis

	No alcohol (n=14)	Alcohol (n=15)
Alcohol drinking (s)	NA	117.8±22.6
Alcohol intake (g/kg)	NA	0.41±0.05
Bite frequency	6.4±1.2	4.7±0.9
Aggression duration (s)	131.7±20.5	109.9±16.5

Resident males confronted a smaller male intruder in their home cage for 10 min. A 10-min alcohol access period preceded 50% of the confrontations. Data are expressed as averages±standard error of the mean. There were no significant differences in aggressive behavior between confrontations with and without alcohol (Student's t-test).
NA not applicable.

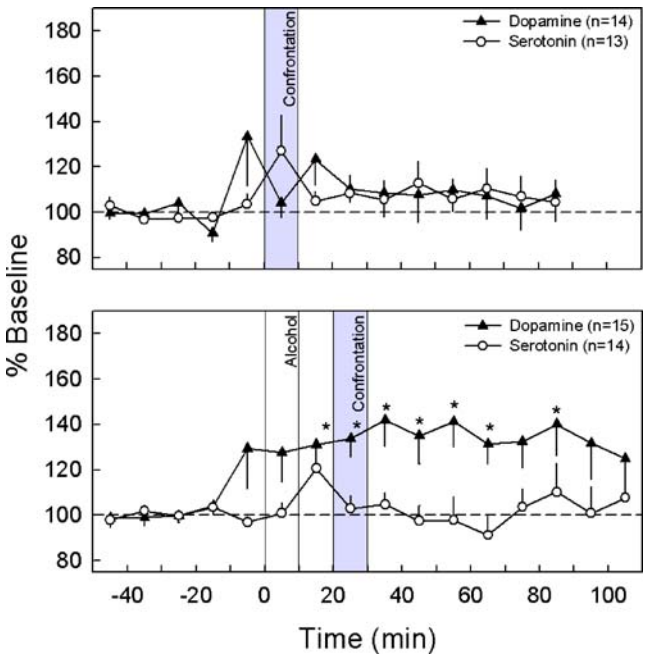

Fig. 3 Monoamine extracellular concentrations in n. accumbens in association with alcohol self-administration and aggressive behavior. Ten-minute microdialysis samples were collected in the n. accumbens of a male resident rat before, during, and after a 10-min confrontation with a smaller male intruder. *Top* Confrontation without alcohol self-administration (n=14). *Bottom* Confrontation preceded by alcohol self-administration (n=15). *Filled symbols* DA; *open symbols* 5-HT. *Vertical bars* indicate the 10-min alcohol access period and the intruder confrontation as indicated. *Asterisks* indicate a significant change from baseline levels, as assessed by one-way RM ANOVA followed by planned paired t-tests ($p<0.05$)

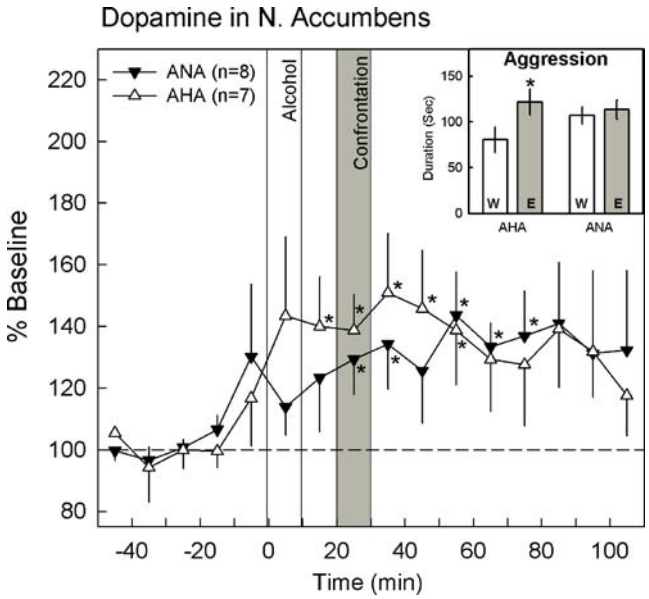

Fig. 4 Dopamine levels during ethanol self-administration and a subsequent confrontation. Dopamine levels increased during alcohol drinking and subsequent aggressive behavior in resident rats that had previously exhibited AHA (*open symbols*) and also in rats that showed ANA (*filled symbols*). No significant differences were observed between the two groups in dopamine or serotonin levels (serotonin not shown). *Inset* Heightened aggression was observed during confrontations preceded by alcohol consumption compared to confrontations preceded by water consumption in a subset of animals (see van Erp and Miczek 2000). Data were obtained during a series of at least ten confrontations prior to probe implantation. *Asterisks* indicate a significant change from baseline levels, as assessed by one-way RM ANOVA followed by planned paired t-tests ($p<0.05$). *AHA* alcohol-heightened aggression, *ANA* alcohol non-heightened aggression, *W* water consumption, *E* ethanol consumption

top). A trend for an increase in DA was observed immediately preceding the intruder confrontation, but this change did not reach statistical significance.

Animals that had a history of AHA showed similar changes in n. accumbens dopamine levels during ethanol drinking and the subsequent confrontation relative to animals that did not have a history of alcohol non-heightened aggression (ANA; Fig. 4). The changes failed to reach significance in either group (one-way RM ANOVA: AHA, df=6, 11; F=1.587; $p>0.10$; ANA, df=7, 11; F=1.065; $p>0.10$) although planned paired t-tests showed significantly increased dopamine levels relative to baseline in both groups at the time of the confrontation (t=20 min: AHA, df=6, T=3.289, $p<0.05$; ANA, df=7, T=2.484, $p<0.05$) and thereafter.

Discussion

The current experimental approach enabled the characterization of behavioral and accumbal DA and 5-HT activity during alcohol self-administration followed by an aggressive encounter in rats. In the current animals with extensive experience of daily alcohol drinking, an anticipatory increase in accumbal DA concentrations immediately before the alcohol self-administration session began to appear. In contrast with previous studies in our laboratory, no significant changes in accumbal DA or 5-HT were observed in association with the aggressive encounter, although the DA increase associated with alcohol consumption was sustained throughout the aggressive encounter and remained elevated for 1 h afterwards, which is well beyond the period during which the relatively low alcohol concentration consumed would be expected to be pharmacologically active.

The tendency for an anticipatory DA increase is reminiscent of a similar anticipatory increase observed in a previous study in our laboratory, in which rats were subjected to a daily intruder confrontation at the same time each day for ten consecutive days (Ferrari et al. 2003). In the absence of actual aggressive behavior on the 11th day, the resident rats showed increased DA immediately preceding the time at which they used to start a confron-

tation on previous days, whereas 5-HT decreased thereafter (Ferrari et al. 2003). In these rats, increased DA reflected neural and behavioral activation in anticipation of the confrontation because the animals became more active in parallel with the DA increase. The 5-HT decrease may reflect inhibition of aggressive behavior because the animals anticipated a confrontation and appeared to be prepared for it—as shown by increased heart rate and motor activity—but did not execute aggressive acts.

Other studies have demonstrated increased accumbal DA in anticipation of alcohol access. For example, Melendez et al. (2002) reported increased accumbal DA levels in alcohol-preferring P-rats expecting access to alcohol as reinforcement for operant responding; no changes were observed in rats expecting saccharine. A small but significant increase in accumbal DA levels was observed during a 20-min waiting period before access to an alcohol solution serving as reinforcement (Katner and Weiss 1999). In contrast, two other studies failed to find accumbal DA increases in anticipation of access to alcohol (Doyon et al. 2003; Nurmi et al. 1998). Our study design resembled the repeated limited access paradigm used by Nurmi et al.; however, we did not provide external cues other than that alcohol access was provided at the same time every day for several months. Increases in accumbal DA activity are readily seen when an animal is exposed to conditioned stimuli associated with and predictive of primary alimentary rewards (Schultz et al. 1998; Bassareo and Di Chiara 1997).

Under the current conditions, accumbal DA levels increased to 140% of baseline levels in animals that drank relatively small amounts of alcohol on a daily basis, leading to blood alcohol levels of up to 80 mg/dl. The maximum increase was observed about 30 min after consumption, at which time the animals were engaged in a confrontation with an intruder. It is possible that the DA peak was not enhanced by the display of aggression but can be explained solely by the uptake, distribution, and pharmacological actions of ethanol because it takes some time before the maximum effect is observed. We did not assess the time-course of the DA response to alcohol consumption in animals without a subsequent aggressive encounter. Although this would have been informative, such data have been reported previously (Weiss et al. 1993; Yoshimoto et al. 1992a,b). A similar delay in DA response after alcohol consumption was observed by Bassareo et al. (2003) who observed a biphasic increase in accumbal DA, with an early rise related to taste and a late rise related to alcohol levels in the dialysate. Moreover, in our previous study, the DA increase related to aggression peaked 20 min after the confrontation ended (van Erp and Miczek 1997), supporting the scenario that the confrontation contributed to sustained increased DA levels over a longer period of time—well past the time period expected to be sustained through alcohol's pharmacological action—but not to a higher DA peak. In contrast to our previous study however, the current study failed to find increased accumbal DA levels during and after aggressive encounters when the encounter was not preceded by alcohol access. The reason for the lack of a DA increase in the current study is not entirely clear but may be related to the fact that the animals were conditioned to daily alcohol consumption and the aggressive confrontation was unexpected.

The current experiments did not reveal significant changes in accumbal 5-HT associated with alcohol consumption or aggression. There is evidence for increased accumbal 5-HT release after alcohol administration (Yan 1999; Yoshimoto et al. 1992b) or self-administration (Weiss et al. 1996), but the amount of alcohol was significantly larger in the latter studies. The lack of change in accumbal 5-HT levels during the aggressive encounter is in agreement with our previous study in resident rats that did not have a history of alcohol consumption (van Erp and Miczek 2000). In the medial prefrontal cortex, 5-HT levels decrease during and after the intruder confrontation (van Erp and Miczek 2000). In addition, we observed decreased accumbal 5-HT in animals that expected a confrontation at the same time as on the previous ten days but did not actually engage in fighting on the 11th day (Ferrari et al. 2003). It is possible that the stimulatory effect of alcohol on accumbal 5-HT and the possible inhibitory effects of aggression have interfered with each other in the current experiment. The differential 5-HT responses in accumbens and prefrontal cortex warrant further research.

Several of the animals included in the current study had a history of heightened aggression after alcohol consumption (van Erp and Miczek 1997). To evaluate whether their history of aggression may have caused a different response, we analyzed the microdialysis results by (1) grouping the animals according to whether they had shown AHA, (2) grouping animals by the amount of alcohol consumed, and (3) grouping animals by the level of aggression displayed during the microdialysis experiment. We found no difference in dopamine response between animals with a history of heightened aggression after alcohol consumption relative to animals without such history. Analyzing the data by the level of alcohol consumed or level of aggression during the microdialysis experiment did not yield meaningful results due to the small sample size. Interestingly, increased basal levels of accumbal DA and decreased basal levels of 5-HT were found in rats that repeatedly drank alcohol; these changes persisted in the absence of alcohol (De Montis et al. 2004). Whether animals that showed AHA differed from animals that did not show heightened aggression with regard to their basal levels of DA and 5-HT remains to be determined.

Methodological issues limit the interpretation of rises in DA, particularly the precise anatomical delineation of the cell groups from which dialysis samples originate and also the sampling interval across which the measured value integrates. It may be possible to detect much larger increases in DA if the measurements could differentiate between core and shell regions of the n. accumbens as, for example, demonstrated with studies on feeding behavior (Bassareo and Di Chiara 1997), and if they had a higher temporal resolution (e.g., Wise et al. 1995). The present study could not accomplish such time resolution and anatomical precision due to the size of the probes.

In addition, the fact that two experimental sessions were held on the same day may have obscured the anticipatory rise in DA to some extent because anticipation should only occur during the session held at 800 hours, regardless of whether the session included alcohol self-administration or not. This may explain why the anticipatory rise in DA levels was not as sharply defined as in our previous study in aggressive rats awaiting a daily intruder confrontation (Ferrari et al. 2003), and why a weaker, non-significant trend for an increase was observed before intruder confrontations without preceding alcohol consumption. Nonetheless, the current trend agrees with our previous study and strengthens the hypothesis that DA increases occur in animals that anticipate a recurring event that takes place at the same time each day, whether it is alcohol consumption or an intruder confrontation.

In conclusion, our data indicate that rats that freely self-administer alcohol for several months show anticipatory changes in accumbal DA but not 5-HT before their regularly scheduled 15-min alcohol access period. DA levels remained elevated during a subsequent intruder confrontation and for at least 1 h thereafter. In contrast with previous studies, we did not observe an accumbal DA increase associated with aggression in the absence of alcohol self-administration. We hypothesize that regular alcohol drinking has caused long-term changes in these animals' brain monoamines, affecting their response to the aggressive challenge. The current study provided evidence for accumbal DA rises in anticipation of and during alcohol drinking both in animals that are prone to increased aggression when under the influence of alcohol and in those that are not.

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References

Abercrombie ED, Keefe KA, DiFrischia DS, Zigmond MJ (1989) Differential effect of stress on in vivo dopamine release in striatum, nucleus accumbens, and medial frontal cortex. J Neurochem 52:1655–1658

Ase AR, Reader TA, Hen R, Riad M, Descarries L (2000) Altered serotonin and dopamine metabolism in the CNS of serotonin 5-HT_{1A} or 5-HT_{1B} receptor knockout mice. J Neurochem 75:2415–2426

Bassareo V, Di Chiara G (1997) Differential influence of associative and nonassociative learning mechanisms on the responsiveness of prefrontal and accumbal dopamine transmission to food stimuli in rats fed ad libitum. J Neurosci 17:851–861

Bassareo V, De Luca MA, Aresu M, Aste A, Ariu T, Di Chiara G (2003) Differential adaptive properties of accumbens shell dopamine responses to ethanol as a drug and as a motivational stimulus. Eur J Neurosci 17:1465–1472

Blanchard RJ, Hori K, Blanchard DC, Hall J (1987) Ethanol effects on aggression of rats selected for different levels of aggressiveness. Pharmacol Biochem Behav 27:641–644

Brodie MS, Shefner SA, Dunwiddie TV (1990) Ethanol increases the firing rate of dopamine neurons of the rat ventral tegmental area in vitro. Brain Res 508:65–69

Bushman BJ, Cooper HM (1990) Effects of alcohol on human aggression: integrative research review. Psychol Bull 107:341–354

Chance MRA, Mackintosh JH, Dixon AK (1973) The effects of ethyl alcohol on social encounters between mice. J Alcohol 8:90–93

Coccaro EF (1992) Impulsive aggression and central serotonergic system function in humans: an example of a dimensional brain–behavior relationship. Int Clin Psychopharmacol 7:3–12

Crowley TJ, Stynes AJ, Hydinger M, Kaufman IC (1974) Ethanol, methamphetamine, pentobarbital, morphine, and monkey social behavior. Arch Gen Psychiatry 31:829–838

De Montis MG, Grappi S, Gambarana C, Leggio B, Nanni G, Scheggi S, Tagliamonte A (2004) Sardinian alcohol-preferring rats show low 5-HT extraneuronal levels in the mPFC and no habituation in monoaminergic response to repeated ethanol consumption in the NAcS. Brain Res 1006:18–27

Di Chiara G, Bassareo V, Fenu S, De Luca MA, Spina L, Cadoni C, Acquas E, Carboni E, Valentini V, Lecca D (2004) Dopamine and drug addiction: the nucleus accumbens shell connection. Neuropharmacology 47(Suppl 1):227–241

Doyon WM, York JL, Diaz LM, Samson HH, Czachowski CL, Gonzales RA (2003) Dopamine activity in the nucleus accumbens during consummatory phases of oral ethanol self-administration. Alcohol Clin Exp Res 27:1573–1582

Ellman GL, Herz MJ, Peeke HVS (1972) Ethanol in a cichlid fish: blood levels and aggressive behavior. Proc West Pharmacol Soc 15:92–95

Everitt BJ, Parkinson JA, Olmstead MC, Arroyo M, Robledo P, Robbins TW (1999) Associative processes in addiction and reward. The role of amygdala-ventral striatal subsystems. Ann N Y Acad Sci 877:412–438

Fadda F, Mosca E, Colombo G, Gessa GL (1989) Effect of spontaneous ingestion of ethanol on brain dopamine metabolism. Life Sci 44:281–287

Ferrari PF, van Erp AMM, Tornatzky W, Miczek KA (2003) Accumbal dopamine and serotonin in anticipation of the next aggressive episode in rats. Eur J Neurosci 17:371–378

Gessa GL, 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

Giancola PR, Zeichner A (2001) The biphasic effects of alcohol on human physical aggression. J Abnorm Psychology 106:598–607

Hajnal A, Smith GP, Norgren R (2004) Oral sucrose stimulation increases accumbens dopamine in the rat. Am J Physiol Regul Integr Comp Physiol 286:R31–R37

Heidbreder C, De Witte P (1993) Ethanol differentially affects extracellular monoamines and GABA in the nucleus accumbens. Pharmacol Biochem Behav 46:477–481

Hernandez L, Hoebel BG (1988) Food reward and cocaine increase extracellular dopamine in the nucleus accumbens as measured by microdialysis. Life Sci 42:1705–1712

Higley JD, Bennett AJ (1999) Central nervous system serotonin and personality as variables contributing to excessive alcohol consumption in non-human primates. Alcohol Alcohol 34:402–418

Hull EM, Eaton RC, Moses J, Lorrain D (1993) Copulation increases dopamine activity in the medial preoptic area of male rats. Life Sci 52:935–940

Hurd YL, Weiss F, Koob GF, And NE, Ungerstedt U (1989) Cocaine reinforcement and extracellular dopamine overflow in rat nucleus accumbens: an in vivo microdialysis study. Brain Res 498:199–203

Ikemoto S, Panksepp J (1999) The role of nucleus accumbens dopamine in motivated behavior: a unifying interpretation with special reference to reward-seeking. Brain Res Rev 31:6–41

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

Imperato A, Angelucci L, Casolini P, Zocchi A, Puglisi-Allegra S (1992) Repeated stressful experiences differently affect limbic dopamine release during and following stress. Brain Res 577:194–199

Katner SN, Weiss F (1999) Ethanol-associated olfactory stimuli reinstate ethanol-seeking behavior after extinction and modify extracellular dopamine levels in the nucleus accumbens. Alcohol Clin Exp Res 23:1751–1760

Katner SN, Kerr TM, Weiss F (1996) Ethanol anticipation enhances dopamine efflux in the nucleus accumbens of alcohol-preferring (P) but not Wistar rats. Behav Pharmacol 7:669–674

Kiianmaa K, Nurmi M, Nykanen I, Sinclair JD (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 GF (2000) Neurobiology of addiction. Toward the development of new therapies. Ann NY Acad Sci 909:170–185

Koob GF, Roberts AJ, Schulteis G, Parsons LH, Heyser CJ, Hyytia P, Merlo-Pich E, Weiss F (1998) Neurocircuitry targets in ethanol reward and dependence. Alcohol Clin Exp Res 22:3–9

Krsiak M, Borgesova M (1973) Effect of alcohol on behaviour of pairs of rats. Psychopharmacologia 32:201–209

Krug EG, Dahlberg LL, Mercy JA, Zwi AB, Lozito R (eds.) (2002) World report on violence and health. World Health Organization, Geneva

Lagerspetz KMJ, Ekqvist K (1978) Failure to induce aggression in inhibited and in genetically non-aggressive mice through injections of ethyl alcohol. Aggress Behav 4:105–113

Linnoila VMI, Virkkunen M (1992) Aggression, suicidality, and serotonin. J Clin Psychiatry 53:46–51

Lister RG, Hilakivi LA (1988) The effects of novelty, isolation, light and ethanol on the social behavior of mice. Psychopharmacology 96:181–187

Louilot A, Le Moal M, Simon H (1986) Differential reactivity of dopaminergic neurons in the nucleus accumbens in response to different behavioral situations. An in vivo voltammetric study in free moving rats. Brain Res 397:395–400

Mann JJ (1999) Role of the serotonergic system in the pathogenesis of major depression and suicidal behavior. Neuropsychopharmacology 21:99S–105S

Mas M, Gonzalez-Mora JL, Louilot A, Sole C, Guadalupe T (1990) Increased dopamine release in the nucleus accumbens of copulating male rats as evidenced by in vivo voltammetry. Neurosci Lett 110:303–308

McBride WJ, Li TK (1998) Animal models of alcoholism: neurobiology of high alcohol-drinking behavior in rodents. Crit Rev Neurobiol 12:339–369

Mehlman PT, Higley JD, Faucher I, Lilly AA, Taub DM, Vickers J, Suomi SJ, Linnoila M (1994) Low CSF 5-HIAA concentrations and severe aggression and impaired impulse control in nonhuman primates. Am J Psychiatry 151:1485–1491

Melendez RI, Rodd-Henricks ZA, Engleman EA, Li TK, McBride WJ, Murphy JM (2002) Microdialysis of dopamine in the nucleus accumbens of alcohol-preferring (P) rats during anticipation and operant self-administration of ethanol. Alcohol Clin Exp Res 26:318–325

Miczek KA (1979) A new test for aggression in rats without aversive stimulation: differential effects of *d*-amphetamine and cocaine. Psychopharmacology 60:253–259

Miczek KA (1982) Ethological analysis of drug action on aggression, defense and defeat. In: Spiegelstein MY (ed) Behavioral models and the analysis of drug action. Elsevier, Amsterdam, pp 225–239

Miczek KA, Barry H III (1977) Effects of alcohol on attack and defensive-submissive reactions in rats. Psychopharmacology 52:231–237

Miczek KA, de Almeida RMM (2001) Oral drug self-administration in the home cage of mice: alcohol-heightened aggression and inhibition by the 5-HT_{1B} agonist anpirtoline. Psychopharmacology 157:421–429

Miczek KA, de Boer SF (2005) Aggressive, defensive, and submissive behavior. In: Whishaw IQ, Kolb B (eds) The behavior of the laboratory rat: a handbook with tests. Oxford University Press, New York, pp 344–352

Miczek KA, O'Donnell JM (1980) Alcohol and chlordiazepoxide increase suppressed aggression in mice. Psychopharmacology 69:39–44

Miczek KA, Weerts EM, Tornatzky W, DeBold JF, Vatne TM (1992) Alcohol and "bursts" of aggressive behavior: ethological analysis of individual differences in rats. Psychopharmacology 107:551–563

Miczek KA, Weerts EM, DeBold JF (1993) Alcohol, benzodiazepine-$GABA_A$ receptor complex and aggression: ethological analysis of individual differences in rodents and primates. J Stud Alcohol Suppl 11:170–179

Miczek KA, Weerts EM, Haney M, Tidey J (1994) Neurobiological mechanisms controlling aggression: preclinical developments for pharmacotherapeutic interventions. Neurosci Biobehav Rev 18:97–110

Miczek KA, Barros HM, Sakoda L, Weerts EM (1998) Alcohol and heightened aggression in individual mice. Alcohol Clin Exp Res 22:1698–1705

Miczek KA, Fish EW, DeBold JF, de Almeida RMM (2002) Social and neural determinants of aggressive behavior: pharmacotherapeutic targets at serotonin, dopamine and γ-aminobutyric acid systems. Psychopharmacology 163:434–458

Mos J, Olivier B (1988) Differential effects of selected psychoactive drugs on dominant and subordinate male rats housed in a colony. Neurosci Res Commun 2:29–36

Mos J, Van Valkenburg CFM (1979) Specific effect on social stress and aggression on regional dopamine metabolism in rat brain. Neurosci Lett 15:325–327

Murphy JM, McBride WJ, Gatto GJ, Lumeng L, Li TK (1988) Effects of acute ethanol administration on monoamine and metabolite content in forebrain regions of ethanol-tolerant and non-tolerant alcohol-preferring (P) rats. Pharmacol Biochem Behav 29:169–174

Nurmi M, Sinclair JD, Kiianmaa K (1998) Dopamine release during ethanol drinking in AA rats. Alcohol Clin Exp Res 22:1628–1633

Olivier B, Mos J, Van Oorschot R, Hen R (1995) Serotonin receptors and animal models of aggressive behavior. Pharmacopsychiatry 28:80–90

Paxinos G, Watson C (1997) The rat brain in stereotaxic coordinates, 3rd edn. Academic, San Diego

Pettijohn TF (1979) The effects of alcohol on agonistic behavior in the Telomian dog. Psychopharmacology 60:295–301

Pettit HO, Justice JB (1991) Effect of dose on cocaine self-administration behavior and dopamine levels in the nucleus accumbens. Brain Res 539:94–102

Pfaus JG, Damsma G, Nomikos GG, Wenkstern DG, Blaha CD, Phillips AG, Fibiger HC (1990) Sexual behavior enhances central dopamine transmission in the male rat. Brain Res 530:345–348

Rada P, Avena NM, Hoebel BG (2005) Daily bingeing on sugar repeatedly releases dopamine in the accumbens shell. Neuroscience 134:737–744

Robinson TE, Berridge KC (2000) The psychology and neurobiology of addiction: an incentive-sensitization view. Addiction 95:S91–S117

Robbins TW, Cador M, Taylor JR, Everitt BJ (1989) Limbic–striatal interactions in reward-related processes. Neurosci Biobehav Rev 13:155–162

Roizen J (1997) Epidemiological issues in alcohol-related violence. In: Galanter M (ed) Recent Developments in alcoholism. Plenum, New York, pp 7–41

Samson HH (1986) Initiation of ethanol reinforcement using a sucrose-substitution procedure in food- and water-sated rats. Alcohol Clin Exp Res 10:436–442

Schultz W (1998) Predictive reward signal of dopamine neurons. J Neurophysiol 80:1–27

Smoothy R, Berry MS (1983) Effects of ethanol on behavior of aggressive mice from two different strains: a comparison of simple and complex behavioral assessments. Pharmacol Biochem Behav 19:645–653

Sorg BA, Kalivas PW (1991) Effects of cocaine and footshock stress on extracellular dopamine levels in the ventral striatum. Brain Res 559:29–36

Tidey JW, Miczek KA (1996) Social defeat stress selectively alters mesocorticolimbic dopamine release: an in vivo microdialysis study. Brain Res 721:140–149

van Erp AMM, Miczek KA (1997) Increased aggression after ethanol self-administration in male resident rats. Psychopharmacology 131:287–295

van Erp AMM, Miczek KA (2000) Aggressive behavior, increased accumbal dopamine, and decreased cortical serotonin in rats. J Neurosci 20:9320–9325

Weiss F, Lorang MT, Bloom FE, Koob GF (1993) Oral alcohol self-administration stimulates dopamine release in the rat nucleus accumbens: genetic and motivational determinants. J Pharmacol Exp Ther 267:250–258

Weiss F, Parsons LH, Schulteis G, Hyytia P, Lorang MT, Bloom FE, Koob GF (1996) Ethanol self-administration restores withdrawal-associated deficiencies in accumbal dopamine and 5-hydroxytryptamine release in dependent rats. J Neurosci 16:3474–3485

WHO (World Health Organization) (1992) International Statistical Classification of Diseases and Related Health Problems. WHO, Geneva

Wilson C, Nomikos GG, Collu M, Fibiger HC (1995) Dopaminergic correlates of motivated behavior: importance of drive. J Neurosci 15:5169–5178

Wise RA, Newton P, Leeb K, Burnette B, Pocock D, Justice JB, Jr. (1995) Fluctuations in nucleus accumbens dopamine concentration during intravenous cocaine self-administration in rats. Psychopharmacology 120:10–20

Yan QS (1999) Extracellular dopamine and serotonin after ethanol monitored with 5-minute microdialysis. Alcohol 19:1–7

Yoshimoto K, McBride WJ (1992) Regulation of nucleus accumbens dopamine release by the dorsal raphe nucleus in the rat. Neurochem Res 17:401–407

Yoshimoto K, McBride WJ, Lumeng L, Li TK (1992a) Alcohol stimulates the release of dopamine and serotonin in the nucleus accumbens. Alcohol 9:17–22

Yoshimoto K, McBride WJ, Lumeng L, Li TK (1992b) Ethanol enhances the release of dopamine and serotonin in the nucleus accumbens of HAD and LAD lines of rats. Alcohol Clin Exp Res 16:781–785

Yoshimoto K, Ueda S, Kato B, Takeuchi Y, Kawai Y, Noritake K, Yasuhara M (2000) Alcohol enhances characteristic releases of dopamine and serotonin in the central nucleus of the amygdala. Neurochem Int 37:369–376

Zeichner A, Pihl RO (1979) Effects of alcohol and behavior contingencies on human aggression. J Abnorm Psychology 88:153–160