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Nicotinic acetylcholine receptors in the anterior, but not posterior, VTA mediate ethanol induced elevation of accumbal dopamine levels

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List of abbreviations:

VTA; ventral tegmental area

nAc; nucleus accumbens

nAChR; nicotinic acetylcholine receptor

GlyR; glycine receptor

DA; dopamine

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ABSTRACT

Ethanol-induced elevations of accumbal dopamine levels have been linked to the reinforcing properties of the drug. However, it has not yet been demonstrated where the primary point of action of ethanol is in the mesolimbic dopamine system and there appears to be conflicting findings depending on methodology (electrophysiology, microdialysis or intra-cranial self-administration). We have suggested that ethanol acts in the nucleus accumbens (nAc) where it activates a neuronal loop involving ventral tegmental nicotinic acetylcholine receptors (nAChR) in order to elevate dopamine levels in the nAc. Application of ethanol in the nAc results in elevated dopamine levels in the same brain region whereas administration in the anterior ventral tegmental area (VTA) fails to influence dopamine output. In the present study we were able to repeat these findings. In addition, application of ethanol in the posterior VTA also failed to influence nAc dopamine levels. Perfusion of the nAChR antagonist mecamylamine in the anterior VTA completely blocked the elevation of accumbal dopamine levels observed after ethanol perfusion in nAc, whereas mecamylamine in the posterior VTA had no effect. In order to detect a possible influence on phasic dopamine release the dopamine transporter inhibitor nomifensine was included in the accumbal perfusate. Also under these conditions ethanol in the anterior or posterior VTA failed to influence dopamine release in the nAc. These results support previous suggestions of distinct functions of the anterior and posterior VTA and give further evidence for our hypothesis of a nAc-anteriorVTA-nAc neuronal circuitry involved in the dopamine activating effects of ethanol.

INTRODUCTION

The mesolimbic dopamine system is suggested to mediate reward-related behaviors and addictive processes, such as alcoholism and drug addiction (Koob, 1992; Wise & Rompre, 1989). Ethanol elevates dopamine levels in the nucleus accumbens (nAc) in several species, including humans (Boileau et al., 2003), and this elevation is believed to be involved in the stimulatory and reinforcing properties of the drug (for reviews see Koob and LeMoal, 2001; Wise, 1998). The mechanisms underlying ethanol´s activation of this dopamine system are not clearly understood, but increased knowledge of these should enable the development of new, more efficient pharmacotherapies against alcohol abuse and alcoholism.

Our previous microdialysis studies demonstrated no effects on nAc dopamine output by local perfusion of ethanol into the ventral tegmental area (VTA; Ericson et al., 2003; Löf et al., 2007). Rather, we have suggested that ethanol elevates extracellular dopamine levels in the nAc by acting at glycine receptors (GlyRs) in or around the nAc (Molander and Söderpalm, 2005a; Molander and Söderpalm, 2005b). However, this effect does not appear to be an isolated local phenomenon since antagonism of VTA nicotinic acetylcholine receptors (nAChRs) prevents this dopamine elevation (Söderpalm et al., 2000; Ericson et al., 2003), indicating the VTA as a secondary site involved in ethanol´s nAc dopamine activating effect and pointing to a neuronal nAc-VTA-nAc loop in this regard (Ericson et al., 2006; Höifödt et al., 2006; Chau et al., 2007, see figure 1 for a simplified schematic drawing over the hypothesized neuronal circuitry). However, the above-mentioned studies investigating the involvement of the VTA with respect to ethanol-induced dopamine response, explored only the anterior VTA.

Literature investigating the dopamine enhancing and reinforcing properties of ethanol is contradictive. Whereas in vivo microdialysis experiments point towards the nAc as a primary target (Wozniak et al., 1991; Yoshimoto et al., 1992; Yim et al., 1997; Ericson et al., 2003; Löf et al., 2007) this does not agree with data from electrophysiological and local self-administration studies. Several findings originating with the study from Gatto et al. (1994) have demonstrated rats to self-administer ethanol into the VTA (Rodd-Henricks et al., 2000; Rodd et al., 2004). More specifically the animals appear to self-administer the drug into the posterior, but not the anterior, VTA (Rodd-Henricks et al., 2000) indicating functional heterogeneity in this brain region. These studies were based on electrophysiological findings that ethanol increases the firing rate of VTA dopamine neurons in the rat, both in vivo (Gessa et al., 1985) and in vitro (Brodie et al., 1990), and microdialysis findings demonstrating that systemic ethanol elevates dopamine levels in the nAc (DiChiara and Imperato, 1988; McBride et al., 1999). In addition, it was recently indicated that microinjections of ethanol into the posterior VTA stimulates dopamine release in the nAc (Rodd et al., 2007), that is, contrary to our previous data investigating the anterior VTA.

The anterior (-4.8 mm to -5.2 mm related to bregma) and posterior (-5.3 mm to -6.3 mm related to bregma) VTA appear to differ not only with respect to drug self-administration but also in $GABA_A$ receptor regulated inhibition (Ikemoto et al., 1997; Ikemoto et al., 1998). Ethanol is suggested to elevate dopamine levels also by either disinhibiting GABAergic inter-neurons due to direct activation of $GABA_A$ receptors, thus removing a tonic inhibitory influence, or by influencing burst firing (Grace, 2000). Floresco et al. (2003) demonstrated that separate afferent pathways to the VTA control the firing properties of the dopamine neurons, which in turn separately can influence tonic and phasic levels of dopamine in the nAc. It is thus possible that the lack of a dopamine response in the nAc following ethanol

administration into the anterior part of the VTA in our previous experiments is due to technical limitations of the in vivo microdialysis technique, a method that rarely detects fast alterations of neurotransmitter release, such as during burst firing.

In the present study we wanted to investigate whether ethanol perfused in the posterior VTA elevates dopamine in the nAc, contrary to our previous findings after perfusion in the anterior VTA. We also wanted to explore whether the ethanol-induced increase in accumbal dopamine levels after local ethanol perfusion in the nAc is antagonized by mecamylamine administered in the posterior VTA (in agreement with our previous findings in the anterior VTA). In addition we investigated the possibility that ethanol perfusion in the posterior or anterior VTA influences phasic dopamine output, something that is not generally detected by in vivo microdialysis unless the system is pharmacologically manipulated with a dopamine transporter inhibitor.

METHODS

Animals

Male Wistar rats (Beekay, Stockholm, Sweden) weighing 270-350 g were housed 4 per cage (55x35x20 cm) at constant room temperature (22°C) and humidity (65%). The animals were kept under regular light-dark conditions (lights on at 7:00 am and off at 7:00 pm) and had free access to “rat standard feed” (Harlan Teklad Europe, UK) and tap water. In all experiments drug naive animals were used. Separate groups of rats were used for each drug/concentration. Animals were allowed to adapt for one week to the novel environment before any experiment was initiated. This study was approved by the Ethics Committee for Animal Experiments, Göteborg, Sweden (5/04, 337/06).

Drugs

Ethanol (Kemetyl AB, Sweden; 300 mM), the non-selective nAChR antagonist mecamylamine (mecamylamine hydrochloride, SIGMA; 100 μM), the dopamine transporter inhibitor nomifensine (nomifensine maleate; SIGMA; 10 μM) and nicotine ((-)nicotine hydrogen tartrate; SIGMA, 1 mM (based on the tartrate salt)) were all dissolved in Ringer solution and administered via reversed microdialysis.

Microdialysis technique

Rats were anaesthetized by isoflurane, mounted into a stereotaxic instrument (David Kopf Instruments) and put on a heating pad to prevent hypothermia during the surgery. Holes were drilled for the placement of two anchoring screws and two I-shaped dialysis probes custom made in the laboratory. The dialysis probes were lowered unilaterally into the nAc (A/P: +1.85, M/L: -1.4 mm relative to bregma and D/V: -7.8 mm relative to dura) and the anterior

VTA (A/P: -5.2, M/L: -0.7 mm relative to bregma and D/V: -8.4 mm relative to dura) or the posterior VTA (A/P: -5.7, M/L: -0.7 mm relative to bregma and D/V: -8.6 mm relative to dura; Paxinos and Watson, 2006). The nAc dialysis probes were placed in the core-shell borderline region (suggesting that sampling was done in both the core and the shell of the nAc) and the probes and the anchoring screws were fixed to the scull with Harvard cement (DAB Dental AB, Sweden). After surgery, the rats were allowed to recover for two days before the dialysis experiments were initiated. Brain microdialysis experiments were performed in awake and freely moving animals. On the experimental day, the sealed inlet and outlet of the probes were cut open and connected to a microperfusion pump (U-864 Syringe Pump, AgnTho's, Sweden) via a swivel allowing the animal to move around freely. The probes were perfused with Ringer solution at a rate of 2 µl/min and dialysate samples (40 µl) were collected every 20 minutes. The rats were perfused with Ringer solution for one hour in order to obtain a balanced fluid exchange before baseline sampling began. Animals were sacrificed directly after the experiment, brains were removed and probe placements were verified using a vibroslicer (Campden Instruments; Fig.2).

Biochemical assay

To analyze the dopamine content of the dialysate samples a high-pressure liquid chromatography system was used for the separation and detection of dopamine as previously described (Ericson et al., 2003). To identify the dopamine peak an external standard was used containing 2.64 fmol/µl of dopamine. When at least three consecutive stable values of dopamine were obtained (± 5%) the first drug was introduced.

Experimental design

In the first set of experiments ethanol (300 mM) was perfused in the nAc (n=8), the anterior VTA (n=7) or the posterior VTA (n=9) for 180 minutes during which dopamine levels in the nAc were monitored. In the second set of experiments mecamylamine (100 μM) was perfused in either the anterior part of the VTA (n=8) or in the posterior part of the VTA (n=10) 40 minutes prior to the initiation of ethanol perfusion (300 mM in the nAc) and concomitant monitoring of extracellular dopamine levels in the nAc. In the third set of experiments nomifensine (10 μM) was administered in the nAc after the stable baseline had been obtained. After 5 samples (100 min) ethanol (300 mM) was applied in the anterior VTA (n=7) or in the posterior VTA (n=8) and accumbal dopamine levels were monitored for an additional 180 min. A subset of 6 animals received nicotine (1 mM) either in the anterior (n=2) or in the posterior part of the VTA (n=4) in order to verify that the neurons were capable of further releasing dopamine in the nAc.

Statistics

Data were statistically evaluated using a two-way ANOVA with repeated measures (treatment group x time) followed by Fisher's protected least significant difference test (PLSD) or paired t-tests. A probability value (P) less than 0.05 was considered statistically significant. All values are expressed as means ± S.E.M.

RESULTS

In the first set of experiments ethanol (300 mM) or Ringer was perfused in the nAc, in the anterior VTA or in the posterior VTA by reversed microdialysis. As previously observed by others and us, ethanol perfused in the nAc elevated the dopamine levels in the same brain region by approximately 40%. However, when perfused in either of the two ventral tegmental regions no ethanol-induced elevation of dopamine in the nAc was observed compared to Ringer perfusion (ANOVA with repeated measures at time 0-80 followed by Fisher's PLSD; ethanol (nAc) vs. Ringer; $p<0.001$, ethanol (nAc) vs. ethanol (aVTA); $p=0.004$, ethanol (nAc) vs. ethanol (pVTA); $p=0.010$; figure 3).

In the second set of experiments we wanted to explore whether nAChRs in the posterior part of the VTA are involved in the ethanol-induced response of nAc dopamine. Thus, the animals were pre-treated with the non-selective nAChR antagonist mecamylamine (100 μM) either in the anterior VTA or in the posterior VTA 40 min before initiation of ethanol perfusion (300 mM in the nAc). Mecamylamine per se did not influence the nAc dopamine output neither when compared to pre-drug baseline levels nor when compared to Ringer treated animals (fig. 4A and 4B). Pre- and co-treatment with mecamylamine (100 μM) in the anterior VTA completely abolished the ethanol-induced elevation of dopamine in the nAc (fig. 4A, ANOVA with repeated measures at time 40-120 min followed by Fisher's PLSD; Ringer vs. mecamylamine(aVTA)+ethanol $p=0.937$, ethanol vs. mecamylamine(aVTA)+ethanol $p=0.005$) whereas pre- and co-treatment with mecamylamine in the posterior VTA did not antagonize the ethanol-induced elevation of dopamine levels in the nAc (fig. 4B, ANOVA with repeated measures at time 40-120 min followed by Fisher's PLSD; Ringer vs.

mecamylamine(pVTA)+ethanol p=0.03, ethanol vs. mecamylamine(pVTA)+ethanol p=0.577).

In the final set of experiments the dopamine transporter inhibitor nomifensine (10 μM perfused in the nAc) was administered to the rats after the initial baseline sampling was satisfactory. This resulted in an increase of extracellular dopamine levels in the nAc by approximately 900% (fig. 5). After 100 min of nomifensine perfusion, ethanol (300 mM) was perfused in either the anterior or the posterior VTA and dopamine levels were monitored for an additional three hours. Ethanol did not influence the nAc dopamine output significantly when applied in any of the two ventral tegmental areas of the brain (fig. 6A) indicating that ethanol, applied by reversed microdialysis in the VTA, does not influence tonic *or* phasic firing of dopamine neurons (ANOVA with repeated measures (at time 100-260 min); p=0.364). In addition, to verify that the dopamine neurons still were able to respond with dopamine release during nomifensine perfusion, some animals received nicotine (1 mM) in the two different ventral tegmental regions. Nicotine increased the dopamine response significantly by approximately 40% after nearly five hours of nomifensine treatment (paired t-test between time points 240 min and 340 min, p=0.038; fig. 6B). All six animals tested with nicotine demonstrated an increased dopamine response.

DISCUSSION

In the present in vivo microdialysis study local ethanol elevated the dopamine levels in the nAc, as previously observed (Wozniak et al., 1991; Yoshimoto et al., 1992; Yim et al., 1997; Ericson et al., 2003; Löf et al., 2007). Also in line with previous results, ethanol perfusion in the anterior VTA did not influence dopamine output in the nAc (Ericson et al., 2003; Löf et al., 2007). We speculated whether the lack of influence on accumbal dopamine levels by ethanol perfusion in the VTA was due to heterogeneity of the VTA and the fact that the previous placements of our dialysis probes were restricted to the anterior VTA. However, in the present study, ethanol perfusion in the posterior VTA also failed to influence nAc dopamine output. This result was not unexpected since it has to be assumed that following 180 min of ethanol perfusion in the anterior VTA (-5.2 mm relative to bregma) the drug would have spread some distance from the dialysis probe, thus reaching also the posterior VTA (-5.3 to -6.3 mm relative to bregma). The current findings are in line with a study performed on genetically selected rat-lines AA/ANA, demonstrating no influence on nAc dopamine levels after ethanol perfusion in the posterior VTA (200, 400 and 800 mM perfused at -5.6 relative to bregma; Tuomainen et al., 2003).

It has been claimed that reversed microdialysis of ethanol is pharmacologically irrelevant since presumably much higher ethanol concentrations reach the tissue immediately surrounding the probe during perfusion than following systemic administration (Gonzales et al., 1998; Tuomainen et al., 2003). There are several factors involved when estimating the concentration of a drug outside of the dialysis probe. For ethanol, perfused at a concentration of 300 mM, we estimate a concentration of approximately 50 mM directly outside the probe

based on a preliminary study in our laboratory (Ericson et al., unpublished data). However, since ethanol is a small molecule with both hydrophilic and lipophilic properties there will be a gradient of ethanol in the surrounding area. Nevertheless, it is estimated that the ethanol concentration that reaches the tissue surrounding the dialysis probe is of a similar magnitude as that self-administered by rats into the VTA (150-300 mg% ethanol; Rodd et al., 2004). More important may, however, be to consider how results obtained by reversed microdialysis compare to findings obtained after systemic ethanol administration. Based on the high degree of pharmacological similarity observed after local, systemic, or voluntary oral ethanol administration we believe that ethanol concentrations up to 300 mM applied by reversed microdialysis in our set-up are relevant. Thus mecamylamine administration in the VTA blocks the dopamine elevation observed following local accumbal perfusion of ethanol (300 mM) in the nAc, after systemic injection of ethanol and during voluntary ethanol intake (Blomqvist et al., 1997; Ericson et al., 1998; Ericson et al., 2003). Similarly, accumbal strychnine blocks the dopamine elevation observed after nAc ethanol perfusion, systemic ethanol and voluntary ethanol intake (Molander and Soderpalm, 2005b; Molander et al., 2005).

In the second part of the study we applied mecamylamine in the two anatomically distinct regions of the VTA and observed whether the nAChR antagonist was able to influence the nAc dopamine elevation after accumbal ethanol perfusion. We were able to repeat our finding that mecamylamine applied in the anterior VTA completely antagonizes the ethanol-induced elevation in accumbal dopamine (Ericson et al., 2003) whereas mecamylamine perfused in the posterior VTA did not influence the ethanol-induced dopamine output. These results provide further support for heterogeneity of the VTA, in line with findings from Ikemoto et al. (1997; 1998) demonstrating rats to self-administer picrotoxin into the anterior, but not the posterior,

VTA and muscimol into the posterior, but not the anterior, VTA. There appears not only to be distinct areas within the VTA but the dopamine neurons in these areas also seem to be differentially regulated.

Microdialysis lacks the ability to monitor fast changes in neurotransmitter release and thus we investigated in a separate set of microdialysis experiments the possibility that ethanol, applied locally in the VTA, influences not tonic, but burst firing of dopamine neurons. In theory, due to a rapid and efficient presynaptic reuptake mechanism, the method of in vivo microdialysis will not detect fast alterations in synaptic neurotransmitter release, as during burst firing. By treating the rats with nomifensine in the nAc, we eliminated the efficient dopamine reuptake mechanism in the last experiment. This manipulation will allow phasically released synaptic dopamine to escape from the synapse and to be detected as elevated extracellular dopamine levels in the dialysate. During treatment with nomifensine we were still not able to detect any significant alterations in nAc dopamine levels during ethanol perfusion in either of the two ventral tegmental regions, indicating that ethanol in the VTA does not produce phasic dopamine release in the nAc. Furthermore, the lack of any ethanol-induced effect on dopamine levels in the nAc did not appear to be due to for example dopamine depletion, resulting from the massive dopamine output that had been monitored for 5 hours, or to compromised neuronal firing, since nicotine perfusion elevated the nAc dopamine response further under the same conditions. It is well established that nicotine increases both tonic and burst dopamine neuronal firing by activating nAChRs in the VTA (Grenhoff et al., 1986; Schilström et al., 2003).

As pointed out in the Introduction, and now further underlined by the present results, the available studies on the dopamine activating and reinforcing properties of ethanol appear

contradictive. Thus, rats apparently self-administer ethanol into the posterior VTA, but not into the anterior VTA (Rodd-Henricks et al., 2000). These intra-cerebral self-administration studies hence indicate that ethanol applied in the VTA is reinforcing to the rat, and this effect is suggested to be mediated via activation of mesolimbic dopamine neurons. This possibility is supported by electrophysiological studies in vivo and in vitro showing an excitatory effect of ethanol on dopamine neurons (Gessa et al., 1985; Brodie et al., 1990). However, it should be noted that the in vivo electrophysiological studies merely demonstrated dopamine neuronal activation after systemic ethanol administration, which could be either indirectly or directly mediated. Further, the in vitro studies were performed in preparations lacking a multitude of afferents which in vivo could be influenced by ethanol in a manner as to cancel out tentative direct excitatory effects, as indicated by the present and previous microdialysis studies failing to detect a dopamine activating effect of ventral tegmental ethanol (Ericson et al., 2003; Löf et al., 2007). The microdialysis studies instead point towards a primary action of ethanol in the nAc as regards the dopamine activating and, in extension, reinforcing effects of ethanol. Whether rats self-administer ethanol into the nAc has to our knowledge not been investigated, but we have previously demonstrated that GlyRs in this area are involved in the dopamine activating effect of ethanol and that local interference with these receptors in the nAc modulates ethanol consumption (Molander et al., 2005).

No doubt ethanol is present both in the nAc and in the VTA after oral consumption and several mechanisms could underlie the reinforcing properties of the drug. The reinforcing effects of intra-VTA administrations could for example be mediated via other mechanisms than dopamine activation, and during oral self-administration both these mechanisms and the dopamine elevating mechanisms could be involved. Another possible explanation to the discrepancy between the present in vivo microdialysis experiments and the intra-VTA self-

administration studies is that the conditions differ in a way as to allow dopamine activation in the latter but not the former set-up. A third possibility is that dopamine would have to be measured exclusively in the core or the shell of the nAc in order to detect dopamine activations after ethanol application in the anterior and posterior VTA, respectively, and/or that the measurements would have had to be made in the posterior nAc after the posterior VTA injections. However, the majority of studies relating oral ethanol consumption to accumbal dopamine activation has used the same nAc co-ordinates as used in the present study (Weiss et al., 1993; Ericson et al., 1998), which in fact was the reason for chosing these. There is no information available indicating whether dopamine in more posterior parts of the nAc relate to oral ethanol intake. A fourth possibility is that the intra-VTA ethanol self-administration findings are irrelevant. It will thus be important to investigate whether the same pharmacology applies to this behavior as to voluntary oral ethanol consumption.

In summary, ethanol elevates nAc dopamine levels after local application in the nAc only but not after perfusion in the anterior or posterior VTA. This is in line with previous microdialysis findings but not in line with in vitro and intra-cerebral self-administration studies. To explore whether the lack of effect of ethanol in the VTA was due to ethanol-induced influence on phasic rather than tonic dopamine neuronal activity, we treated the animals with the dopamine transporter inhibitor nomifensine. Ethanol applied in either part of the VTA failed to influence nAc dopamine levels also under these conditions. Finally, local administration of the nAChR antagonist mecamylamine in the anterior but not the posterior VTA completely antagonized the accumbal dopamine elevation observed during ethanol perfusion in the nAc. These results support previous suggestions of distinct functions of the anterior and posterior VTA and give further evidence for our hypothesis of a nAc-anteriorVTA-nAc neuronal circuitry involved in the dopamine activating effect of ethanol. These findings would thus encourage further

studies on the mechanism(s) of action of ethanol in the nAc and of the nAc-anterior VTA-nAc neuronal circuitry in order to find new treatment strategies for alcohol use disorders.

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Footnotes

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Legends for figures

Figure 1. Simplified schematic figure of the hypothesized neuronal circuitry which ethanol interacts with in its reinforcing properties (nAc = nucleus accumbens, VTA = ventral tegmental area, LDTg = laterodorsal tegmental nucleus, DA = dopaminergic neuron, GABA = GABAergic neuron, ACh = cholinergic neuron, GlyR = glycine receptor, DAR = dopamine receptor, GABAR = GABA receptor, nAChR = nicotinergic acetylcholine receptor).

Figure 2. Histology. Location of the microdialysis probes in a) the nucleus accumbens, b) the anterior ventral tegmental area and c) the posterior ventral tegmental area. The black line indicates the tracks of the microdialysis probes. Numbers beside each plate represent distance from bregma.

Figure 3. Effect of ethanol 300 mM or Ringer perfused in the nAc, the anterior VTA (aVTA) or the posterior VTA (pVTA) on extracellular dopamine levels in the nAc as measured by in vivo microdialysis in awake freely moving rats. Drug administration was initiated as indicated by the arrow. Data are presented as means±SEM, n= 7-9. Statistics; ANOVA with repeated measures (0-60 min) followed by Fisher's PLSD, Ringer vs Ethanol (nAc) $p<0.001$, Ethanol (nAc) vs Ethanol (aVTA) $p=0.004$, Ethanol (nAc) vs Ethanol (pVTA) $p=0.010$.

Figure 4. Effect of mecamylamine (100 μM) perfused in the anterior (A) or posterior (B) ventral tegmental area alone or concomitant with ethanol (300 mM in the nAc) on

extracellular dopamine levels in the nAc as measured by in vivo microdialysis in awake freely moving rats. Drug administration was initiated as indicated by the arrows. Data are presented as means±SEM, n= 6-10. Statistics; ANOVA with repeated measures over time points 40-120, followed by Fisher's PLSD, Ringer vs. mecamylamine (aVTA)+ethanol p=0.937, ethanol vs. mecamylamine(aVTA)+ethanol p=0.005, Ringer vs. mecamylamine(pVTA)+ethanol p=0.029, ethanol vs. mecamylamine(pVTA)+ethanol p=0.577.

Figure 5. Effect of the dopamine transporter inhibitor nomifensine (10 μM) after local application in the nAc on extracellular dopamine levels in the nAc as measured by in vivo microdialysis in awake freely moving rats. Drug administration was initiated as indicated by the arrow. Data are presented as means±SEM, n=29.

Figure 6. A) Effect of ethanol 300 mM perfused in the nAc, the anterior VTA (aVTA) or the posterior VTA (pVTA) on extracellular dopamine levels in the nAc after nomifensine pre-treatment (10 μM, 100 min. in the nAc) as measured by in vivo microdialysis in awake freely moving rats. Data are presented as means±SEM, n= 7-8. Statistics; ANOVA with repeated measures over time points 100-260, p=0.364. B) Effect of nicotine (1 mM) perfused in either the anterior (n=2) or posterior (n=4) VTA after 100 min of nomifensine (10 μM in the nAc) and 180 min of ethanol perfusion (300 mM in the corresponding VTA region; paired t-test between time points 240 and 340, p=0.038).

Figure 1.

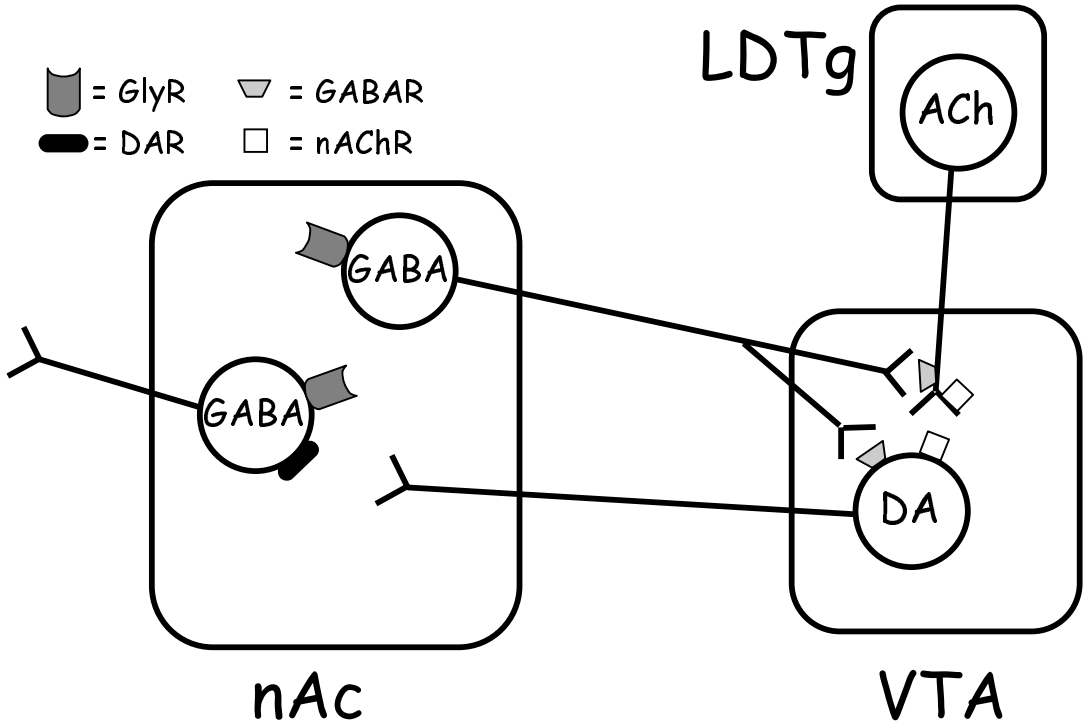
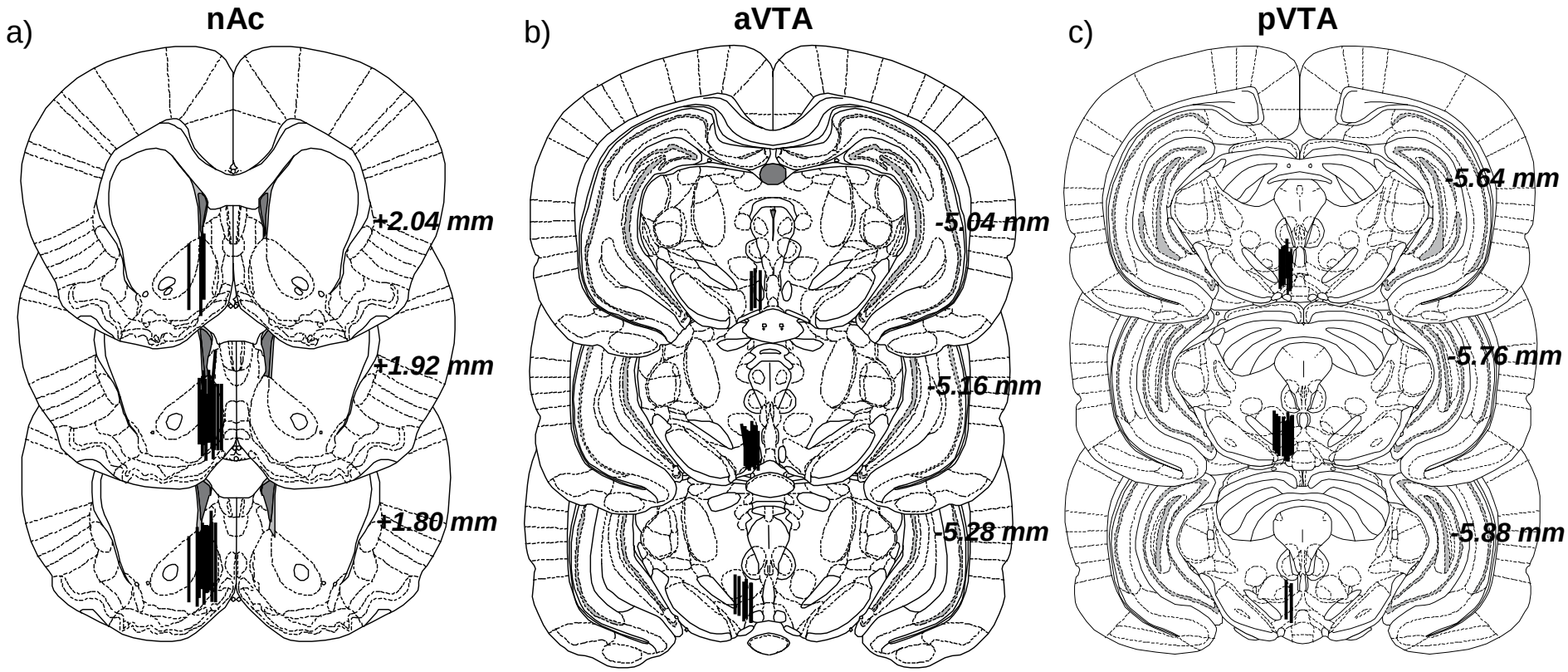


Figure 2.



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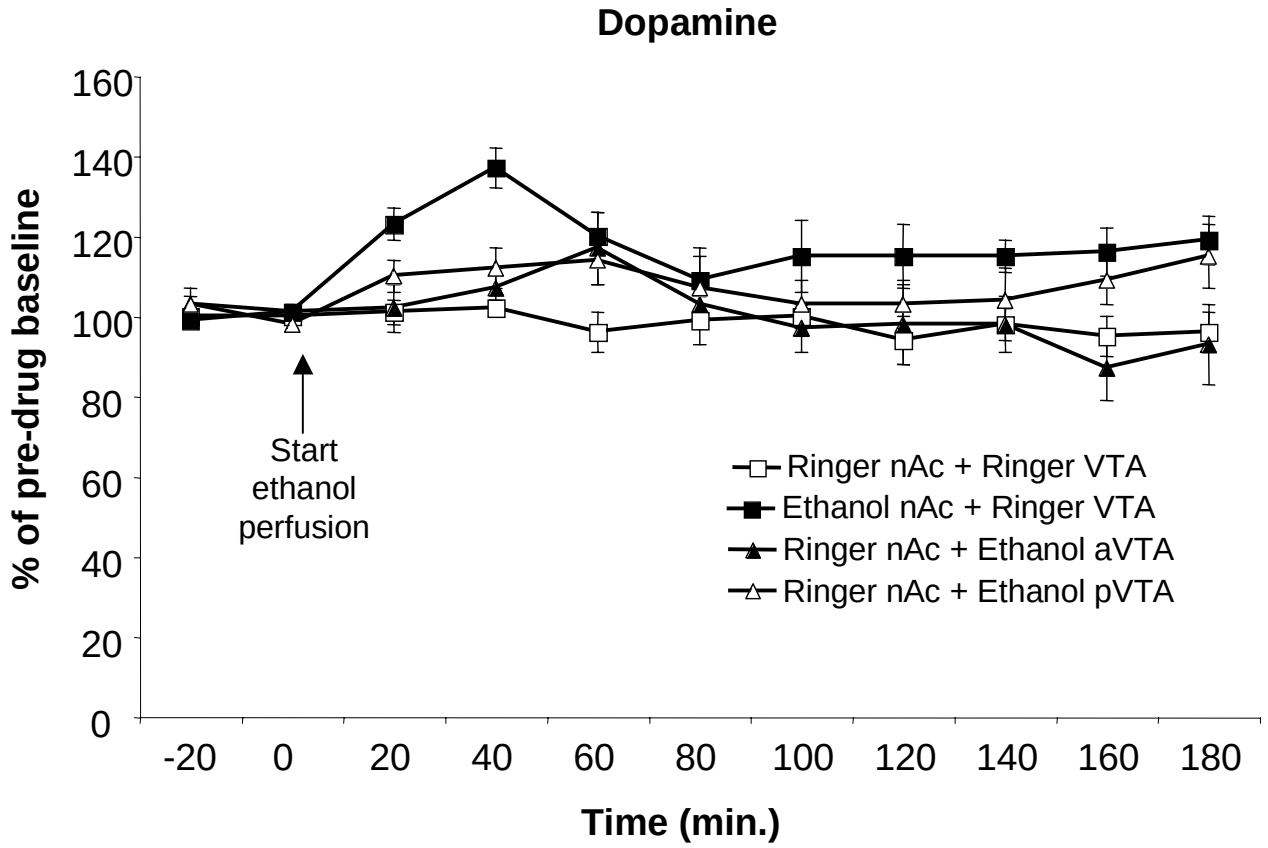
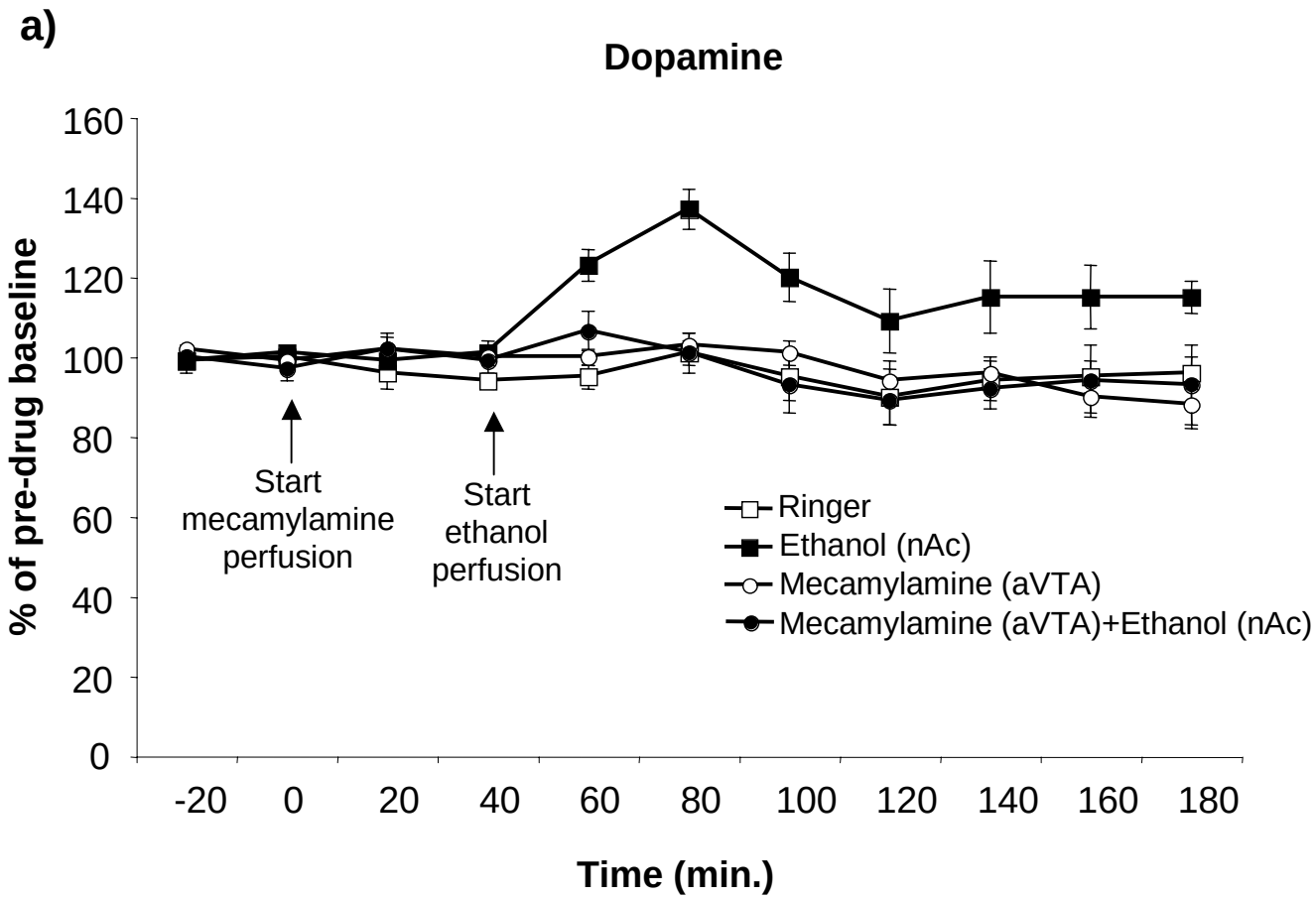


Figure 4.



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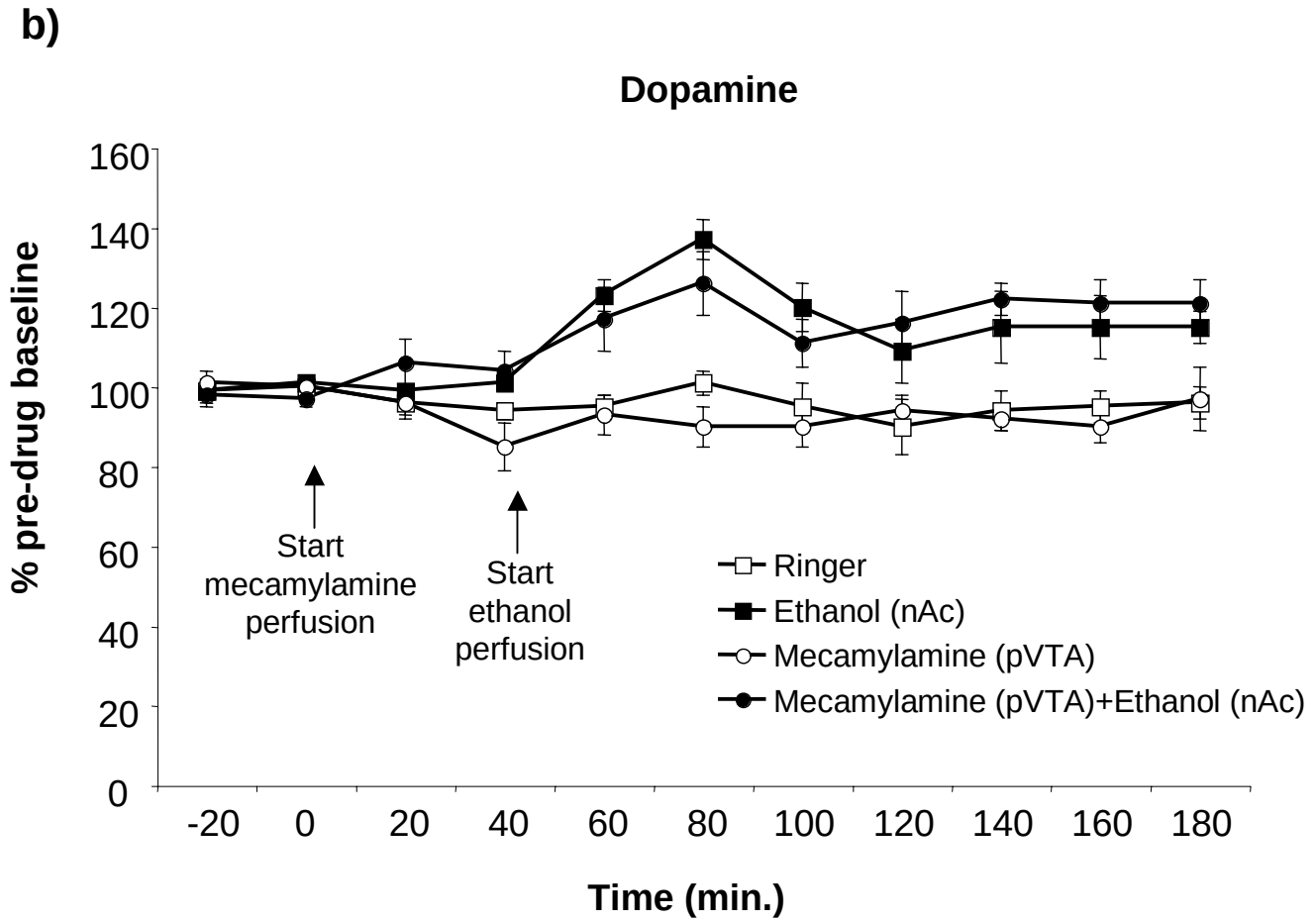

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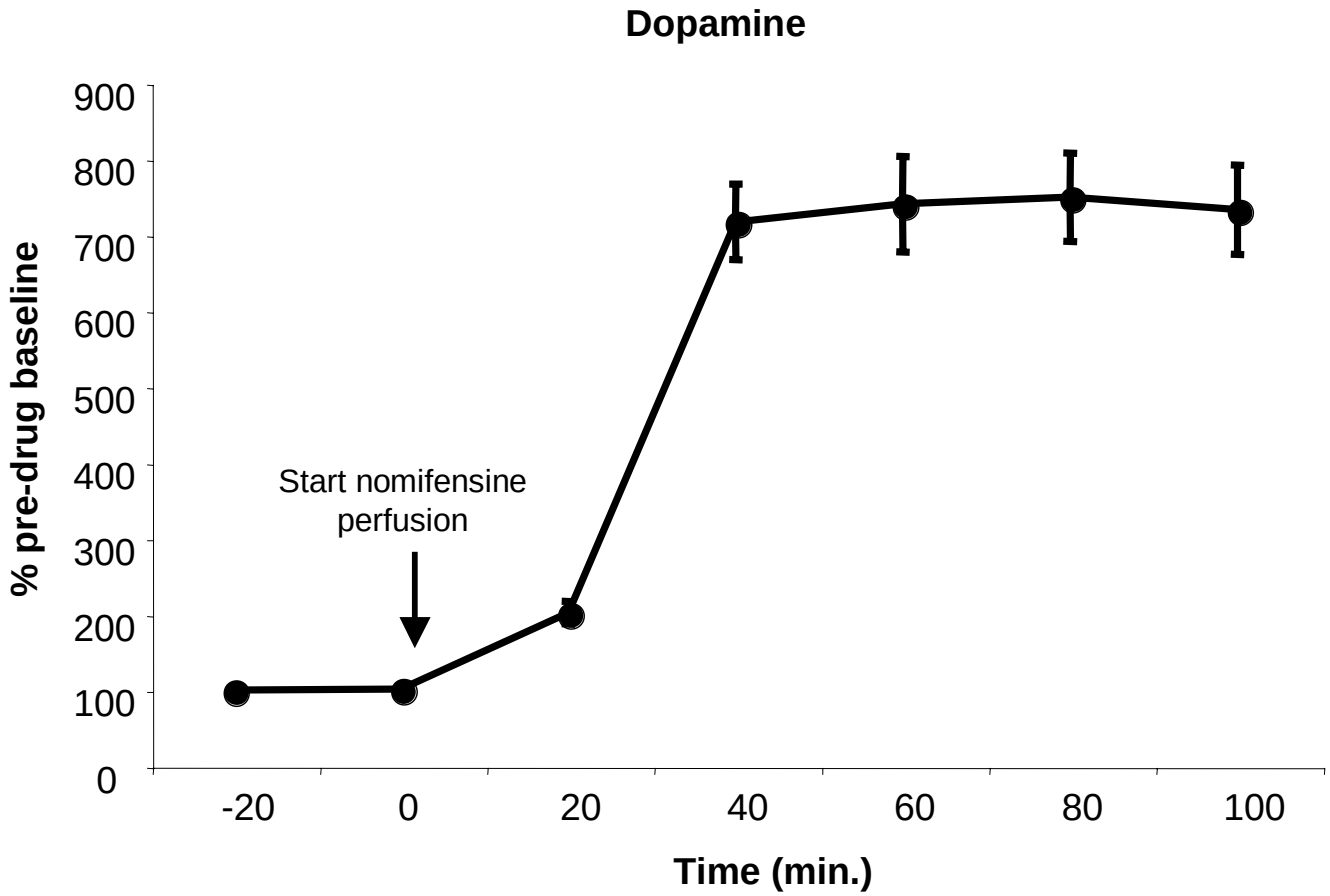

Figure 6.

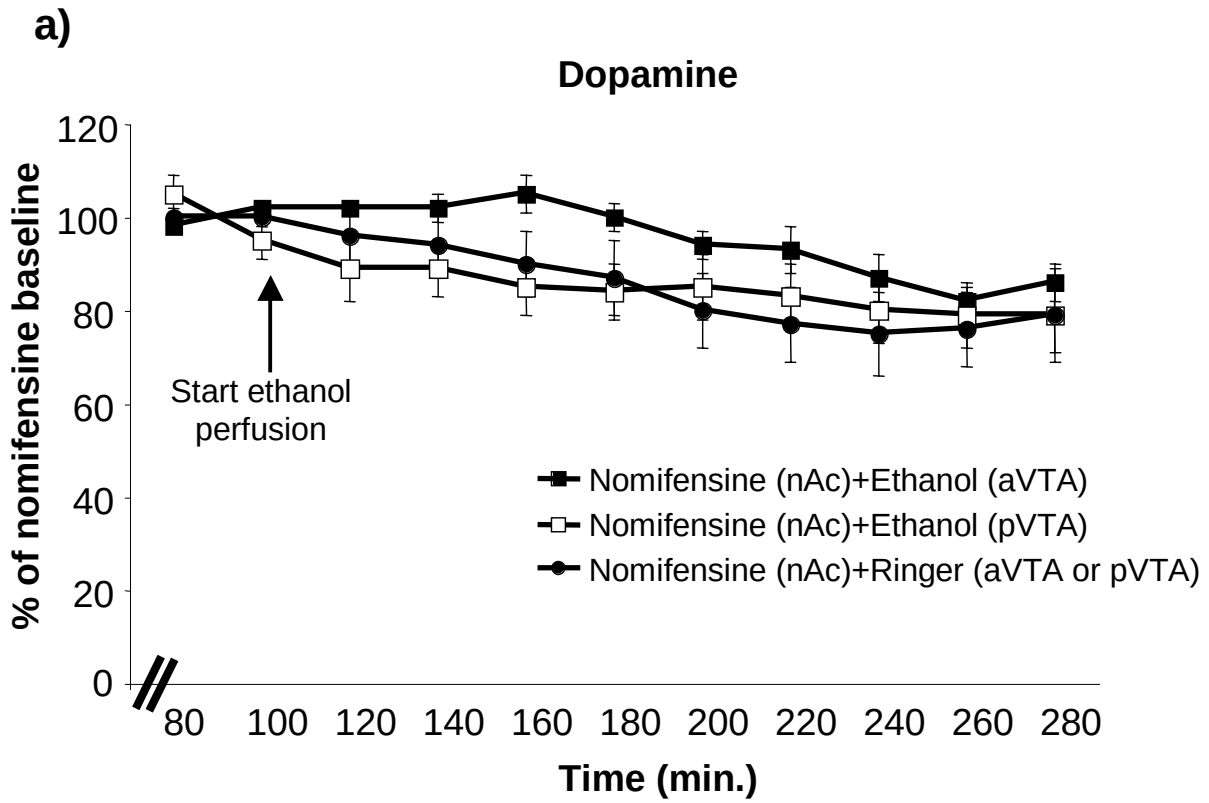

Figure 6.