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Differential Muscarinic Modulation of Synaptic Transmission in Dorsal and Ventral Regions of the Rat Nucleus Accumbens Core

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Summary

The nucleus accumbens (NAc) core is critical in the control of motivated behaviors. The muscarinic acetylcholine receptors (mAChRs) modulating the excitatory inputs into the NAc core have been reported to impact such behaviors. Recent studies suggest that ventral and dorsal regions of the NAc core seem to be innervated by distinct populations of glutamatergic projection neurons. To further examine mAChRs modulation of these glutamatergic inputs to the NAc core, we employed intracellular recordings in rat NAc coronal slice preparation to characterize: 1) the effects of muscarine, an mAChRs agonist, on membrane properties of the NAc core neurons; 2) depolarizing synaptic potentials (DPSP) elicited by ventral and dorsal focal electrical stimuli; and 3) paired-pulse response with paired-pulse stimulation. Here we report that the paired-pulse ratio (PPR) elicited by dorsal stimuli was greater than that elicited by ventral stimuli. Bath application of muscarine (1-30 μM) decreased both ventral and dorsal DPSP in a concentration-dependent manner, with no effect on electrophysiological properties of NAc core neurons. Muscarine at 30 μM also elicited larger depression of dorsal DPSP than ventral DPSP. Moreover, muscarine increased the PPR of both dorsal and ventral DPSP. These data indicate that the glutamatergic afferent fibers traversing the dorsal and ventral NAc are separate, and that differential decrease of distinct afferent excitatory neurotransmission onto NAc core neurons may be mediated by presynaptic mechanisms.

Key words

Muscarine • Nucleus accumbens • Postsynaptic potentials • Paired-pulse ratio • *In vitro*

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Introduction

The nucleus accumbens (NAc) plays a vital role in the generation of motivated behaviors (Berridge 2007) and is generally divided into core and shell subregions based primarily on immunohistochemical characteristics (Zahm 1999). The NAc core is thought to translate limbic sensory information, including reward-related stimuli, the context-dependent cues, into motor output (Meredith *et al.* 2008), forming part of a network crucial for appetitive instrumental learning implicated in drug addiction and certain psychiatric diseases (Kelley 2004, Shirayama and Chaki 2006).

The functions of NAc core are regulated by glutamate transmission (Kalivas *et al.* 2009, LaLumiere and Kalivas 2008). Glutamatergic inputs onto the NAc core primarily arise from basolateral amygdala (BLA), prefrontal cortex (PFC), hippocampus and thalamus. Recent studies suggest that distinct subregions of the NAc core are innervated by different glutamatergic projections (Britt *et al.* 2012, Humphries and Prescott 2010, Voorn *et al.* 2004). Neuroanatomical studies have

demonstrated that dorsal agranular insular cortex and dorsal prelimbic cortex project primarily to the dorsal NAc core, while the ventral NAc core is mainly innervated by pathways originating in the ventral prelimbic cortex, ventral agranular insular cortex and olfactory tubercle (Groenewegen *et al.* 1999, Stuber *et al.* 2011, Voorn *et al.* 2004, Yuan *et al.* 1992). These heterogeneously distributed afferents generate distinct physiological and behavioral responses when activated selectively (Britt *et al.* 2012, Goto and Grace 2005, Stuber *et al.* 2012) and may be differently regulated by the interneurons, for example, the cholinergic interneurons, in the NAc core.

Acetylcholine (ACh) transmission by local cholinergic interneurons is widespread in the NAc. ACh regulates glutamatergic synaptic transmission in the NAc and has a critical role in NAc-mediated behavior (Crespo *et al.* 2006, Hikida *et al.* 2003, Pratt and Kelley 2004). ACh inhibits the release of neurotransmitter by activating presynaptic muscarinic acetylcholine receptors (mAChRs) (Pennartz and Lopes da Silva 1994, Zhang and Warren 2002) and increases the excitability of NAc neurons by acting on postsynaptic mAChRs (Uchimura and North 1990). In this study, we performed intracellular recordings in rat NAc coronal slice preparation to identify any differential effects of mAChR signaling on synaptic transmission in the dorsal and ventral NAc core.

Materials and Methods

Animals

Experiments were performed on 3-8 week old Sprague-Dawley rats of male and female. Rats were obtained from the Experimental Animal Center of Zhejiang Province, China. All procedures were approved by the Animal Care and Use Committee of the Institute of Psychology, Chinese Academy of Sciences, and all efforts were made to minimize the rats' suffering and the number of rats used.

NAc slice preparation

Rat NAc slices were prepared as follows. Rats were anaesthetized with diethyl ether and decapitated, and the brain was quickly transferred to cold (0-4 °C) artificial cerebrospinal fluid (ACSF) of the following composition (in mM): NaCl 126, KCl 2.5, NaH_2PO_4 1.25, $MgCl_2$ 1, $CaCl_2$ 2, $NaHCO_3$ 26, and glucose 20. A tissue block containing the NAc was glued to an agar chunk. Coronal brain slices (350-400 μm thick) containing the NAc were sectioned by Vibratome 1000 or 1500 series tissue slicers (Technical Products International Inc., USA). Slices were incubated in oxygenated ACSF at room temperature (22-28 °C) for 60 min, after which a single slice was transferred to a recording chamber (0.5 ml). In the recording chamber, the slice was superfused by oxygenated ACSF continuously at room temperature.

Intracellular recordings

Borosilicate, filament containing capillary glass micropipettes (FSG12, OD: 1.2 mm, ID: 0.6 mm) were pulled into sharp glass microelectrodes using a Micropipette Puller (Model P-97, Sutter Instrument Co., USA). Intracellular recordings were performed in NAc core neurons with microelectrodes filled with 3 M potassium acetate (input impedance of 70-120 MΩ), using an AxoClamp 2B amplifier (Axon Instrument Inc., USA) in current-clamp mode. The location of the recorded neurons was visualized by a stereo microscope, using the anterior commissure (AC), neostriatum, septum and lateral ventricles as landmarks based on a rat brain stereotaxis atlas (Paxinos and Watson 1986).

Data acquisition was performed with pClamp7.0 software (Axon Instruments) and off-line analysis was performed with Clampfit software (Axon Instruments).

Presynaptic stimulation

At resting potential, depolarizing synaptic potentials (DPSP) were evoked by focal stimulation with a concentric bipolar tungsten stimulating electrode, placed onto the surface of the slice dorsal or ventral to the AC (D-DPSP and V-DPSP). The distance between the stimulating electrode and the recording electrode was 0.3-0.5 mm. Electrical stimulation (single square wave, 20-100 V, 0.1 ms duration) was delivered at 0.2 Hz, and stimulus intensity was kept invariant throughout the recording period. Paired-pulse stimulation was delivered with interpulse intervals (IPIs) of 50, 100 and 150 ms, and other stimulation parameters were the same as above. Paired-pulse ratio (PPR) was evaluated as the ratio of the second DPSP amplitude to the first, and was used to distinguish pre- and/or postsynaptic mechanisms.

Drug application

Drugs were prepared in stock solutions and perfused at set concentrations *via* a pump. After recording a stable DPSP for at least 15 min, ACSF containing muscarine was superfused for 3-10 min, which was

sufficient for depression of the synaptic potential to achieve a steady state, and was then followed by washout with control ACSF. Muscarine, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and DL-2-amino-5-phosphonovaleric acid (APV) were purchased from Sigma-Aldrich Co., USA, and tetrodotoxin (TTX) was purchased from Fisheries Science and Technology Development Company of Hebei Province, China.

Statistics

All averaged values are expressed as mean ± SEM. Statistical comparisons were performed using Student's unpaired or paired *t* test, one-way ANOVA followed by *post-hoc* Holm-Sidak test, and $p<0.05$ was considered statistically significant.

Table 1. Electrophysiological parameters of NAc core neurons before and during muscarine superfusion.

	Control (n=22)	Muscarine (μM)			
		1 (n=22)	3 (n=21)	10 (n=20)	30 (n=21)
RMP (mV)	−82.9±1.7	−83.9±2.0	−80.7±2.4	−79.5±1.6	−78.6±1.6
Membrane resistance (MΩ)	63.9±4.7	70.2±5.1	59.1±6.6	65.8±4.8	73.6±6.7
Spike amplitude (mV)	58.5±1.8	58.4±1.4	56.0±2.3	57.8±2.6	52.2±1.9

One-way ANOVA: $p>0.05$ in all parameters

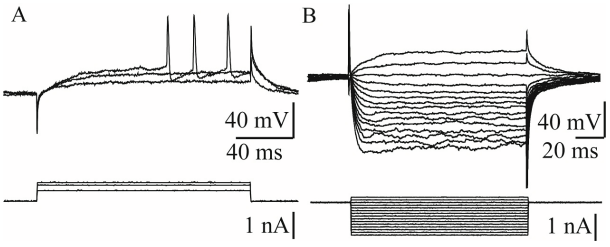

Fig. 1. Typical membrane properties of NAc core neurons, evaluated by recording responses to current steps. (**A**) The electrotonic membrane potential (top) was obtained with a step of the depolarizing current injected, 0.4 nA, 0.6 nA and 0.7 nA, respectively (bottom), indicating a slowly depolarizing ramp. The resting potential of this neuron was −78 mV. (**B**) Electrotonic membrane potentials induced by a series of hyperpolarization current steps, in which the most negative one was −1.2 nA with decrements of current pulses of 0.1 nA, showing the inward rectification. The resting potential was −74 mV.

Results

Effects of muscarine on membrane properties of NAc core neurons

Results were obtained from 53 neurons using intracellular recordings. These neurons generally exhibited electrophysiological characteristics of medium spiny neurons (MSNs), *i.e.*, relatively negative average RMP of −79.5±1.0 mV, input resistance of 63.4±2.6 MΩ, spike amplitude of 61.8±1.1 mV, spontaneous spike firing and spontaneous synaptic activity were not observed, a slow depolarizing ramp (Fig. 1A) was observed in more than half of the neurons (29/53) and inward rectification (Fig. 1B) was observed in nearly one third of the neurons (17/53).

After bath application of the selective mAChR agonist muscarine at 1 μM, 3 μM, 10 μM and 30 μM, there was no change in the intrinsic membrane properties of the NAc core neurons in which ventral or dorsal DPSP were evoked, so we pooled them (RMPs: $F_{(4,56)}=1.4$, $p>0.05$; membrane resistance: $F_{(4,64)}=0.8$, $p>0.05$; spike amplitude: $F_{(4,58)}=1.6$, $p>0.05$) (Table 1).

Muscarine produces a differential dose-dependent decrease in V-DPSP and D-DPSP

At RMPs, focal electrical stimulation of the ventral and dorsal NAc evoked DPSP in 29 and 34 neurons, respectively, defined as V-DPSP and D-DPSP. The amplitudes of both DPSPs were graded with the intensity of the stimulation (ventral n=10, dorsal n=4) (Fig. 2A), decreased with membrane potential depolarization and increased with membrane potential hyperpolarization (ventral n=3, dorsal n=3) (Fig. 2B). These synaptic potentials were also reversibly abolished by low Ca^{2+} / high Mg^{2+} ACSF solution (ventral n=3, dorsal n=2) or by TTX (0.5 μM) (ventral n=2, dorsal n=3) (Fig. 2C). The amplitudes of V-DPSP and D-DPSP were reduced to 15-17 % and 14-25 % of control, respectively, when CNQX (10 μM) was applied (ventral n=3, dorsal n=2) (Fig. 2D), and there was minimal further reduction when CNQX (10 μM) and APV (30 μM) were co-applied

(Fig. 2D). We found no difference between V-DPSP and D-DPSP at the same stimulus range (unpaired *t* test in all parameters, $p>0.05$), and the quantified parameters of both responses are summarized in Table 2. The latencies of V-DPSP and D-DPSP remained constant when the stimulus intensity was changed, indicating that the two types of DPSPs were both monosynaptic.

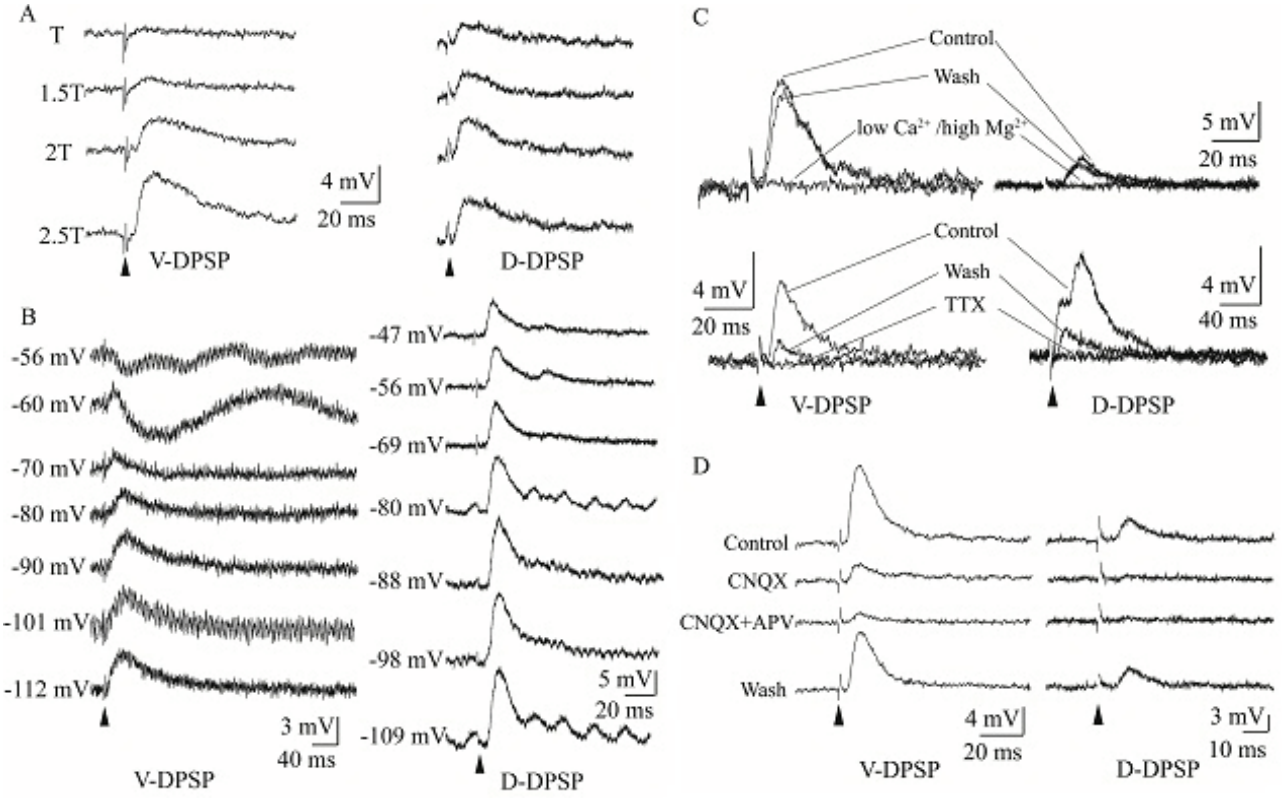

Fig. 2. Cell electrophysiological properties of postsynaptic potentials evoked by ventral and dorsal focal electrical stimuli in NAc core neurons. (**A**) Both DPSP amplitudes were graded with stimulus intensities. T shows the threshold intensity of stimulus to evoke DPSP. (**B**) Responses to the same electrical stimulus at different membrane potentials by injecting hyperpolarizing and depolarizing currents into the neurons. The more negative the membrane potential, the greater the DPSP amplitude. (**C**) The top traces show that both DPSPs were reversibly abolished by low Ca^{2+} / high Mg^{2+} ACSF solution, and the bottom traces indicate that both DPSPs were abolished by TTX (0.5 μM) with partial recovery. (**D**) Effect of a quisqualate/kainate receptor antagonist on both DPSPs. 10 μM CNQX almost completely abolished the DPSPs, and CNQX superfusion along with APV (50 μM) had minimal further inhibitory effect, with recovery on washout.

Table 2. Cell electrophysiological parameters of ventral and dorsal stimulus evoked depolarizing synaptic potentials.

	V-DPSP (n=29)	**D-DPSP (n=34)**
Amplitude (mV)	6.4±0.8	8.6±0.9
*Area under curve (mV*ms)*	171.1±29.2	231.6±31.1
Duration (ms)	84.6±8.3	77.5±5.3
Latency (ms)	4.4±0.2	4.8±0.3
Half-width (ms)	16.3±1.6	18.1±1.5
Rise time (ms)	6.1±0.7	5.8±0.5
Decay time (ms)	37.0±5.4	38.5±4.2

Unpaired *t* test: $p>0.05$ in all parameters

At RMPs, muscarine attenuated the amplitude of V-DPSP in 18 tested cells in a dose-dependent manner ($F_{(4,53)}=18.2$, $p<0.05$), with partial recovery after wash. Uniformly, muscarine also reversibly decreased the amplitude of D-DPSP in 14 neurons in a dose-dependent manner ($F_{(4,35)}=30.6$, $p<0.05$). The areas under the curves of V-DPSP and D-DPSP were also decreased (ventral $F_{(4,52)}=6.9$, $p<0.05$; dorsal $F_{(4,35)}=21.1$, $p<0.05$). The normalized parameters of both responses are summarized in Table 3. The time course of V-DPSP, evaluated by duration, half-width, rise time and decay time, were not altered during superfusion of muscarine at all concentrations except at 30 μM relative to control. However, the duration, half-width and decay time of D-DPSP were shortened compared to control with application of 30 μM muscarine (paired *t* test, $p<0.05$).

Table 3. Comparisons of concentration-dependent inhibition of muscarine on V-DPSP and D-DPSP.

	Amplitude (%)		Area under curve (%)	
	V-DPSP	D-DPSP	V-DPSP	D-DPSP
1 μM	72.1±6.5*(10)	73.0±7.0*(6)	73.7±11.2(10)	64.9±6.0*(6)
3 μM	63.0±11.5*(7)	53.3±6.0*(6)	60.6±14.9*(7)	47.9±8.6*(6)
10 μM	61.1±4.6*(13)	52.3±7.2*(9)	62.0±7.4*(13)	46.1±11.9*(9)
30 μM	52.6±4.3*(9)	32.4±5.4*(5)	54.4±9.2*(9)	18.0±4.2*(5)

One-way ANOVA followed by *post-hoc* Holm-Sidak test, * $p<0.05$ vs. control

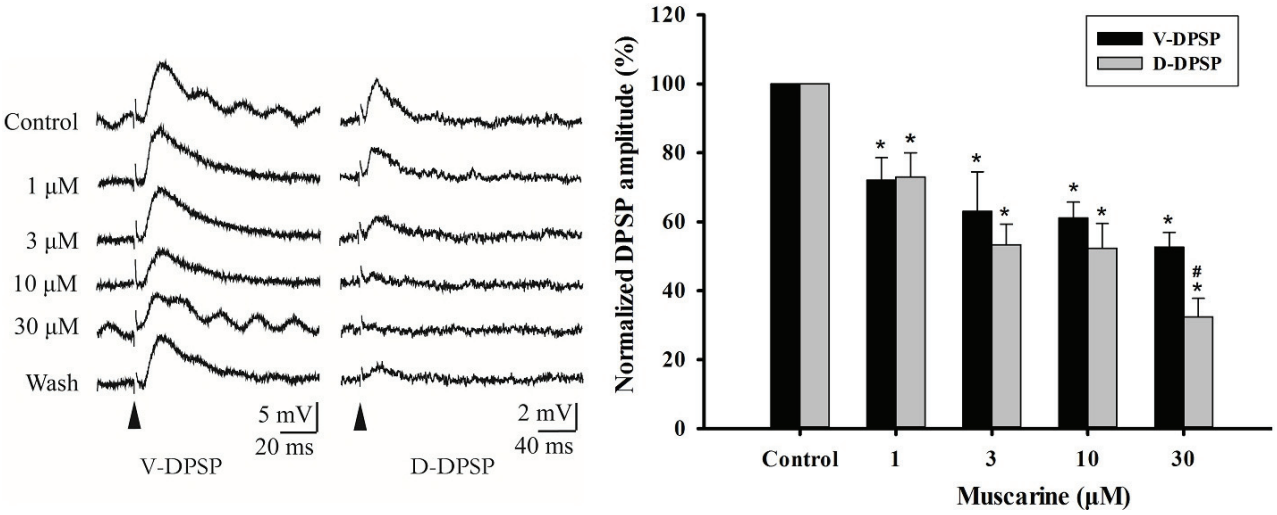

Fig. 3. Muscarine inhibited V-DPSP and D-DPSP to a different degree. Left, representative DPSPs obtained from ventral and dorsal focal electrical stimuli were depressed by muscarine at several concentrations. Arrow head indicates the electrical stimulus artifact. Right, summary of normalized V-DPSP (black) and D-DPSP (gray) amplitudes at several concentrations of muscarine. Bars represent mean proportions (± SEM) determined in two independent experiments. * $p<0.05$ compared with untreated control (one-way ANOVA followed by the Holm-Sidak test). Muscarine elicited a stronger inhibitory effect on D-DPSP amplitude than V-DPSP amplitude, # $p<0.05$ compared with V-DPSP (unpaired *t* test).

We also observed that the inhibition ratio of D-DPSP amplitude and area under curve by muscarine at 30 μM was greater than that of V-DPSP (unpaired *t* test, $p<0.05$), but not at lower concentrations of muscarine (Fig. 3).

Muscarine increases the PPR of V-DPSPs and D-DPSPs

Paired-pulse facilitation of ventral or dorsal focal electrical stimulation evoked DPSP was observed in most of the tested neurons when IPIs was 50 ms, 100 ms and 150 ms. The PPR of V-DPSPs (i.e. V-PPR) and D-DPSPs (i.e. D-PPR) were both decreased as the IPIs extended (ventral n=13, $p<0.05$; dorsal n=21, $p<0.05$) (Fig. 4A). V-PPR was 1.4±0.1, 1.2±0.1 and 1.1±0.1 at IPIs 50 ms, 100 ms and 150 ms, respectively, while D-PPR was 1.6±0.1, 1.5±0.1 and 1.4±0.1. As shown in Figure 4A, D-PPR was statistically greater than V-PPR (unpaired *t* test, $p<0.05$) at 100 ms and 150 ms intervals.

To assess whether presynaptic mechanisms were involved in the muscarinic inhibition of DPSP, V-DPSPs and D-DPSPs evoked by paired-pulse stimulation at 100 ms intervals were examined before and after muscarine superfusion. The PPR of V-DPSPs and D-DPSPs was increased in most neurons examined (paired *t* test, ventral n=6, dorsal n=3, $p<0.05$) (Fig. 4B).

Discussion

In this study, we investigated the effects of the mAChR agonist muscarine on the DPSP and PPR evoked by ventral or dorsal local circuits in NAc core. We found that muscarine elicited a reduction of both dorsal and ventral DPSP but had no influence on membrane properties of the NAc core neurons. Moreover, we found

that the inhibitory effect on D-DPSP was significantly larger than that on V-DPSP at high muscarine concentration (30 μM). Furthermore, muscarine increased both D-PPR and V-PPR. Our results suggest the existence of distinct dorsal and ventral subregions in the NAc core that are differentially modulated by muscarine.

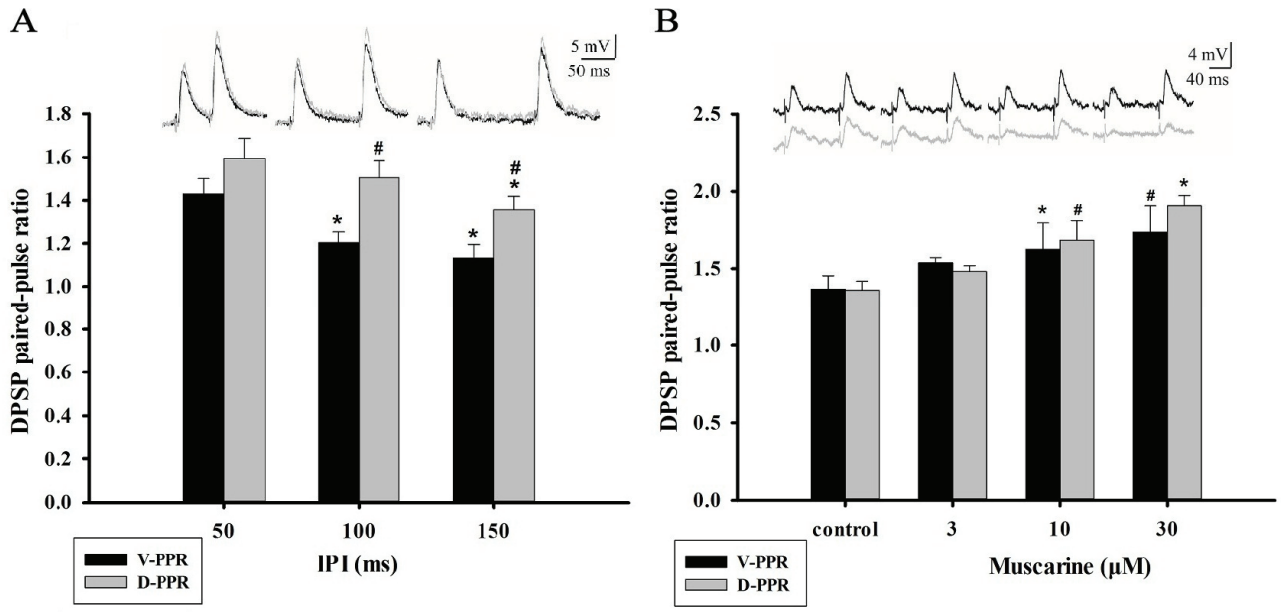

Fig. 4. PPR was increased by muscarine. (**A**) paired-pulse facilitation was induced by paired-pulse stimulation at different IPIs. V-PPR (black, n=13) and D-PPR (gray, n=21) were decreased along with the increase of IPIs. D-PPR was greater than V-PPR. * $p<0.05$ compared with 50 ms (paired *t* test). # $p<0.05$ compared with V-PPR (unpaired *t* test). Inset shows the representative paired-pulse facilitation evoked from ventral and dorsal focal electrical stimuli (ventral, black; dorsal, gray). (**B**) summary of V-PPR (black) and D-PPR (gray) at several concentration of muscarine. Error bars represent mean proportions (± SEM). * $p<0.05$; # $p=0.07$ compared with untreated control (paired *t* test).

Neuroanatomical studies have suggested that the main origin of glutamatergic afferent fibers traversing the ventral and dorsal NAc is separate (Groenewegen *et al.* 1999, Humphries and Prescott 2010, Voorn *et al.* 2004). In the present study, both V-DPSP and D-DPSP were mainly mediated by AMPA/KA receptors (Fig. 2) and showed similar electrophysiological characteristics. These data were in agreement with previous reports (O'Donnell and Grace 1993, Pennartz *et al.* 1991, Pennartz and Lopes da Silva 1994, Uchimura *et al.* 1989). Moreover, we found that D-PPR was greater than V-PPR (Fig. 4A), suggesting that glutamatergic afferent properties are different between dorsal and ventral afferent fibers within the NAc core. The specific pathways of the ventral and dorsal NAc core await further examination.

Axonal arborization originating from cholinergic interneurons is distributed throughout the NAc core, and ACh plays critical roles in the functional regulation of NAc core neurons *via* the activation of mAChRs. Muscarine has been used as a specific agonist of mAChRs in many brain regions including the superior colliculus, dorsal striatum, suprachiasmatic nucleus and hippocampus (Lin *et al.* 2004, Sooksawate and Isa 2006, Vogt and Regehr 2001, Yang *et al.* 2010). In agreement with previous studies (Pennartz and Lopes da Silva 1994, Sugita *et al.* 1991, Zhang and Warren 2002), we found that muscarine inhibits V-DPSP and D-DPSP in a dose-dependent manner. This suppressive effect of muscarine on DPSP may be mainly *via* presynaptic mechanisms. Firstly, PPR were increased after bath application of muscarine, indicating that muscarine likely decreases the probability of glutamatergic transmitter release (Schulz 1997, Stuber *et al.* 2011). Secondly, muscarine depressed DPSP but did not change the RMPs or the membrane input resistance, which rules out the possibility of somatic and proximal dendritic activity.

Considering that the effectiveness of bioactive substances and neuromodulators might vary depending on the nature and state of the neural network in which they operate (Pennartz and Lopes da Silva 1994), our results showing that the inhibitory effect of 30 μM muscarine in D-DPSP amplitude and area under the curve was greater than that in V-DPSP suggests the dorsal and

ventral NAc core are differentially innervated by glutamatergic afferents and exhibit different modulation by muscarine. The density or amount of muscarinic receptors located on presynaptic glutamate terminals in dorsal afferents might be higher than that in ventral afferents, since muscarine attenuated DPSP mainly by decreasing glutamatergic transmitter release (Pennartz and Lopes da Silva 1994, Zhang and Warren 2002). Supporting this hypothesis, similar phenomena have been found in anatomical studies showing that the distributions of muscarinic receptors are different between the NAc core and shell (Wirtshafter and Osborn 2004). Moreover, behavioral studies suggest that the roles of muscarinic receptors in the NAc core and shell differ, as muscarinic receptors in NAc shell modulate drug seeking selectively whereas those receptors in the NAc core modulate both food and drug seeking (Yee *et al.* 2011). We note that intrinsic postsynaptic neural elements such as distal dendritic inhibitory transmission shunt cannot be excluded, since the reduction of D-DPSP time course was contingent upon the attenuation of its amplitude. With the knowledge that glutamatergic striatal afferents are topographically organized, the findings from this study further clarify how these subregions are anatomically and functionally modulated in the NAc core.

In conclusion, the present data indicate that the activation of mAChRs by muscarine decreases excitatory neurotransmission of the dorsal and ventral pathway in the NAc core to different degrees, and this effect might be mediated by presynaptic mechanisms. Given that the NAc core is implicated in motivated behaviors, our characterization of structural and functional subdivisions of the NAc core might clarify different behavioral contributions of these regions.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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