Dopamine modulates synaptic transmission in the premotor nuclei of songbirds

Songhua Wang, Shaoyi Liu, Congshu Liao, Wei Meng, Dongfeng LI

Songbirds, such as zebra finches, contribute to explore behaviors underlying neural activities. Birdsong is controlled by the song system. The robust nucleus of the arcopallium (RA) is a key nucleus for producing birdsong in the song system. The RA receives dopaminergic （DArgic） inputs from the midbrain, however, the function of these inputs involved excitatory synaptic transmission is still unclear. Excitatory synaptic transmission is critical in the signal integration activities of the brain. We examined the effects of dopamine (DA) on excitatory synaptic transmission of the projection neurons in the RA of adult male zebra finches, using whole-cell recording technique. We found that DA (100 μM) decreases the frequency of spontaneous and miniature excitatory postsynaptic currents (sEPSCs/mEPSCs). In our further study, these effects of DA were reversed by the D1-like dopamine receptor (D1R) antagonist and stimulated by a D1R agonist. However, a D2-like dopamine receptor (D2R) has no influence on the effects of DA. These results demonstrate that DA can inhibit excitatory synaptic transmission mainly via activation of D1R in adult male zebra finches.

PeerJ PrePrints | https://doi.org/10.7287/peerj.preprints.1563v1 | CC-BY 4.0 Open Access | rec: 4 Dec 2015, publ: 4 Dec 2015

Dopamine Modulates Synaptic Transmission in the Premotor Nuclei of Songbirds

Songhua Wang[1], Shaoyi Liu[1], Congshu Liao, Wei Meng, Dongfeng Li

School of Life Science, South China Normal University, Guangzhou 510631, China

Corresponding author: Dongfeng Li.

Tel.: +86 20 8521372, E-mail: dfliswx@126.com

[1]These authors contributed equally to this work.

Abstract

Songbirds, such as zebra finches, contribute to explore behaviors underlying neural activities. Birdsong is controlled by the song system. The robust nucleus of the arcopallium (RA) is a key nucleus for producing birdsong in the song system. The RA receives dopaminergic（DArgic） inputs from the midbrain, however, the function of these inputs involved excitatory synaptic transmission is still unclear. Excitatory synaptic transmission is critical in the signal integration activities of the brain. We examined the effects of dopamine (DA) on excitatory synaptic transmission of the projection neurons in the RA of adult male zebra finches, using whole-cell recording technique. We found that DA (100 μM) decreases the frequency of spontaneous and miniature excitatory postsynaptic currents (sEPSCs/mEPSCs). In our further study, these effects of DA were reversed by the D1-like dopamine receptor (D1R) antagonist and stimulated by a D1R agonist. However, a D2-like dopamine receptor (D2R) has no influence on the effects of DA. These results demonstrate that DA can inhibit excitatory synaptic transmission mainly via activation of D1R in adult male zebra finches.

Keywords: Zebra finches; Dopamine; sEPSCs/mEPSCs; The robust nucleus of the arcopallium

Introduction

The song of oscines is a complex behavior, which is regulated by interconnected brain nuclei, the so-

called song system (Nottebohm et al. 1976). The robust nucleus of the arcopallium (RA) is the pivotal site receiving afferent input from both HVC (High Vocal Center) and lateral magnocellular nucleus of the anterior neostriatum (LMAN) (Mooney & Konishi 1991). It is well-known that the RA activity is significantly correlated with acoustic features (such as pitch, amplitude, and spectral entropy) of syllables (Sober et al. 2008). The RA has two cell types: the projection neurons (PNs) and the inter-neurons, and both of these neurons receive excitatory glutamatergic input from the HVC and LMAN, but have distinct postsynaptic properties. As previously described, the HVC-RA input is mainly mediated by the α-amino-3-hydroxy -5-methyl-4-isoxazolepropionic acid receptor (AMPAR) and N-methyl-D-aspartic acid receptor (NMDAR), whereas the LMAN-RA input is mostly mediated by the NMDAR (Mooney & Konishi 1991). PNs also receive excitatory glutamatergic projections from the collateral axons of other RA PNs and inhibitory GABAergic projections from inter-neurons (Sizemore & Perkel 2008).

Neurons are connected by synaptic transmission. The role of glutamate receptors is essential in excitatory synaptic transmission, they have two main excitatory postsynaptic currents (EPSCs) mediated through NMDARs and AMPARs. NMDARs are responsible for normal brain function; they are involved in slow excitatory synaptic transmission and long-term plasticity as well as pathological mechanisms (Gardoni & Bellone 2015; Szczurowska & Mares 2013; Vyklicky et al. 2014). Contrary to NMDARs, the AMPARs mediate most of the fast excitatory synaptic transmission in the brain. AMPARs also have a role in enhancing or weakening the activity-dependent synaptic function (Stafford et al. 2014).

The dopaminergic (DArgic) system contributes to cognitive and motor activities, including reward behaviors, learning, memory and motor control by regulating glutamatergic inputs in both mammals and songbirds (Ding et al. 2003; Durstewitz et al. 1999; Gardoni & Bellone 2015). In songbirds, the ventral tegmental area (VTA) sends a dense DArgic input to the Area X (Bottjer et al. 1989; Lewis 1981). Moreover, dopamine (DA) can directly modulate intrinsic excitability and synaptic activity of Area X neurons (Ding & Perkel 2002; Ding et al. 2003). The RA also receives DArgic inputs and expresses D1-like DA receptors (D1R) and D2-like DA receptors (D2R) (Kubikova et al. 2010), our previous study showed that DA modulates the excitability of RA PNs (Liao et al. 2013) and activation of the D1R can increase NMDAR-induced gain modulation (Wang et al. 2015). But the function of DA affecting synaptic activity in the RA is still unknown. Understanding of how activation of DA can affect excitatory synaptic activity in the RA will provide a foundation for better understanding of the neural control of song behavior. In this study, we examined the effects of DA on the spontaneous and mininature excitatory synaptic transmission (sEPSCs/mEPSCs) on RA PNs using the whole-cell technique. The meaning of sEPSCs and mEPSCs have been described in previous work (Behr et al. 2000; Cooke & Woolley 2005; Tian et al. 2012; Wang et al. 2014). Briefly, the sEPSCs are represented of functional excitatory synaptic activity. The mEPSCs reflect the quantal release of excitatory transmitters. Analysis of the mEPSCs frequency predicates mechanism about changes in the presynaptic sites, while change in the amplitudes of the mEPSCs reflects postsynaptic sites (Behr et al. 2000).

Material and Methods

Preparation of Brain Slices

The experimental protocols were approved by the Institutional Animal Care Committee at South China Normal University (scnu20070033). Coronal brain slices (250 μm thick) were obtained from adult male zebra finches (>90 day) as previously described in our work (Hou et al. 2012; Liao et al. 2013; Liao et al. 2012).

Patch clamp recordings

For electrophysiological recordings, we followed the methods of our previous work (Wang et al. 2014). In order to ensure the recordings were excitatory glutamatergic currents, after recording sEPSCs or mEPSCs, both the NMDA receptor antagonist D(−)-2-Amino-5-phosphonopentanoic acid (APV, 50 μM) and the AMPA/KA receptor antagonist 6,7-Dinitroquinoxaline-2,3(1H,4H)-dione (DNQX, 20 μM) were added to the recording chamber. If APV and DNQX did not completely inhibit sEPSCs/mEPSCs, the data would be rejected.

Drug application

All agents were applied by changing the bath solution from standard ACSF to modified ACSF in which various drugs were simply added. All drugs were purchased from Sigma (St. Louis, MO, USA).

Data analysis

Data were acquired and analyzed according to methods of our previous work (Wang et al. 2014). To compare pooled data under the control and drug conditions, we followed the methods described previously

(Tian et al. 2012).

Results

The RA neurons were observed under DIC-IR optics. Stable whole-cell recordings were obtained in 122 RA PNs from 45 male zebra finches. PNs were identified by a large soma, and time-dependent inward rectifier induced by hyperpolarizing current (Liao et al. 2011; Spiro et al. 1999).

I. DA decreases the frequency of sEPSCs in the RA PNs

To understand the actions of DA (100 μM) on excitatory synaptic transmission, sEPSCs were recorded from RA PNs (Tian et al. 2012). We first compared the frequency of sEPSCs in control and DA application conditions. The frequency of sEPSCs was significantly higher after DA application than that in control as shown in Fig. 1A. Cumulative probability plots of inter-event interval (IEI) and amplitude in both the control and DA-treated are also shown in Fig. 1B and 1C, respectively. DA increased the proportion of longer IEI and decreased frequency from 2.81 ±0.17 Hz to 2.27±0.16 Hz (n = 49; $p < 0.01$, Fig 1D). The amplitude of sEPSCs was unchanged after DA treating (control: 20.37 ± 0.96 pA; DA: 19.23 ± 0.78 pA; n = 49; $p > 0.05$, Fig. 1E). Furthermore, the rise and decay times of the sEPSCs were not altered by DA (Table 1).

Ⅱ. DA depresses the frequency of mEPSCs in the RA PNs.

In order to further investigate whether DA exhibits its effects through presynaptic or postsynaptic sites, we then examined the effects of DA (100 μM) on the frequency and amplitude of mEPSCs. Similar to the results of sEPSCs, mEPSCs were affected after the application of DA (Fig. 2A). Cumulative probability plots of IEI were shown in Fig. 2B. DA increased the proportion of longer IEIs. Fig. 2C showed the cumulative probability plots of amplitude in control and after DA application, demonstrating that DA has not changed the amplitude distribution. The bar graphs in Fig. 2D and 2E present the mean of frequency and amplitude. DA decreased the mEPSCs frequency from 2.07 ± 0.17 Hz to 1.78 ± 0.17 Hz ($p<0.01$; n = 25) (Fig 2D), while the amplitude of the mEPSCs was unchanged by DA (control: 18.19 ± 0.73pA; DA: 17.77 ± 0.71pA; $p > 0.05$; n = 25) (Fig 2E). The rise and decay times of the mEPSCs were also not changed by DA (Table 1).

Ⅲ. DA via activation of D1R inhibited excitatory synaptic transmission.

DA has main two receptor subtypes: D1R and D2R. Both D1R and D2R are found in the RA (Kubikova & Kostal 2010). To define which the receptor subtype was modulating DA-induced reductions in mEPSCs, the effects of specific DA receptor agonists and antagonists were investigated.

We first examined the effect of D1R in RA PNs, we applied the D1R agonist SKF-38393 (10 μM). Sample trace of mEPSCs with SKF was shown in Fig. 3A. Cumulative probability plots of IEI were shown in Fig. 3B. Like DA, SKF increased the proportion of longer IEIs. Cumulative probability plots in Fig. 3C shows the amplitude of mEPSCs recordings in both the control and SKF-applied slices, and revealed that SKF has not changed the distribution of the amplitude. SKF inhibited the frequency of mEPSCs from 1.94 ± 0.19 Hz to 1.76 ± 0.16 Hz ($p < 0.01$; n=12) (Fig. 3D); while the amplitude of the mEPSCs was no change by SKF (control: 19.08 ± 1.45 pA; SKF: 18.44 ± 1.21 pA; $p > 0.05$; n = 12) (Fig 3E). To further examine whether D1R contributes to the effect of DA, we applied DA and the D1R antagonist SCH-23390 (20 μM). Sample traces of mEPSCs are shown in Fig. 4A. Cumulative probability plots of IEI and amplitude are shown in Fig. 4B and 4C, respectively. DA and SCH had no effect on the distribution of the IEI and amplitude. DA and SCH did not change the frequency of mEPSCs (control: 1.61 ± 0.24 Hz; DA +SCH: 1.56 ± 0.22 Hz; $p > 0.05$; n=13) (Fig 4D); the amplitude of the mEPSCs was also not altered by DA and SCH (control: 21.29 ± 1.13 pA; DA +SCH: 21.53 ± 0.89 pA; $p > 0.05$; n = 13) (Fig 4E). These results indicated that D1R are one of the main factors contributing to the inhibited effect of DA on the mEPSCs of RA PNs.

To examine whether D2R is involved in the effect of DA, we applied the D2R agonist quinpirole (10 μM). Sample traces of mEPSCs were shown in Fig. 5A. Cumulative probability plots of IEI and amplitude were shown in Fig. 5B and 5C, respectively. Contrary to SKF, quinpirole had no effect on the distribution of the IEIs and amplitude. Quinpirole also did not change the frequency of mEPSCs (control: 2.03 ± 0.18 Hz; Quinpirole: 1.95 ± 0.22 Hz; $p>0.05$; n=7) (Fig 5D); the amplitude of the mEPSCs was also not altered by quinpirole (control: 20.30 ± 1.05 pA; quinpirole: 19.80 ± 0.93 pA; $p > 0.05$; n =7) (Fig 5E). Next we applied DA and D2R antagonist sulpiride (10 μM) to further examine these effects. Sample traces of mEPSCs were shown in Fig. 6A. Cumulative probability plots of IEI were shown in Fig. 6B. It showed that DA and sulpiride increase the proportion of longer IEIs. Cumulative probability plots in Fig. 6C showed the amplitude of mEPSCs recordings in both the control, DA and sulpiride -applied slices, and indicated that DA and sulpiride had no change on the distribution of the amplitudes. DA and sulpiride decreased the frequency of mEPSCs (from 1.93 ± 0.34 Hz to 1.52 ± 0.27 Hz; $p<0.01$; n=6) (Fig 6D); while, the amplitude of the mEPSCs was not changed by DA and sulpiride (control: 21.06 ± 0.97 pA; DA + sulpiride: 20.91 ± 0.88 pA; $p > 0.05$; n = 6) (Fig 6E). These results indicated that D2R are not involved in the inhibited effect of DA on the mEPSCs of RA PNs.

Discussion

In our results, DA reduces the ionotropic glutamate (NMDARs and AMPAR)-mediated excitatory synaptic transmission of RA PNs. Our results showed that DA efficiently decreases the frequency of sEPSCs but had no effect on the amplitude of sEPSCs. These suggested that the DA can significantly modulate functional synaptic transmission between HVC/LMAN neurons and RA PNs (Tian et al. 2012). We then examined mEPSCs to distinguish whether presynaptic or postsynaptic effects of DA contributed to these effects. In our results, DA also decreased the frequency but not amplitude of mEPSCs, suggesting that DA acts on presynaptic sites to inhibit the release of glutamate (Basavarajappa et al. 2008; Chavez-Noriega & Stevens 1994; Nelson et al. 2008). In addition to those results, DA had no change of the kinetic properties (the rise and decay times) of sEPSCs and mEPSCs, which further confirmed the presynaptic effects of DA.

The pharmacological analysis of the synaptic actions of DA indicated that the receptor mediating the decrease in glutamate release may be the D1R. Since the D1R agonist, but not D2R agonist mimicked the actions of DA, and D1R antagonist but not D2R antagonist inhibited the actions of DA, which indicated that the observed effects of DA on synaptic transmission is the result of DA binding to D1R. These results are similar to results seen in rats and mice, in which activation of D1R induces presynaptic depression of evoked EPSC in the core and shell region of nucleus accumbens, and subicular neurons (Behr et al. 2000; Harvey & Lacey 1996; Nicola et al. 1996; Pennartz et al. 1992).

Previous research has shown that DA can modulate excitability in spiny neurons (SNs) in area X (Ding & Perkel 2002) and PNs in RA (Liao et al. 2013) of songbirds, suggesting that DA can influence information processing in the song system by altering the input-output functions of the song control system. Furthermore, D1R activation suppresses glutamatergic synaptic responses in SNs in area X of adult zebra finches (Ding et al. 2003) and subicular neurons of rats (Behr et al. 2000), which are similar to our findings here. The present study indicates that DA modulates glutamatergic synaptic transmission in the RA PNs of adult zebra finches, which test the effect of DA in the synaptic level following to our previous work (Liao et al. 2013). The RA is a sensorimotor nucleus, which receives a direct projection from HVC and LMAN (Doupe & Konishi 1991; Mooney & Konishi 1991). A series of evidence has demonstrated that RA intrinsic circuitry controls premotor output, thus producing birdsong (Bottjer et al. 1989; Margoliash 1997; Nottebohm et al. 1976; Yu & Margoliash 1996). We found that DA decreased excitatory inputs to RA PNs. Previous studies have shown that DA modulates the key processes involved in the sensorimotor integration of song and/or the amount, speed and intensity of song production (Soha et al. 1996). Thus, information that is first processed within the motor and the forebrain pathways and then transmitted to RA via HVC and LMAN, respectively, could be modulated by DA before they are transmitted and integrated within the premotor song nuclei. Combined with results in Area X (Ding & Perkel 2002; Ding et al. 2003) and our previous work (Liao et al. 2013), we show that DA can exhibit complex control on the signal transformation of excitatory inputs to RA principal from HVC and

LMAN, and finally modulate the production of birdsong. These experiments give a better understanding of DA functions in modulating the excitatory synaptic transmission of RA.

In conclusion, the present work demonstrates that DA can inhibit excitatory synaptic transmission, mainly via activation of D1R in the RA of adult male zebra finch.

Competing interests

The authors have declared that they have no conflicts of interests.

Acknowledgments

The authors thank Dr. Paul Michael Anderson and Dr. Gangyi Wu for critical reading and helpful comments on this paper. This work was supported by the National Natural Science Foundation of China (31172092) and (31472002).

References

Basavarajappa BS, Ninan I, and Arancio O. 2008. Acute ethanol suppresses glutamatergic neurotransmission through endocannabinoids in hippocampal neurons. *J Neurochem* 107:1001-1013.

Behr J, Gloveli T, Schmitz D, and Heinemann U. 2000. Dopamine depresses excitatory synaptic transmission onto rat subicular neurons via presynaptic D1-like dopamine receptors. *J Neurophysiol* 84:112-119.

Bottjer SW, Halsema KA, Brown SA, and Miesner EA. 1989. Axonal connections of a forebrain nucleus involved with vocal learning in zebra finches. *J Comp Neurol* 279:312-326.

Chavez-Noriega LE, and Stevens CF. 1994. Increased transmitter release at excitatory synapses produced by direct activation of adenylate cyclase in rat hippocampal slices. *J Neurosci* 14:310-317.

Cooke BM, and Woolley CS. 2005. Sexually dimorphic synaptic organization of the medial amygdala. *J Neurosci* 25:10759-10767.

214 Ding L, and Perkel DJ. 2002. Dopamine modulates excitability of spiny neurons in the avian basal ganglia. *J*
215 *Neurosci* 22:5210-5218.

216 Ding L, Perkel DJ, and Farries MA. 2003. Presynaptic depression of glutamatergic synaptic transmission by D1-like
217 dopamine receptor activation in the avian basal ganglia. *J Neurosci* 23:6086-6095.

218 Doupe AJ, and Konishi M. 1991. Song-selective auditory circuits in the vocal control system of the zebra finch.
219 *Proc Natl Acad Sci U S A* 88:11339-11343.

220 Durstewitz D, Kroner S, and Gunturkun O. 1999. The dopaminergic innervation of the avian telencephalon. *Prog*
221 *Neurobiol* 59:161-195.

222 Gardoni F, and Bellone C. 2015. Modulation of the glutamatergic transmission by Dopamine: a focus on Parkinson,
223 Huntington and Addiction diseases. *Front Cell Neurosci* 9:25.

224 Harvey J, and Lacey MG. 1996. Endogenous and exogenous dopamine depress EPSCs in rat nucleus accumbens in
225 vitro via D1 receptors activation. *J Physiol* 492 (Pt 1):143-154.

226 Hou GQ, Pan X, Liao CS, Wang SH, and Li DF. 2012. SK channels modulate the excitability and firing precision of
227 projection neurons in the robust nucleus of the arcopallium in adult male zebra finches. *Neurosci Bull*
228 28:271-281.

229 Kubikova L, and Kostal L. 2010. Dopaminergic system in birdsong learning and maintenance. *J Chem Neuroanat*
230 39:112-123.

231 Kubikova L, Wada K, and Jarvis ED. 2010. Dopamine receptors in a songbird brain. *J Comp Neurol* 518:741-769.

232 Lewis MJ. 1981. Age-related decline in brain stimulation reward: rejuvenation by amphetamine. *Exp Aging Res*
233 7:225-234.

234 Liao C, Wang S, Pan X, Hou G, and Li D. 2013. Dopamine modulates the excitability of projection neurons in the
235 robust nucleus of the arcopallium in adult zebra finches. *PLoS ONE* 8:e82497.

236 Liao SQ, Hou GQ, Liu XL, Long C, and Li DF. 2011. Electrophysiological properties of neurons in the robust
237 nucleus of the arcopallium of adult male zebra finches. *Neurosci Lett* 487:234-239.

238 Liao SQ, Hou GQ, Pan X, Liao CS, and Li DF. 2012. The gain modulation by N-methyl-D-aspartate in the
239 projection neurons of robust nucleus of the arcopallium in adult zebra finches. *Neural Plast* 2012:931780.

240 Margoliash D. 1997. Distributed time-domain representations in the birdsong system. *Neuron* 19:963-966.

241 Mooney R, and Konishi M. 1991. Two distinct inputs to an avian song nucleus activate different glutamate receptor

242 subtypes on individual neurons. *Proc Natl Acad Sci U S A* 88:4075-4079.

243 Nelson ED, Kavalali ET, and Monteggia LM. 2008. Activity-dependent suppression of miniature neurotransmission
244 through the regulation of DNA methylation. *J Neurosci* 28:395-406.

245 Nicola SM, Kombian SB, and Malenka RC. 1996. Psychostimulants depress excitatory synaptic transmission in the
246 nucleus accumbens via presynaptic D1-like dopamine receptors. *J Neurosci* 16:1591-1604.

247 Nottebohm F, Stokes TM, and Leonard CM. 1976. Central control of song in the canary, Serinus canarius. *J Comp*
248 *Neurol* 165:457-486.

249 Pennartz CM, Dolleman-Van der Weel MJ, Kitai ST, and Lopes da Silva FH. 1992. Presynaptic dopamine D1
250 receptors attenuate excitatory and inhibitory limbic inputs to the shell region of the rat nucleus accumbens
251 studied in vitro. *J Neurophysiol* 67:1325-1334.

252 Sizemore M, and Perkel DJ. 2008. Noradrenergic and GABA B receptor activation differentially modulate inputs to
253 the premotor nucleus RA in zebra finches. *J Neurophysiol* 100:8-18.

254 Sober SJ, Wohlgemuth MJ, and Brainard MS. 2008. Central contributions to acoustic variation in birdsong. *J*
255 *Neurosci* 28:10370-10379.

256 Soha JA, Shimizu T, and Doupe AJ. 1996. Development of the catecholaminergic innervation of the song system of
257 the male zebra finch. *J Neurobiol* 29:473-489.

258 Spiro JE, Dalva MB, and Mooney R. 1999. Long-range inhibition within the zebra finch song nucleus RA can
259 coordinate the firing of multiple projection neurons. *J Neurophysiol* 81:3007-3020.

260 Stafford BK, Manookin MB, Singer JH, and Demb JB. 2014. NMDA and AMPA receptors contribute similarly to
261 temporal processing in mammalian retinal ganglion cells. *J Physiol* 592:4877-4889.

262 Szczurowska E, and Mares P. 2013. NMDA and AMPA receptors: development and status epilepticus. *Physiol Res*
263 62 Suppl 1:S21-38.

264 Tian Y, Lei T, Yang Z, and Zhang T. 2012. Urethane suppresses hippocampal CA1 neuron excitability via changes
265 in presynaptic glutamate release and in potassium channel activity. *Brain Res Bull* 87:420-426.

266 Vyklicky V, Korinek M, Smejkalova T, Balik A, Krausova B, Kaniakova M, Lichnerova K, Cerny J, Krusek J,
267 Dittert I, Horak M, and Vyklicky L. 2014. Structure, function, and pharmacology of NMDA receptor
268 channels. *Physiol Res* 63 Suppl 1:S191-203.

269 Wang S, Liao C, Meng W, Huang Q, and Li D. 2015. Activation of D1-like dopamine receptors increases the
270 NMDA-induced gain modulation through a PKA-dependent pathway in the premotor nucleus of adult zebra

finches. *Neurosci Lett* 589:37-41.

Wang S, Meng W, Liu S, Liao C, Huang Q, and Li D. 2014. Sex differences of excitatory synaptic transmission in RA projection neurons of adult zebra finches. *Neurosci Lett* 582:75-80.

Yu AC, and Margoliash D. 1996. Temporal hierarchical control of singing in birds. *Science* 273:1871-1875.

	n	rise time (ms)		decay time (ms)	
		Control	DA	Control	DA
sEPSC	49	4.01±0.12	4.07±0.10 (NS)	6.25±0.22	6.40±0.27 (NS)
mEPSC	28	3.55±0.09	3.79±0.10 (NS)	5.36±0.30	5.43±0.24 (NS)

Table

Table 1. DA has no effect of the kinetics of sEPSC and mEPSC.

Values are presented mean ±SEM. NS means not significant. DA not alters the rise and decay times constants of sEPSCs and mEPSCs recorded from RA PNs.

Figure Legends

Figure 1. Effects of DA on sEPSCs in the RA PNs. (A) Sample traces represent sEPSCs recordings before (control) and after DA application. (B) Cumulative probability plots IEI distributions for sEPSCs show that DA prolong inter-event interval ($p < 0.01$). (C) Cumulative amplitude distributions for sEPSCs showed that DA did not change the amplitude of sEPSCs ($p > 0.05$). (D and E) DA significantly reduces the frequency but not amplitude in the RA PNs (** mean $p < 0.01$).

Figure 2. Effects of DA on mEPSCs in the RA PNs. (A) Sample traces represent mEPSCs recorded from RA PNs before and after application of DA. (B and C) Cumulative probability plots IEI and amplitude distributions of mEPSCs under the control and DA application, respectively. DA prolonged the intervals between mEPSCs events ($p < 0.01$) but had no change on their amplitudes ($p > 0.05$). (D and E) DA significantly reduces the frequency but not amplitude in the RA PNs (** mean $p < 0.01$).

Figure 3. Effects of D1R agonist SKF-38393 on mEPSCs in the RA PNs. (A) Recordings of mEPSCs in the absence and presence of SKF38393. (B) Cumulative IEI distributions for mEPSCs in control and after application of SKF ($p < 0.01$). (C) Cumulative amplitude distributions of mEPSCs in control and after application of SKF. (D and E) SKF significantly decreases the frequency but not amplitude of mEPSCs in the RA PNs (**$p < 0.01$).

Figure 4. Effects of DA and D1R antagonist SCH-23390 (20 μM) on mEPSCs in the RA PNs. (A) Sample trace of mEPSCs in the control and after application of SCH and DA. (B) Cumulative IEI distributions for mEPSCs in control and after DA and SCH ($p > 0.05$). (C) Cumulative amplitude distributions of mEPSCs in control and after DA and SCH ($p > 0.05$). (D and E) DA and SCH did not change the frequency and amplitude of mEPSCs in the RA PNs.

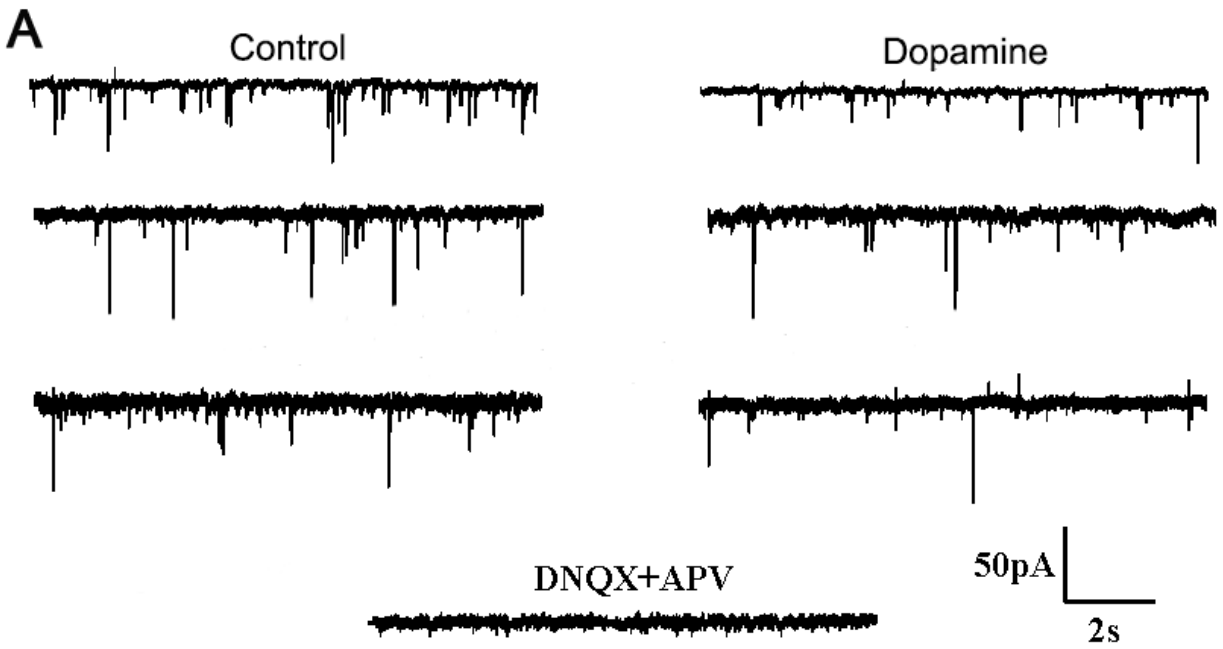
Figure 5. Effects of D2R agonist quinpirole on mEPSCs in the RA PNs. (A) Sample trace of mEPSCs in the absence and presence of quinpirole. (B) Cumulative IEI distributions for mEPSC in control and after quinpirole ($p > 0.05$). (C) Cumulative amplitude distributions of mEPSCs in control and after quinpirole ($p > 0.05$). (D and E) Quinpirole did not change the frequency and amplitude of mEPSCs in the RA PNs.

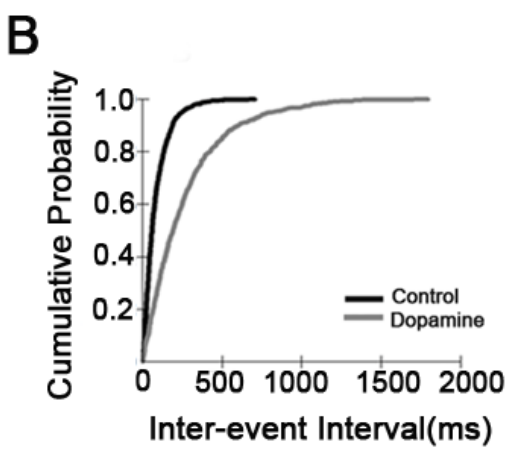
Figure 6. Effects of DA and D2R blocker, sulpiride, of DA on mEPSCs in the RA PNs. (A) Sample trace of mEPSCs in the absence and presence of sulpiride and DA. (B) Cumulative distributions of the IEI for mEPSCs in control and after DA and sulpiride ($p < 0.01$). (C) Cumulative distributions of the amplitude for mEPSCs in control and after DA and sulpiride ($p > 0.05$). (D and E) DA and sulpiride applying significantly decreases the frequency but not amplitude of mEPSCs in the RA PNs (**$p < 0.01$).

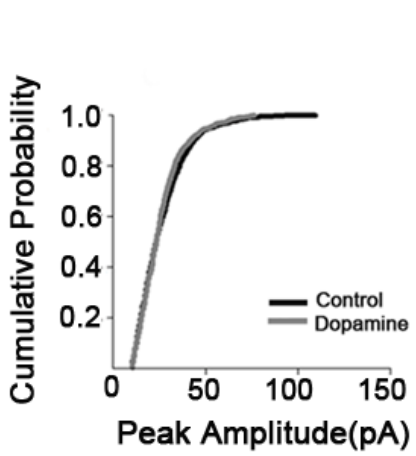
1

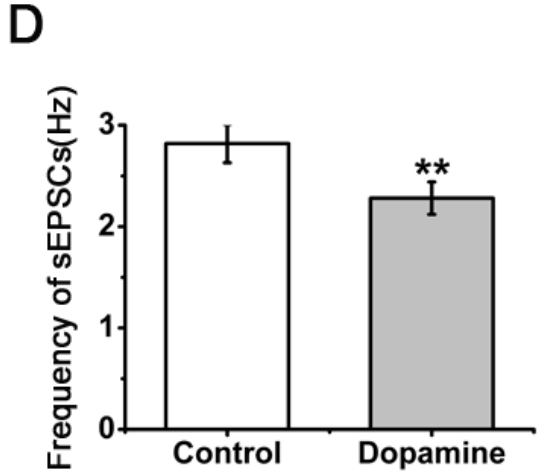
Figure 1

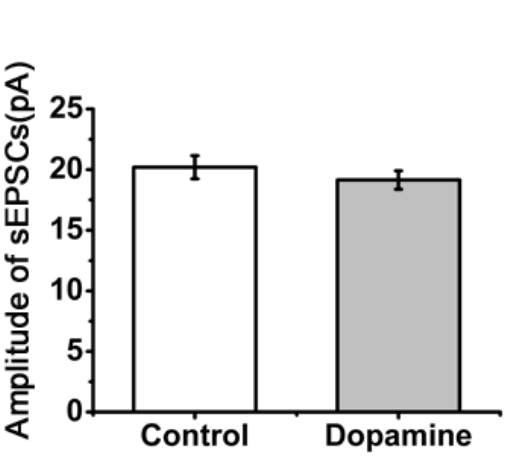
PeerJ PrePrints | https://doi.org/10.7287/peerj.preprints.1563v1 | CC-BY 4.0 Open Access | rec: 4 Dec 2015, publ: 4 Dec 2015





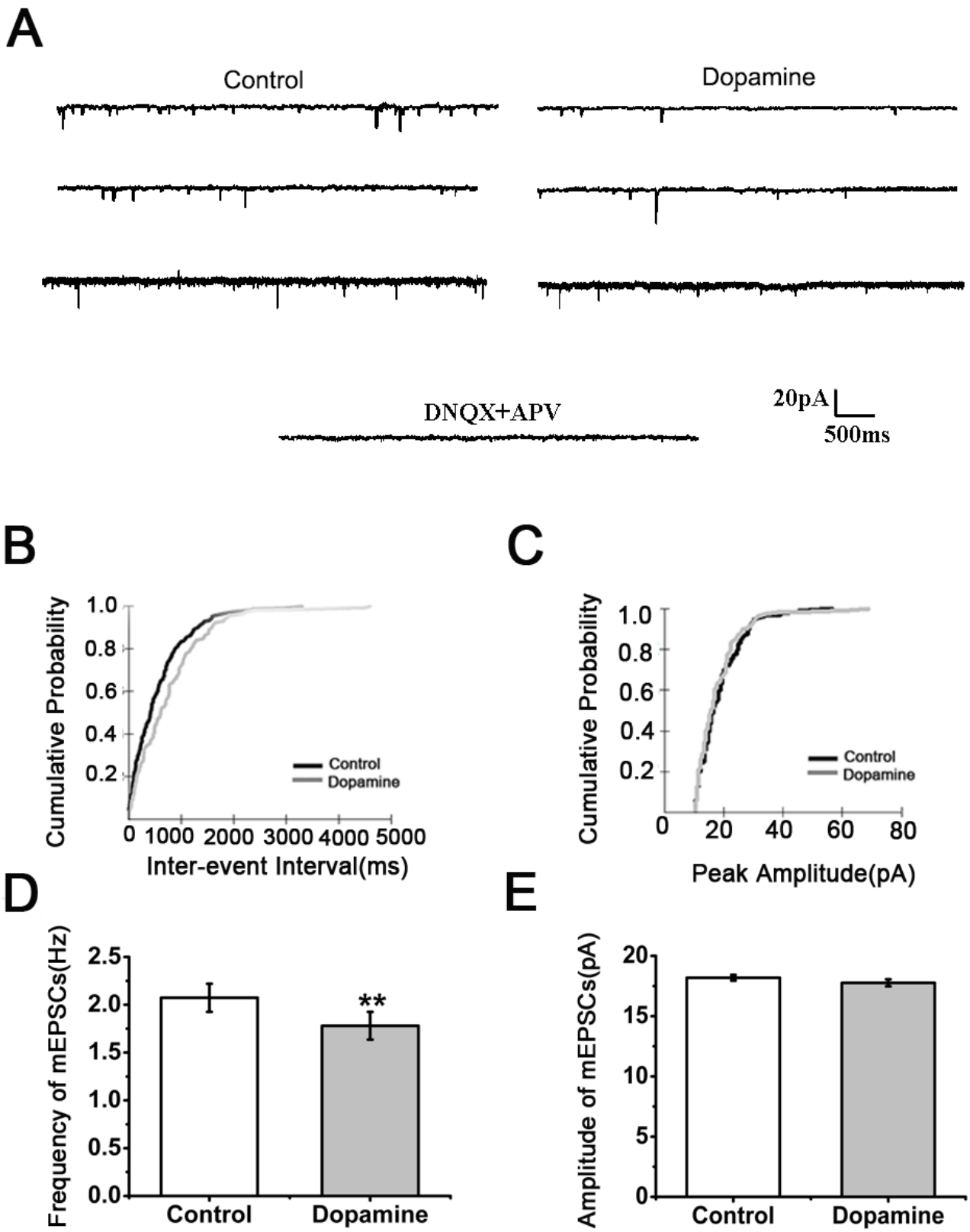






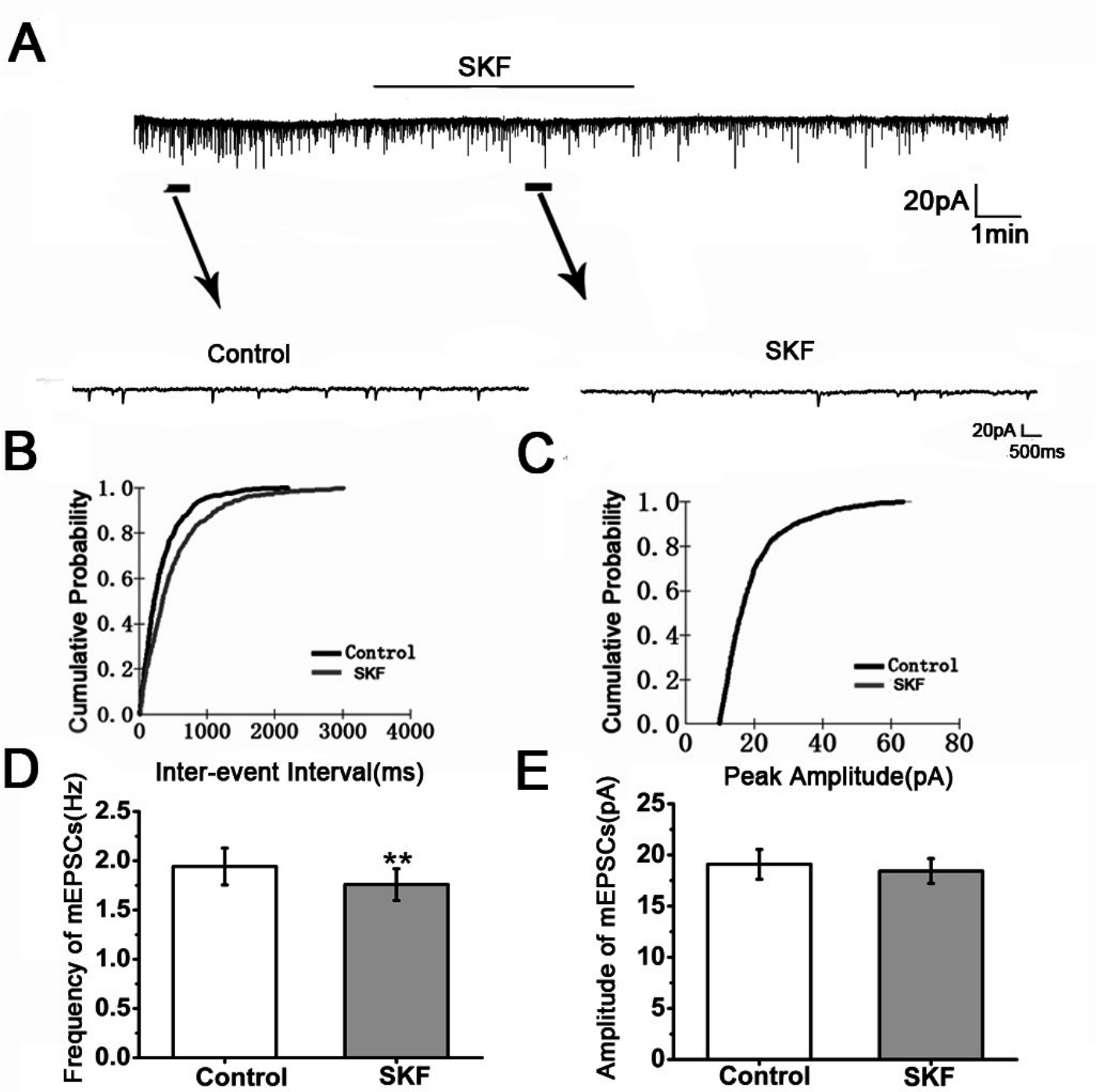
2

Figure 2



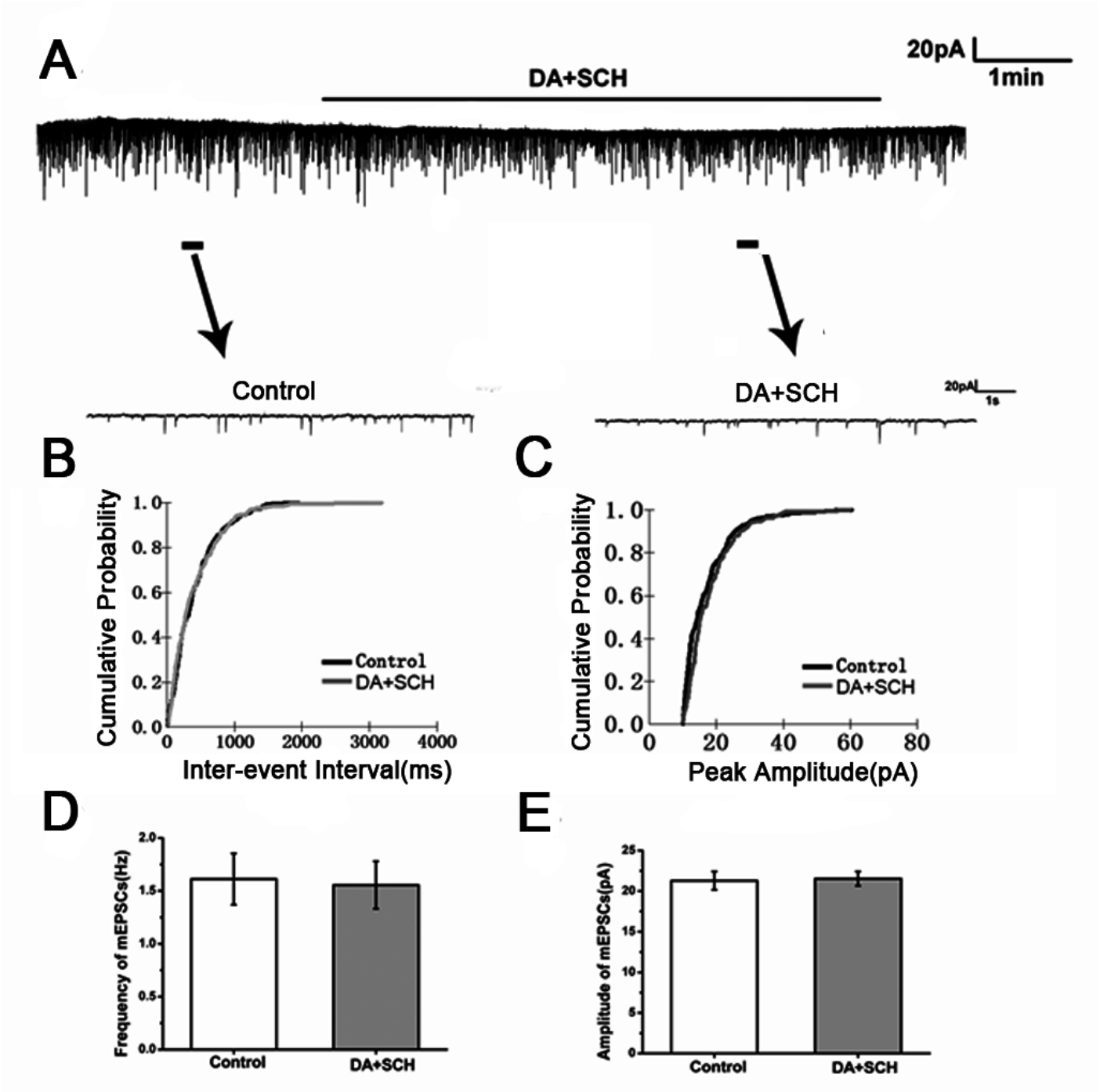
3

Figure 3



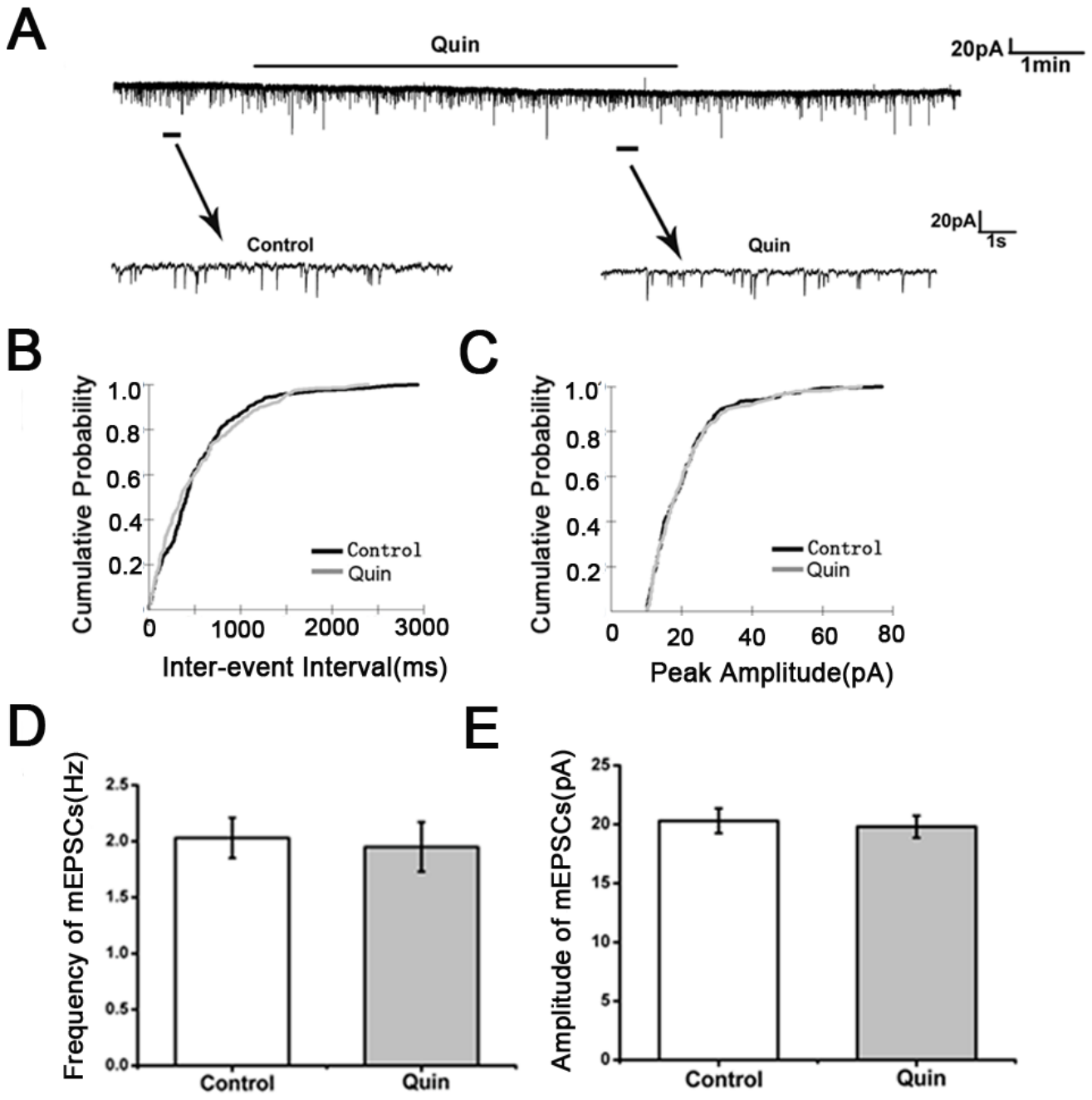
4

Figure 4


A
20pA
1min
DA+SCH
Control
DA+SCH
20pA
1s
B
Cumulative Probability
1.0
0.8
0.6
0.4
0.2
0.0
0
1000
2000
3000
4000
Inter-event Interval(ms)
Control
DA+SCH
C
Cumulative Probability
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
Peak Amplitude(pA)
Control
DA+SCH
D
Frequency of mEPSCs(Hz)
2.0
1.5
1.0
0.5
0.0
Control
DA+SCH
E
Amplitude of mEPSCs(pA)
25
20
15
10
5
0
Control
DA+SCH

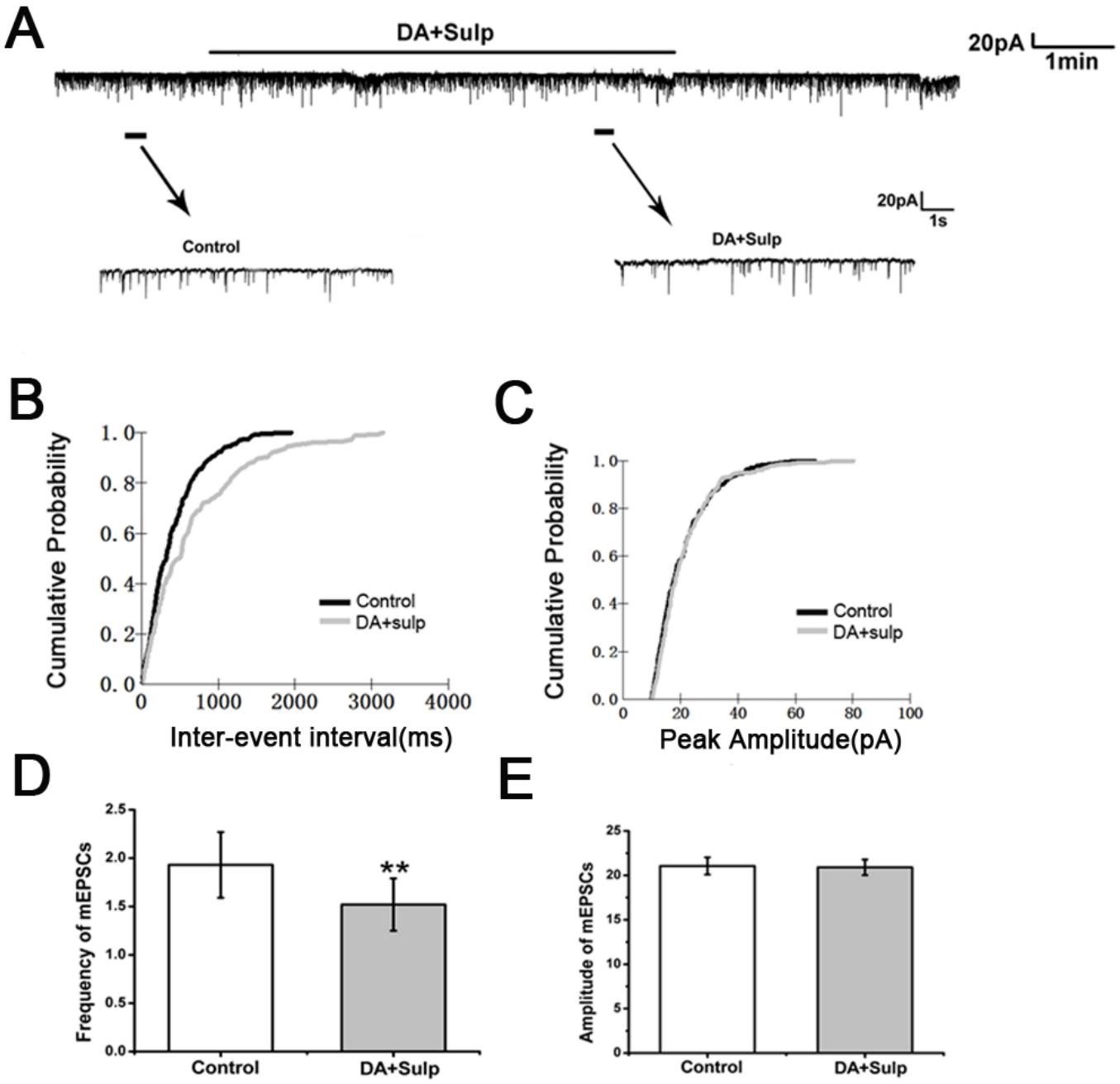
5

Figure 5



6

Figure 6


A
DA+Sulp
20pA
1min
20pA
1s
Control
DA+Sulp
B
Cumulative Probability
1.0
0.8
0.6
0.4
0.2
0.0
0
1000
2000
3000
4000
Inter-event interval(ms)
Control
DA+sulp
C
Cumulative Probability
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
Peak Amplitude(pA)
Control
DA+sulp
D
Frequency of mEPSCs
2.5
2.0
1.5
1.0
0.5
0.0
**
Control
DA+Sulp
E
Amplitude of mEPSCs
25
20
15
10
5
0
Control
DA+Sulp