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# Differential effects of dopamine $D_2$ and $GABA_A$ receptor antagonists on dopamine neurons between the anterior and posterior ventral tegmental area of female Wistar rats

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ABSTRACT

Previous findings indicated differences in neuronal circuitries mediating drug reinforcement between the anterior and posterior ventral tegmental area (VTA). The objective of the present study was to examine the effects of the dopamine $D_2$ antagonist sulpiride and the $GABA_A$ antagonist picrotoxin administered in the anterior and posterior VTA on the activity of mesoaccumbal dopamine neurons in female Wistar rats. Sulpiride and picrotoxin were administered in the anterior and posterior VTA. Extracellular dopamine levels were measured in sub-regions of the VTA and nucleus accumbens (ACB). Reverse-microdialysis of sulpiride (100 μM) into the posterior VTA increased extracellular dopamine levels locally (80% above baseline) and in the ACB shell and core (70% above baseline), whereas reverse-microdialysis into the anterior VTA produced a much smaller effect locally (30% above baseline) and in the ACB shell and core. In contrast, microinjection of picrotoxin (80 and 160 μM) into the anterior, but not posterior VTA, increased dopamine release in the ACB shell. The results suggest that dopamine neurons in the posterior VTA, compared to the anterior VTA, may be under greater $D_2$ receptor-mediated tonic inhibition, whereas dopamine neurons in the anterior VTA, compared to the posterior VTA, may be under greater $GABA_A$ receptor-mediated tonic inhibition.

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## 1. Introduction

The ventral tegmental area (VTA), located within the ventral mesencephalon, is the origin of the mesolimbic dopamine system projecting to forebrain limbic areas including the nucleus accumbens (ACB) (Oades and Halliday, 1987; Swanson, 1982). The mesolimbic system has been implicated in motivation, positive reinforcement, learning of reward (Wise, 2004), and pathology of substance abuse and addiction (Kalivas and Volkow, 2005; Koob, 1992; McBride et al., 1999; Melis et al., 2005). Drugs of abuse, including opioids (Bozarth and Wise, 1981), cocaine (Rodd et al., 2005a) and ethanol (Gatto et al., 1994; Rodd et al., 2004) can be directly self-infused into the VTA, indicating that the VTA mediates the reinforcing effects of drugs of abuse.

Evidence has emerged suggesting that the VTA is a functionally heterogeneous brain region, with different sub-regions along the anterior–posterior axis mediating reinforcing effects of different agents. Intracranial self-administration studies showed that the $GABA_A$ receptor antagonist picrotoxin was self-infused into the anterior, but not posterior, VTA (Ikemoto et al., 1997b), whereas the $GABA_A$ receptor agonist muscimol was self-infused into the posterior, but not anterior, VTA (Ikemoto et al., 1998). A follow-up study revealed that microinjection of $GABA_A$ antagonists into the anterior VTA (the only sub-region of the VTA that was examined) increased dopamine release in the ACB (with most probe placements in the core and core plus shell) (Ikemoto et al., 1997a), suggesting a tonic $GABA_A$ receptor-mediated inhibition on dopamine neurons within the anterior VTA. It has also been demonstrated that the posterior, but not anterior, VTA, mediated the reinforcing effects of several drugs of abuse, including cocaine (Rodd et al., 2005a) and ethanol (Rodd-Henricks et al., 2000; Rodd et al., 2004, 2005b), and that activation of local dopamine neurons were involved. Taken together, the above studies suggest that there are differences between the anterior and posterior VTA in (a) $GABA_A$ receptor regulation of dopamine neurons, and (b) the intrinsic/extrinsic reward circuitries.

Dopamine neurons in the VTA are under tonic $D_2$ receptor-mediated inhibition (Kalivas, 1993; White, 1996). Intra-VTA application of $D_2$ receptor antagonists increased dopamine neuronal firing (Wang, 1981; White and Wang, 1984b) and dopamine release in the VTA and ACB (Kohl et al., 1998; Westerink et al., 1996). However, there is evidence that not all VTA dopamine neurons are regulated by $D_2$ autoreceptors to the same degree (Chiodo et al., 1984; Margolis et al.,

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2006; White and Wang, 1984a; Yim and Mogenson, 1980). Because previous studies did not examine differences in $D_2$ autoreceptor regulation of dopamine neuronal activity between the anterior and posterior VTA, one objective of the present study was to determine if dopamine neurons in different sub-regions of the VTA are under similar or different $D_2$ autoreceptor inhibition. A dual-probe microdialysis technique was employed to examine the effects of local inhibition of $D_2$ autoreceptors in both sub-regions of the VTA on extracellular dopamine levels in the VTA and ACB. A second objective was to extend the study of Ikemoto et al. (1997a) and determine if there are differences in $GABA_A$ receptor regulation of dopamine neuronal activity between the anterior and posterior VTA. A microinjection–microdialysis technique was used to examine the effects of local inhibition of $GABA_A$ receptors in each sub-region of the VTA on extracellular dopamine levels in the ACB. This procedure was applied because it was used in a previous study (Ikemoto et al., 1997a) and would allow a better comparison of the results between the present and previous study. The overall hypothesis to be tested is that dopamine neurons in the anterior and posterior VTA are differentially regulated by local $D_2$ autoreceptors and $GABA_A$ receptors.

## 2. Materials and methods

### 2.1. Animals

Adult female Wistar rats (body weight 270 to 320 g, Harlan Sprague Dawley, Inc., Indianapolis IN, USA) were double-housed upon arrival in plastic cages in temperature- and humidity-controlled rooms for at least 2 weeks to be acclimated with housing environment. Rats were kept in a regular 12-h light–dark cycle room (light on 7:00 am) and food and water were available *ad libitum*. All experimental procedures, including surgery and microdialysis, were conducted during the light phase. Female rats were used because these rats maintain their head size better than male rats for more accurate stereotaxic placements (Ikemoto et al., 1997a,b, 1998; Rodd-Henricks et al., 2000). The estrous cycle was not monitored in this study. However, counterbalanced experiments were conducted on different days so that any effect of a given phase of the estrous cycle was distributed across experimental conditions. Protocols used were approved by the Institutional Animal Care and Use Committee of Indiana University School of Medicine. All experiments were conducted in accordance with principles outlined in the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996).

### 2.2. Chemical agents

NaCl, $Na_2HPO_4 \cdot 7H_2O$, $MgCl_2$, ascorbate, acetonitrile, *d*-glucose, ethylenediaminetetraacetic acid (EDTA), and sodium octanesulfonic salt (SOS) were purchased from Sigma (St. Louis, MO, USA). KCl, $CaCl_2$, $MgSO_4$ and sodium acetate were purchased from Fisher Scientific (Fair Lawn, NJ, USA). $KH_2PO_4$ and $NaHCO_3$ were purchased from Acros Organics (NJ, USA). The dopamine $D_2$ receptor antagonist (−)-sulpiride and the $GABA_A$ receptor antagonist picrotoxin were obtained from Sigma (St. Louis, MO, USA) and were dissolved in the artificial cerebrospinal fluid (aCSF) to the desired concentrations.

### 2.3. General stereotaxic surgery procedures

Rats were stereotaxically implanted ipsilaterally with two guide cannulae (Plastics One, Inc., Roanoke, VA, USA) under 2% isoflurane inhalation anesthesia. One cannula was aimed above the anterior or posterior VTA, and the other above the ACB shell or core. As previously reported, the 18-gauge cannulae were used for microdialysis (Campbell and McBride, 1995; Kohl et al., 1998), and the 22-gauge cannulae were used for microinjection (Gatto et al., 1994; Ikemoto et al., 1997a). The cannulae and mounting screws were secured and fixed on the skull with cranio-plastic cement. The coordinates for the target areas were: the ACB shell: AP +1.7 mm, L +2.3 mm, DV −8.2 mm; the ACB core: AP +1.7 mm, L +2.7 mm, DV −8.0 mm; the anterior VTA: AP −4.8 mm, L +2.1 mm, DV −9.0 mm; the posterior VTA: AP −5.6 mm, L +2.1 mm, DV −9.0 mm (Paxinos and Watson, 1998). All cannulae were implanted at a 10° angle to the vertical, and the incisor bar was set at −3.3 mm. Stylets were inserted into cannulae when no experiments were being conducted. Rats were singly housed following surgery. Each rat was allowed to recover from surgery for at least 5 days, during which they were habituated to the microdialysis chambers and handled on a daily basis. Loop-style dialysis probes (1.5 mm active membrane length for the sub-regions of both the VTA and ACB, Spectra/Por RC, I.D: 200 um, MWCO: 13,000, Spectrum Laboratories, Inc, Rancho Dominguez, CA, USA) were used to increase the size of the dialysis membrane area and increase recovery of dopamine (Benveniste and Huttemeier, 1990). These probes were constructed and inserted into the targeted brain regions as previously described (Ikemoto et al., 1997a; Kohl et al., 1998; Perry and Fuller, 1992). The length of the microdialysis probes used for the VTA was similar to the length used in previous studies (Kohl et al., 1998; Liu et al., 2006).

**Experiment 1.** Determine the effects of reverse-microdialysis of the $D_2$ receptor antagonist sulpiride into the anterior or posterior VTA on extracellular dopamine levels both locally and in the ACB shell and core.

Microdialysis probes were used in both regions to allow concurrent measurement of the effects of sulpiride on dopamine release in both regions. Two probes were inserted, one in the anterior or posterior VTA, the other in the ACB shell or core. One day later, microdialysis was conducted. General procedures were described previously (Engleman et al., 2003; Kohl et al., 1998). Briefly, rats were placed into Plexiglas chambers (40 cm × 28 cm × 40 cm) and connected to a Harvard pump with PE20 tubing (I.D. 0.38 mm, Becton Dickinson & Co., MD, USA). Microdialysis aCSF (140.0 mM NaCl, 3.0 mM KCl, 1.2 mM $CaCl_2$, 2.0 mM $Na_2HPO_4 \cdot 7H_2O$, 1.0 mM $MgCl_2$, and 0.2 mM Ascorbate, pH 7.2–7.4) was perfused through probes at a rate of 1.0 µl/min. After a 90-min washout, four to five 20-min baseline samples were collected. Then, 100 µM sulpiride was delivered via reverse-microdialysis into the VTA for 100 min before being switched back to aCSF. Eight 20-min samples were collected following the initiation of reverse-microdialysis of sulpiride. A previous study (Engleman et al., 2003) indicated that 100 µM sulpiride was the optimal concentration for inducing dopamine release. All samples were collected into vials containing 5 µl of 0.1-N perchloric acid, were frozen immediately on dry ice, and were stored at −70 °C. A previous study indicated no degradation of dopamine up to one month using this procedure (Campbell and McBride, 1995). The microdialysis technique has been applied in studies with local delivery of drugs into the anterior and posterior VTA to differentiate pharmacological responses to these drugs between these two sub-regions (Ericson et al., 2008; Liu et al., 2006).

Dopamine was analyzed with a reversed-phase high performance liquid chromatography coupled with an electrochemical detection system, as described previously (Engleman et al., 2003; Kohl et al., 1998). Briefly, samples were loaded into a 10-µl loop and injected into a microbore analytical column (BDS Hypersil C18 pioneer, 100 mm × 1 mm, Thermo). The mobile phase (77.0 mM sodium acetate, 0.5 mM EDTA, 3.4 mM SOS, 9.9 mM NaCl, and 6.0% acetonitrile, pH 4.0) was delivered by an ESA 582 solvent delivery system (Chelmsford, MA, USA). Dopamine was detected by a unijet glassy carbon electrode (MF-1016, BAS, West Lafayette, IN, USA) and an amperometric detector (EG&G Princeton Applied Research, Princeton, NJ, USA) with potential set at 400 mV and sensitivity set at 2.0 nA/V. The outputs from the detector were sent to a ChromPerfect (Version 4.4.0, Justice Innovations, Inc., Palo Alto, CA,

USA) chromatography data analysis system. The lower detection limit for dopamine was approximately 0.1 nM.

**Experiment 2.** Determine the effects of microinjecting the $GABA_A$ receptor antagonist picrotoxin into the anterior or posterior VTA on extracellular dopamine levels in the ACB shell.

The microinjection–microdialysis technique was used in this experiment because this procedure was used in the previous study (Ikemoto et al., 1997a) and would allow a better comparison of the results between the present and the previous study. Because the study of Ikemoto et al. (1997a) had placements mainly in the ACB core, the present study determined the effects of picrotoxin on dopamine release in the ACB shell. Microdialysis probes were inserted into the ACB shell the day before dialysis. Microdialysis was conducted following general procedures previously described (Engleman et al., 2003; Kohl et al., 1998) in Experiment 1, with the exception of 15-min collection interval instead of 20-min interval. After collection of four or five baseline samples, aCSF (120.0 mM NaCl, 4.7 mM KCl, 1.2 mM $KH_2PO_4$, 1.2 mM $MgSO_4$, 25.0 mM $NaHCO_3$, 2.5 mM $CaCl_2$, 10.0 mM *d*-glucose, pH 7.2–7.4) or picrotoxin (80, 160 μM in aCSF) was injected in a volume of 0.25 μl into the anterior or posterior VTA over a 30-sec period. The 2.5 mM concentration of $CaCl_2$ was commonly used in electrophysiological studies with brain slices (Bonci and Malenka, 1999; Brodie et al., 1990), and with the intracranial self-administration technique (Ikemoto et al., 1997b; Rodd-Henricks et al., 2000). The 80 and 160 μM picrotoxin concentrations were the same as previously tested (Ikemoto et al., 1997a). The injector was removed 30 s after injection. Seven samples were collected after microinjection. Dopamine was determined as described in the Experiment 1.

### 2.4. Histology

At the end of each experiment, rats were euthanized with an overdose of $CO_2$ inhalation. For experiment 1, 1% bromophenol blue was perfused through probes both in the VTA and the ACB. For experiment 2, 1% bromophenol blue was injected into the VTA and perfused through probes in the ACB shell. Brains were removed quickly and frozen immediately on dry ice and stored at −20 °C. Sections (40 μm) were sliced on a cryostat microtome and stained with cresyl violet for the verification of the injection sites and the placements of probes with reference to the rat brain atlas of Paxinos and Watson (1998).

### 2.5. Statistical analysis

All data are presented as the means ± SEM. The last three baseline samples prior to drug delivery were averaged and used to normalize data. Effects on extracellular dopamine levels are presented as percent of baseline. ANOVAs with repeated measures on time were employed for data analysis. If significant differences were detected with ANOVAs, post-hoc test or student *t* test were employed to determine the individual differences. The significance was set when *p* value is less than 0.05.

## 3. Results

### 3.1. Histology

Fig. 1 shows the representative placements of probes and injection sites. The anterior VTA extends from 4.8 mm to 5.2 mm posterior to bregma, whereas, the posterior VTA extends from 5.3 mm to 6.0 mm posterior to the bregma (Rodd-Henricks et al., 2000). For experiment

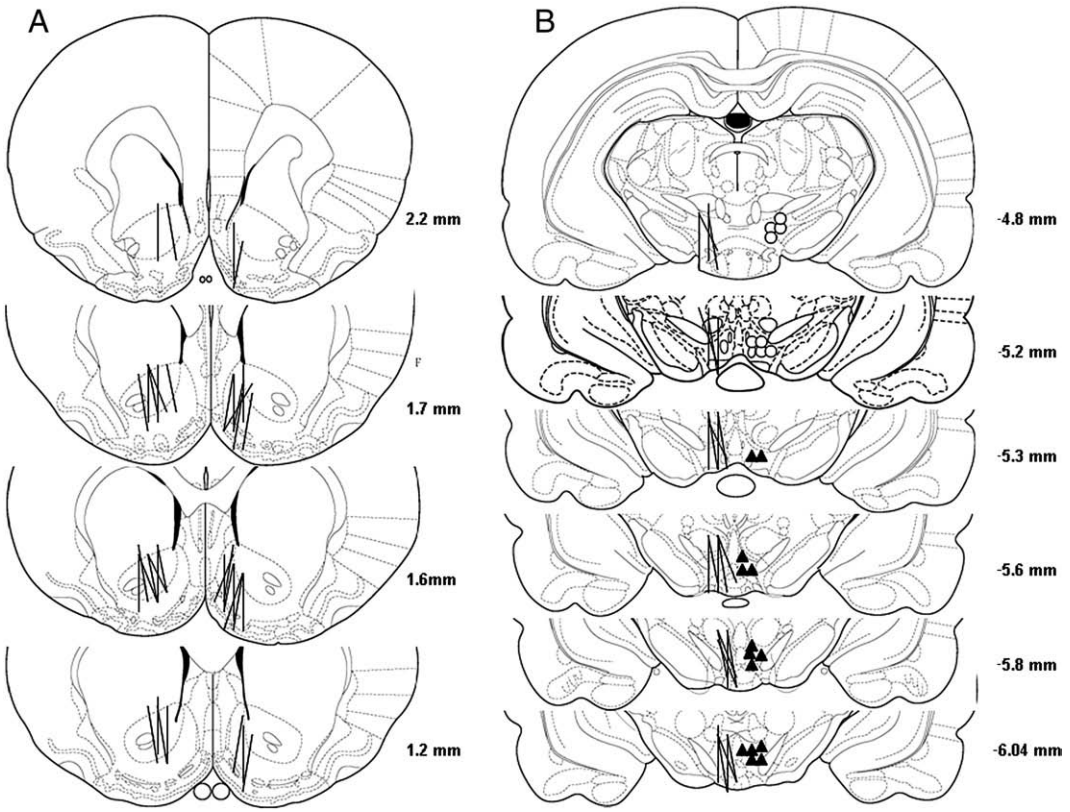


**Fig. 1.** The placements of microdialysis probes and injection sites in different sub-regions of the VTA and ACB. In the experiments, all probes were implanted in the right hemisphere of rat brains. For illustration convenience, probe placements in the ACB core and VTA are drawn on the left side. A: The lines represent the 1.5 mm length of microdialysis probes in the ACB shell (right side) and core (left side). B: The lines in brain slices from −4.8 mm to −5.2 mm indicate microdialysis probes in the anterior VTA, and the lines in slices from −5.3 mm to −6.04 mm indicate probes in the posterior VTA. The open circles represent for injection sites within the anterior VTA and the filled triangles for injection sites in the posterior VTA.

1, the correct placement for the sub-regions of the VTA should have probes implanted in the target regions. There are two sources of dopamine in the midbrain, the VTA and substantial nigra (Dahlstrom and Fuxe, 1964; Halliday and Tork, 1986). Rats with probes in the substantial nigra were not included in the analysis. Probes in the VTA also covered a portion of the red nucleus dorsally and/or interpeduncular nucleus ventrally. Since there are no dopamine neurons in these two areas, the VTA is the main source of dopamine collected in the current study. The correct placement for the ACB should have at least 75% of the probe within the ACB shell or core; the probes implanted into the ACB shell also covered a portion of the olfactory tubercle. In addition, the results from the ACB shell and core were included only if the placements of probes in the corresponding sub-regions of the VTA were correct. For experiment 2, the criteria for correct placements in the ACB shell was the same as applied in Experiment 1. For the VTA, only injectors within the anterior or the posterior VTA were counted as correct placements. The rats with injection sites not clearly in either the anterior or posterior VTA were not included in the analysis. Approximate 80% of animals that underwent surgery had correct placements. This figure is not a quantitative representation of all the probes and injection sites.

### *3.2. Effects of reverse-microdialysis of sulpiride into the anterior and posterior VTA on local extracellular dopamine levels*

The baseline extracellular dopamine levels were not different between the anterior and posterior VTA ($1.5 \pm 0.2$ nM and $1.2 \pm 0.2$ nM, respectively, $p = 0.45$). The levels observed in the current study are within the range of extracellular dopamine levels previously reported for the VTA (Liu et al., 2006; Yan et al., 2005).

Fig. 2 shows the effects of reverse-microdialysis of 100-μM sulpiride into the anterior and posterior VTA on local extracellular dopamine levels within each sub-region. The overall ANOVA with repeated measure of time revealed a significant time effect ($F_{10,\ 350} = 19.03$, $p<0.001$) and region effect ($F_{1,\ 35} = 9.41$, $p<0.01$), as well as a significant interaction ($F_{10,\ 350} = 3.96$, $p<0.001$). Reverse-microdialysis of sulpiride into both the anterior and posterior VTA increased extracellular dopamine levels locally.

In the posterior VTA, extracellular dopamine levels increased within the first 20 min after reverse-microdialysis of sulpiride ($144 \pm 8\%$ of baseline, $p<0.05$) and reached a peak level ($180 \pm 12\%$ of baseline) by 40 min. Extracellular dopamine levels remained elevated throughout reverse-microdialysis of sulpiride and gradually returned toward baseline after removing sulpiride from the perfusion medium (Fig. 2).

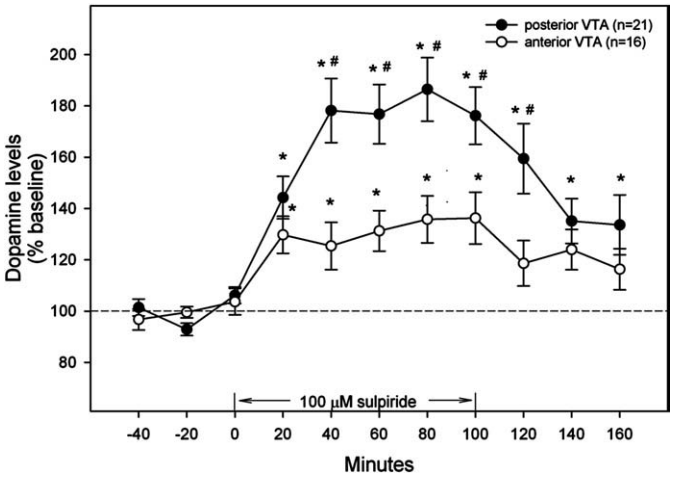


**Fig. 2.** The effects of reverse-microdialysis of 100-μM sulpiride into the anterior and posterior VTA on local extracellular dopamine levels. Time-course plots are corrected for delays due to the length of the inlet and outlet lines. *: significantly higher from baselines, $p<0.05$. #: significantly higher than the anterior VTA, $p<0.05$.

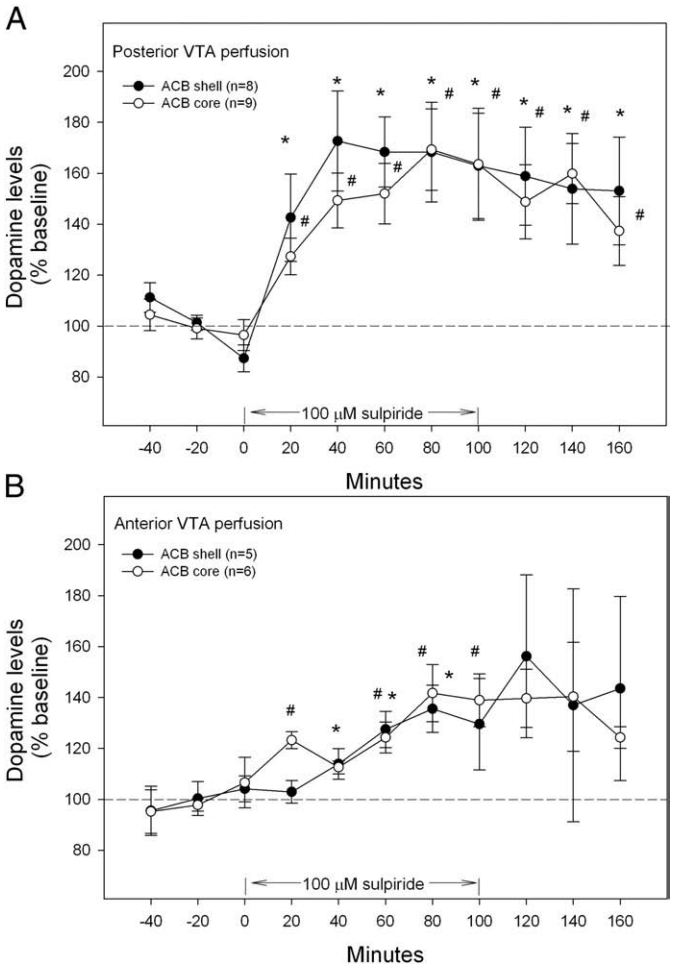


**Fig. 3.** A: Time course of extracellular dopamine levels in the ACB shell and core after perfusing sulpiride into the posterior VTA. *, #: significantly different from baseline level, $p<0.05$; *: ACB shell data; #: ACB core data. B: Time course of extracellular dopamine levels in the ACB shell and core after perfusing sulpiride into the anterior VTA. *, #: significantly different from baseline level, $p<<0.05$; *: ACB shell data; #: ACB core data.

Reverse-microdialysis of sulpiride into the anterior VTA increased local extracellular dopamine levels within the first 20 min ($130 \pm 7\%$ of baseline, $p<0.05$) and maintained this level ($136 \pm 10\%$ of baseline, $p<0.05$) throughout the reverse-microdialysis of sulpiride. Extracellular dopamine levels returned toward baseline when the perfusion medium was changed to aCSF alone. The extracellular dopamine levels during 40–120 min after the initiation of reverse-microdialysis of sulpiride were significantly greater in the posterior VTA than that in the anterior VTA ($p<0.05$) (Fig. 2).

### *3.3. Effects of reverse-microdialysis of sulpiride into the anterior and posterior VTA on extracellular dopamine levels in the ACB shell and core*

The basal extracellular dopamine concentration was higher in the ACB core ($0.9 \pm 0.1$ nM, $n = 15$) than shell ($0.6 \pm 0.1$ nM, $n = 13$) ($p<0.05$). The basal extracellular dopamine levels in the current study are within the range of extracellular dopamine levels reported for the ACB (Engleman et al., 2000; Melendez et al., 2002).

Fig. 3A shows changes in extracellular dopamine levels in the ACB shell and core after reverse-microdialysis of sulpiride into the posterior VTA. Individual repeated measure ANOVA revealed a significant time effect ($F_{10,\ 150} = 11.22$, $p<0.001$), but no significant

region effect ($F_{1,\ 15}=0.26$, $p>0.05$) or interaction ($F_{10,\ 150}=0.39$, $p>0.05$). In the ACB shell, extracellular dopamine levels significantly increased within the first 20 min after the initiation of administration of sulpiride (143 ± 17% of baseline, $p<0.05$), reached the peak levels within 40 min (173 ± 19% of baseline, $p<0.05$), and remained elevated throughout the experiment, even after sulpiride had been removed from the perfusion media (Fig. 3A). The time-course effects of perfusing sulpiride into the posterior VTA on extracellular dopamine levels in the ACB core were very similar to the effects observed in the ACB shell (Fig. 3A).

Fig. 3B shows the effects of reverse-microdialysis of sulpiride into the anterior VTA on extracellular dopamine levels in the ACB shell and core. Repeated measure ANOVA analysis revealed a significant time effect ($F_{10,\ 90}=3.47$, $p<0.01$), but no significant region effect ($F_{1,\ 9}=0.01$, $p>0.05$) and interaction ($F_{10,\ 90}=0.33$, $p>0.05$). Reverse-microdialysis of 100-μM sulpiride into the anterior VTA had a delayed effect on increasing extracellular dopamine levels in the ACB shell; no significant effect was observed after 20 min, and only a small effect (about 115% of baseline, $p<0.05$) was observed after 40 min perfusion of the anterior VTA with sulpiride, which gradually increased to around 135% of baseline by 80 min. In the case of ACB core, reverse-microdialysis of sulpiride into the anterior VTA had a modest effect on extracellular levels of dopamine (about 120% of baseline, $p<0.05$) within the first 20 min, which gradually increased to about 140% of baseline by the end of the 100 min perfusion period with sulpiride.

Fig. 4 showed the average extracellular dopamine levels as percent of baseline in the anterior and posterior VTA and the ACB shell and core during the reverse-microdialysis of sulpiride in the anterior or posterior VTA. Reverse-microdialysis of sulpiride in the posterior VTA produced significantly greater increases in extracellular dopamine levels both locally in the VTA and in the ACB shell and core than reverse-microdialysis in the anterior VTA ($p<0.05$).

### *3.4. Effects of microinjecting picrotoxin in the anterior and posterior VTA on extracellular dopamine levels in the ACB shell*

Microinjection of picrotoxin into the anterior VTA concentration-dependently increased extracellular dopamine levels in the ACB shell ($F_{2,\ 15}=27.73$, $p<0.001$) (Fig. 5A). Extracellular dopamine levels significantly increased within 30 min after microinjection of 80 μM picrotoxin into the anterior VTA (130 ± 7% of baseline), peaked within 45 min post injection (141 ± 9% of baseline), then returned to baseline by 75 min post microinjection. Microinjection of 160 μM picrotoxin into the anterior VTA increased extracellular dopamine levels within the first 15 min (150 ± 20% of baseline, $p<0.05$), which reached a peak within 45 min after microinjection (187 ± 12% of baseline, $p<0.05$). Dopamine levels returned to baseline by 75 min.

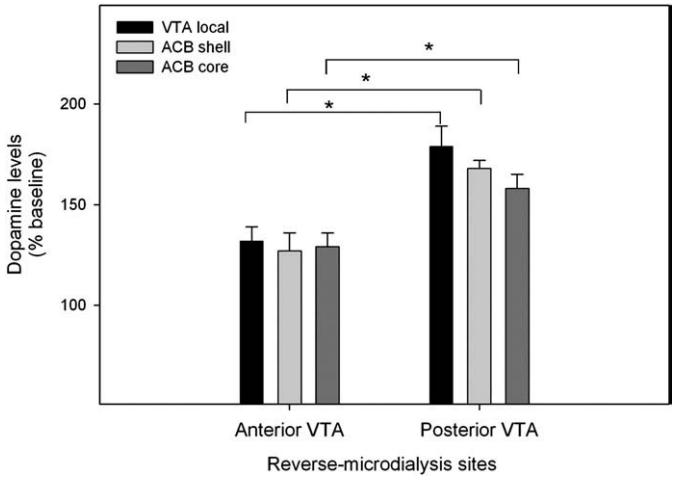


**Fig. 4.** The average extracellular dopamine levels in three collection areas during 40- to 100-min reverse-microdialysis of sulpiride into the anterior or posterior VTA. *: significant difference between reverse-microdialysis in the anterior and posterior VTA, $p<0.05$.

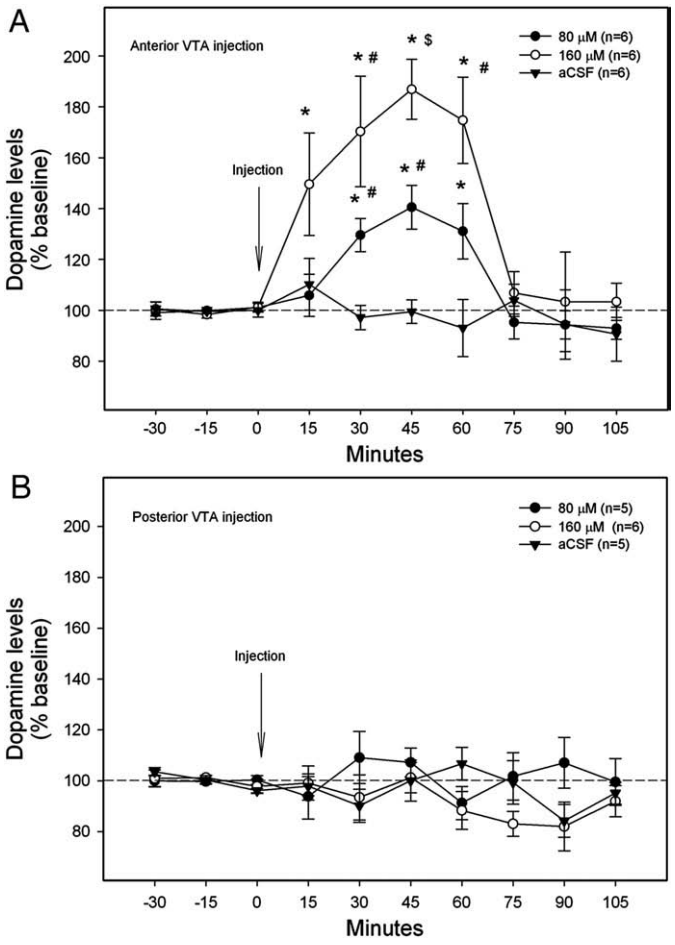


**Fig. 5.** A: The effects of microinjecting picrotoxin into the anterior VTA on extracellular dopamine levels in the ACB shell. *: significant difference from baseline, $p<0.05$. #: different from aCSF-injection group, $p<0.05$; $: different from aCSF- and 80 μM picrotoxin-injection groups, $p<0.05$. B: The effects of microinjection of picrotoxin into the posterior VTA on extracellular dopamine levels in the ACB shell. There was no significant effect of picrotoxin.

In contrast to the effects observed in the anterior VTA, injection of picrotoxin into the posterior VTA had no significant effect on extracellular dopamine levels in the ACB shell ($F_{2,\ 14}=0.22$, $p>0.05$) (Fig. 5B). Analysis of average dopamine levels during the 60-min period after injection indicated that picrotoxin produced a greater effect after being injected into the anterior compared to posterior VTA (80 μM: 127 ± 4% and 100 ± 4% of baseline, respectively, $p<0.01$; 160 μM: 170 ± 8% and 96 ± 6% of baseline, respectively, $p<0.001$).

## 4. Discussion

The results of this study indicate that there may be different mechanisms regulating dopamine neuronal activity between the anterior and posterior VTA of female Wistar rats with greater dopamine $D_2$ autoreceptor-mediated tonic inhibition in the posterior than anterior VTA and greater $GABA_A$ receptor-mediated tonic inhibition in the anterior than posterior VTA. These conclusions are based upon the findings that local perfusion of sulpiride (a $D_2$ receptor antagonist) increased extracellular dopamine levels greater in the posterior than anterior VTA (Fig. 2 and 4), and picrotoxin (a $GABA_A$ receptor antagonist) increased dopamine release in the ACB shell when administered into the anterior but not the posterior VTA (Fig. 5).

In addition, the results that sulpiride increased dopamine release in the ACB shell and core after being perfused into the posterior VTA (Fig. 3A) suggest that the posterior VTA projects to both the ACB shell and core; the results of elevated dopamine release in the ACB shell after picrotoxin microinjection into the anterior VTA (Fig. 5A), along with the finding in the study of Ikemoto et al. (1997a), which reported an increased dopamine release mainly in the ACB core after microinjection of picrotoxin in the anterior VTA, suggest that dopamine neurons in the anterior VTA also project to the ACB shell and core. Finally, the higher basal extracellular dopamine concentrations in the ACB core compared to the shell suggest higher dopamine activity in the core, and/or more dopamine projections to the ACB core versus the shell. However, quantitative microdialysis studies with no-net-flux technique are needed to appropriately determine possible differences in extracellular concentrations of dopamine between these two sub-regions.

Dopamine $D_2$ receptors are expressed in the VTA (Bouthenet et al., 1987; Pickel et al., 2002; Sesack et al., 1994), and mediate tonic inhibition of somatodendritically released dopamine (Adell and Artigas, 2004; Beart et al., 1979; Kalivas et al., 1989). Administration of $D_2$ receptor antagonists increased VTA dopamine neuronal firing (Aghajanian and Bunney, 1977; Groves et al., 1975; Wang, 1981; White and Wang, 1984b) and extracellular dopamine levels in both the VTA and its projecting areas, including the ACB and prefrontal cortex (Kohl et al., 1998; Westerink et al., 1996, 1998). The present results are in agreement with these studies. However, these previous studies did not examine the regional differences within the VTA and ACB, although the study of Kohl et al. (1998) indicated that most probes were in the posterior VTA. The current study suggests that the tonic inhibition by $D_2$ autoreceptors is more robust in the posterior than anterior VTA. Possible explanations for this finding are there may be fewer dopamine neurons with $D_2$ autoreceptors in the anterior than posterior VTA and/or the sensitivities of $D_2$ autoreceptors may be different between the anterior and posterior VTA. It is also possible that dopamine neurons in the anterior VTA may be under tonic inhibitory control by other mechanisms and, as a result, perfusion with sulpiride has smaller effect in the anterior than posterior VTA.

There are studies suggesting there may be fewer dopamine neurons with $D_2$ autoreceptors and/or less sensitive $D_2$ autoreceptors in the anterior than posterior VTA (Chiodo et al., 1984; Olson et al., 2005; Pickel et al., 2002; Swanson, 1982; White, 1996). VTA dopamine neurons send mesocortical projections to cortical regions and mesolimbic projections to limbic areas (Bannon and Roth, 1983; Oades and Halliday, 1987). Mesocortical pathway originates mainly from dopamine neurons in the parabrachial pigmented nucleus (Pickel et al., 2002; Williams and Goldman-Rakic, 1998), which is distributed throughout the VTA, with a relatively greater volume and number of cells within the anterior than posterior VTA (Halliday and Tork, 1986; Ikemoto, 2007). The mesolimbic pathway originates mainly from dopamine neurons in the paranigral nucleus, the interfascicular nucleus and central linear nucleus (Ikemoto, 2007; Pickel et al., 2002; Williams and Goldman-Rakic, 1998), which are located mainly within the posterior VTA (Halliday and Tork, 1986; Ikemoto, 2007). Some other retrograde-tracing studies also indicate that more mesocortical projection neurons are located in the anterior portion of the VTA and ACB-projecting neurons are mainly in the posterior VTA (Olson et al., 2005; Swanson, 1982). If this is the case, it may provide an explanation for the results from the sulpiride experiments in the current study (Fig. 2). Mesocortical dopamine neurons have been suggested to have lower density and/or sensitivity of somatodendritic $D_2$ autoreceptors compared to mesolimbic dopamine neurons (Bannon and Roth, 1983; Chiodo et al., 1984; White, 1996; White and Wang, 1984a). These neurons exhibited significantly faster basal firing rate and more intense burst firing activity compared to mesolimbic and nigrostriatal dopamine neurons (Chiodo et al., 1984; Cooper et al., 2003; Elsworth and Roth, 1997; White, 1996; White and Wang, 1984a). These neurons were also significantly less sensitive to the inhibition of intravenously administrated dopamine agonist apomorphine, and were unresponsive to microiontophoretically applied dopamine (Chiodo et al., 1984; White and Wang, 1984a). Although several electrophysiological studies did not find similar differences between the mesocortical and mesolimbic dopamine neurons (Diana et al., 1998; Gariano et al., 1989), two subsequent studies supported possible differences in $D_2$ autoreceptor density and function between the mesocortical and mesolimbic dopamine neurons (Chen and Pan, 2000; Pickel et al., 2002). Perfusing a low dose of the $D_2$ autoreceptor antagonist eticlopride into the VTA had no effects on extracellular dopamine levels in the medial prefrontal cortex but significantly increased dopamine release in the ACB (Chen and Pan, 2000). Furthermore, mesocortical dopamine neurons exhibited a significantly lower percentage of $D_2$ receptor immunoreactivity than mesolimbic dopamine neurons (Pickel et al., 2002). Taken together, these studies favor the tentative hypothesis that the anterior VTA may have fewer and/or less sensitive $D_2$ autoreceptors than the posterior VTA, so that the anterior VTA responded less robustly than the posterior VTA to local perfusion of sulpiride (Fig. 2).

Several studies suggest that the anterior VTA may have fewer dopamine neurons than the posterior VTA (German and Manaye, 1993; Ikemoto, 2007; Swanson, 1982). Studies with immunohistochemical staining of tyrosine hydroxylase – a marker of dopamine neurons, revealed that the greatest density of dopamine neurons in the VTA was in the posterior portion of the VTA at the level of the interpeduncular nucleus, whereas the anterior portion of the VTA at the level of the mammillary body had less dense dopamine neurons (German and Manaye, 1993; Ikemoto, 2007; Swanson, 1982). Given these findings, it would be expected that the basal extracellular dopamine level in the anterior VTA might be less than that in the posterior VTA. However the results in the current study and the study of Liu et al. (2006) do not support this assumption, because both studies revealed no significant difference in extracellular basal dopamine levels between the anterior and posterior VTA ($1.5 \pm 0.2$ nM vs $1.2 \pm 0.2$ nM in the current study and $1.1 \pm 0.2$ nM vs $1.0 \pm 0.2$ nM in Liu et al. 2006 study). The comparable basal extracellular dopamine levels in the anterior and posterior VTA may be due to higher dopamine neuronal activity in the anterior than posterior VTA, due to lower $D_2$ autoreceptor function in the anterior VTA (Fig. 2). However, further quantitative microdialysis experiments will need to be conducted in the VTA to confirm this interpretation.

$GABA_A$ receptors and mRNAs for various $GABA_A$ receptor subunits are located within the VTA (Churchill et al., 1992; Pirker et al., 2000). Specifically, VTA dopamine neurons express mRNAs for major $GABA_A$ receptor subunits, such as $\alpha$, $\beta$, and $\gamma$ subunits (Okada et al., 2004). Previous electrophysiological and pharmacological studies demonstrated that $GABA_A$ receptors mediate tonic inhibition of VTA dopamine neurons (Johnson and North, 1992; Kalivas, 1993). Local application of $GABA_A$ receptor antagonists depolarized VTA dopamine neurons (Johnson and North, 1992; Yim and Mogenson, 1980), and increased extracellular dopamine levels in the ACB (Ikemoto et al., 1997a; Westerink et al., 1996). The study of Westerink et al. (1996) had probes mainly in the border of the anterior and the posterior VTA ($-5.3$ mm relative to the bregma), and the study of Ikemoto et al. (1997a) examined only the anterior VTA ($-4.8$ mm relative to the bregma) and had most probes in the ACB core and ACB core plus shell. The current study confirmed that picrotoxin microinjection into the anterior VTA concentration-dependently increased extracellular dopamine levels in the ACB and these effects could be observed with probes in the ACB shell (Fig. 5A). Furthermore, the present study demonstrated that blockade of $GABA_A$ receptors with picrotoxin in the posterior VTA did not change extracellular dopamine levels in the ACB shell (Fig. 5B). This is consistent with the ICSA study with $GABA_A$ antagonists, in which the anterior VTA supported self-infusion and

reinforcing effects of picrotoxin, whereas the posterior VTA did not (Ikemoto et al., 1997b). In addition, microinjection of picrotoxin into the anterior VTA increased locomotor activity, whereas the same treatment in the posterior VTA caused mild sedation (Arnt and Scheel-Kruger, 1979). Taken together, these reports suggest that the ACB shell-projecting dopamine neurons in the anterior VTA may be under tonic $GABA_A$ receptor-mediated inhibition, whereas their counterparts in the posterior VTA may not. The apparent lack of effect of picrotoxin in the posterior VTA on dopamine release in the ACB shell suggests that $GABA_A$ receptors in the posterior VTA may not be tonically active; and/or $GABA_A$ receptors may not form an inhibitory circuitry on VTA dopamine neurons in the posterior VTA.

The present results suggest that both the anterior and posterior VTA dopamine neurons project to the ACB shell and core (Figs. 3 and 5). The apparent 'upward-drift' of extracellular dopamine levels in Fig. 3B was likely due to sulpiride perfusion, and not a result of 'baseline drift'. No 'baseline drift' was observed in the ACB shell with aCSF administration (Fig. 5). Also previous studies did not observe 'baseline drift' in the ACB with aCSF perfusion over a similar time course (Engleman et al., 2000; Yoshimoto et al., 1991). This finding is consistent with previous neuroanatomical observations, in which injection of retrograde-tracers into the ACB shell or core labeled dopamine neurons both in the anterior and posterior VTA (Ikemoto, 2007; Swanson, 1982). Although ACB shell and core receive dopaminergic innervations from dopamine neurons across the VTA, these two sub-regions of the ACB differ from each other in a number of ways, including immunohistochemical markers (Voorn et al., 1989), afferent innervations, and efferent projections (Brog et al., 1993; Zahm, 2000). The current study observed a significantly higher basal extracellular dopamine level in the core than shell, which may suggest higher dopamine activity in the core than shell. The present finding agrees with several microdialysis studies (Acquas et al., 2007; Hedou et al., 1999a,b; Pierce and Kalivas, 1995), but disagree with two other studies reporting no difference between the two sub-regions (Pontieri et al., 1995; Sokolowski et al., 1998). Two recent studies, which measured basal extracellular dopamine levels in the sub-regions of the ACB, reported higher basal dopamine levels in the core than shell (Hipolito et al., 2008; McKittrick and Abercrombie, 2007). McKittrick and Abercrombie (2007) proposed that the inconsistency among different studies was likely due to the rostrocaudal differences in probe placements in the ACB shell, because higher dopamine levels were reported in the rostral shell. Another possibility may be due to the variability of *in vivo* recovery of microdialysis probes. The basal dopamine levels measured with a conventional microdialysis technique (employed in the present and previous studies) are dependent upon the recovery of the probe (Justice, 1993). To appropriately compare basal extracellular dopamine concentrations in these two sub-regions, the quantitative microdialysis technique is needed.

Several studies, utilizing the microinjection and microdialysis techniques indicated that it was possible to differentiate behavioral and neurochemical effects between the anterior and posterior VTA (Arnt and Scheel-Kruger, 1979; Ericson et al., 2008; Ikemoto et al., 1997b, 1998, 2006). Local perfusion of a serotonin-3 receptor agonist produced a significantly greater increase of extracellular dopamine levels in the posterior than anterior VTA (Liu et al., 2006). Perfusion of a nicotinic acetylcholine receptor antagonist mecamylamine into the anterior, but not posterior VTA, blocked ethanol-induced dopamine release in the ACB (Ericson et al., 2008). In addition, more and more evidence emerges suggesting anatomical and behavioral heterogeneity in the VTA along the anterior–posterior axis. The anterior VTA has more GABA interneurons (Olson and Nestler, 2007), whereas the posterior VTA has more dopamine neurons and specifically more ACB-shell-projecting dopamine neurons (German and Manaye, 1993; Ikemoto, 2007; Swanson, 1982). Endomorphine 1, nicotine and serotonin-3 receptor agonist 1-(*m*-chlorophenyl)-biguanide are more reinforcing in the posterior VTA than in the anterior VTA (Ikemoto et al., 2006; Rodd et al., 2007; Zangen et al., 2002). Over-expression of either one of the GluR1 subunit of the AMPA receptor, phospholipase $C_\gamma$ subunit, or cAMP-response element - binding protein in the anterior VTA enhanced the rewarding effects of morphine and cocaine, whereas the same manipulations in the posterior VTA caused aversion to morphine and cocaine (Bolanos et al., 2003; Carlezon et al., 2000; Olson et al., 2005).

The differential regulation of dopamine neurons by $D_2$ autoreceptors and $GABA_A$ receptors may contribute to the differential reinforcing effects of ethanol between the anterior and posterior VTA (Rodd-Henricks et al., 2000; Rodd et al., 2004). Dopamine neurons appear to be under tonic $GABA_A$ receptor mediated inhibition in the anterior but not posterior VTA (Fig. 5). Since ethanol can increase the function of $GABA_A$ receptors (Allan et al., 1987; Ticku et al., 1986), then the actions of ethanol in the anterior VTA would be to reduce dopamine neuronal activity, which would not produce reinforcing effects. In the posterior VTA, this tonic $GABA_A$ mediated inhibition of dopamine neurons does not appear to be evident. Therefore, ethanol could be acting through excitatory receptors to stimulate dopamine neurons and produce reinforcing effects. There is evidence that the reinforcing effects of ethanol in the posterior VTA are regulated by 5-$HT_3$ (Rodd et al., 2004, 2005a) and 5-$HT_{2A}$ (Ding et al., in press) receptors. Furthermore, chronic alcohol drinking reduces $D_2$ autoreceptor function (Engleman et al., 2003), increases dopamine neuronal activity in the mesolimbic system (Thielen et al., 2004), and increases the reinforcing effects of ethanol in the posterior VTA (Rodd et al., 2005c). Therefore, in the posterior VTA, the reduction in tonic $D_2$ regulated negative feedback with ethanol exposure could increase the reinforcing effects of alcohol and contribute to alcohol addiction.

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