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Nanomolar propofol stimulates glutamate transmission to dopamine neurons: a possible mechanism of abuse potential?

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ABBREVIATIONS: D₁Rs, dopamine D₁ receptors; DNQX, 6,7-dinitroquinoxaline-2, 3-dione; eEPSCs, evoked excitatory postsynaptic currents; GABA, γ -aminobutyric acid; mEPSCs, miniature excitatory postsynaptic currents; NAcc, nucleus accumbens; sEPSCs, spontaneous excitatory postsynaptic currents;

VTA, ventral tegmental area;

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ABSTRACT

Anesthesiologists among physicians are on the top of the drug abuse list, and the mechanism is unclear. Recent studies suggest occupation-related second hand exposure to intravenous drugs including propofol may play a role. Growing evidence indicates that propofol is one of the choices of drugs being abused. Here we show that propofol at minute concentrations increases glutamatergic excitatory synaptic transmission and discharges of dopamine neurons in the ventral tegmental area (VTA). We found that acute application of propofol (0.1-10 nM) to the VTA in midbrain slices of rats increased the frequency but not the amplitude of spontaneous EPSCs-mediated by AMPA (α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid) receptors. We observed that propofol increased the amplitude but decreased the paired-pulse ratio of EPSCs evoked by stimulation in the absence and the presence of GABA_A receptor antagonist. Moreover, the propofol-induced facilitation of EPSCs was mimicked by SKF38393, an agonist of dopamine D₁ receptor, and by GBR12935, a dopamine reuptake inhibitor, but blocked by SKF83566, a D₁ antagonist, or by depleting dopamine stores with reserpine. Finally, 1 nM propofol increased the spontaneous discharge rate of dopamine neurons. These findings suggest that propofol at minute concentrations enhances presynaptic D₁ receptor-mediated facilitation of glutamatergic synaptic transmission and the excitability of VTA dopamine neurons, probably by increasing extracellular dopamine levels. These changes in synaptic plasticity in the VTA, an addiction-related brain area might contribute to the development of propofol abuse and the increased susceptibility to addiction of other drugs.

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Drug abuse is a major social and health concern. Although physicians fully understand the consequences, some still abuse drugs. Interestingly, the drug abuse rate among physicians tends to be the highest for anesthesiologists (McAuliffe et al., 2006). A recently proposed hypothesis on second hand exposure might explain this trend (McAuliffe et al., 2006). Chronic exposure to low levels of aerosolized intravenously administered drugs, such as propofol, can potentially lead to a sensitization and later abuse of drugs (McAuliffe et al., 2006). However, it is not clear how this exposure induces addiction.

Propofol is the most widely used intravenous drug for induction of general anesthesia. Although propofol has not traditionally been considered as a drug of abuse, growing evidence suggests that it may have an abuse potential. Patients anesthetized with propofol report experienced euphoria during recovery (Gepts et al., 1985). Besides medical use, propofol is abused for recreational purpose. Nine human cases of abuse and dependency in anesthesiologists, nurses, and laypersons have been reported since 1992. Death occurred in four among these nine cases. Note that this number is certainly not representative of propofol abuse, as only serious cases have been reported (Roussin et al., 2007). According to a recent survey, propofol abuse in academic anesthesiology likely has increased over the last 10 years. 18% of the anesthesiology programs in the United States have experienced one or more cases of propofol abuse or diversion in the past 10 years, and this percentage is likely an underestimate because the challenges of detecting propofol abuse, as propofol is not a drug routinely tested for on urine (Wischmeyer et al., 2007). Propofol abuse potential has also been supported by studies in human volunteers. Under blind conditions, 50% of the volunteers (six of twelve) chose propofol over placebo, and four of those six subjects chose propofol exclusively across three repeated trials (Zacny et al., 1993a; Zacny et al., 1993b). Propofol abuse potential has also been supported by animal studies. Propofol is self-administered by baboons (Weerts et al., 1999) and rats (LeSage et al., 2000). Conditioned-place preference for propofol has been established in rats (Pain et al., 1996).

The mesolimbic dopamine system is a critically important component of the neuronal circuits mediating addiction. This system originates in the ventral tegmental area (VTA) and terminates in the nucleus accumbens (NAcc). All drugs of abuse enhance the activity

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of this dopamine circuit of reward (Nestler, 2005). In previous rat studies, propofol has been found to alter dopamine levels in the NAcc (Pain et al., 2002), suggesting that propofol may modify the activity of VTA dopamine neurons. However, the cellular mechanisms of the effects of propofol remain to be determined. Glutamatergic inputs to the VTA are the primary excitatory control of dopamine neurons. The purpose of current investigation was to determine whether minute concentrations of propofol can alter glutamatergic transmission and dopamine neuronal activity.

Materials and Methods

All experiments were performed in accordance with the guidelines outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committee of the University of Medicine and Dentistry of New Jersey. The experiments were done on brains from Sprague Dawley rats (P10 to P20).

Slice preparation. The midbrain slices were prepared as described previously (Ye et al., 2004). In brief, rats were anesthetized and then sacrificed by decapitation. Coronal midbrain slices (300 μ m thick) were cut using a VF-200 Slicer (Precisionary Instruments Inc., Greenville, NC). Slices were prepared in ice-cold glycerol-based artificial cerebrospinal fluid (GACSF) – containing (in mM): 250 glycerol, 2.5 KCl, 1.2 NaH₂PO₄, 1.2 MgCl₂, 2.4 CaCl₂, 26 NaHCO₃, and 11 glucose, and saturated with 95% O₂/5% CO₂ (carbogen). Slices (two per animal) were allowed to recover for at least 1 hr in a holding chamber at 32 °C in carbogen-saturated regular ACSF, which has the same composition as GACSF, except that glycerol was replaced by 125 mM NaCl.

Electrophysiological recording. Electrical signals were obtained in whole-cell patch clamp configurations with MultiClamp 700A amplifiers (Molecular Devices Corp, Union city, CA, USA), a Digidata 1322A A/D converter (Molecular Devices Corp) and pCLAMP 9.2 software (Molecular Devices Corp). Data were filtered at 1 kHz and sampled at 5 kHz. Neurons were voltage clamped at -70 mV to record AMPA (α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid) receptor-mediated spontaneous and miniature excitatory postsynaptic currents (sEPSCs and mEPSCs). Current-clamp mode was used to record spontaneous firing. The patch electrodes had a resistance of 4-6 M Ω

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when filled with a pipette solution containing (in mM): 135 potassium gluconate, 5 KCl, 2 MgCl₂, 10 HEPES, 2 Mg ATP, 0.2 GTP. The pH of the pipette solution was adjusted to 7.2 with tris-base. The experiments presented on Figure 9 were done with a CsF based pipette solution, which has the same composition as the potassium gluconate-based solution, except that potassium gluconate was replaced by 135 mM CsF. A single slice was transferred into a 0.4 ml recording chamber, and was stabilized by a round platinum ring. Throughout the experiments, the bath was continually perfused with carbogenated ACSF (1.5 - 2.0 ml/min). Cells were visualized with an upright microscope (E600FN, Nikon) and near-infrared illumination. To evoke monosynaptic EPSCs (eEPSCs), the tip of a glass stimulating pipette filled with 1 M NaCl was placed 50-100 μm from the recorded neuron. Electrical stimuli (100-200 μs in duration) were applied at the rate of 0.05 Hz. Near the start of recording, an input/output curve was obtained. The stimulation was set to 20-30% of maximum, an intensity that evoked stable responses with no failures. Paired eEPSCs were elicited with a pair of identical stimuli separated by an interval of 20 ms. The series resistance (15-30 MΩ) or input resistance (300-500 MΩ) was monitored throughout the whole-cell recording and data were discarded if the resistance changed by more than 20%. All recordings were made at 32 °C, maintained by an automatic temperature controller (Warner Instruments, Hamden, USA). In some experiments, brain slices were pretreated by reserpine (10 μM) for 90 min at 32 °C to deplete endogenous monoamines (including dopamine).

All recordings were obtained from putative dopamine neurons identified by their pharmacological and physiological properties. Specifically, VTA dopamine neurons were identified by the hyperpolarization response to U69593, an agonist of kappa-opioid receptor (5 μM, Figure 1A, B). A previous study has showed that U69593 induced postsynaptic hyperpolarization in a subset of tyrosine hydroxylase staining positive neurons (Margolis et al., 2003). Moreover, a prominent voltage-sag (equivalent to the hyperpolarization current, I_h, recorded in voltage-clamp mode) recorded in response to a hyperpolarizing current pulse (-130 pA) (Figure 1C) confirmed the identity of dopamine neurons. Prominent I_h is present in ~60% of VTA dopamine neurons, whereas VTA GABAergic neurons have no prominent I_h (Lacey et al., 1990; Johnson and North, 1992).

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Chemicals and applications. The chemicals, including 2,6-diisopropylphenol (propofol), GABA_Azine (SR-95531), 6,7-dinitroquinoxaline-2, 3-dione (DNQX), tetrodotoxin (TTX), U69593, methyl-11,17 α -dimethoxy-18 β -[(3,4,5-trimethoxybenzoyl) (reserpine) and cadmium chloride (CdCl₂) were obtained from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). (\pm)-7-Bromo-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride ((\pm)-SKF 83566), 6-phenyl-4-azabicyclo[5.4.0]undeca-7,9,11-triene-9,10-diol (SKF 38393) and 1-[2-(Diphenylmethoxy)ethyl]-4-(3-phenylpropyl)piperazine dihydrochloride (GBR 12935) were from TOCRIS bioscience (Ellisville, MO, USA). Drugs were added to the superfusate.

Data analysis. Spontaneous excitatory postsynaptic currents (sEPSCs) and spontaneous firing were counted and analyzed using the MiniAnalysis program (Synaptosoft, Leonia, NJ). Spontaneous events (sEPSCs, and mEPSCs) were screened automatically (5 pA amplitude threshold), checked visually, and accepted or rejected according to their rise and decay times. The frequency and amplitude of all events, during and after drug applications, were normalized to the mean of the values observed during the initial control period. Cumulative probability plots of the incidence of various inter-event intervals and amplitudes, recorded in control conditions and during pharmacological manipulations from the same neuron, were analyzed with the Kolmogorov-Smirnov (K - S) test. For other plots, data obtained over a 1 - 2 min period at the peak of a drug response were normalized to the average values of sEPSC (or mEPSC) frequency and amplitude during the initial control period (1 - 2 min). Data were expressed as means (\pm SEM). The statistical significance of drug effects was assessed by a paired two-tailed t test. Values of $p < 0.05$ were considered significant.

Results

Propofol at minute concentrations enhances evoked EPSCs (eEPSCs) of dopamine neurons in the VTA. We first examined the effect of propofol on the EPSCs evoked by a local stimulating electrode from putative dopamine neurons at a holding potential (V_H) of -70 mV and in the presence of 10 μ M GABA_Azine. These eEPSCs were mediated by glutamate AMPA (α -amino-3-hydroxy-5-methylisoxazole-4- propionic

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acid) receptors, as indicated by the completely blockage by 10 μ M DNQX (Figure 2A). Unexpectedly, 1 nM propofol reversibly enhanced the amplitude of such eEPSCs by $32 \pm 6\%$ ($n = 5$, $p = 0.006$, by paired t test, Figure 2B, C₁). This enhancement suggests an increase in glutamatergic transmissions, which could be caused either by facilitation of presynaptic glutamate release, potentiation of postsynaptic glutamate receptors, or a combination of these. To distinguish between pre- and post-synaptic effects, we examined the paired-pulse ratio (PPR = EPSC₂/EPSC₁). Propofol (1 nM) significantly reduced the PPR, by $27 \pm 3\%$, from 2.0 ± 0.3 before to 1.5 ± 0.3 after propofol (Figure 2C₂, $n = 5$, $p = 0.02$, by paired t test). Changes in transmitter release generally affect the PPR, as observed in several brain regions including the VTA (Ye et al., 2004).

Propofol at minute concentrations increases the frequency but not the amplitude of spontaneous EPSCs (sEPSCs) in VTA dopamine neurons. The above results suggest that propofol increases presynaptic glutamate release. To further test this possibility, we examined sEPSCs recorded at V_H -70 mV in the presence of 10 μ M GABazine from putative dopamine neurons in brain slices. As illustrated in Figure 3A, 10 μ M DNQX eliminated the sEPSCs, indicating that they were mediated by AMPA receptors. Addition of 1 nM propofol significantly increased the frequency of sEPSCs (Figure 3A, B₁, D₁) by $113 \pm 32\%$ (from 0.5 ± 0.1 to 1.0 ± 0.2 Hz, $n = 5$, $p = 0.02$, by paired t test). The change in frequency is further shown by the significant increase in the probability of shorter intervals between successive sEPSCs (K-S test, $p < 0.001$, Figure 3B₁). After washout of propofol, the sEPSC frequency returned to the control level (Figure 3A, C). However, 1 nM propofol did not alter the cumulative probability plot of sEPSC amplitudes (K-S test: $p > 0.5$, Figure 3B₂). Additionally, propofol-induced increases in sEPSC frequency are dose-dependent: for propofol concentrations up to 1 nM, there was a progressive increase in sEPSC frequency (0.1 nM: $23 \pm 5\%$, $n = 4$, $p < 0.05$; 0.3 nM: $55 \pm 14\%$, $n = 6$, $p < 0.05$); but at doses higher than 1 nM, propofol induced less increase in frequency (3 nM: $46 \pm 15\%$, $n = 4$, $p < 0.05$; 10 nM: $21 \pm 8\%$, $n = 6$, $p < 0.05$). At even higher concentrations, propofol (1 μ M) suppressed sEPSC frequency by $18 \pm 2\%$ ($n = 3$, $p < 0.05$; Figure D₁). On the other hand, all concentrations of propofol (from 0.1 nM to 1 μ M) did not significantly alter the mean amplitudes of sEPSCs (Figure D₂, 0.1 nM: $-0.4 \pm 7\%$, $n = 4$, $p > 0.8$; 0.3 nM: $6 \pm 6\%$, $n = 6$, $p = 0.6$; 1

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nM: $4 \pm 6\%$, $n = 5$, $p = 0.8$; 3 nM: $5 \pm 7\%$, $n = 4$, $p = 0.4$; 10 nM: $10 \pm 3\%$, $n = 6$, $p = 0.07$; 1 μM : $-3 \pm 4\%$, $n = 3$, $p = 0.6$). These data further support a presynaptic mechanism in the enhancement of glutamatergic transmission by minute concentrations of propofol.

Propofol facilitation of spontaneous EPSCs depends on sodium and calcium channels. We next tested the effect of propofol on spontaneous EPSCs recorded in the presence of GABA_A (10 μM) and tetrodotoxin (TTX, 1 μM), a blocker of sodium channels. Application of tetrodotoxin (1 μM) only slightly, albeit significantly, reduced the frequency of the spontaneous events (1.3 ± 0.3 Hz in control, 1.0 ± 0.5 Hz in 1 μM tetrodotoxin, $p < 0.05$, $n = 4$, by paired t test), but not their amplitude (18.1 ± 1.8 pA in control, 17.0 ± 1.9 pA in tetrodotoxin, $p = 0.22$, $n = 4$, by paired t test). In the presence of tetrodotoxin, propofol (1 nM) did not significantly increase the frequency of the miniature EPSCs (by $9 \pm 7\%$, $n = 5$, $p = 0.27$; Figure 4C₁). We also tested the effect of cadmium (100 μM), a non-selective blocker of voltage-gated calcium channels. The application of cadmium significantly reduced sEPSC frequency, by $58 \pm 8\%$ (2.0 ± 0.4 Hz in control, 0.8 ± 0.2 Hz in cadmium, $p = 0.02$, $n = 5$, by paired t test), but not their amplitude (23.3 ± 4.0 pA in control, 24.2 ± 4.8 pA in cadmium, $p = 0.46$, $n = 5$, by paired t test). In the presence of cadmium, propofol (1 nM) had no significant effect on sEPSC frequency (by $8.1 \pm 20.6\%$, $n = 5$, $p = 0.63$; Figure 4C₂). These data indicate that propofol action depends on both tetrodotoxin-sensitive sodium channels and voltage-gated calcium channels.

Propofol facilitation of sEPSCs is eliminated by dopamine D₁R antagonist. Dopamine D₁Rs are expressed on VTA glutamatergic axons (Lu et al., 1997). Previous in vivo study has shown that activation of D₁Rs increases glutamate levels in the VTA (Kalivas and Duffy, 1995). To test a possible involvement of D₁Rs in propofol-induced facilitation of glutamatergic transmission, we compared the effects of propofol on sEPSC frequency in the absence and the presence of SKF 83566 (10 μM), a D₁R antagonist. All sEPSCs were recorded in the presence of 10 μM GABA_A. As illustrated in Fig. 5, though ineffective when applied alone, SKF 83566 (10 μM) suppressed the effects of propofol (1 nM). On five cells, propofol (1 nM) slightly decreased sEPSC frequency by $0.3 \pm 4.0\%$ ($p = 0.95$; Figure 5C₁) and amplitude by $12 \pm 5\%$ ($p = 0.06$; Figure 5C₂) in the

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presence of SKF 83566 (10 μ M). These results strongly suggest that D₁Rs are an essential element in the mechanism of propofol-induced facilitation of glutamatergic transmission.

D₁R agonist mimics propofol-induced facilitation of sEPSCs. To further test the involvement of D₁Rs, we applied D₁R agonist (SKF 38393) to the VTA in slices. As illustrated in Figure 6, SKF 38393 (10 nM) significantly increased sEPSC frequency, thus shortening intervals between successive sEPSCs (K-S test, $p < 0.01$, Figure 6B). The effect of SKF 38393 was reversible. The sEPSC frequency soon returned to its control level after washout (Figure 6C). As illustrated in Figure 6D, the effects of SKF 38393 on sEPSC frequency was dose-dependent: 10, 100, 1000 and 3000 nM SKF 38393 increased sEPSC frequency by $45 \pm 8\%$ ($n = 10$, $p < 0.01$), $57 \pm 19\%$ ($n = 5$, $p = 0.01$), $101 \pm 22\%$ ($n = 6$, $p < 0.007$), and $96 \pm 16\%$ ($n = 5$, $p = 0.004$), respectively. No corresponding changes in sEPSC amplitude were produced by 10, 100, 1000 and 3000 nM SKF 38393: $-2 \pm 7\%$ ($n = 10$, $p = 0.44$); $6 \pm 12\%$ ($n = 5$, $p = 0.88$), and $-0.1 \pm 10\%$ ($n = 6$, $p = 0.5$), and $-3 \pm 8\%$ ($n = 5$, $p = 0.74$), respectively. These results, which closely parallel the effects of propofol, provide additional evidence for the presence of functional D₁Rs on glutamatergic terminals.

Dopamine release is essential for propofol-induced facilitation of eEPSCs. To determine whether dopamine release is necessary for propofol-induced facilitation of glutamatergic transmission, we applied propofol to slices in which dopamine was depleted by reserpine. As illustrated in Figure 7, 1 nM propofol was ineffective when applied to slices pretreated for 90 min with 10 μ M reserpine (Figure 7A), propofol influenced neither the amplitudes of eEPSCs (by $-7 \pm 15\%$, $n = 5$, $p = 0.67$), nor their paired-pulse ratio (by $10 \pm 15\%$, from 1.4 ± 0.1 to 1.5 ± 0.1 , $n = 5$, $p = 0.7$).

Propofol-induced facilitation of sEPSCs is mimicked by a dopamine reuptake blocker. Another, perhaps more important mechanism is suggested by previous evidence that acute administration of propofol (intraperitoneal injection) elevates extracellular dopamine levels (Pain et al., 2002). To elicit a rise in local dopamine levels in slices, we applied GBR 12935, a selective blocker of dopamine transporter. As illustrated in Figure 8A, 10 nM GBR 12935 significantly and reversibly increased the frequency of sEPSCs recorded in VTA dopamine neurons (by $55 \pm 10\%$, $n = 12$, $p = 0.002$). Like propofol,

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GBR 12935 was ineffective in the presence of the D₁R antagonist SKF 83566 (10 μM) (the sEPSC frequency changed by $-7 \pm 6\%$, $n = 5$, $p = 0.16$, Figure 8D-F). This finding shows that a rise in local dopamine levels also mimics the action of propofol.

Propofol at minute concentrations enhances eEPSCs in the absence of GABA_Azine. GABA_A receptor is a major target of propofol (Krasowski et al., 1998; Orser et al., 1998). However, the above experiments were conducted in the presence of GABA_Azine, a GABA_A receptor antagonist. To determine whether GABA_Azine may alter the effect of propofol on EPSCs, we reexamined the effect of propofol on EPSCs in the absence of GABA_Azine, using the CsF-based internal solution (see method) and at a V_H of -60 mV. The postsynaptic responses to GABA or glycine were suppressed under these experimental conditions (Bormann et al., 1987), allowing us to monitor propofol effect on EPSC. As illustrated in Fig. 9A, the evoked postsynaptic currents recorded under these experimental conditions were completely blocked by 10 μM DNQX, indicating that they were the EPSCs mediated by AMPA receptors. Fig. 9B and 9C illustrate that propofol (1 nM) enhanced the amplitude of evoked EPSCs and reduced the paired pulse ratio (PPR = EPSC₂/EPSC₁). These effects depend on the concentrations of propofol: 0.1, 0.3, 1, 3 and 1000 nM propofol increased the amplitude of evoked EPSCs (Figure 9D₁) by: $15 \pm 7\%$ ($n = 4$, $p = 0.12$), $30 \pm 9\%$ ($n = 6$, $p = 0.02$), $41 \pm 6\%$ ($n = 5$, $p = 0.002$), $27 \pm 4\%$ ($n = 5$, $p = 0.003$), and $-16 \pm 2\%$ ($n = 7$, $p < 0.001$), respectively, and reduced the PPR (Figure 9D₂) by: $5 \pm 2\%$ ($n = 4$, $p = 0.046$), $19 \pm 6\%$ ($n = 6$, $p = 0.03$), $31 \pm 7\%$ ($n = 5$, $p < 0.01$), $21 \pm 6\%$ ($n = 5$, $p = 0.02$), and $-15 \pm 6\%$ ($n = 7$, $p = 0.05$), respectively. Thus, propofol induces similar changes in EPSCs in the absence and the presence of GABA_Azine.

Propofol (1 nM) increases the frequency of spontaneous firing of VTA dopamine neurons. Having established that minute concentrations of propofol facilitate glutamatergic transmission, we next assessed the physiological consequences of this action of propofol by examining the spontaneous firing of VTA dopamine neurons. As shown in Figure 10, 1 nM propofol reversibly increased the frequency of spontaneous firing of VTA dopamine neurons in brain slices by $33 \pm 7\%$ (from 1.5 ± 0.3 Hz in control to 1.9 ± 0.4 Hz in propofol, $n = 7$, $p = 0.005$, paired *t* test).

Discussion

Our major finding is that propofol at nanomolar concentrations increases glutamatergic transmissions to, and the excitability of VTA dopamine neurons. This finding is surprising since propofol is an anesthetic agent acts as a GABAergic drug (Krasowski et al., 1998; Orser et al., 1998) and has generally inhibitory effects on the brain. Furthermore, this intriguing action of propofol is mediated by D₁R in glutamate-releasing terminals on VTA dopamine neurons.

Although a recent study found propofol concentration in the expired breath of patients is close to that in the plasma (Takita et al., 2007), propofol concentration in the air of operating rooms has not been quantified. We speculate that it is very low, and that the minute concentrations tested in current study coincide with the possibly very low concentrations of propofol in the air of operating rooms. Our finding may offer a plausible mechanism to the proposed hypothesis of second hand exposure (McAuliffe et al., 2006). Since glutamatergic transmissions play a major role in mediating behavioral manifestations of drug abuse (Kalivas, 1993), propofol induced enhancement of glutamatergic transmission in the VTA could be an important aspect underlying the development of propofol abuse.

Several lines of evidence indicate that propofol acts presynaptically to enhance glutamatergic transmission. Propofol increased sEPSC frequency but had no effect on the amplitude of sEPSCs and mEPSCs. Propofol enhanced the amplitude of stimulation-evoked EPSCs and decreased their paired pulse ratio. All these effects point to a presynaptic mechanism of propofol action.

Dopamine receptors, including both the D₂ family (in particular D₂R) (Blum et al., 1990) and D₁R are associated with drug dependence and abuse. For example, administration of D₁R antagonist (Liu and Weiss, 2002) or disruption of D₁R gene expression (El-Ghundi et al., 1998) prevents or attenuates alcohol-seeking behavior. Activation of D₁R can increase glutamatergic transmission (Kalivas and Duffy, 1995). Consistent with this report, in current investigation, SKF 38393, a D₁R agonist dose-dependently increased the frequencies of sEPSCs without affecting their amplitudes. A

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similar effect was produced by an endogenous dopamine, induced by GBR 12935, a dopamine reuptake blocker. All these changes were abolished by SKF 83566, a D₁R antagonist, which also eliminated the effects-induced by propofol: increases the amplitude, but decreases the paired pulse ratio of evoked EPSCs. In keeping with a crucial involvement of dopamine was the suppression of the action of propofol when endogenous monoamines (including dopamine) were depleted by reserpine. All these support the notion that propofol increases glutamate transmission via the activation of D₁Rs.

How does propofol activate presynaptic D₁Rs? In the midbrain, dopamine is released from the somatodendritic region of dopamine neurons (Bjorklund and Lindvall, 1975). Dopamine molecules travel retrogradely across the synapse to bind to the D₁Rs in the glutamate-releasing terminals. In reserpine treated slices, in which dopamine was depleted, propofol failed to alter the amplitude and paired pulse ratio of evoked EPSCs. This excludes the possibility that propofol directly activates presynaptic D₁Rs. Propofol may raise local dopamine level. This possibility is supported by the similar effects of propofol and of dopamine reuptake blocker.

A rise in local dopamine levels is also suggested by the bell-shaped dose-dependence of propofol action. The diminishing potency of concentrations > 1 nM cannot be explained by saturation of D₁Rs at the site of action on glutamatergic terminals, because the selective D₁R agonist was effective over a wide range of concentrations. One possible explanation is suggested by opposite effects of dopamine (the non-selective endogenous agonist) acting on different dopamine receptor subtypes. At low concentrations, dopamine appears to bind predominantly to D₁Rs and at higher concentrations to D₂Rs. For instance, Trantham-Davidson and colleagues (Trantham-Davidson et al., 2004) found that low concentrations of dopamine (< 0.5 μM) enhance IPSCs by activating mainly D₁Rs; whereas higher concentrations of dopamine (> 1 μM) have the opposite effect of reducing IPSCs by predominantly activating D₂Rs. In comparable experiments on EPSCs, Koga and Momiyama (Koga and Momiyama, 2000) found that dopamine inhibits non-NMDA receptor-mediated evoked EPSCs in VTA dopamine neurons through presynaptic D₂Rs with a high IC₅₀ of 16 μM. The bell-shaped concentration dependence of propofol action in our slices is thus consistent with

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potentiation of dopamine release and opposite presynaptic actions via D₁Rs and then D₂Rs as the local dopamine rises. Our assumption that with minute concentrations of propofol local dopamine concentrations remain below the micromolar level, which predominantly activates D₁Rs, is supported by previous evidence that electrical stimulation in the VTA does not raise extracellular dopamine levels above the submicromolar range (Kalivas and Duffy, 1991; Adell and Artigas, 2004).

Propofol increases excitation of VTA dopamine neurons. VTA dopamine neurons receive numerous inputs that can modulate their eventual output. Integration of the synaptic inputs and the intrinsic properties sets the frequency and pattern of firing (Alberto et al., 2006). As mentioned, glutamatergic afferents are the major excitatory inputs. Thus, the increase in glutamatergic transmission may contribute to the excitatory effect of minute concentrations of propofol on the dopamine neurons in the VTA.

Although many factors modulate glutamate release onto VTA dopamine neurons, the current study focused on the mechanism operating via presynaptic D₁Rs because these receptors are involved in the rewarding action of abused drugs such as ethanol, and D₁R activation enhances glutamate releases in the VTA. Furthermore, the fact that a selective D₁R antagonist eliminated propofol-induced elevation of glutamate transmission, demonstrated the predominant role of D₁Rs in propofol-induced increase in glutamate transmission in the VTA.

Although the main action of propofol is to potentiate GABAergic transmission (Krasowski et al., 1998; Orser et al., 1998), the effect of minute concentrations of propofol on the EPSCs in the VTA seems independent of GABAergic action, since the effects of propofol on EPSCs were similar in presence and absence of GABA_Azine, the GABA_A receptor antagonist. While the underlying mechanisms warrant further study, we propose that propofol at nanomolar concentrations may not be able to activate GABA_A receptors, as a previous study in brain slices indicates that propofol activates GABA_A receptors at concentrations $\geq 1 \mu\text{M}$ (Shirasaka et al., 2004). Nevertheless, our finding is in general agreement with a previous report that propofol effect on dopamine release in the NAcc is independent of GABA_A receptors (Schulte et al., 2000).

As mentioned, previous clinical trials performed by Zacny and colleagues (Zacny et al., 1993a; Zacny et al., 1993b) on healthy human volunteers have shown that the

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rewarding effect of propofol was observed only in a subgroup of treated people (half). In these studies, a discrete-trial procedure was used. This procedure has been used with well known drugs of abuse such as alcohol, amphetamines, delta-9 THC or methylphenidate. As observed with propofol, although to a different extent, these other drugs were not clearly served as a reinforcer in a good number of volunteers tested (Uhlenhuth et al., 1981; de Wit et al., 1986; Stern et al., 1989; Chait and Zacny, 1992).

Our *in vitro* data are in general agreement with a previous *in vivo* study regarding propofol alters the activity of the mesolimbic dopamine system. Nevertheless, in that *in vivo* microdialysis study, propofol increases extracellular dopamine levels in the NAcc only in subanesthetic and anesthetic doses (Pain et al., 2002), which are in apparent conflict with our *in vitro* electrophysiological data in the VTA, where only minute concentrations of propofol increase EPSCs. We have no firm answer to the difference. Note that whereas electrophysiology measures synaptic release with high temporal and spatial resolution, microdialysis measures extrasynaptic outflow and reuptake. As suggested, propofol increased dopamine in the NAcc might be resulted from inhibition of dopamine reuptake (Pain et al., 2002). Furthermore, several brain imaging mapping of human and primate functioning found no activity in any midbrain area or limbic structures after the use of threshold, sub or maximal doses of anesthetics (Heinke and Schwarzbauer, 2002). Note that in these imaging studies the effect of minute concentrations of propofol has not been tested.

The high sensitivity to propofol of the D₁R-glutamatergic pathway unveiled in the current investigation may have particular significance in that anesthesiologists are exposure to trace amounts of general anesthetics in the air of the operating room. Furthermore, the changes in synaptic plasticity in the VTA, an addiction-related brain area shown in the current investigation may not only contribute to the mechanism of the development of propofol abuse, but also the mechanism of the higher rate of drug abuse among anesthesiologists, since a history of exposure to one drug (such as propofol) can facilitate self-administration of another drug (such as cocaine) (Young, 1986).

In conclusion, the present study shows that propofol at minute concentrations enhances presynaptic D₁ receptor-mediated facilitation of glutamatergic synaptic transmission and the excitability of VTA dopamine neurons, probably by increasing

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extracellular dopamine levels. The stimulating effect of minute concentrations of propofol on VTA dopamine neurons may contribute to the mechanisms underlying the development of propofol abuse. Furthermore, the proposed mechanism supports the hypothesis that physicians may become addicted to drugs through second hand exposure. Through further study, preventive measures can be adopted to protect physicians and healthcare professionals.

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Legends for Figures

Fig. 1. Identification of VTA dopamine neurons. A, Traces of spontaneous firings of a putative dopamine neuron in the VTA in slice show that 5 μ M U69593 reversibly hyperpolarized the membrane potential and ceased the firings. B, Corresponding time course 5 μ M U69593-induced suppression of spontaneous firings in A. C, Superimposed traces of membrane potential elicited by hyperpolarizing and depolarizing current pulses. Note the prominent voltage “sag” of a putative dopamine neuron during a large hyperpolarizing pulse.

Fig. 2. Propofol (1 nM) enhances evoked EPSCs (eEPSCs) in VTA dopamine neurons in midbrain slices. A, EPSCs evoked by pulse stimulation within the VTA and recorded from a putative dopamine neuron. 1 nM propofol significantly and reversibly enhanced the amplitude but reduced the paired pulse ratio (EPSC₂/EPSC₁) of eEPSCs. These currents were completely blocked by 10 μ M DNQX (far right panel). Data are averaged of 10 traces. B, C, Time courses of an increase in eEPSC amplitude (mean \pm SEM, n = 5 neurons, B), and a decrease in paired pulse ratio (EPSC₂/EPSC₁, mean \pm SEM, n = 5, C) induced by 1 nM propofol. For all figure, the bar (open or filled) above the time course indicates the duration of the application of the chemical indicated. D, Summary of changes in eEPSC amplitude and paired pulse ratio (PPR) from 5 cells. ** p < 0.01, paired t-test for propofol application vs. pre-propofol control. All EPSCs were recorded at a V_H of -70 mV. Whereas the EPSCs shown in Figures 1-8 were recorded in the presence of 10 μ M GABAzine, those in Figure 9 in the absence of GABAzine.

Fig. 3. Propofol at nanomolar concentrations increases the frequency of spontaneous EPSC (sEPSC) in VTA dopamine neurons. A, 1 nM propofol significantly (and reversibly) increased sEPSC frequency. These sEPSCs were completely blocked by 10 μ M DNQX. B, Representative cumulative probability plots show increased incidence of shorter intervals between sEPSCs (B₁), but no change in the sEPSC amplitude (B₂). C, Time course of the increase in sEPSC frequency (means \pm SEM, n = 5) induced by 1 nM propofol. D, Dose-response relationship of propofol induced changes in the frequency

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(D₁), and amplitude of sEPSCs (D₂) (means ± SEM). Number of cells in each group is indicated. * p < 0.05, paired t-test for propofol application vs. pre-propofol control. All sEPSCs were recorded at a V_H of -70 mV and in the presence of 10 μM GABA_Azine.

Fig. 4. Propofol-induced increase in sEPSC frequency depends on sodium and calcium channels. A, In the presence of 1 μM tetrodotoxin (TTX), 1 nM propofol did not change the incidence of the miniature EPSCs (mEPSCs). B, Time course shows that in the presence of TTX, 1 nM propofol induced no significant change in mEPSC frequency (mean ± SEM, n = 5). C, Cumulative probability plots and the accompanied insets show in the presence of TTX, 1 nM propofol induced no significant changes in interevent intervals (C₁) and amplitudes (C₂) of mEPSCs. D, In the presence of 100 μM cadmium, 1 nM propofol did not change the incidence of sEPSCs. E, Time course shows that in the presence of 100 μM cadmium, 1 nM propofol induced no significant changes in sEPSC frequency (means ± SEM, n = 4). F, Cumulative probability plots and the inset panels show that in the presence of cadmium, 1 nM propofol induced no significant change in interevent interval (F₁) and amplitude (F₂) of sEPSCs.

Fig. 5. A dopamine D₁ receptor antagonist eliminates propofol-induced increase in sEPSCs. A, In the presence of 10 μM SKF 83566, a D₁ antagonist 1 nM propofol did not change the incidence of sEPSCs. B, Time course shows that 1 nM propofol did not change sEPSC frequency (means ± SEM) in the presence of SKF 83566. C, Representative cumulative probability plots show in the presence of 10 μM SKF 83566, 1 nM propofol induced no change in interevent interval (C₁) and amplitude (C₂) of sEPSCs.

Fig. 6. A D₁R agonist mimics the effects of propofol on sEPSCs. A, 100 nM SKF 38393, a D₁R agonist increased the sEPSC frequency in a putative dopamine neuron in the VTA. B, Representative cumulative probability plots show 100 nM SKF 38393 increased incidence of shorter intervals between sEPSCs (left panel), but there was no associated change in sEPSC amplitudes (right panel). C, Time course of 10 nM SKF 38393-induced increase in sEPSC frequency. D, Dose-response relationship for SKF 38393-induced increase in sEPSC frequency. Each circle represents the mean (± SEM) of results from 4

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to 6 cells. * $p < 0.05$, ** $p < 0.01$, paired t-test for SKF 38393 application vs. pre-SKF 38393.

Fig. 7. Propofol-induced increase in the eEPSCs is suppressed by dopamine depletion. 1 nM propofol has no effect on the amplitude (A) and paired pulse ratio (EPSC₂/EPSC₁, B) of evoked EPSCs recorded in the VTA DA neurons in slices pretreated with reserpine for 90 min from 5 cells. C, Summary of data from 5 cells in slices.

Fig. 8. A dopamine uptake blocker mimics the effects of propofol on sEPSCs. A, Traces show 20 nM GBR 12935 increased sEPSC frequency (A_b). B, Time course of 20 nM GBR 12935 increased sEPSC frequency in one cell. C, Representative cumulative probability plots and the inset panel (mean \pm SEM, $n = 12$) show 20 nM GBR 12935 increased incidence of short intervals between sEPSCs. **, $p < 0.01$, 20 nM GBR 12935 vs. pre-GBR control. D-b, 10 μ M SKF 83566, a D₁ antagonist blocked GBR 12935 (20 nM) induced increase in sEPSC frequency. E, Time course shows that in the presence of 10 μ M SKF 83566, 20 nM GBR 12935 had no significant effect on sEPSC frequency. F, Representative cumulative probability plots of interevent interval, and the inset panel (mean \pm SEM, $n = 5$) indicate that in the presence of 10 μ M SKF 83566, 20 nM GBR 12935 induced no significant effect on sEPSC frequency.

Fig. 9. Propofol enhances EPSCs of VTA dopamine neurons in the absence of GABA_A receptors. A, DNQX (10 μ M) abolished the evoked postsynaptic currents recorded from a putative dopamine neuron using CsF-based pipette solution in the absence of GABA_A receptors. B, 1 nM propofol enhanced the amplitude and decreased the paired-pulse ratio (EPSC₂/EPSC₁) of evoked EPSCs. C₁₋₂, Time course of 1 nM propofol induced increase in amplitude (mean \pm SEM, $n = 5$, C₁) and decrease in paired pulse ratio (mean \pm SEM, $n = 5$, C₂). D, Dose-response relationship of propofol induced changes (means \pm SEM) in EPSC amplitude (D₁), and paired pulse ratio (D₂). Numbers of cells in each group are indicated. * $p < 0.05$, ** $p < 0.01$, paired t-test for propofol application vs. pre-propofol control.

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Fig. 10. Propofol increases the firing rate of VTA dopamine neurons. A, Traces of spontaneous firings of a putative dopamine neuron show that 1 nM propofol significantly and reversibly increases the firing rate of this neuron. B, A plot of means (\pm SEM) shows the time course of 1 nM propofol induced an increase in the normalized frequency of spontaneous firings in 7 dopamine neurons in the VTA.

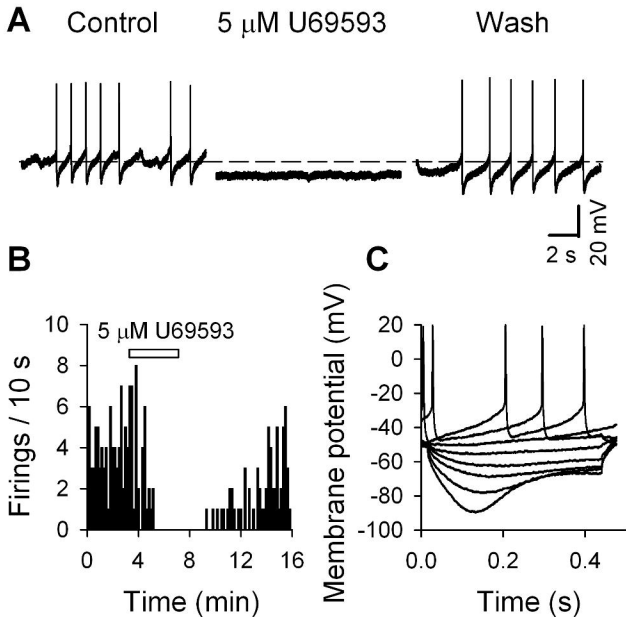


Figure 1

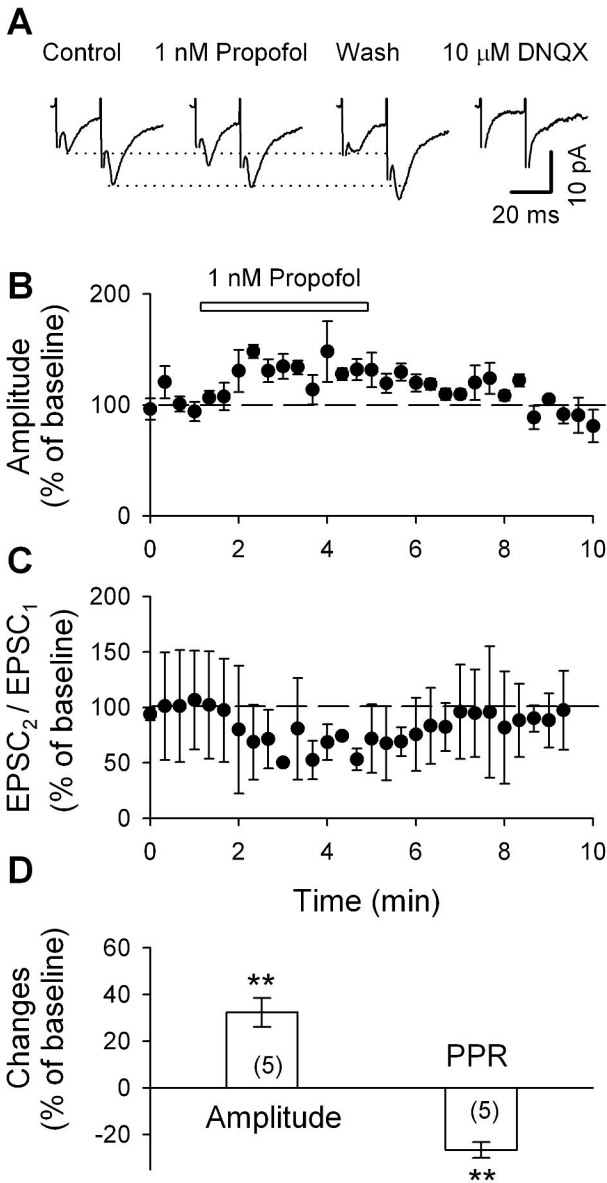


Figure 2

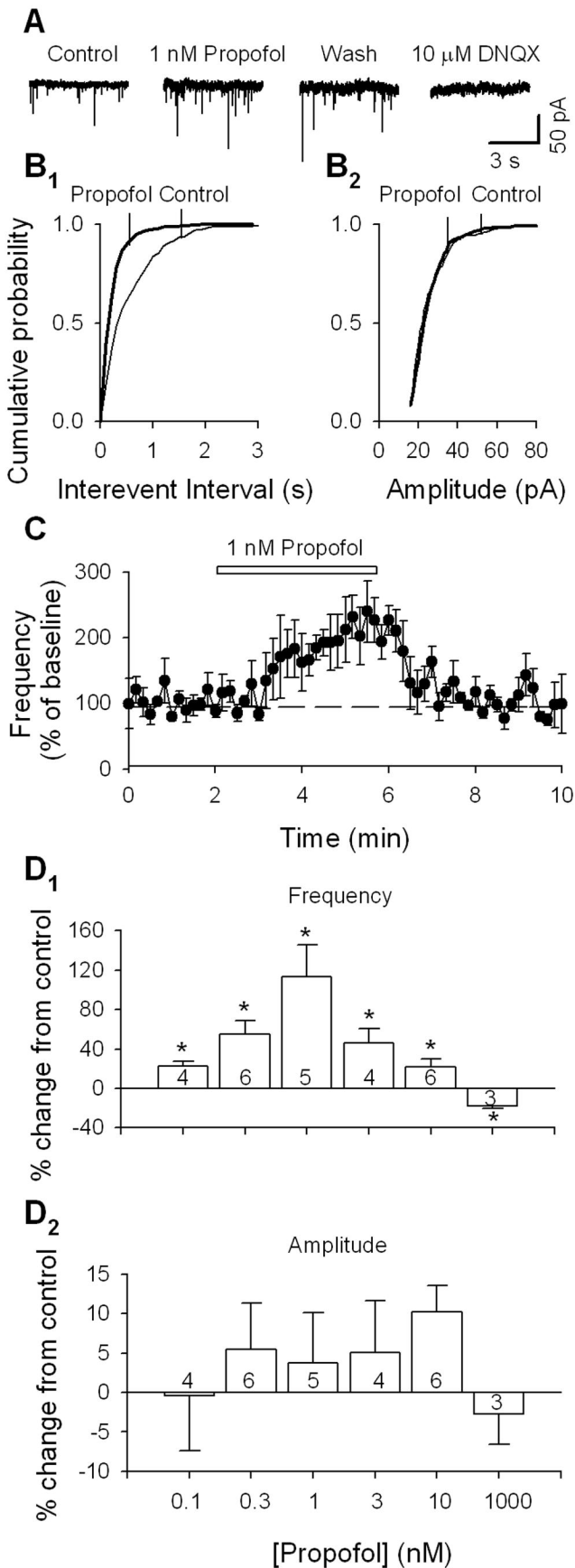


Figure 3

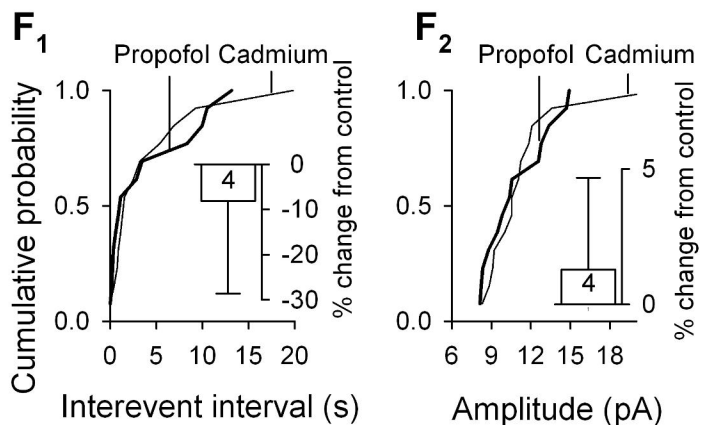
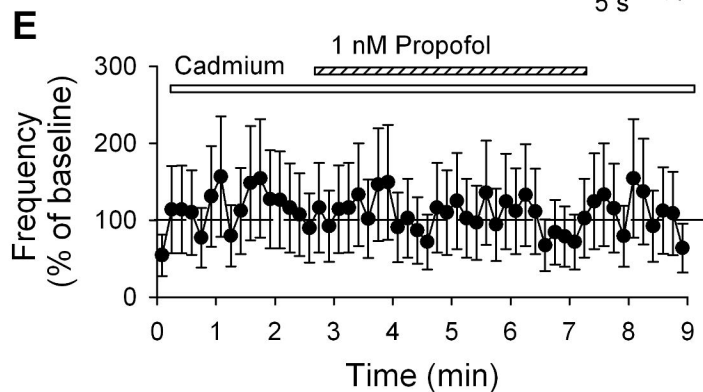
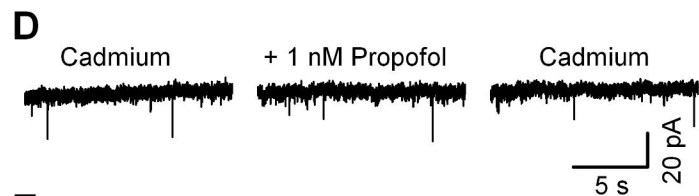
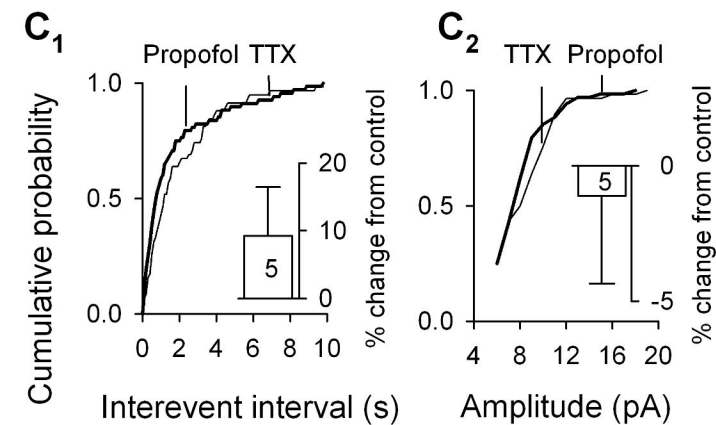
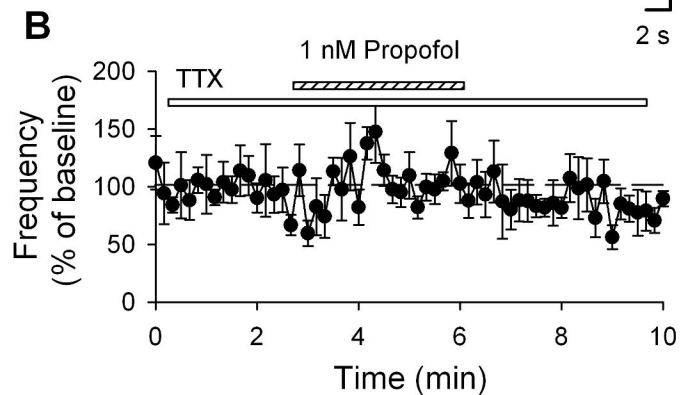
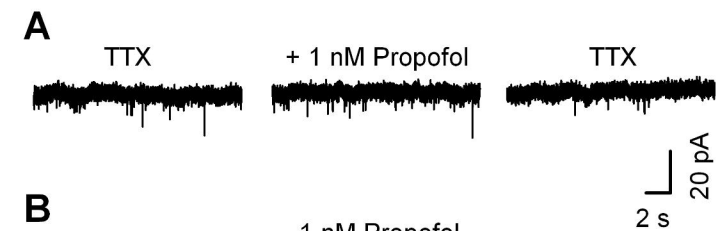


Figure 4

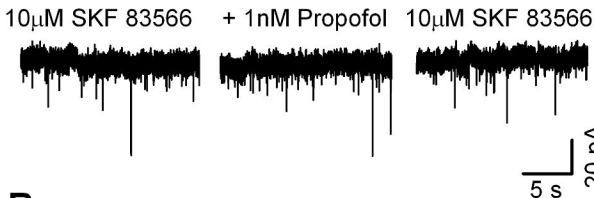
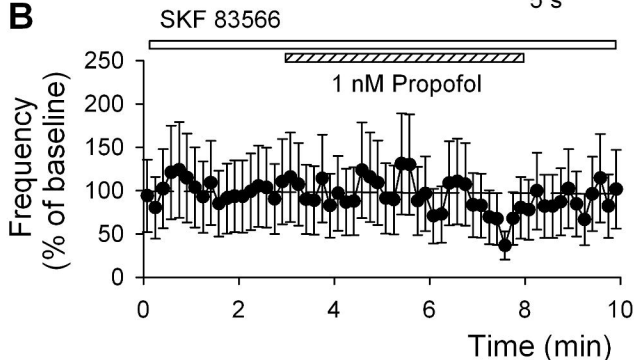
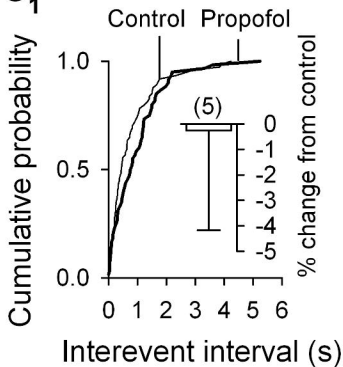
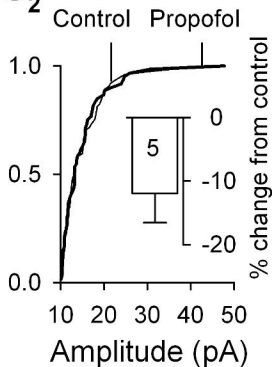
A**B****C₁****C₂**

Figure 5

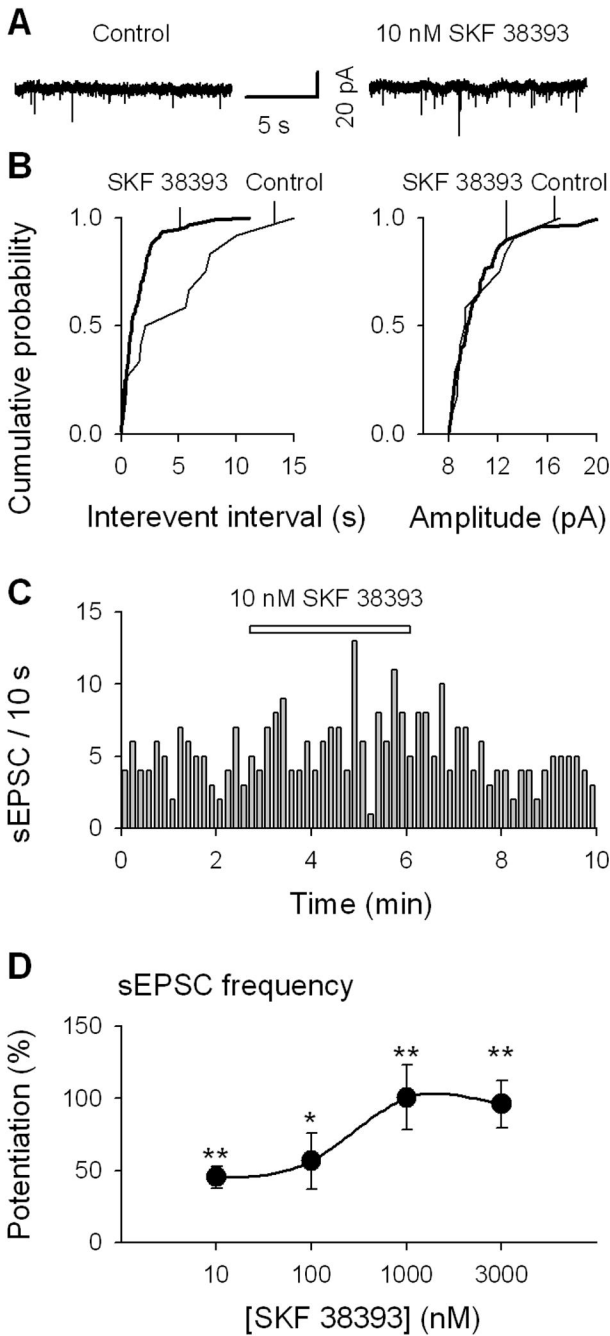


Figure 6

Reserpine-treated slices

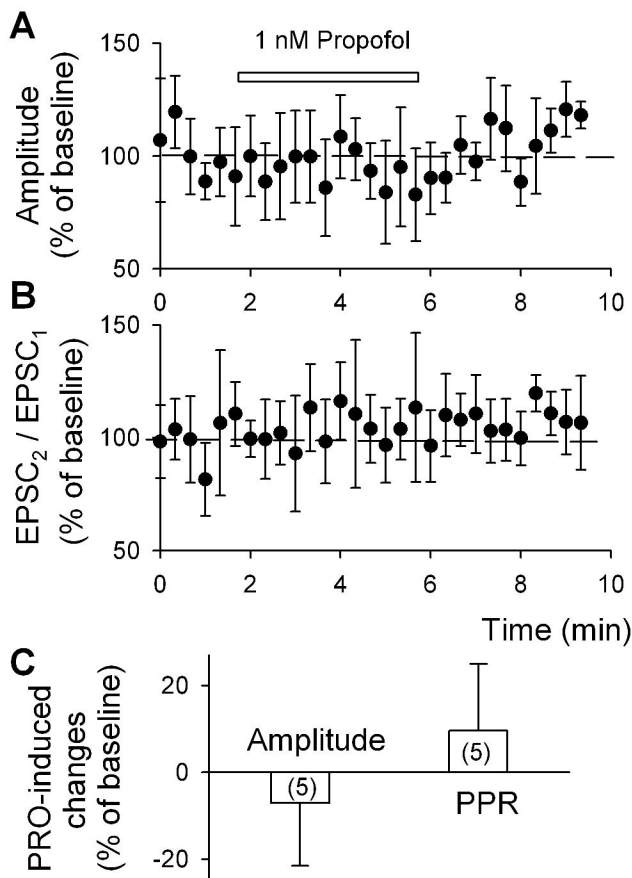


Figure 7

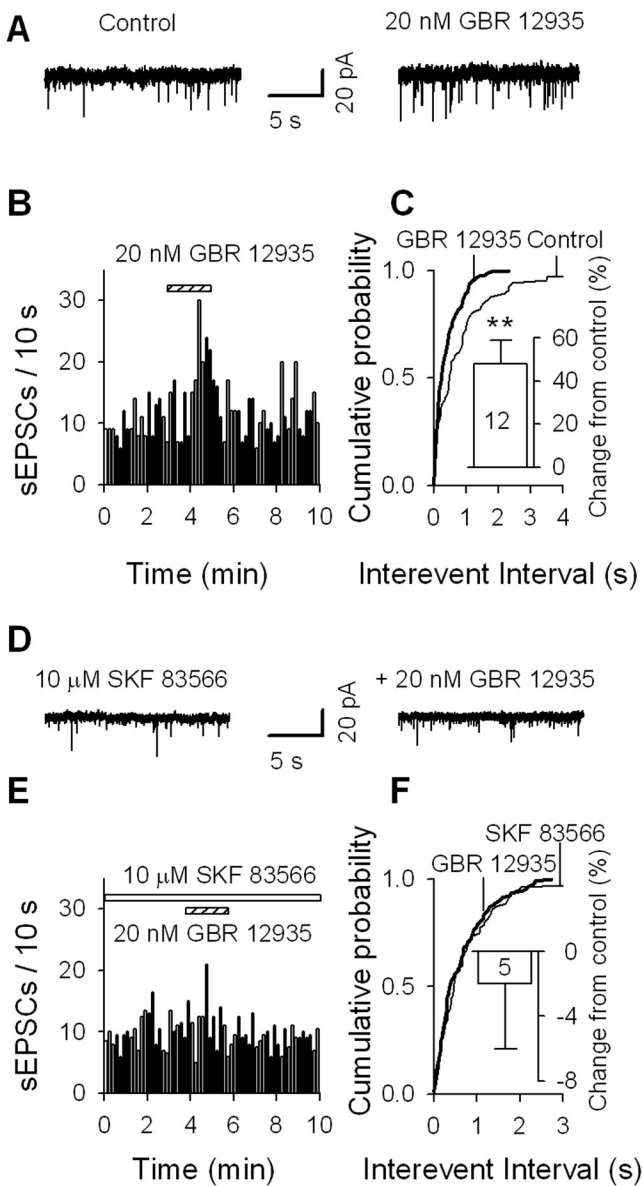


Figure 8

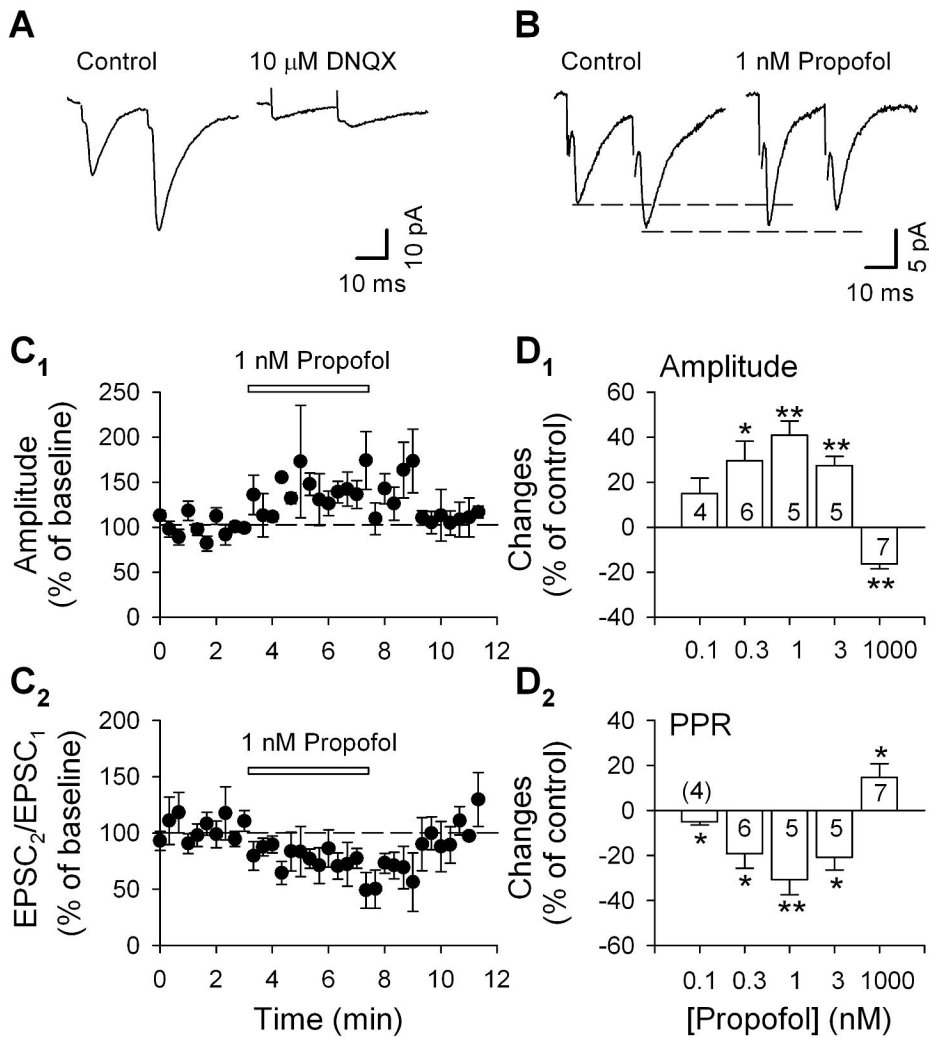


Figure 9

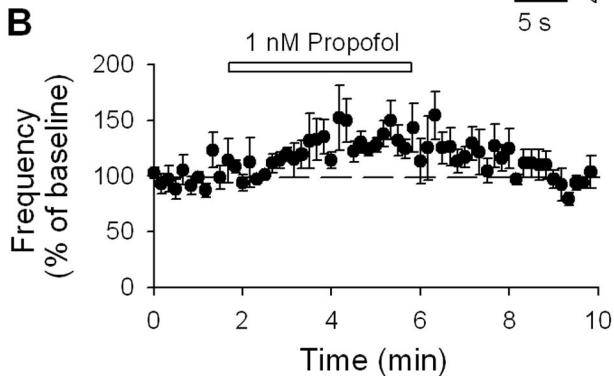
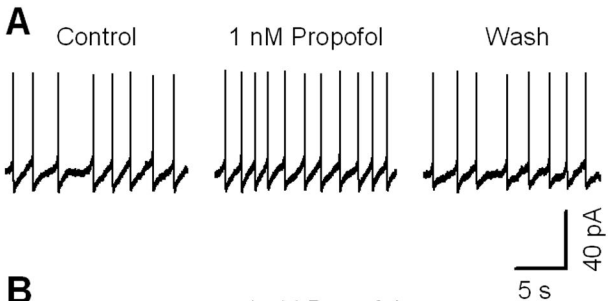


Figure 10