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Regulation of inhibitory synapses by presynaptic D_4 dopamine receptors in thalamus

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Running Head: Modulation of pallido-thalamic GABAergic transmission

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ABSTRACT

Dopamine (DA) receptors are the principal targets of drugs used in the treatment of schizophrenia. Among the five DA receptor subtypes, the D_4 subtype is of particular interest because of the relatively high affinity of the atypical neuropleptic clozapine for D_4 compared to D_2 receptors. Gamma-amino butyric acid (GABA)-containing neurons in the thalamic reticular nucleus (TRN) and globus pallidus (GP) express D_4 receptors. TRN neurons receive GABAergic afferents from globus pallidus (GP), substantia nigra pars reticulata (SNr), basal forebrain, as well as neighboring TRN neuron collaterals. In addition, TRN receives dopaminergic innervations from substantia nigra pars compacta (SNc); however, the role of D_4 receptors in neuronal signaling at inhibitory synapses is unknown. Using whole cell recordings from *in vitro* pallido-thalamic slices, we demonstrate that DA selectively suppresses $GABA_A$ receptor-mediated inhibitory postsynaptic currents (IPSCs) evoked by GP stimulation. The D_2-like receptor ($D_{2, 3, 4}$) agonist, quinpirole, and selective D_4 receptor agonist, PD168077, mimicked the actions of DA. The suppressive actions of DA and its agonists were associated with alterations in paired pulse ratio (PPR) and a decrease in the frequency of miniature IPSCs (mIPSCs), suggesting a presynaptic site of action. $GABA_A$ receptor agonist, muscimol, induced postsynaptic currents in TRN neurons were unaltered by DA or quinpirole, consistant with the presynaptic site of action. Finally, DA agonists did not alter intra-TRN inhibitory signaling. Our data demonstrate that the activation of presynaptic D_4 receptors regulates GABA release from GP efferents, but not TRN collaterals. This novel and selective action of D_4 receptor activation on GP-mediated inhibition may provide insight to potential functional significance of atypical antipsychotic agents. These findings suggest a potential heightened TRN neuron activity in certain neurological conditions, such as schizophrenia and attention deficit hyperactive disorders.

INTRODUCTION

The thalamus relays sensory and motor information to the cerebral cortex and receives strong modulatory input back from the cortex. Both thalamocortical and corticothalamic projection neurons send collaterals to ventral thalamic nuclei (Jones, 1975). Gamma-amino-butyric acid (GABA)-containing thalamic reticular nucleus (TRN) neurons provide a major source of inhibitory synaptic input to thalamocortical (TC) neurons (Huguenard and McCormick, 2007;Pinault, 2004;Schofield et al., 2009). By modulating the flow of information through the thalamus, TRN has been hypothesized to play a major role in the control of attention and sensory processing (Guillery et al., 1998;Mayo, 2009;McAlonan et al., 2008;Rees, 2009;Yu et al., 2009). The TRN is also a key player in various types of rhythmic activity associated with certain arousal mechanisms and epileptiform activities (Huguenard and McCormick, 2007;Huntsman et al., 1999;McCormick and Bal, 1997;Sohal et al., 2000;Steriade, 1992;Steriade et al., 1993;Steriade, 1997;vonKrosigk et al., 1993;Hughes et al., 2002).

Dopamine (DA) is a major neuromodulator in the brain, and its dysfunction has been implicated in multiple human neurological and psychiatric disorders (Di Chiara G, 2002;Takahashi et al., 2006). Within thalamic circuitry, DA-dependent actions are thought to play a potentially significant role in emotion, attention, cognition, and complex somatosensory and visual processing (Takahashi et al., 2006). Alterations in thalamic DA receptors are also implicated in various neurological and psychiatric disorders (Behrendt, 2006;Buchsbaum et al., 2006;Di Chiara G, 2002;Kane et al., 2009;Takahashi et al., 2006;Yasuno et al., 2004). Anatomical studies have shown that TRN receives a dopaminergic innervation from the substantia nigra pars compacta (SNc; Anaya-Martinez et al., 2006;Gandia et al., 1993;Garcia-Cabezas et al., 2007;Garcia-Cabezas et al., 2009;Sanchez-Gonzalez et al., 2005) and expresses

DA receptors (Khan et al., 1998;Mrzljak et al., 1996). In addition, TRN receives GABAergic projections from globus pallidus (GP), substantia nigra pars reticulata (SNr) and basal forebrain (Anaya-Martinez et al., 2006;Asanuma and Porter, 1990;Asanuma, 1994;Bickford et al., 1994;Gandia et al., 1993;Hazrati and Parent, 1991;Pare et al., 1990). Inhibitory innervations within TRN arise from local collaterals (Deleuze and Huguenard, 2006;Lam et al., 2006;Shu and McCormick, 2002). GABAergic terminals within TRN are hypothesized to express D_4 receptors (Ariano et al., 1997;Defagot et al., 1997;Mrzljak et al., 1996); however, the action of DA on GABAergic inhibitory signaling in TRN has not been explored. In this study, we found that DA, via presynaptic D_4 receptors, selectively suppresses $GABA_A$-receptor mediated inhibition arising from GP efferents without altering intra-TRN inhibitory signaling.

MATERIALS AND METHODS

The present study was performed on Sprague Dawley rats (postnatal age 11–20 days). All experimental procedures were carried out in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and approved by the University of Illinois Animal Care and Use committee. Animals were maintained with 12 h ON-OFF light/dark schedule in a temperature-controlled environment, and food and water were provided ad libitum.

Brain slices containing thalamus and GP were prepared as previously described with modifications (Lee et al., 2007). Rats were deeply anesthetized with sodium pentobarbital (50 mg/kg) and decapitated. The brains were quickly removed and placed into chilled (4°C), oxygenated (5% CO_2-95% O_2) slicing medium containing (in mM): 2.5 KCl, 1.25 NaH_2PO_4, 10.0 $MgCl_2$, 2.0 $CaCl_2$, 26.0 $NaHCO_3$, 11.0 glucose, and 234.0 sucrose. Brain slices (250 to 300 μm thick) were cut in the horizontal plane using a vibrating tissue slicer (Leica, Germany). The slices were transferred to a holding chamber containing oxygenated, physiological solution and incubated (32°C) for ≥1 h prior to recording. The physiological solution contained (in mM): 126.0 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 2.0 $MgCl_2$, 2.0 $CaCl_2$, 26.0 $NaHCO_3$, and 10.0 glucose. The solution was continuously gassed with 95% O_2-5% CO_2 to a final pH of 7.4. Individual slices were transferred to a submersion type recording chamber maintained at 30±1°C and continuously superfused (3 ml/min) with oxygenated solution.

Recording procedures

Whole-cell recordings were obtained using a microscope equipped with differential interference contrast (DIC) optics (Axioskop 2FS, Carl Zeiss) similar to that previously used (Govindaiah and Cox, 2006a;Govindaiah and Cox, 2006b). Specific thalamic nuclei were

distinguished using a low-power objective, and a high-power water-immersion objective was used to identify individual neurons. Recording pipettes were pulled from borosilicate glass tubing and had tip resistances of 4–7 MΩ. For voltage clamp recordings, the pipette solution contained (in mM): 117.0 Cs-gluconate, 13.0 CsCl, 1.0 $MgCl_2$, 0.07 $CaCl_2$, 0.1 EGTA, 10.0 HEPES, 2.0 Na_2-ATP and 0.4 Na-GTP. For current clamp experiments, the pipette solution contained (in mM): 117.0 K-gluconate, 13.0 KCl, 1.0 $MgCl_2$, 0.07 $CaCl_2$, 0.1 EGTA, 10.0 HEPES, 2.0 Na_2-ATP, 0.4 Na-GTP, and 0.3% biocytin. The pH and osmolarity of internal solution were adjusted to 7.3 and ~290 mosm, respectively. The internal solution resulted in a 10 mV junction potential that has been corrected in the voltage measures. After forming whole cell configuration, the recording was allowed to stabilize for at least 5 minutes prior to data acquisition. Inhibitory postsynaptic currents (IPSCs) were optimized by using the cesium (Cs^+)-based internal solution and a 0 mV holding potential. All signals were obtained using a Multiclamp 700 amplifier (Molecular Devices, Foster City, CA). For current-clamp recordings, an active bridge circuit was continuously monitored and, if necessary, adjusted to balance the drop in potential produced by passing current through the recording electrode. Recordings included in this study had initial access resistances ranging from 5-12 MΩ and typically remained stable throughout the recording. Data were omitted from the analyses if initial access resistance changed by >20%. Only neurons that exhibited stable baseline with resting membrane potentials greater than -55 mV and overshooting action potentials were included for data analyses.

Stimulation procedures

IPSCs were evoked in TRN neurons by electrically stimulating GP (> 0.5 mm lateral to TRN border, Fig. 1A) using monopolar electrode (200-700 μA, 50 μs duration). The stimulus

intensity typically ranged from 200-400 μA, but on occasions up to 700 μA was used if no response was observed at the lower intensities. The actions of the dopaminergic agonists were reversible thereby suggesting a lack of damage to afferent fibers from the stimulus paradigm. IPSCs were pharmacologically isolated using NMDA and non-NMDA receptor antagonists, (±3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP, 10μM) and 6,7-dinitroquinoxaline-2,3-dione (DNQX; 20 μM), respectively. In some neurons, focal application of glutamate via pressure ejection was used to evoke IPSCs in TRN neurons by neighboring neurons.

Drug administration

Concentrated stock solutions were originally prepared in appropriate solvents and diluted in physiological saline to final concentration just prior to use. Agonists were applied via a short bolus into an input line using a syringe pump (Govindaiah and Cox, 2005). DA and SKF38393 were prepared fresh prior to application, and DA was prepared with 0.08% ascorbic acid to prevent oxidation. All antagonists were bath applied at least 5-10 min prior to subsequent agonist application. DA was purchased from Sigma (St. Louis, MO), whereas all remaining compounds were purchased from Tocris (Ellisville, MO).

Data acquisition and analyses

Quantification of evoked IPSC amplitude was performed on 5-10 consecutive responses in each experimental condition using pClamp software (Molecular Devices, Sunnyvale, CA USA). Miniature IPSCs (mIPSCs) were detected and analyzed using MiniAnalysis software (Synaptosoft, Leonia, NJ). The threshold for mIPSC detection was 10 pA and automatic detection was verified *post hoc* by visual analysis. The threshold for mIPSC detection was

established from the baseline noise level recorded in the presence of $GABA_A$ receptor antagonist (SR95531, 10 μM) and glutamate receptor antagonists, CPP and DNQX. For quantification of mIPSCs, the average mIPSC frequency was calculated from 60-second time windows: 1 minute prior to agonist application, and 30 seconds following agonist application (Govindaiah and Cox, 2006b). To analyze glutamate evoked IPSCs in TRN, the frequency and amplitude of IPSCs were quantified from 1-second windows for 5 consecutive sweeps for each condition. Due to lack of clear baseline in these cases, the events were manually detected and subsequently the frequency and amplitude of IPSCs quantified using Minianalysis software. Data are expressed as mean ± standard deviation, unless otherwise noted. Most analyses consisted of Student's t-test, paired test if appropriate, unless otherwise noted. P-values <0.05 were considered statistically significant.

Histology

In order to confirm that the recordings were obtained from TRN neurons, the morphology of recorded TRN neurons was recovered by filling neurons with a fluorescent dye (Alexa-594, 50 μM). Alexa-594 was included in the recording pipette and after recording, a z-stack of images was captured using custom-made two-photon laser scanning microscopy (Prairie Technologies, Middleton, WI USA) and collapsed using ImageJ software (NIH).

RESULTS

The results presented in the present study were obtained from 80 TRN neurons. Inhibitory postsynaptic currents (IPSCs) in TRN neurons were evoked by GP stimulation (Fig. 1A) in the presence of NMDA and AMPA receptor glutamatergic antagonists, CPP (10 μM) and DNQX (20 μM), respectively. The evoked IPSCs (eIPSCs) were completely attenuated by the antagonist SR95531 (10 μM), indicating that these events are mediated by $GABA_A$ receptors (Fig. 1C).

DA suppresses $GABA_A$ receptor- mediated inhibition in TRN neurons

We initially tested the effects of DA on $GABA_A$ receptor- mediated IPSCs evoked by GP stimulation. Short duration DA application (2.5-25 μM, 20-30 s) reversibly suppressed the eIPSCs amplitudes in a reversible manner (Fig. 2Ai). Overall, DA produced a concentration-dependent suppression of the eIPSC amplitude up to 43 ± 11% with an IC_{50} of 5.1 μM (Boltzman fit). As illustrated in figure $2A_2$, DA reduced the eIPSC amplitude by 9 ± 4% (control: 496±143 pA, DA: 455±144 pA, n=5, $p<0.001$), 37 ± 14% (control: 481±128 pA, DA: 306±108 pA, n=7, $p<0.0007$), and 43 ± 11% (control: 469±124 pA, DA: 275±100 pA, n=8, $p<0.0004$) at 2.5, 10, and 25 μM, respectively. There was no significant differences between 10 μM and 25 μM DA.

Suppressive actions of DA are mediated via activation of D_4 receptors

We next used selective receptor agonists and antagonists to determine the receptor subtype(s) mediating the DA effect. Similar to DA, the D_2-like receptor ($D_{2,3,4}$) agonist quinpirole (5-10 μM) and selective D_4 receptor agonist PD168077 (25-50 μM) reversibly

suppressed eIPSCs in TRN neurons (Fig. 2B). The suppressive action of quinpirole (10 μM) was reproducible to similar degree when applied at the interval of 5-10 minutes between each application (Fig.2C). Short bath application (45 s) of quinpirole significantly reduced the eIPSC amplitude from 544±245 pA to 347±140 pA (35.1±6.5%, n=10, $p<0.0001$, Fig. 2B). The magnitude of the quinpirole effects did not significantly differ from the DA-mediated effects ($p > 0.2$). The selective D_4 receptor agonist PD168077 (25-50 μM) also suppressed the eIPSC by 37±12% (control: 565±179 pA, PD168077: 355±122 pA, n=5, $p<0.0001$, Fig. 2B2). In contrast, the D_1-like receptor agonist SKF38393 (10 μM) did not alter eIPSC amplitude in all cells tested (3.4±5%, control: 506±182 pA, SKF38393: 501±174 pA, n=4, $p<0.2$, Fig. 2B). We further confirmed the suppressive affects of D_2-like and selective D_4 receptor agonists on eIPSCs using selective antagonists. As illustrated in figure 3A, quinpirole produced a significant reduction in the eIPSC amplitude from 366±66 pA to 233±33 pA (36.3± 5%, n=5, $p<0.002$), which recovered following washout (360±70 pA). In the presence of D_2-like receptor antagonist sulpiride (10 μM), subsequent quinpirole application significantly reduced eIPSC amplitude by 28.3±8.8% (Fig. 3A, $n = 5$, $p<0.02$). The quinpirole-mediated suppression of the eIPSC amplitude did not significantly differ between control and sulpiride conditions (Fig. 3A, p=0.2). Considering the repeated quinpirole applications in this experiment, in a different population of TRN cells, we made repeated quinpirole applications (10 μM, 10 minutes interval) in control conditions and found that the eIPSC amplitude was suppressed to a similar degree with each quinpirole application, suggesting no desensitization with repeated applications (n=4, p=0.27, Fig. 2C). Of the D_2-like receptors (D_2, D_3, D_4), sulpiride has a higher affinity for D_2 and D_3 receptors (Werner et al., 1996), suggesting the suppression may be mediated via D_4 receptor activation.

To confirm the selective D_4 receptor activation by agonist PD168077, we next tested the actions of PD168077 in the presence of selective D_4 receptor antagonist L745870 (Patel et al., 1997). PD168077 (25 μM) reduced the eIPSC amplitude by 39.0±13.1% in control conditions (Fig. 3B, control: 563±207 pA, PD168077: 341±137 pA, n=4, $p<0.001$). In the presence of L745870 (50 μM), the subsequent application of PD168077 did not alter the eIPSC amplitude (Fig. 3B, L7458706: 485±260 pA, PD168077+L7458706: 493±254 pA, n=4, $p=0.1$). We further examined the sensitivity of the DA-mediated suppression of eIPSCs to the D_4 receptor antagonist. In these neurons, DA (10 μM) significantly suppressed the eIPSC amplitude (Fig. 3C, control: 522±127 pA, DA: 314±191pA, n=5, $p<0.007$). Following recovery, the D_4 receptor antagonist L745870 (25 μM) was bath applied for 7-10 minutes. In the presence of L745870, the subsequent DA application did not alter the eIPSC amplitude significantly (Fig.3C, control: 519±126 pA, L745870+DA: 483±120 pA, n=5, $p<0.03$). Overall, these data indicate that activation of D_4 receptors attenuates evoked inhibitory synaptic transmission arising from GP stimulation.

Pre-synaptic D_4 receptors modulates GABAergic signaling in TRN

To examine whether the inhibitory actions of DA on IPSCs is mediated by a pre- or post-synaptic action, we studied the effect of DA and quinpirole on eIPSCs produced by paired pulse stimulation as well as their effect on miniature IPSCs (mIPSCs). Paired pulse stimulation within GP (75-125 ms inter-stimulus intervals, ISI) resulted in paired pulse depression of the eIPSCs in TRN neurons (Fig. 4). The paired pulse ratio (PPR: $IPSC_2$/ $IPSC_1$) was calculated prior to and after agonist application. DA (25 μM) attenuated the eIPSC and the PPR was significantly increased from 0.67±0.08 to 0.91±0.10 (Fig. 4A2; n=6, $p<0.002$), indicating a decrease in paired

246 pulse depression. Similar to DA, quinpirole (10 μM) also significantly increased the PPR from
247 0.63±0.04 to 0.79±0.09 (Fig. 4B; n=8, $p<0.002$). The alteration in PPR is consistent with a
248 presynaptic site of action such as a change in release properties (Zucker and Regehr, 2002).
249 We next tested the effects of DA and dopaminergic agonists on mIPSC activity in TRN
250 neurons. The mIPSCs were pharmacologically isolated using 1 μM TTX, 10 μM CPP and 10 μM
251 DNQX, and recorded using a holding potential of 0 mV. Under these conditions, quinpirole (10
252 μM, n=9) and DA (25 μM, n=3) reduced mIPSC frequency, but not mIPSC amplitude.
253 Cumulative probability analyses indicate that quinpirole significantly increased the inter-event
254 interval (Fig. 5B,C) without significant alterations in mIPSC amplitude (Fig. 5D,E). Overall,
255 quinpirole decreased the frequency of mIPSCs by 37.0±12.5% (control: 1.0±0.4 Hz, quinpirole:
256 0.6±0.2 Hz, n=9, $p<0.001$, Fig. 5C). In contrast, quinpirole did not significantly alter mISPC
257 amplitude (control: 15.6±1.0 pA; quinpirole: 14.7±3.2 pA, n=9; p=0.5, Fig. 5E). The selective
258 alteration in mIPSC frequency, but not mIPSC amplitude, is consistent with a presynaptic site of
259 action.
260 Previous studies have demonstrated that activation of D_2-like receptors inhibits Ca^{2+}
261 currents through a pertussis toxin (PTX)-sensitive G protein ($G_{i/o}$, Yan et al, 1997). To determine
262 whether the presynaptic D_4 receptor-mediated effect involves this G protein subtype, we
263 examined the effect of the sulfhydryl alkylating agent *N*-ethylmaleimide (NEM), which disrupts
264 coupling of PTX-sensitive $G_{i/o}$ type G proteins to Ca^{2+} channels (Shapiro et al, 1994). The
265 suppressive action of D_4 receptor agonist PD168077 on eIPSC was blocked by NEM (Fig. 6). In
266 control conditions, PD168077 (25 μM) reduced the eIPSC amplitude by 36.2 ± 13.7% (n=5,
267 $p<0.01$). Bath application of NEM (50 μM) alone had no effect on the evoked IPSCs. In the
268 presence of NEM, PD168077 produced a smaller suppression of the eIPSC (9.9 ±4.1%), which is

significantly smaller than that in the absence of NEM ($p < 0.02$), indicating that NEM-sensitive Gi proteins are coupled to presynaptic D_4 receptors.

As a measure of postsynaptic sensitivity, we tested if the DA and its agonists could alter $GABA_A$ receptor-mediated currents in TRN neurons. $GABA_A$ receptor-mediated currents were evoked by brief, focal application of the $GABA_A$ receptor agonist muscimol at 20-second intervals. Muscimol (50 μM, 0.5 s duration) elicited repeatable outward currents (Fig. 7A) that were completely attenuated by SR95531 (10 μM, not shown). The muscimol-induced currents were unaltered by 25 μM DA (Fig. 7B, control: 691±167 pA, DA: 667±170, n=3; p=0.2,) or 10 μM quinpirole (Fig. 7B, control: 766±222, quinpirole: 729±227, n=6; $p<0.05$). These data suggest that DA does not regulate postsynaptic $GABA_A$ receptors in TRN neurons. Overall, these data indicate that the dopamine-mediated suppression of eIPSCs is due to a presynaptic action, thereby reducing GABA release from their terminals originating from GP.

DA-mediated suppression of inhibition is restricted to GP-TRN pathway

Our results clearly demonstrate that the activation of DA D_4 receptors suppress inhibitory synaptic transmission resulting from electrical stimulation of presumed GP efferents, but it is unclear if this is a general action affecting all inhibitory inputs onto TRN neurons. We next tested if DA could modulate intra-TRN inhibition considering these neurons form chemical synapses with each other (Deleuze and Huguenard, 2006;Lam et al., 2006;Shu and McCormick, 2002). In order to evoke intra-TRN inhibitory responses, we focally applied glutamate via pressure ejection within TRN near our recording. As shown in figure 8A, glutamate (500 μM, 50 ms) produced an inward current (likely via direct depolarization and/or electrical coupling) along with an increase in spontaneous IPSCs (sIPSCs). These sIPSCs were completely antagonized by

292 a $GABA_A$ receptor antagonist SR95531 (Fig. 8A, SR95531). The glutamate-evoked IPSCs had
293 an average frequency of 23.7±11.4 Hz (Fig. 8B, n=6). After obtaining a consistent IPSCs
294 resulting from glutamate application, quinpirole (10 μM, 45-60 s) was applied via bolus.
295 Quinpirole did not alter the frequency of glutamate-evoked sIPSCs (Fig.8B, control: 25.8±11.3
296 Hz, quinpirole: 24.3±10.9 Hz, n=5, p=0.3). Similarly, D_4 receptor agonist PD168077 (25-50 μM)
297 did not alter sIPSC frequency (Fig.8B, control: 23.3±12.7 Hz, PD168077: 20.6±11.5 Hz, n=5,
298 p=0.1). Likewise, sIPSC amplitudes were also unaltered by either quinpirole (control: 37.7±11.8
299 pA, quinpirole: 39.7±12.8 pA, n=5, p=0.9) or PD168077 (Fig.8C, control: 35.9±12.3 pA,
300 PD168077: 33.9±11.7 pA, n=5, p=0.4). The data suggests that the DA-mediated reduction in
301 inhibitory synaptic transmission in TRN neurons is limited to the GP-TRN inhibition and not
302 intra-TRN inhibition.

DISCUSSION

We demonstrate that the activation of presynaptic D_4 receptors selectively suppresses $GABA_A$ receptor-mediated inhibition at pallido-thalamic innervation without altering intra-TRN inhibition (Fig. 7). The reduction in GP-thalamic inhibition is by reducing GABA release at presynaptic terminals of GP neurons via D_4 receptor activation. Despite the recognized association of D_4 receptors with schizophrenia, attention deficit hyperactivity disorder, and other mental disorders (Oak et al., 2000;Seeman et al., 1993), the cellular mechanisms by which D_4 receptors modulate neuronal functions remain elusive. Anatomical studies have shown that D_2-like dopamine receptors are expressed by GABAergic neurons including TRN and GP (Mrzljak et al., 1996;Khan et al., 1998), however, their functional role on inhibitory synaptic transmission has not been explored.

TRN receives GABAergic projections from GP, SNr, and basal forebrain (Anaya-Martinez et al., 2006;Asanuma and Porter, 1990;Asanuma, 1994;Bickford et al., 1994). In addition, TRN neurons form intra-TRN connections via axon collaterals (Huntsman et al., 1999;Shu and McCormick, 2002). Although, the precise origin of GABA terminals within the TRN containing the D_4 receptors remains unclear, GP neurons have been shown to express D_4 receptor and its mRNA (Ariano et al., 1997;Defagot et al., 1997;Mrzljak et al., 1996). Anatomical evidence suggests that TRN neurons receive dopaminergic innervations from substantia nigra pars compacta (Anaya-Martinez et al., 2006;Garcia-Cabezas et al., 2007;Garcia-Cabezas et al., 2009;Sanchez-Gonzalez et al., 2005) and expresses DA receptors (Ariano et al., 1997;Defagot et al., 1997;Khan et al., 1998;Mrzljak et al., 1996). In this study, we have demonstrated a functional role of D_4 receptors exclusively found on GABAergic neurons

(Mrzljak et al., 1996). Our present findings are supported by a evidence suggesting that the activation of D_4 receptors can modulate GABA release in TRN (Floran et al., 2004).

The dopaminergic system in TRN is thought to play crucial role in sensory gating, and has been postulated that some of the manifestations of disorders of dopaminergic transmission may be caused by abnormal TRN function. For example, the TRN plays a central role in the control of attention (Guillery et al., 1998;Mayo, 2009;McAlonan et al., 2008;Rees, 2009;Yu et al., 2009), and attention deficit hyperactive disorder (ADHD) is associated with genetic abnormalities of dopamine D_4 receptors (LaHoste et al., 1996;Castellanos and Tannock, 2002). Moreover, TRN lies at the interface of thalamocortical circuits between prefrontal cortex and associated thalamic relay nuclei, thus alterations in TRN signaling will influence the gating properties between these two structures, which could underlie the hypothesized role of TRN in attention mechanisms (Zikopoulos and Barbas, 2006). Additionally, abnormal dopaminergic function in the TRN may also contribute to some of the manifestations of Parkinson's disease, such as the sleep disorders (Rye and Jankovic, 2002) and the abnormal processing of proprioceptive signals (Dietz, 2002). By its connections with motor-related structures, TRN is thought to play integrative role in motor functions (Anaya-Martinez et al., 2006;Kane et al., 2009;Obeso et al., 2008;Piggott et al., 2007). Thus, alterations in dopaminergic system in TRN may lead to abnormal motor functions found in Parkinson's disease. In fact, the D_4 receptor-containing GABAergic neurons of the SNr and GP are thought to constitute major links in the basal ganglia loop circuits that regulate the motor thalamus and the cortex in sequence. We propose that activation of SNc neurons lads to release of DA in TRN and this in turn activates D4 receptor on GP terminals leading to reduced GABA release. The reduced inhibition by activation of SNc neurons may lead to increased excitability of TRN neurons and decrease in

output of thalamocortical neurons. Thus, activation of D4 receptor can influence the thalamocortical circuit activity.

Thalamic inhibitory mechanisms have been shown to play crucial role in thalamocortical oscillations associated with arousal and sleep mechanisms (Huguenard and McCormick, 2007;McCormick and Bal, 1997;Sohal et al., 2000; Sohal et al., 2003; Steriade, 1992;Steriade, 1997;vonKrosigk et al., 1993). Schizophrenic patients have been reported to show abnormalities in slow-wave sleep that are correlated with the state of spindle activity and synchronization of cortical and thalamic activity (Keshavan et al., 1995). Thus, thalamic D_2–like receptors are thought to play key roles in pathophysiology of schizophrenia (Buchsbaum et al., 2006). Elevated levels of D_4 receptors have been reported in schizophrenics (Seeman et al., 1993). In addition, alterations in thalamic D_2 receptors have been reported in schizophrenia (Takahashi et al., 2006). Clearly, there are a number of different alterations in thalamocortical activities that may result in alterations in sensory processing and attentional modulation, the underlying mechanisms leading to such alterations and further connection to the manifestation of specific schizophrenic behaviors remains speculative (Behrendt, 2006). Nonetheless, malfunctioning of DA receptors in TRN is one mechanism that would lead to abnormalities in thalamocortical rhythms and thalamic gating that could contribute to some symptoms of schizophrenia. Additional studies are required to further unravel the functional significance of DA in the thalamus under normal and pathological conditions such as schizophrenia.

FIGURE LEGENDS

Figure 1: ***A:*** Image of horizontal brain slice at the level of GP and TRN illustrating electrode placement. GP, globus pallidus; IC, internal capsule; TRN, thalamic reticular nucleus; VB, ventrobasal; R, recording pipette, S, stimulation electrode. ***B1:*** **P**hotomicrograph of a representative TRN neuron. The morphology of neuron was recovered by including a fluorescent dye Alexa-594 in recording pipette. ***B2:*** Characteristic responses of a TRN neuron to hyperpolarizing and depolarizing current steps. TRN neurons display characteristic low-threshold calcium spikes (LTS) in response to hyperpolarizing current steps. ***C:*** Paired pulse stimulation of GP evokes IPSCs recorded in TRN neuron. The IPSCs were isolated by using NMDA receptor antagonist CPP (10 μM) and non-NMDA receptor antagonist DNQX (20 μM), and were blocked by $GABA_A$ receptor antagonist SR95531 (10 μM).

Figure 2: DA suppresses IPSCs in TRN neurons**.** ***A1*****:** In a representative neuron, the IPSC is reversibly attenuated by short application of DA at different concentrations (2.5 and 10 μM) Traces above are representative IPSCs and below is the time course of DA-mediated actions. ***A2***: Population data indicating a significant concentration-dependent suppression of eIPSC by DA. *,$p < 0.001$, **$p < 0.0001$. The number of cells tested for each group is shown in parenthesis. ***B:*** D2-like receptor agonist quinpirole and D_4 receptor agonist PD168077 mimic the actions of DA. ***B1***: In a representative TRN neuron, quinpirole (10 μM) reversibly suppresses IPSC amplitude. In a different neuron, PD168077 (25 μM) also reversibly suppresses IPSC amplitude. ***B2***: Population data indicate a significant suppression of IPSC amplitude by quinpirole (n=10) and PD168077 (n=5). ** $p < 0.0001$.

Figure 3: Suppressive actions of DA on IPSCs mediated via D_4 receptor activation. ***A1:*** Representative synaptic responses (top) and time course (bottom) illustrating that quinpirole10 μM) suppresses the IPSC in a reversible manner. Following washout, the D_2-like receptor antagonist sulpiride (10 μM) was bath applied, and the subsequent application of quinpirole still significantly attenuates IPSC amplitude. ***A2:*** Population data illustrating the suppressive actions of quinpirole (**p<0.002, n=5) and this effect is not completely antagonized by sulpiride (*p<0.02, n=5). ***B1:*** In a different TRN neuron, PD168077 (25 μM) reversibly suppresses the IPSC similar to quinpirole. Following recovery, the selective D_4 receptor antagonist L745870 (50 μM) was bath applied, and the subsequent application of PD168077 does not alter IPSC amplitude. ***B2:*** Population data illustrating reversible suppressive actions of PD168077 (*p < 0.001, n=4). In the presence of L745870, PD168077 no longer alters the IPSC (p=0.1, n=4). ***C1:*** L745870 also blocks the DA-mediated suppression of the IPSC. In different TRN neurons, DA strongly attenuates the IPSC. In the presence of L745870 (50 μM), the DA-mediated suppression is greatly reduced. ***C2:*** Population data illustrating the suppressive actions of DA alone (**p < 0.007, n=5) and its reduced action in presence of L745870 (*p < 0.03, n=5).

Figure 4: DA reduces paired pulse depression of IPSC in TRN neurons. ***A1***: Representative IPSCs recorded from TRN neuron using paired-pulse stimulation (100 ms ISI) of GP. DA (25 μM) reduces the amplitude of the first IPSC ($IPSC_1$) with little alteration in the second IPSC ($IPSC_2$), leading to an increase in paired pulse ratio (PPR). Scaling of the first IPSC clearly illustrates the reduction in paired pulse depression. ***A2***: Population data illustrate a significant increase in PPR by DA (*p < 0.002, n=6) indicating a reduction in paired pulse depression. ***B1***:

In a different neuron, quinpirole (10 μM) produces a similar alteration in PPR as DA. ***B2***: Population data illustrate a significant increase in PPR by quinpirole (**p* < 0.002, n=8).

Figure 5: Activation of D_2 receptors increases miniature IPSC frequency, but not mIPSC amplitude in TRN neurons. Miniature IPSCs (mIPSCs) were recorded in presence of TTX (1 μM). ***A:*** Representative current traces reveal mIPSCs in control and following quinpirole (10 μM) application. ***B:*** Cumulative probability plots for neuron in ***A*** illustrating the increase in inter-event intervals by quinpirole. ***C:*** Population data revealing that quinpirole significantly reduces mIPSC frequency (**p*<0.001, n=9). ***D:*** Cumulative probability plot for neuron in ***A*** illustrating that mIPSC amplitude is unaltered by quinpirole. ***E:*** Population data reveal quinpirole does not alter mIPSC amplitude (*p*=0.5, n=9).

Figure 6: The D_4-mediated suppression of inhibition in TRN involves presynaptic inhibitory G proteins (Gi). ***A:*** In a representative TRN neuron, eIPSCs are reversibly attenuated by PD168077 (25 μM); however the suppressive action of PD168077 is blocked in the presence of Gi-protein inhibitor NEM (50 μM). ***B:*** Time course of the experiment illustrated in A. *C:* Population data summarizing the effects of PD with and without NEM. n= 5, *p < 0.01, **p<0.02.

Figure 7: DA does not alter post-synaptic $GABA_A$ receptor mediated currents in TRN neurons. ***A:*** Current recording from a TRN neuron showing repeatable outward response to focal pressure application of $GABA_A$ receptor agonist muscimol (50 μM, 50 ms). Bath application of either DA (25 μM) or quinpirole (10 μM) did not alter the muscimol-mediated outward currents. Representative examples are shown below. ***B:*** Population data illustrating amplitude of the muscimol-induced currents following DA (n= 3) or quinpirole (n= 6) exposure.

Figure 8: Activation of DA receptors does not alter intra-TRN inhibition. ***A:*** Current recording from a TRN neuron reveals that focal glutamate application via pressure ejection within TRN (500 μM, 50 ms, 30s interval) elicits short outward current and long-lasting inward current along with increase in spontaneous IPSCs (sIPSCs, *). After repeating this several times, either quinpirole (10 μM) or PD168077 (50 μM) was bath applied (45 s). The sIPSCs evoked by glutamate application are not altered by the DA agonists. Subsequent application of $GABA_A$ receptor antagonist SR95531 (10 μM) completely blocked sIPSCs. ***B, C:*** Population data indicating no significant change in sIPSC frequency (B) or sIPSC amplitude (C) by quinpirole or PD168077.

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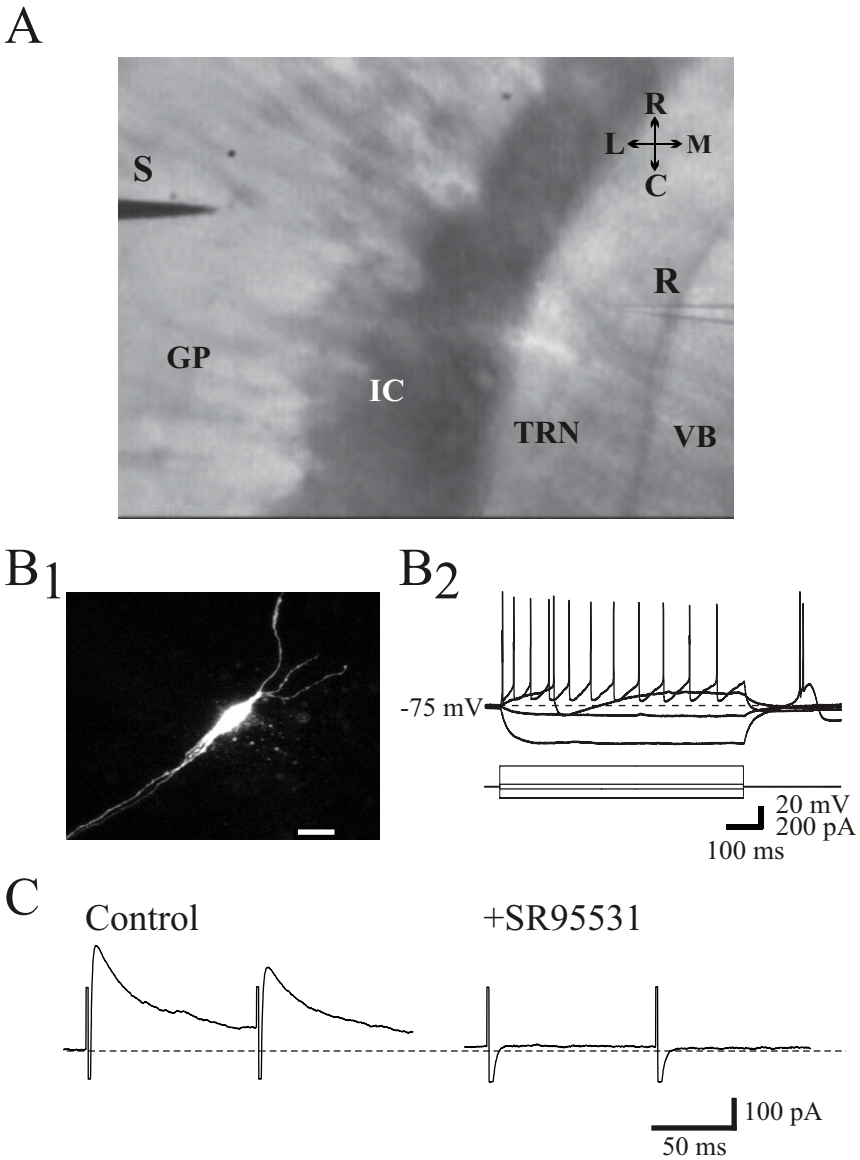
A
S
R
L
M
C
R
GP
IC
TRN
VB
B1
B2
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20 mV
200 pA
100 ms
C
Control
+SR95531
100 pA
50 ms

Figure 1

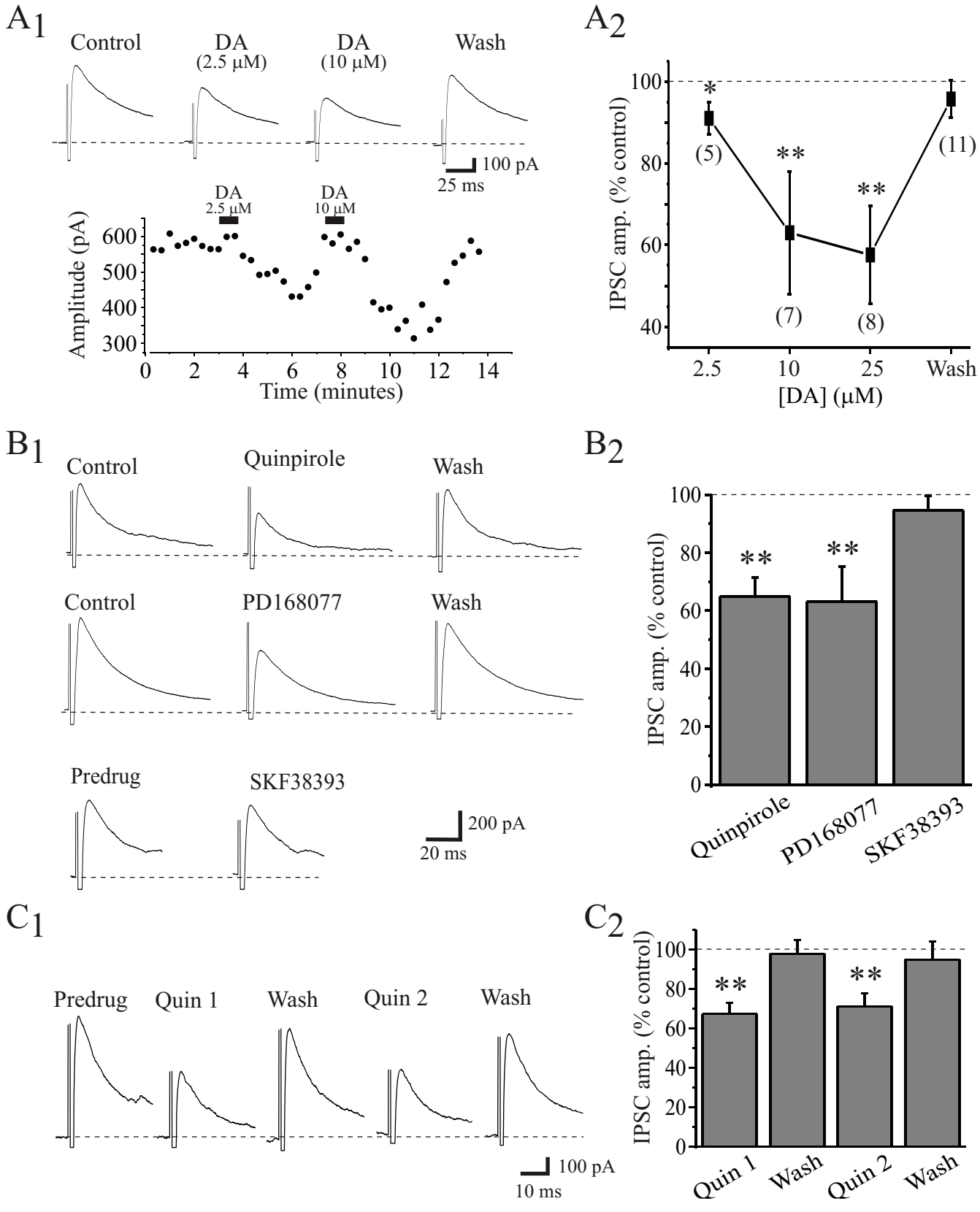
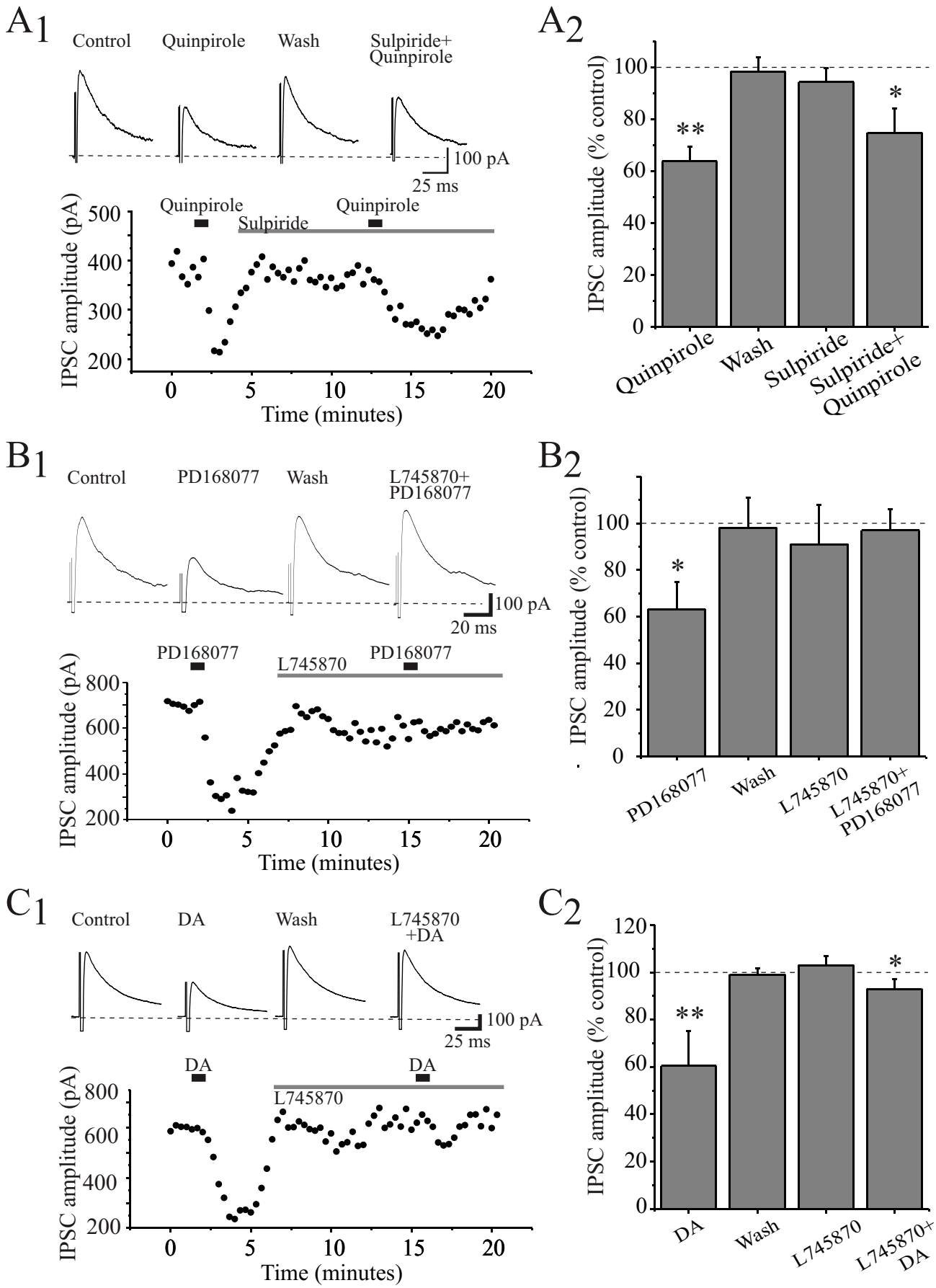

A1
Control
DA
(2.5 μM)
DA
(10 μM)
Wash
100 pA
25 ms
DA
2.5 μM
DA
10 μM
Amplitude (pA)
600
500
400
300
0
2
4
6
8
10
12
14
Time (minutes)
A2
IPSC amp. (% control)
100
80
60
40
*
**
**
(5)
(7)
(8)
(11)
2.5
10
25
Wash
[DA] (μM)
B1
Control
Quinpirole
Wash
Control
PD168077
Wash
Predrug
SKF38393
200 pA
20 ms
B2
IPSC amp. (% control)
100
80
60
40
20
0
**
**
Quinpirole
PD168077
SKF38393
C1
Predrug
Quin 1
Wash
Quin 2
Wash
100 pA
10 ms
C2
IPSC amp. (% control)
100
80
60
40
20
0
**
**
Quin 1
Wash
Quin 2
Wash

Figure 2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Control
Quinpirole
Wash
Sulpiride+
Quinpirole
100 pA
25 ms
Quinpirole
Sulpiride
Quinpirole
IPSC amplitude (pA)
500
400
300
200
0
5
10
15
20
Time (minutes)
A2
IPSC amplitude (% control)
100
80
60
40
20
0
**
*
Quinpirole
Wash
Sulpiride
Sulpiride+
Quinpirole
B1
Control
PD168077
Wash
L745870+
PD168077
100 pA
20 ms
PD168077
L745870
PD168077
IPSC amplitude (pA)
800
600
400
200
0
5
10
15
20
Time (minutes)
B2
IPSC amplitude (% control)
100
80
60
40
20
0
*
PD168077
Wash
L745870
L745870+
PD168077
C1
Control
DA
Wash
L745870
+DA
100 pA
25 ms
DA
DA
L745870
IPSC amplitude (pA)
800
600
400
200
0
5
10
15
20
Time (minutes)
C2
IPSC amplitude (% control)
120
100
80
60
40
20
0
**
*
DA
Wash
L745870
L745870+
DA

Figure 3

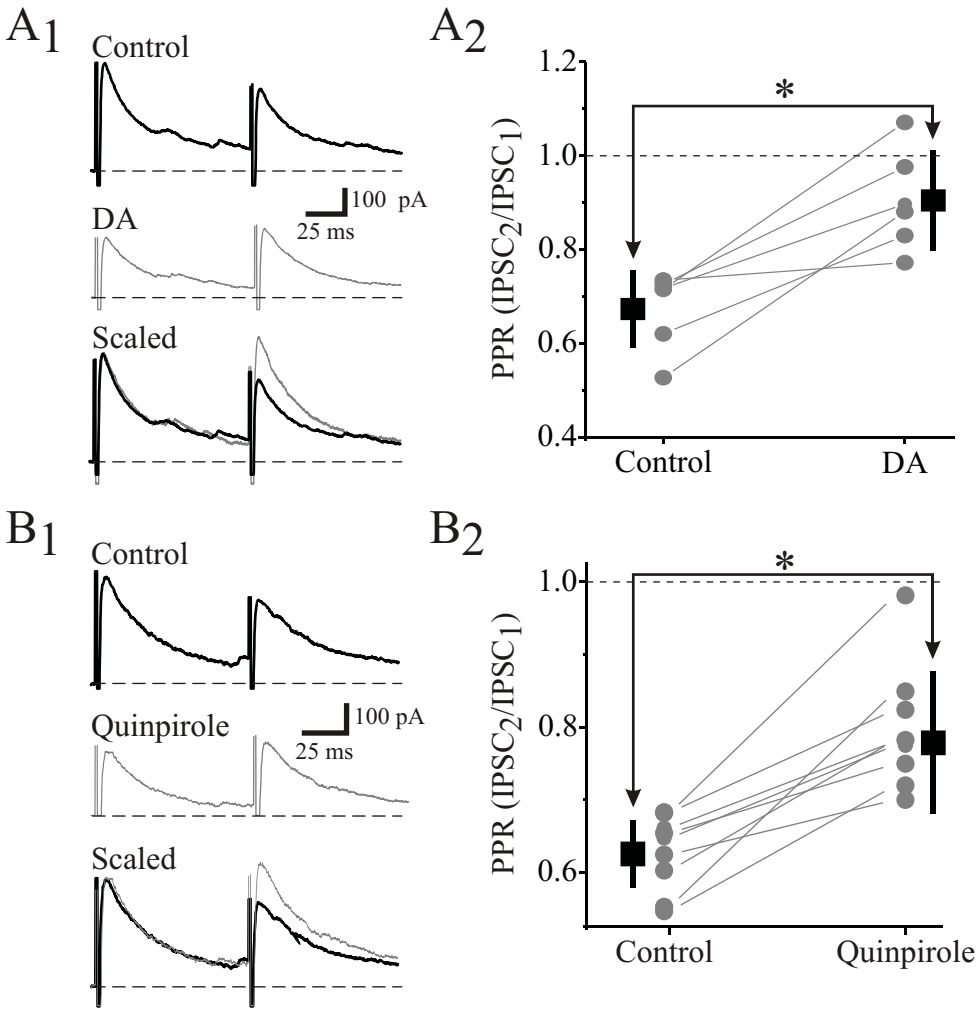
A1
Control
DA
Scaled
100 pA
25 ms
A2
PPR (IPSC2/IPSC1)
1.2
1.0
0.8
0.6
0.4
*
Control
DA
B1
Control
Quinpirole
Scaled
100 pA
25 ms
B2
PPR (IPSC2/IPSC1)
1.0
0.8
0.6
*
Control
Quinpirole

Figure 4

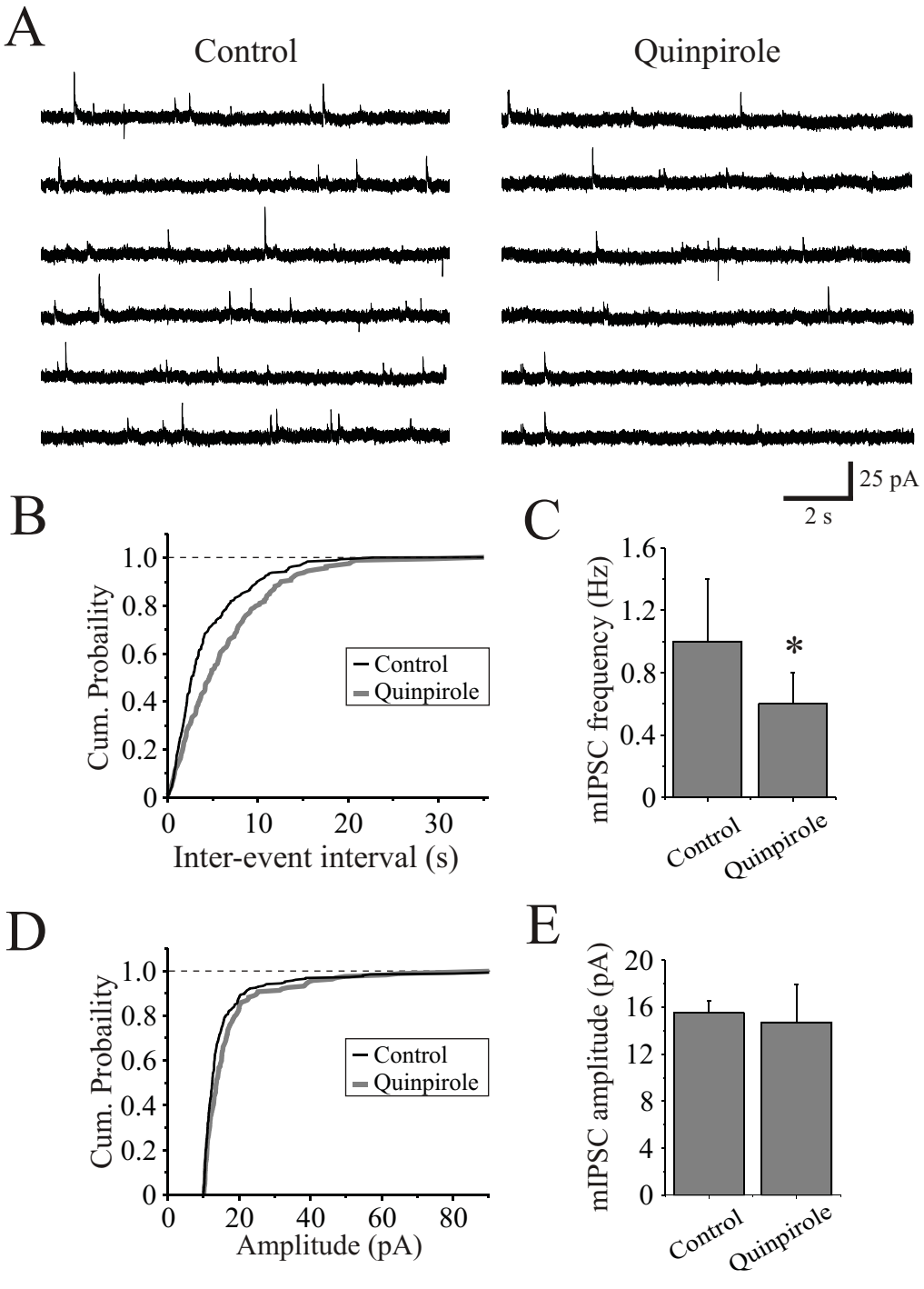
A
Control
Quinpirole
25 pA
2 s
B
Cum. Probaility
1.0
0.8
0.6
0.4
0.2
0
0
10
20
30
Inter-event interval (s)
Control
Quinpirole
C
mIPSC frequency (Hz)
1.6
1.2
0.8
0.4
0
*
Control
Quinpirole
D
Cum. Probaility
1.0
0.8
0.6
0.4
0.2
0
0
20
40
60
80
Amplitude (pA)
Control
Quinpirole
E
mIPSC amplitude (pA)
20
16
12
8
4
0
Control
Quinpirole

Figure 5

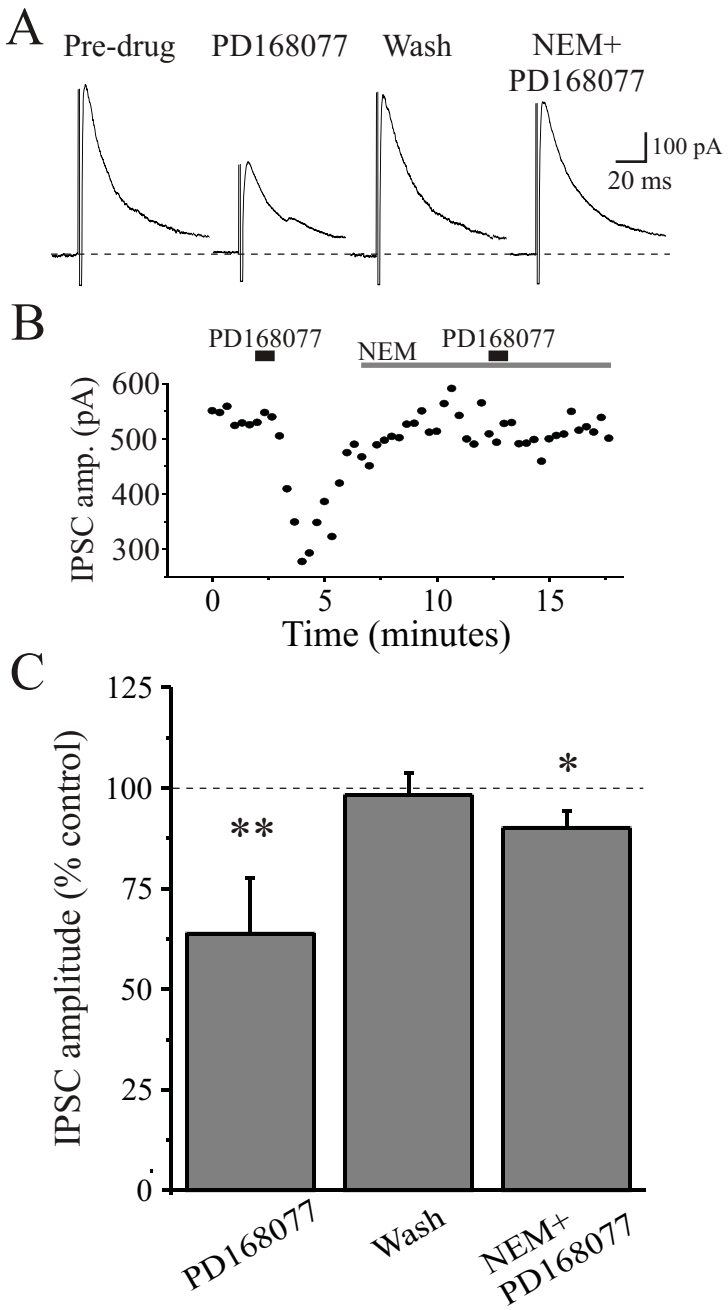
A
Pre-drug
PD168077
Wash
NEM+
PD168077
100 pA
20 ms
B
PD168077
NEM
PD168077
IPSC amp. (pA)
600
500
400
300
0
5
10
15
Time (minutes)
C
IPSC amplitude (% control)
125
100
75
50
25
0
**
*
PD168077
Wash
NEM+
PD168077

Figure 6

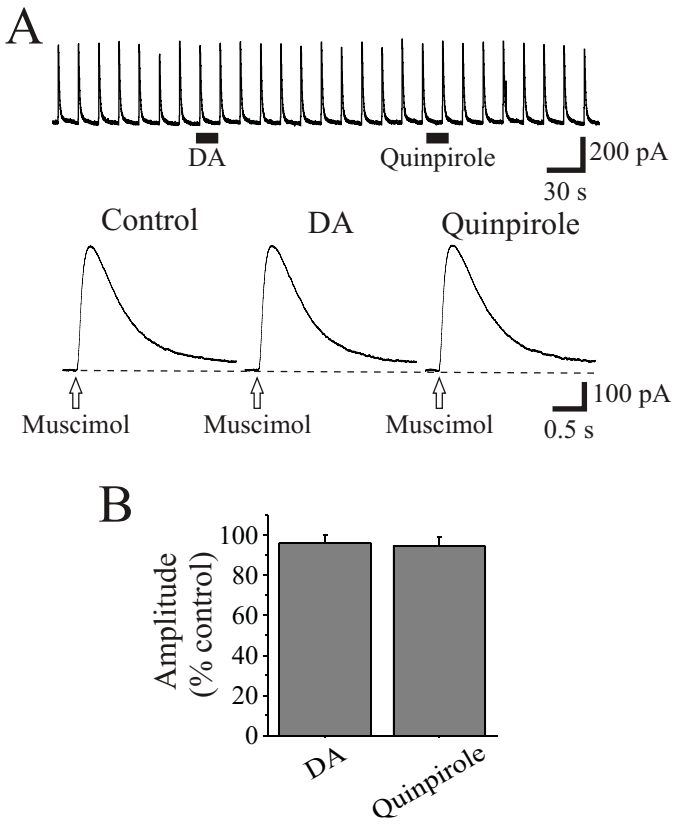
A
DA
Quinpirole
200 pA
30 s
Control
DA
Quinpirole
Muscimol
Muscimol
Muscimol
100 pA
0.5 s
B
Amplitude
(% control)
100
80
60
40
20
0
DA
Quinpirole

Figure 7

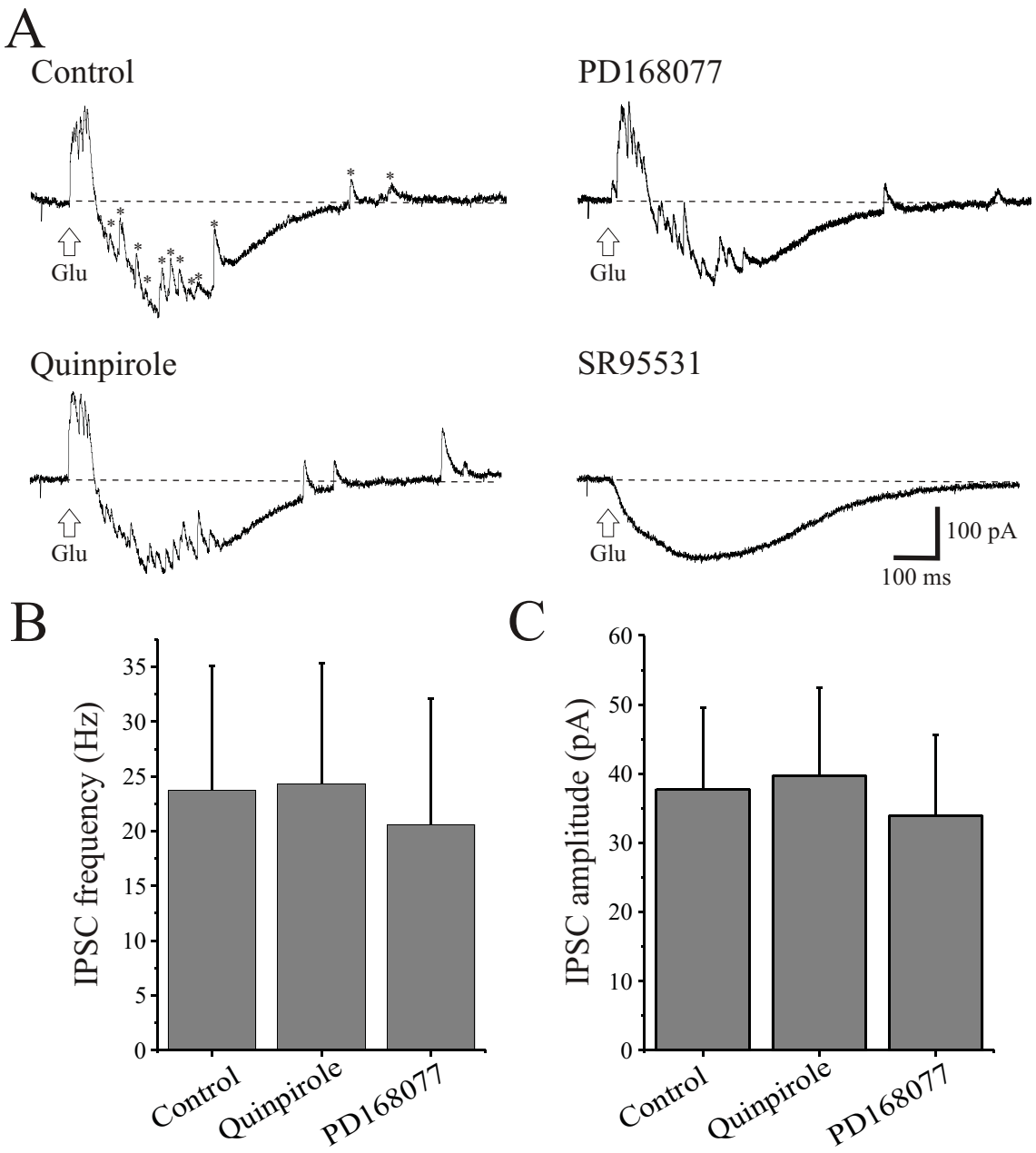
A
Control
PD168077
Quinpirole
SR95531
Glu
100 pA
100 ms
B
IPSC frequency (Hz)
0
5
10
15
20
25
30
35
Control
Quinpirole
PD168077
C
IPSC amplitude (pA)
0
10
20
30
40
50
60
Control
Quinpirole
PD168077

Figure 8