Available online at www.sciencedirect.com
SCIENCE DIRECT
European Journal of Pharmacology 523 (2005) 40–45
www.elsevier.com/locate/ejphar

Local effects of acute ethanol on dopamine neurotransmission in the ventral striatum in C57BL/6 mice

Evgeny A. Budygin, Tiffany A. Mathews, Gennady B. Lapa, Sara R. Jones *

Department of Physiology and Pharmacology, Wake Forest University Health Sciences, School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157, United States

Received 26 May 2005; received in revised form 23 August 2005; accepted 1 September 2005
Available online 14 October 2005

Abstract

In this study, fast-scan cyclic voltammetry in brain slices was used to evaluate the effects of acute ethanol on dopamine terminal release and uptake in the nucleus accumbens of C57BL/6 mice. We found that pharmacologically relevant concentrations of ethanol (20 and 100 mM) did not alter electrically evoked dopamine release, while the highest concentration (200 mM) significantly decreased release (~45%). No significant changes were observed in the rate of dopamine uptake after ethanol (20, 100 or 200 mM). In addition, it was established that a moderate dose (2 g/kg, i.p.) of ethanol did not alter the rate of dopamine synthesis, measured as L-dihydroxyphenylalanine (L-DOPA) accumulation. However, a high dose (5 g/kg, i.p.) of ethanol significantly increased the levels of L-DOPA to 60% above the control value. These data are consistent with earlier findings obtained in brain slices from rats; dopamine release, but not clearance, is affected by acute ethanol.

Keywords: Dopamine; Ethanol; C57BL/6 mouse; Nucleus accumbens; Voltammetry

1. Introduction

Ethanol in low or moderate doses is known to be reinforcing and locomotor-stimulating, similar to many other addictive drugs. These low to moderate concentrations of ethanol activate the dopaminergic pathways of the brain (Noble, 1996; Weiss and Porrino, 2002) through stimulation of cell firing (Mereu et al., 1984; Gessa et al., 1985; Brodie et al., 1999; Brodie and Appel, 2000). Conversely, high doses of ethanol, which produce anesthetic and toxic effects, can suppress dopaminergic activity (Mereu et al., 1984; Imperato and Di Chiara, 1986; Blanchard et al., 1993; Budygin et al., 2001a,b). This is consistent with the hypothesis that dopamine is positively linked to "behavioral alertness" (Schultz, 1994). Dopamine changes with ethanol administration have been observed in several species including rat (Mereu et al., 1984; Gessa et al., 1985; Imperato and Di Chiara, 1986; Brodie et al., 1999; Budygin et al., 2001a,b), mice (Brodie and Appel, 2000; Yavich and Tiihonen, 2000; Hungund et al., 2003; Tang et al., 2003; Zocchi et al., 2003; Robledo et al., 2004), monkey (Bradberry, 2002) and human (Boileau et al., 2003). In rats, it was found that acutely administered ethanol increases the firing rate of dopamine neurons, acting at the level of the cell bodies (Mereu et al., 1984; Gessa et al., 1985), and leads to an increase in dopamine release in the terminal fields (Imperato and Di Chiara, 1986). In contrast, high doses have a tendency to decrease striatal dopamine levels (Blanchard et al., 1993) and this has been suggested to secondarily activate dopamine biosynthesis through disinhibition of autoreceptors (Budygin et al., 2001a,b). In the rat striatum, acute ethanol has no effect on uptake of endogenous dopamine (Budygin et al., 2001a,b). However, acute ethanol may increase the clearance of exogenous dopamine applied directly to the striatum in high concentrations (Wang et al., 1997; Sabeti et al., 2003).

Recent progress in molecular biology has allowed the production of many strains of transgenic, knockout, recombinant inbred and other genetically defined mice which can help to further clarify the neurochemical mechanisms of addictive drugs, including ethanol. Therefore, it is necessary to characterize ethanol-induced changes in dopamine neurotransmission in mouse models with a variety of techniques.

* Corresponding author. Tel.: +1 336 716 8533; fax: +1 336 716 8501.
E-mail address: srjones@wfubmc.edu (S.R. Jones).

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2005.09.006

In addiction research, microdialysis and voltammetry are traditional tools for characterizing the dynamics of brain dopamine (Jones et al., 1999; Budygin et al., 2000). The different parameters of dopamine transmission obtained by these techniques are complementary and together provide a complete picture of extracellular dopamine dynamics. The microdialysis technique samples basal levels of neurotransmitters in the extracellular fluid on the minute time scale, and the results from this approach reveal the steady-state changes that occur in response to a pharmacological challenge (Imperato and Di Chiara, 1986; Blanchard et al., 1993; Budygin et al., 1999, 2000; Bradberry, 2002; Boileau et al., 2003). In contrast, voltammetry, which provides information on the millisecond time scale, is more commonly used to examine presynaptic dopamine release and uptake evoked by depolarizing stimuli and the effects of pharmacological agents on these parameters (Jones et al., 1999; Budygin et al., 1999, 2000, 2001a,b; Yavich and Tiihonen, 2000; Phillips et al., 2003). Recently, several microdialysis studies aimed at characterizing the mechanisms of ethanol-induced changes in dopamine neurotransmission have been performed in the mouse models (Hungund et al., 2003; Tang et al., 2003; Zocchi et al., 2003; Robledo et al., 2004).

A concurrent microdialysis analysis of dopamine and ethanol concentrations across time showed the dopamine response declined faster than ethanol concentrations in the ventral striatum in C57BL/6 mice (Tang et al., 2003). Mechanisms postulated to contribute to the dissociation between the dopamine response and ethanol concentrations were decreased dopamine release and/or increased dopamine uptake.

In the present study, fast-scan cyclic voltammetry was used to clarify the effects of acute ethanol on dopamine release and uptake in the ventral striatum in C57BL/6 mice. In addition, the effect of ethanol on dopamine biosynthesis in this brain region was studied. The primary aim of these studies was to explore the mechanisms of ethanol-induced changes in nucleus accumbens dopamine neurotransmission in the C57BL/6 mouse.

2. Materials and methods

2.1. Animals

C57BL/6 mice (Jackson Laboratories, Bar Harbor, Maine) were housed in groups of three to four per cage with food and water ad libitum on a 12-h light–dark cycle. Experiments were performed in male mice (8–12 weeks old). Experimental protocols adhered to National Institutes of Health Animal Care guidelines and were approved by the Wake Forest University Institutional Animal Care and Use Committee.

2.2. Fast-scan cyclic voltammetry in brain slices

Mice were sacrificed by decapitation and the brains rapidly removed and cooled in ice-cold, pre-oxygenated (95% O_2/5% CO_2) artificial cerebrospinal fluid (artificial CSF). The tissue was then sectioned into 400-μm thick coronal slices containing the nucleus accumbens with a vibrating tissue slicer (Leica VT1000S, Leica Instruments, Wetzlar, Germany). Slices were kept in a reservoir of oxygenated artificial CSF with HEPES buffer (20 mM) at room temperature until required (Budygin et al., 2001b). Thirty minutes before each experiment, a brain slice was transferred to a submersion recording chamber, perfused at 1 ml/min with 32 °C oxygenated artificial CSF, and allowed to equilibrate. Once the extracellular dopamine response to electrical stimulation was stable for three successive stimulations, ethanol (0, 20, 100 or 200 mM) was applied to the nucleus accumbens via the superfusate. Each test was performed in one slice, which served as its own precondition control. Dopamine release was evoked by individual electrical pulses. The rectangular (300 μA, 2 ms/phase, biphasic) pulses were applied every 5 min.

Dopamine was detected using voltammetry as described earlier (Budygin et al., 2002; Phillips et al., 2003). Briefly, voltage sweeps from −400 to 1200 mV and back to −400 mV at a scan rate of 300 V/s were applied every 100 ms. The current obtained at the oxidation potential for dopamine (typically 600 mV) was monitored. Background subtracted cyclic voltammograms were constructed by subtracting the background current obtained before release from the current measured after release. In each case, dopamine was the substance detected, and it was identified by its characteristic cyclic voltammograms. The oxidation current for dopamine was converted to concentration by electrode calibration with 10 μM dopamine at the end of the experiment. Measured time courses of extracellular dopamine before and after ethanol (20, 100 or 200 mM) were analyzed with a Michaelis–Menten based set of kinetic equations (Phillips et al., 2003) to determine the maximal concentration of dopamine released by stimulation and the kinetics of dopamine uptake.

2.3. Biosynthesis experiments

To measure dopamine synthesis rates, mice were injected with the *l*-aromatic acid decarboxylase inhibitor NSD 1015 (Budygin et al., 1999; 2001a, b). Ethanol (2.0 or 5.0 g/kg i.p.) or saline was administered 10 min before 3-hydroxybenzylhdrazine dihydrochloride {NSD 1015, from Sigma-Aldrich (St. Louis, MO)} (50 mg/kg i.p.). The animals were sacrificed 30 min later. The whole brain was quickly removed and placed on a glass plate over ice. The ventral striatum was dissected and homogenized in 0.1 M perchloric acid containing 0.1 mM EDTA and 250 nM 3,4-dihydroxybenzylamine as an internal standard. Homogenates were centrifuged for 10 min at 10,000 ×*g*. Supernatants were filtered through a 0.2-μm filter and analyzed for levels of L-dihydroxyphenylalanine (L-DOPA) using HPLC with electrochemical detection. Samples (10 μl) were injected onto a microbore column (50×2 mm, Kromasil 100 C-18, 3.5 μm, Phenomenex) for separation, followed by detection with a glassy carbon electrode (+0.70 V, Bioanalytical Systems, Inc.). The L-DOPA determination was performed with a mobile phase consisting of 0.1 M monochloracetic acid, 0.05 mM sodium octylsulfanate, 0.1 mM EDTA and 5% acetonitrile (pH=2.90).

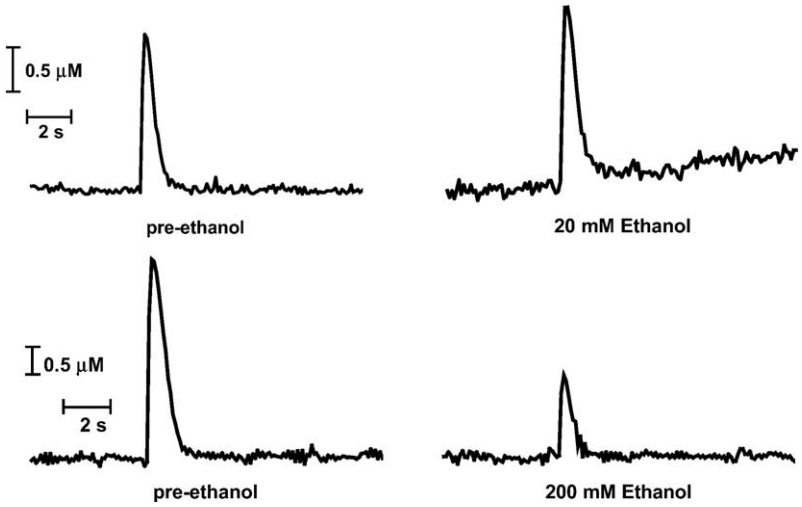

Fig. 1. Dopamine efflux measured by voltammetry in representative mouse nucleus accumbens slices. Single pulses of electrical simulation were applied at 5 min to evoke dopamine efflux. Rising portion of curve indicates release while falling portion is due to uptake through the dopamine transporter. Ethanol at a low concentration did not alter stimulated dopamine release compared with predrug value, but there was significant decrease in this parameter following application of a high ethanol concentration.

2.4. Statistical analysis

Statistical analyses were carried out by a one-way ANOVA and Dunnett's Multiple Comparison Test with GraphPad Prism (Graph Pad Software Inc., San Diego, CA, USA). The data are presented as mean±S.E.M. The criterion of significance was set at $P<0.05$.

3. Results

Electrical stimulation induced a transient increase in extracellular dopamine concentrations (Fig. 1). The rising portion of the dopamine efflux curve is controlled by release and uptake mechanisms, while uptake alone controls the falling portion of the curve. Therefore, the peak amplitude ($[\text{dopamine}]_{max}$) is an index of the balance between release and uptake. In the nucleus accumbens, where all measurements were taken, $[\text{dopamine}]_{max}$ following a single electrical stimulation pulse was 1.85±0.39 μM ($n=9$) for predrug values (Fig. 2). Acutely applied ethanol did not significantly change the $[\text{dopamine}]_{max}$ at pharmacologically relevant concentrations (20 and 100 mM), but there was a significant decrease in this parameter following application of the high ethanol concentration (200 mM) (1.84±0.43, $n=7$, 20 mM; 2.06±0.21, $n=6$, 100 mM and 0.89 ±0.12, $n=9$, 200 mM ethanol) (Fig. 2). For baseline dopamine uptake the responses fit well when K_m was set to 0.16 μM, a value determined in brain synaptosomes (Near et al., 1988), and when V_{max} was set close to 2 μM/s (1.86±0.34 μM/s, $n=8$) (Fig. 3). The effect of 20 and 100 mM ethanol on uptake was best fit without any changes in K_m or V_{max} (1.77±0.38 μM/s, $n=7$ and 2.12±0.18 μM/s, $n=6$, respectively) (Fig. 3). Similar results were obtained with 200 mM ethanol, but in some cases, together with decreased $[\text{dopamine}]_{max}$, V_{max} values appeared to be slightly decreased (1.2±0.2 μM/s, $n=8$) (Fig. 3). However, this effect did not reach significance ($P>0.05$).

To determine whether a decrease in dopamine biosynthesis could explain the ethanol-induced decrease in evoked dopamine,

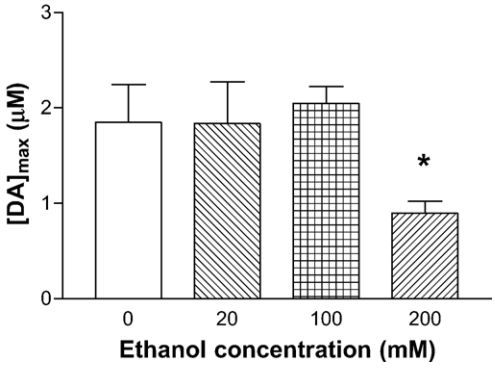

Fig. 2. Summary of effect of ethanol on dopamine release in nucleus accumbens core slices. Ethanol at pharmacologically relevant concentrations (20 and 100 mM) did not alter stimulated dopamine release compared with predrug value; however, dopamine release was decreased following high concentration (200 mM) of ethanol. *$P<0.05$. Data shown are mean±S.E.M. values from 6–9 mice.

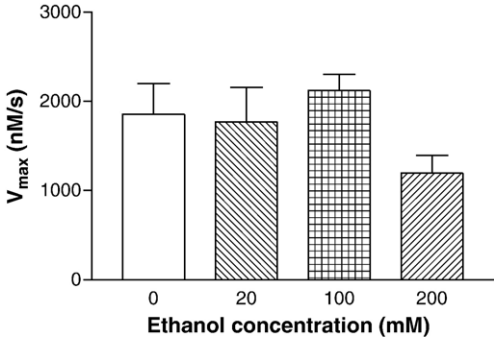

Fig. 3. Summary of effect of ethanol on dopamine uptake in nucleus accumbens core slices. There was no significant change in clearance rate at low or high concentrations of ethanol. Data are mean±S.E.M. values from 6–9 mice.

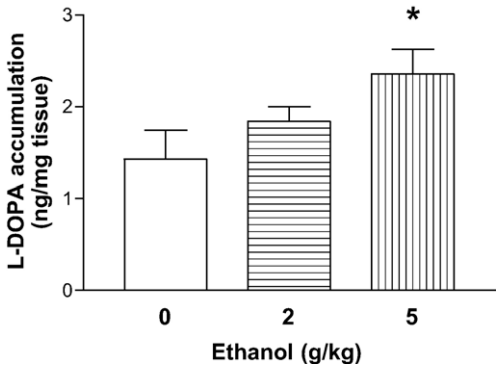

Fig. 4. L-DOPA accumulation in tissue of ventral striatum 40 min after ethanol or saline administration (30 min after NSD 1015). L-DOPA accumulation was significantly greater after 5 g/kg ethanol compared with saline. *$P<0.05$. Data are mean±S.E.M. values from 5 mice.

L-DOPA accumulation was measured in the ventral striatum (Fig. 4). A moderate dose (2 g/kg) of ethanol did not alter L-DOPA accumulation following NSD 1015 in comparison with the saline group (1.84±0.16 and 1.43±0.31ng/mg tissue; $n=5-6$, respectively). The high dose (5 g/kg) of ethanol significantly increased the levels of L-DOPA (2.36±0.27 ng/mg tissue) to 60% above the control value ($P<0.05$, Dunnett's Multiple Comparison Test).

4. Discussion

In the present study, the direct effect of ethanol on dopamine parameters in terminal fields was evaluated. Voltammetry results indicated that electrically evoked dopamine release in mouse nucleus accumbens is affected by acute ethanol in a manner similar to that observed in striatal slices from rat (Budygin et al., 2001a, b) and monkey (Budygin et al., 2003). In the nucleus accumbens, a high concentration of ethanol suppressed dopamine release, whereas low and moderate concentrations had no effect on this parameter. Neither concentration of acute ethanol induced a significant dopamine uptake change. A high dose of ethanol increased dopamine synthesis, as measured by the accumulation of tissue levels of L-DOPA following inhibition of aromatic acid decarboxylase in the ventral striatum of mice. However, no effect of a moderate ethanol dose on dopamine synthesis was observed. Similar results regarding the acute effect of ethanol on striatal L-DOPA accumulation were previously obtained in rats (Budygin et al., 2001a).

These data are consistent with the current hypothesis that the primary mechanism underlying the increase in striatal dopamine with low and moderate doses of ethanol is an increase in the firing rate of dopamine cell bodies located in the ventral tegmental area **or** substantia nigra. In contrast to the effect of ethanol on cell body regions, direct actions of high concentrations of ethanol on terminals may lead to decreases in dopamine release. In fact, the concentration of ethanol (200 mM), which was used in our in vitro experiments, may be toxic or even lethal following systemic injection in naïve animals. However, the presynaptic terminal is quite insensitive to ethanol (Yim et al., 1998; Budygin et al., 2001a; Budygin et al., 2003). For example, local perfusion with several ethanol concentrations showed that low ethanol concentrations, 170 mM or below, failed to change basal dopamine concentration in the nucleus accumbens of freely moving rats (Yim et al., 1998). Therefore, supra-physiological concentrations are required to induce changes in dopamine dynamics when the drug is applied at the level of the nerve terminal. Such results serve to emphasize the fact that ethanol's primary effects on dopamine are not on terminals but on cell bodies.

It is unlikely that the decreases in dopamine release are due to reduced dopamine biosynthesis, because a high dose of ethanol actually enhanced rates of dopamine synthesis in the ventral striatum of mice. Moreover, these changes in biosynthesis following high dose ethanol are probably secondary to a decreased amount of dopamine in the synaptic cleft, resulting in relief of inhibition from synthesis-modulating autoreceptors on the dopamine nerve terminals. Conversely, it has been demonstrated that increasing synaptic dopamine concentrations, for example after uptake inhibition, can lead to a decrease in dopamine synthesis (Budygin et al., 1999). The suppressed dopamine release observed with a high concentration of ethanol in our experiments may correlate with either direct or indirect actions of ethanol on calcium influx (Harris and Hood, 1980; Fredholm and Dunwiddie, 1988; Crews et al., 1996; Budygin et al., 2001b). In this regard, endogenous gangliosides, which are crucially involved in intracellular calcium homeostasis (Wu et al., 2001), could be an important target for ethanol. This suggestion is based on the fact that even a single acute dose of ethanol can significantly decrease the level of brain gangliosides (Klemm and Foster, 1986). This may be important in the anesthetic and toxic effects of ethanol.

Two opposing processes predominantly regulate extracellular dopamine concentrations: presynaptic release of dopamine and its subsequent uptake via the dopamine transporter. The time course of the extracellular dopamine response to ethanol, measured by microdialysis in the ventral striatum of mice, did not match the time course of ethanol concentrations in dialysate (Tang et al., 2003). The mechanism underlying the rapid decrease in dopamine response could be an increase in clearance and/or a decrease in release. Since it is unlikely that acute ethanol exposure could deplete the releasable pool of dopamine, there was ruled out a possibility that decreased dopamine release may be explanation for this dissociation (Tang et al., 2003). Nevertheless, a rapid activation of release-inhibiting autoreceptors in response to the elevated release of dopamine that is induced by ethanol may occur. In the present experiments, in vitro evoked dopamine release was unaffected by ethanol at similar concentrations to those observed by Tang et al. in the ventral striatum of mice (Tang et al., 2003). Since cell bodies were removed in the present slice preparations, there was no ethanol-induced stimulation of dopamine release in the terminal field. However, when dopamine dynamics were measured in vivo in freely moving rats (Budygin et al., 2001a, b) or in anaesthetized BALB/C mice (Yavich and Tiihonen, 2000), evoked dopamine release was decreased by ethanol at pharmacologically relevant concentrations. The difference in

evoked dopamine release could be explained by autoreceptor regulation, at least in part. Therefore, changes in dopamine release could be involved in the rapid neural adaptation to ethanol-induced dopamine responses.

Another possible mechanism that may contribute to the decrease in dopamine response after acute ethanol over time may be dopamine uptake. Our data does not support such a possibility. In fact, ethanol concentrations of 20 mM (close to the 10 mM reported by Tang and co-workers, Tang et al., 2003) and 100 mM, had no effect on dopamine uptake. These results are in agreement with data obtained in anaesthetized BALB/c mice, where no changes in dopamine uptake following acute ethanol injections were reported (Yavich and Tiihonen, 2000). In the case of the highest ethanol concentration (200 mM), dopamine responses were best fit when dopamine release alone was decreased, although a slight decrease in V_{max} could not be ruled out. It is important to note once more that this ethanol concentration is supra-physiological and may induce pronounced intoxication. Therefore, it seems unlikely that dopamine uptake is involved in the dissociation between dopamine and ethanol concentrations that was observed in the ventral striatum of mice by microdialysis (Tang et al., 2003).

These data are consistent with our earlier findings obtained in brain slices from rats and monkeys; dopamine terminal release, but not clearance, is affected by acute ethanol. In addition, increased dopamine biosynthesis may be a secondary effect of high doses of acute ethanol on dopamine dynamics in the ventral striatum. These studies emphasize the utility of voltammetric techniques in providing detailed information on mesolimbic dopamine alterations induced by alcohol, which complement and explain some aspects of existing microdialysis studies. In addition, the alcohol research community has created tremendous behavioral and genetic informational resources from selectively bred, inbred and recombinant inbred mouse strains. Because C57BL/6 mice are commonly used, it is essential to elucidate the details of the neurochemistry of alcohol actions in this mouse strain. Therefore, the C57BL/6 mouse strain is a promising model to study neurochemical mechanisms of ethanol regulation of mesolimbic dopamine neurotransmission.

Acknowledgments

This work was supported by Wake Forest University School of Medicine Venture Funds and National Institutes of Health Grants AA014686-01A1 and AA014091. T.A.M was supported by NIAAA training grant AA0765.

References

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

Boileau, I., Assaad, J.M., Pihl, R.O., Benkelfat, C., Leyton, M., Diksic, M., Tremblay, R.E., Dagher, A., 2003. Alcohol promotes dopamine release in the human nucleus accumbens. Synapse 49, 226–231.

Bradberry, C.W., 2002. Dose-dependent effect of ethanol on extracellular dopamine in mesolimbic striatum of awake rhesus monkeys: comparison with cocaine across individuals. Psychopharmacology 165, 67–76.

Brodie, M.S., Pesold, C., Appel, S.B., 1999. Ethanol directly excites dopaminergic ventral tegmental area reward neurons. Alcohol., Clin. Exp. Res. 23, 1848–1852.

Brodie, M.S., Appel, S.B., 2000. Dopaminergic neurons in the ventral tegmental are of C57BL/6J and DBA/2J mice differ in sensitivity to ethanol excitation. Alcohol., Clin. Exp. Res. 24, 1120–1124.

Budygin, E.A., Gainetdinov, R.R., Kilpatrick, M., Rayevsky, K., Mannisto, P., Wightman, R.M., 1999. Effect of tolcapone, a catechol-O-methyltransferase inhibitor, on striatal dopaminergic transmission during blockade of dopamine uptake. Eur. J. Pharmacol. 370, 125–131.

Budygin, E.A., Kilpatrick, M., Gainetdinov, R.R., Wightman, R.M., 2000. Correlation between behavior and extracellular dopamine levels in rat striatum: comparison of microdialysis and fast-scan cyclic voltammetry. Neurosci. Lett. 281, 9–12.

Budygin, E.A., Phillips, P.E., Robinson, D.L., Kennedy, A.P., Gainetdinov, R.R., Wightman, R.M., 2001a. Effect of acute ethanol on striatal dopamine neurotransmission in ambulatory rats. J. Pharmacol. Exp. Ther. 297, 27–34.

Budygin, E.A., Phillips, P.E.M., Wightman, R.M., Jones, S.R., 2001b. Terminal effects of ethanol on dopamine dynamics in rat nucleus accumbens: An in vitro voltammetric study. Synapse 42, 77–79.

Budygin, E.A., John, C.E., Mateo, Y., Jones, S.R., 2002. Lack of cocaine effect on dopamine clearance in the core and shell of the nucleus accumbens of dopamine transporter knock-out mice. J. Neurosci. 22, 1–4.

Budygin, E.A., John, C.E., Mateo, Y., Daunais, J.B., Friedman, D.P., Grant, K.A., Jones, S.R., 2003. Chronic ethanol exposure alters presynaptic dopamine function in the striatum of monkeys: a preliminary study. Synapse 50, 266–268.

Crews, F.T., Morrow, A.L., Criswell, H., Breese, G., 1996. Effects of ethanol on ion channels. Int. Rev. Neurobiol. 39, 283–367.

Fredholm, B.B., Dunwiddie, T.V., 1988. How does adenosine inhibit transmitter release? Trends Pharmacol. Sci. 9, 130–134.

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.

Harris, R.A., Hood, W.F., 1980. Inhibition of synaptosomal calcium uptake by ethanol. J. Pharmacol. Exp. Ther. 213, 562–568.

Hungund, B.L., Szakall, I., Adam, A., Basavarajappa, B.S., Vadasz, C., 2003. Cannabinoid CB1 receptor knockout mice exhibit markedly reduced voluntary alcohol consumption and lack alcohol-induced dopamine release in the nucleus accumbens. J. Neurochem. 84, 698–704.

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.

Jones, S.R., Gainetdinov, R.R., Caron, M.G., 1999. Application of microdialysis and voltammetry to assess dopamine function in genetically altered mice: correlation with locomotor activity. Psychopharmacology 147, 30–32.

Klemm, W.R., Foster, D.M., 1986. Alcohol, in a single pharmacological dose, decreases brain gangliosides. Life Sci. 39, 897–902.

Mereu, G., Fadda, F., Gessa, G.L., 1984. Ethanol stimulates the firing rate of nigral dopaminergic neurons in unanesthetized rats. Brain Res. 292, 63–69.

Near, J.A., Bigelow, J.C., Wightman, R.M., 1988. Comparison of uptake of dopamine in rat striatal chopped tissue and synaptosomes. J. Pharmacol. Exp. Ther. 245, 921–927.

Noble, E.P., 1996. Alcoholism and the dopaminergic system: a review. Addiction Biol. 1, 333–348.

Phillips, P.E., Johns, J.M., Lubin, D.A., Budygin, E.A., Gainetdinov, R.R., Lieberman, J.A., Wightman, R.M., 2003. Presynaptic dopaminergic function is largely unaltered in mesolimbic and mesostriatal terminals of adult rats that were prenatally exposed to cocaine. Brain Res. 961, 63–72.

Robledo, P., Mendizabal, V., Ortuno, J., de la Torre, R., Kieffer, B.L., Maldonado, R., 2004. The rewarding properties of MDMA are preserved in mice lacking mu-opioid receptors. Eur. J. Neurosci. 20, 853–858.

Sabeti, J., Gerhardt, G.A., Zahniser, N.R., 2003. Chloral hydrate and ethanol, but not urethane, alter the clearance of exogenous dopamine recorded by chronoamperometry in striatum of unrestrained rats. Neurosci. Lett. 343, 9–12.

Schultz, W., 1994. Behavior-related activity of primate dopamine neurons. Rev. Neurol. 150, 634–639.

Tang, A., George, M.A., Randall, J.A., Gonzales, R.A., 2003. Ethanol increases extracellular dopamine concentration in the ventral striatum in C57BL/6 mice. Alcohol., Clin. Exp. Res. 27, 1083–1089.

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., Porrino, L.J., 2002. Behavioral neurobiology of alcohol addiction: Recent advances and challenges. J. Neurosci. 22, 3332–3337.

Wu, G., Xie, X., Lu, Z.-H., Ledeen, R.W., 2001. Cerebellar neurons lacking complex gangliosides degenerate in the presence of depolarizing levels of potassium. Proc. Natl. Acad. Sci. U. S. A. 98, 307–312.

Yavich, L., Tiihonen, J., 2000. Ethanol modulates evoked dopamine release in mouse nucleus accumbens: dependence on social stress and dose. Eur. J. Pharmacol. 401, 365–373.

Yim, H.L., Schallert, T., Randall, P.K., Gonzales, R.A., 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.

Zocchi, A., Girlanda, E., Varnier, G., Sartori, I., Zanetti, L., Wildish, G.A., Lennon, M., Mugnaini, M., Heidbreder, C.A., 2003. Dopamine responsiveness to drugs of abuse: a shell-core investigation in the nucleus accumbens of the mouse. Synapse 50, 293–302.