

Chapter 18

Rising Taurine and Ethanol Concentrations in Nucleus Accumbens Interact to Produce the Dopamine-Activating Effects of Alcohol

Mia Ericson, PeiPei Chau, Louise Adermark,
and Bo Söderpalm

Abstract Alcohol misuse and addiction is a worldwide problem causing enormous individual suffering as well as financial costs for the society. To develop pharmacological means to reduce suffering, we need to understand the mechanisms underlying the effects of ethanol in the brain. Ethanol is known to increase extracellular levels of both dopamine and taurine in the nucleus accumbens (nAc), a part of the brain reward system, but the two events have not been connected. In previous studies we have demonstrated that glycine receptors in the nAc are involved in modulating both basal- and ethanol-induced dopamine output in the same brain region. By means of in vivo microdialysis in freely moving rats we here demonstrate that the endogenous glycine receptor ligand taurine mimics ethanol in activating the brain reward system. Furthermore, administration of systemic ethanol diluted in an isotonic (0.9% NaCl) or hypertonic (3.6% NaCl) saline solution was investigated with respect to extracellular levels of taurine and dopamine in the nAc. We found that ethanol given in a hypertonic solution, contrary to an isotonic solution, failed to increase concentrations of both taurine and dopamine in the nAc. However, a modest, non-dopamine elevating concentration of taurine in the nAc disclosed a dopamine elevating effect of systemic ethanol also when given in a hypertonic solution. We conclude that the elevations of taurine and dopamine in the nAc are closely related and that in order for ethanol to induce dopamine release, a simultaneous

M. Ericson (✉) • P. Chau • L. Adermark
Addiction Biology Unit, Section of Psychiatry and Neurochemistry,
Institute of Neuroscience and Physiology, The Sahlgrenska Academy at University
of Gothenburg, Gothenburg, Sweden
e-mail: Mia.Ericson@neuro.gu.se

B. Söderpalm
Addiction Biology Unit, Section of Psychiatry and Neurochemistry,
Institute of Neuroscience and Physiology, The Sahlgrenska Academy at University
of Gothenburg, Gothenburg, Sweden

Beroendekliniken, Sahlgrenska University Hospital, Gothenburg, Sweden

increase of extracellular taurine levels in the nAc is required. These data also provide support for the notion that the nAc is the primary target for ethanol in its dopamine-activating effect after systemic administration and that taurine is a prominent participant in activating the brain reward system.

Abbreviations

nAc	Nucleus accumbens
GlyR	Glycine receptor
VTA	Ventral tegmental area
nAChR	Nicotinic acetylcholine receptor
DA	Dopamine

18.1 Introduction

Alcoholism is a worldwide chronic disease causing enormous individual suffering as well as socioeconomic costs. The underlying mechanism for development of this disabling disease remains unknown, which is why it is of great importance to study the actions of alcohol in the brain in order to find potential new targets for treating alcohol addiction.

Alcohol, as well as other drugs of abuse, activates the mesolimbic dopamine (DA) system, a central part of the brain reward system, resulting in increased DA release in the nucleus accumbens (nAc) (DiChiara and Imperato 1988; Wise and Rompre 1989; Drevets et al. 1999; Boileau et al. 2003), which has been associated with the reinforcing properties of the drugs. In a series of studies we have demonstrated that glycine receptors (GlyR) located in the nAc are involved in modulating both basal- and ethanol-induced dopamine output in the same brain region (Molander and Söderpalm 2005a, b). We found that ethanol as well as glycine increased DA in the nAc and that this is executed in a GlyR-dependent manner. These effects are not local but involve activation of nicotinic acetylcholine receptors (nAChRs) in the ventral tegmental area (VTA), possibly due to inhibition of GABAergic projection neurons modulating acetylcholine release in the VTA (Ericson et al. 2003; Larsson et al. 2005; for review see Söderpalm et al. 2009).

Besides glycine there are several amino acids with affinity for the GlyR (Pan and Slaughter 1995). Taurine has been demonstrated to act as an agonist or a partial agonist at the GlyR and has been attributed inhibitory, neuromodulatory, neuroprotectant and osmoregulatory properties, to mention a few (Huxtable 1989; 1992; Saransaari and Oja 2000; Olive 2002). Interestingly, taurine levels in the nAc increase after systemic and local ethanol administration (De Witte et al. 1994; Dahchour et al. 1996; Adermark et al. 2011), an increase that is reduced and abolished by increased osmolarity of the ethanol solution (Quertemont et al. 2003). Taurine administration also decreases ethanol intake and alters ethanol aversion (Quertemont et al. 1998).

In addition, chronic ethanol administration decreases taurine levels in the whole brain, an effect that returns to normal upon ethanol withdrawal (Iwata et al. 1980). Whether ethanol-induced taurine and dopamine release are connected events or separate from each other has not been determined.

In the present study we aimed to explore whether taurine per se can influence DA output and if manipulation of the ethanol-induced elevation of extracellular taurine levels influences the DA output after ethanol administration. To this end we used *in vivo* microdialysis in freely moving Wistar rats while monitoring both DA and taurine.

18.2 Methods

18.2.1 *In Vivo Microdialysis*

Male Wistar rats were implanted with a custom made I-shaped dialysis probe in the nAc alone or in combination with a probe in the VTA, as previously described (Lidö et al. 2009). Two days after surgery the sealed inlet and outlet of the probes were cut open and connected to a microperfusion pump via a swivel allowing the animal to move around freely. The probes were perfused with Ringer solution at a rate of 2 μ l/min and dialysate samples (40 μ l) were collected every 20 min. The rats were perfused with Ringer solution for 1 h in order to obtain a balanced fluid exchange before baseline sampling began. Dopamine and taurine were analyzed in the dialysis samples by means of HPLC as previously described (Lidö et al. 2009). Animals were sacrificed directly after the experiment, brains were removed, and probe placements were verified using a vibroslicer. Only rats with correctly placed dialysis probes were included in statistical analysis.

18.2.2 *Experimental Design*

In the first set of experiments rats were perfused with vehicle (Ringer) or taurine (1, 10, or 100 mM) via reversed dialysis in the nAc. Following this the rats received pretreatment with either the GlyR antagonist strychnine (2 μ M perfused in the nAc) or the nAChR antagonist mecamylamine (100 μ M perfused in the VTA) before administration of 10 mM taurine via the nAc dialysis probe. Extracellular levels of DA were monitored in the nAc for 3 h after drug administration.

In the second set of experiments four groups of drug naïve rats received an acute injection of 0.9% NaCl (i.p.), 3.6% NaCl (i.p.), ethanol 2.5 g/kg diluted in 0.9% NaCl (i.p.), or ethanol 2.5 g/kg diluted in 3.6% NaCl (i.p.). Half of the animals in each group received the addition of 50 μ M taurine in the perfusate (nAc) at the time of injection. Extracellular levels of DA and taurine were monitored in the nAc for 3 h after the systemic injection.

18.2.3 *Statistical Analysis*

Data were statistically evaluated using Student's *t*-test or a one-way ANOVA followed by Fishers PLSD. A probability value (*P*) less than 0.05 were considered statistically significant. All values are expressed as means \pm SEM.

18.3 Results

18.3.1 *Taurine Mimics the Dopamine Elevating Properties of Ethanol*

In the first study, where different concentrations of taurine were administered into the nAc by reversed microdialysis, the two higher concentrations (10 or 100 mM in the perfusate) increased nAc DA output while the low dose (1 mM) had no effect. The medium concentration, 10 mM, elevated DA in a pattern similar to ethanol over the 3 h of measuring, which is why this concentration was selected for further studies (Fig. 18.1).

Administration of the GlyR antagonist strychnine (2 μ M locally in the nAc) alone did not influence the DA levels. However, strychnine perfusion 40 min prior to co-perfusion with taurine (10 mM in the nAc) completely abolished the DA elevating effects of taurine, just as previously demonstrated with ethanol (Ericson et al. 2003). Also in line with studies on the DA elevating effects of ethanol, administration of the nAChR antagonist mecamylamine (100 μ M locally in the VTA) prevented taurine (10 mM in the nAc) from increasing accumbal DA levels (Fig. 18.1).

18.3.2 *Ethanol, Dopamine, and the Necessity of Taurine*

In the second set of experiments, we explored the extracellular DA as well as taurine response to ethanol when administered in a normal (0.9%) or hypertonic (3.6%) saline solution. In line with the findings from Quertemont et al. (2003), we found that ethanol diluted in a hypertonic saline solution (3.6%) completely prevented the ethanol-induced increase of taurine 40 min after the administration, whereas systemic ethanol diluted in an isotonic saline solution (0.9%) elevated the extracellular levels of taurine by approximately 50%. None of the saline solutions influenced the taurine levels per se (Fig. 18.2a). Furthermore, concomitant measurement of DA in the same samples revealed an ethanol-induced DA response similar to that of taurine. Ethanol diluted in a hypertonic saline solution was unable to increase DA output in the nAc, whereas when administered in an isotonic saline solution ethanol produced the expected increase (Fig. 18.2b).

In a final set of rats we added a small amount of taurine (50 μ M in the nAc perfusate), unable to influence DA per se, at the time of the systemic injection (saline

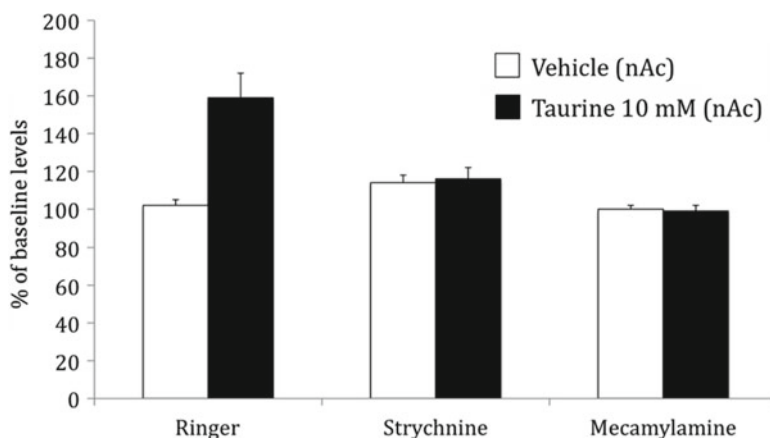


Fig. 18.1 Effect of taurine (10 mM) or vehicle (Ringer) administration on extracellular dopamine levels in the nucleus accumbens 40 min after initiation of taurine/vehicle administration as measured by in vivo microdialysis. The rats received no pretreatment (Ringer), 40 min pretreatment with the glycine receptor antagonist strychnine (2 μ M perfused locally in the nAc) or 40 min pretreatment with the nAChR antagonist mecamylamine (100 μ M perfused locally in the VTA). Taurine increased dopamine levels, an effect that was prevented by both accumbal strychnine treatment and ventral tegmental mecamylamine treatment. Data are presented as means \pm SEM, $n=8-12$

or ethanol). The addition of taurine completely restored ethanol's ability to increase DA when administered in a hypertonic (3.6%) saline solution (Fig. 18.3b). However, the small amount of taurine did not influence DA levels observed after administration of ethanol in normal (0.9%) saline solution (Fig. 18.3a).

18.4 Discussion

In the present study we found that taurine increases DA in the mesolimbic DA system, a part of the brain reward pathway. More specifically, taurine elevated DA levels in the nAc, a phenomenon that has been linked to positive reinforcement and perhaps also to the development of addiction (Koob 1992; Spanagel 2009). Since ethanol is known to produce enhanced extracellular levels of both taurine and DA it is interesting to note that taurine on its own can raise DA levels. Further studies demonstrated that taurine appears to use the same mechanisms as ethanol to influence DA, since, as with ethanol, pretreatment with either strychnine in the nAc or mecamylamine in the VTA completely abolished both ethanol- and taurine-induced elevations of DA.

In a series of studies we have previously demonstrated the importance of both accumbal GlyRs and ventral tegmental nAChRs for the reinforcing and DA elevating effects of ethanol. Based on these studies we have suggested that ethanol influences DA via a neuronal nAc-VTA-nAc circuitry (Söderpalm et al. 2009) and the data presented here suggest that taurine exerts its effect on DA via the same mechanism.

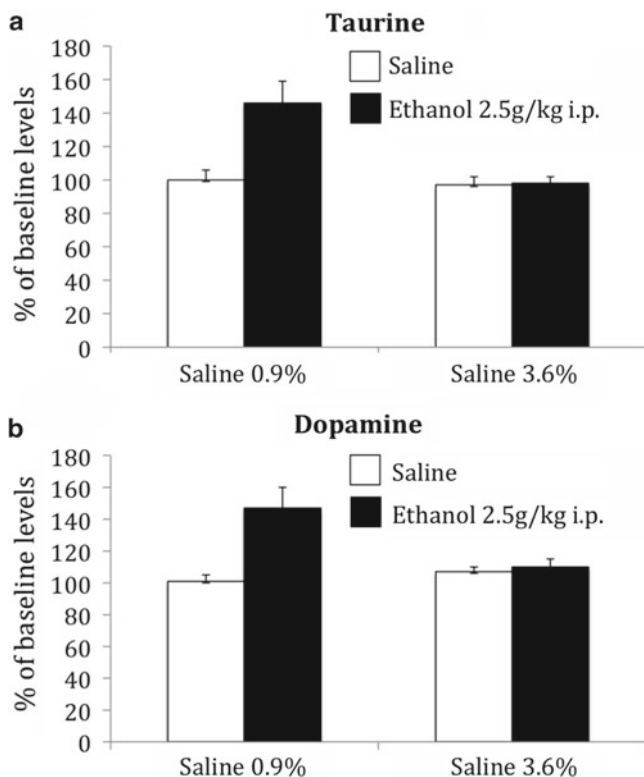


Fig. 18.2 Effect of a systemic injection (i.p.) of 0.9% NaCl, 3.6% NaCl, ethanol (2.5 g/kg) diluted in 0.9% NaCl or ethanol (2.5 g/kg) diluted in 3.6% NaCl on extracellular (a) taurine levels or (b) dopamine levels in the nucleus accumbens 40 min after the systemic injection. Administration of ethanol in a hypertonic saline solution prevented the drug from increasing both taurine and dopamine. Data are presented as means \pm SEM, $n=6-12$ and were detected by using *in vivo* microdialysis in freely moving Wistar rats

An interesting study by Quertemont et al. (2003) demonstrated that the ethanol-induced increase in taurine levels could be modified by altering the osmolarity of the saline solution that ethanol was diluted in. Here we repeated this finding and found a similar phenomenon for taurine and DA when concomitantly measured in the same animal. Several studies have demonstrated the osmoregulatory properties of taurine, where, for example, a change in the sodium milieu surrounding the cells greatly influences taurine release (Korpi and Oja 1983). Ethanol has also been shown to induce cell swelling in astrocytes, which leads to an increased release of taurine into the extracellular space (Kimmelberg et al. 1993; Allansson et al. 2001). In fact, a recent study found that inhibition of ethanol-induced astrocyte cell swelling also prevents the increase in microdialysate concentration of taurine and DA induced by local administration of ethanol in the nAc (Adermark et al. 2011). It is thus possible that taurine is released in response to ethanol-mediated cell swelling, and that this swelling is counterbalanced when ethanol is administered in a hyperosmotic solution. However, it could also be speculated that the administration of high

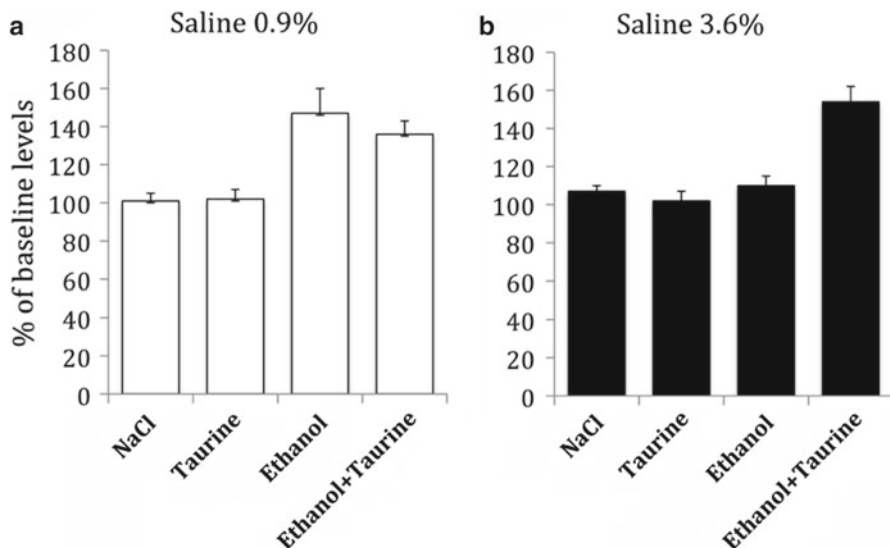


Fig. 18.3 Administration of vehicle (NaCl i.p.), taurine (50 μ M perfused locally in the nAc), ethanol (2.5 g/kg i.p.), or the combination of taurine and ethanol using (a) normal saline solution (0.9% NaCl) or (b) hypertonic saline solution (3.6% NaCl) measuring nucleus accumbens dopamine levels by means of in vivo microdialysis in male Wistar rats. Data are presented as means \pm SEM, $n=6-8$, 40 min after the systemic injection

amounts of sodium disrupts neurotransmission in general, leaving, e.g., the cells unable to release taurine and/or DA or compromising the GlyR previously shown to be involved in the DA releasing effect of ethanol. To address this issue we mimicked the taurine elevation normally induced by ethanol by perfusing a relatively low concentration of taurine in the nAc concomitantly with systemic administration of the hypertonic ethanol solution. The addition of the inert amount of taurine appeared to be the missing component for ethanol to produce the elevation of DA. Overall, these results demonstrate that the hypertonic solution does not compromise mechanisms involved in DA release and indicate that GlyR function is intact under these conditions. Since the concentration of taurine used did not influence DA release per se the results moreover suggest that a concomitant rise in ethanol and taurine concentrations is required in order to obtain DA release after ethanol and that the two substances act in synergy at GlyRs in the nAc. It should be noted that taurine also can act as a ligand at GABA_A receptors, and thus modulate DA output by influencing GABAergic neurotransmission in the nAc. However, taurine-induced currents in medium spiny neurons are only partially depressed by the GABA_A receptor antagonist gabazine indicating that taurine primarily affect neurotransmission in the nAc by interacting with GlyR (Sergeeva and Haas 2001).

These results also may have implications for the debate concerning the site of action of ethanol in its DA activating and reinforcing effects. Based on pharmacological in vivo studies using microdialysis with or without concomitant monitoring

of systemic ethanol intake, we have suggested that the primary site of action is in the nAc (cf. Söderpalm et al. 2009), whereas other investigators, based mainly on *in vitro* studies and on studies of intracerebral self-administration of ethanol argue that the VTA is the important site in this respect (Brodie et al. 1999; McBride et al. 1999). The present finding that local perfusion of an inert concentration of taurine in the nAc completely rescues the DA activating effect of systemic ethanol shows that an ethanol-induced event, i.e., taurine elevation, in this particular area in combination with ethanol itself is extremely important, and, again, points to GlyR in the nAc as the primary site of action for ethanol in this context. The alternative, more far-fetched interpretation would be that a concomitant elevation of taurine in the nAc is a prerequisite for obtaining DA release after some ethanol interaction in the VTA, but this alternative, if true, would still require ethanol-induced taurine release in the nAc.

18.5 Conclusion

Taurine has the ability to increase DA output in the nAc on its own, via mechanisms similar to ethanol. In addition it appears that in order for systemic ethanol to increase DA in the nAc, a phenomenon that has been related to the reinforcing properties of the drug, a concomitant extracellular increase of accumbal taurine is required. The studies presented here suggest that taurine could be a target for development of new pharmacotherapies against alcoholism.

Acknowledgments The authors are thankful for the technical assistance from Mrs Rosita Stomberg. This work was supported by Swedish Medical Research Council (Grants No: 2009-2289, 2009-4477, 2010-3100), governmental support under the LUA/ALF agreement, Wilhelm and Martina Lundgrens Scientific Foundation, the Swedish Brain foundation.

References

- Adermark L, Clarke RBC, Olsson T, Hansson E, Söderpalm B, Ericson M (2011) Implications for glycine receptors and astrocytes in ethanol-induced elevation of dopamine levels in the nucleus accumbens. *Addict Biol* 16(1):43–54
- Allansson L, Khatibi S, Olsson T, Hansson E (2001) Acute ethanol exposure induces $[Ca^{2+}]_i$ transients, cell swelling and transformation of actin cytoskeleton in astroglial primary cultures. *J Neurochem* 76:472–479
- Boileau I, Assaad JM, Pihl RO, Benkelfat C, Leyton M, Diksic M, Tremblay RE, Dagher A (2003) Alcohol promotes dopamine release in the human nucleus accumbens. *Synapse* 49:226–231
- Brodie MS, Pesold C, Appel SB (1999) Ethanol directly excites dopaminergic ventral tegmental area reward neurons. *Alcohol Clin Exp Res* 23(11):1848–1852
- Dahchour A, Quertemont E, De Witte P (1996) Taurine increases in the nucleus accumbens microdialysate after acute ethanol administration to naive and chronically alcoholised rats. *Brain Res* 735:9–19
- De Witte P, Dahchour A, Quertemont E (1994) Acute and chronic alcohol injections increase taurine in the nucleus accumbens. *Alcohol Alcohol Suppl* 2:229–233

- DiChiara G, Imperato A (1988) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc Natl Acad Sci USA* 85:5274–5278
- Drevets WC, Price JC, Kupfer DJ, Kinahan PE, Lopresti B, Holt D, Mathis C (1999) PET measures of amphetamine-induced dopamine release in ventral versus dorsal striatum. *Neuropsychopharmacology* 21(6):694–709
- Ericson M, Molander A, Löf E, Engel JA, Söderpalm B (2003) Ethanol elevates accumbal dopamine levels via indirect activation of ventral tegmental nicotinic acetylcholine receptors. *Eur J Pharmacol* 467:85–93
- Huxtable RJ (1992) Physiological actions of taurine. *Physiol Rev* 72:101–163
- Huxtable RJ (1989) Taurine in the central nervous system and the mammalian actions of taurine. *Prog Neurobiol* 32:471–533
- Iwata H, Matsuda T, Lee E, Yamagami S, Baba A (1980) Effect of ethanol on taurine concentration in the brain. *Experientia* 36:332–333
- Kimelberg HK, Cheema M, O'Connor ER, Tong H, Goderie SK, Rossman PA (1993) Ethanol-induced aspartate and taurine release from primary astrocyte cultures. *J Neurochem* 60:1682–1689
- Koob GF (1992) Neural mechanisms of drug reinforcement. In: Kalivas PW, Samson HH (eds) *The neurobiology of drug and alcohol addiction*, vol 654. The New York Academy of Sciences, New York, pp 171–191
- Korpi ER, Oja SS (1983) Characteristics of taurine release from cerebral cortex slices induced by sodium-deficient media. *Brain Res* 289:197–204
- Larsson A, Edström L, Svensson L, Söderpalm B, Engel JA (2005) Voluntary ethanol intake increases extracellular acetylcholine levels in the ventral tegmental area in the rat. *Alcohol* 40:349–358
- Lidö HH, Stomberg R, Fagerberg A, Ericson M, Söderpalm B (2009) Glycine reuptake inhibition: a novel principle for prevention of ethanol-induced dopamine release. *Alcohol Clin Exp Res* 33(7):1–7
- McBride WJ, Murphy JM, Ikemoto S (1999) Localization of brain reinforcement mechanisms: intracranial self-administration and intracranial place-conditioning studies. *Behav Brain Res* 101(2):129–152
- Molander A, Söderpalm B (2005a) Glycine receptors regulate dopamine release in the rat nucleus accumbens. *Alcohol Clin Exp Res* 29:17–26
- Molander A, Söderpalm B (2005b) Accumbal strychnine-sensitive glycine receptors: an access point for ethanol to the brain reward system. *Alcohol Clin Exp Res* 29:27–37
- Olive MF (2002) Interactions between taurine and ethanol in the central nervous system. *Amino Acids* 23:345–357
- Pan ZH, Slaughter MM (1995) Comparison of the actions of glycine and related amino acids on isolated third order neurons from the tiger salamander retina. *Neuroscience* 64:153–164
- Quertemont E, Devitgh A, De Witte P (2003) Systemic osmotic manipulations modulate ethanol induced taurine release: a brain microdialysis study. *Alcohol* 29:11–19
- Quertemont E, Goffaux V, Vlamincq AM, Wolf C, De Witte P (1998) Oral taurine supplementation modulates ethanol-conditioned stimulus preference. *Alcohol* 16:201–206
- Saransaari P, Oja SS (2000) Taurine and neural cell damage. *Amino Acids* 19:509–526
- Sergeeva OA, Haas HL (2001) Expression and function of glycine receptors in striatal cholinergic interneurons from rat and mouse. *Neuroscience* 104(4):1043–1055
- Söderpalm B, Löf E, Ericson M (2009) Mechanistic studies of ethanol's interaction with the mesolimbic dopamine reward system. *Pharmacopsychiatry* 42(Suppl 1):S87–S94
- Spanagel R (2009) Alcoholism: a systems approach from molecular physiology to addictive behavior. *Physiol Rev* 89(2):649–705
- Wise RA, Rompre PP (1989) Brain dopamine and reward. *Annu Rev Psychol* 40:191–225