Loss of the preferential control over the striato-nigral direct pathway by striatal NMDA receptors in a rat model of Parkinson's disease

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ABBREVIATIONS

DA	dopamine
DLS	dorsolateral striatum
GLU	glutamate
GP	globus pallidus
6-OHDA	6-hydroxydopamine
SNr	substantia nigra reticulata

Abstract

By using multi-probe microdialysis we previously demonstrated that endogenous glutamate differentially regulates the activity of the striatal output pathways in vivo, through N-methyl-D-aspartate (NMDA) receptors containing the GluN2A or GluN2B subunits. Using the same approach, we presently investigate whether reverse dialysis of NMDA in striatum differentially affects GABA release in striatum and in striatal target areas, i.e. globus pallidus (GP) and substantia nigra reticulata (SNr). Moreover, we ask whether this control is altered under parkinsonian conditions. Intrastriatal NMDA perfusion (10 min) evoked GABA release more potently in SNr (1-100 µM) than other regions (10-100 µM), suggesting a preferential control over striato-nigral projection neurons. Intrastriatal NMDA more potently stimulated glutamate levels in striatum (1-100 µM) and SNr (1-10 µM) than GP (10 µM). Striatal dopamine denervation with 6-hydroxydopamine caused a leftward shift in the NMDA concentration-response curve. Intrastriatal NMDA elevated GABA levels at 0.1 µM (all regions) and 1 µM (striatum and GP only), but not higher concentrations, indicating that, compared to naïve animals, the GABA response in SNr was attenuated. Attenuation of the glutamate response was also observed in SNr (NMDA effective only at 0.1 µM). Conversely, the glutamate response in GP was widened (NMDA effective in the 0.1-1 µM range).

We conclude that NMDA preferentially stimulates the activity of the striato-nigral direct pathway under physiological conditions. In Parkinson's disease, dopamine loss compromises the NMDA ability to stimulate striato-nigral neurons, thus shifting the NMDA control towards the striato-pallidal ones.

Key words: direct pathway, GABA, glutamate, microdialysis, NMDA, 6-OHDA.

Introduction

Striatal N-methyl-D-aspartate (NMDA) receptors are a promising target in the therapy of neuro-psychiatric disorders such as Parkinson's disease (PD) [1-3], Huntington's disease [4], dystonia [5], and drug addiction [6]. The striatal region is particularly enriched of NMDA receptors, which appear to be unevenly distributed across the different neuronal populations [7-9]. The NMDA receptor is a heteromer composed of two obligatory GluN1 subunits and two GluN2 (A-D) or, less commonly, GluN3 (A-C) subunits [10-12]. The most abundant subtype combinations (GluN1/GluN2A and GluN1/GluN2B) are expressed to approximately the same extent on medium-sized spiny neurons (MSNs) projecting to substantia nigra reticulata (SNr) and globus pallidus (GP) [8], i.e. those neurons giving rise to the so-called direct and indirect striatal output pathways, respectively [13-15].

According to the current model of basal ganglia (BG) functioning [13-15], these two pathways exert a functionally opposing influence over nigro-thalamic neurons (i.e. the basal ganglia output) and motor function. MSNs which preferentially express D1 receptors and co-release GABA with substance P and dynorphin (the direct pathway), monosynaptically inhibit tonically-active nigro-thalamic GABA neurons, causing disinhibition of thalamo-cortical afferents, and movement initiation [16, 17]. Conversely, MSNs which express D2 receptors and co-release GABA with enkephalins inhibit GABA neurons in GP (GP externalis in primates), which, in turn, disinhibit glutamate (GLU) neurons located in subthalamic nucleus (STN). These neurons send excitatory projections to SNr, stimulating the firing of nigro-thalamic neurons and inhibiting movement [17]. More recent evidence indicates that simultaneous, rather than alternate, activation of these two pathways is essential to a correct action selection and expression of motor function, since the direct pathway promotes movement while the indirect one inhibits competing motor programmes [18-20].

Striatal output neurons are activated by cortical and thalamic glutamatergic inputs [21, 22], but the contribution of NMDA receptors under both physiological and pathological in vivo conditions is yet to be clarified. To this purpose, we have adopted dual or triple-probe microdialysis technique in awake rats, implanting one probe in dorsolateral striatum (DLS) and the others in GP and/or SNr.

This approach allowed us to locally deliver NMDA or NMDA receptor antagonists (either broad-spectrum or subunit-selective) into the striatum, and simultaneously measure changes of GABA release in target areas [23-26]. Indeed, previous dual- probe microdialysis studies had shown that manipulation of striatal environment resulted in coherent changes of GABA release in GP and SNr, suggesting that GABA dynamics could probe the activity of striato-pallidal and striato-nigral MSNs [27-31]. To integrate our previous studies, we now specifically examine whether reverse dialysis of increasing NMDA concentrations in DLS causes differential activation of striato-nigral or striato-pallidal MSNs, and whether such control is altered under parkinsonian conditions.

Materials and Methods

Male Sprague-Dawley rats (150 g; Harlan Italy, S. Pietro al Natisone, Italy) were kept under controlled lighting conditions (12 hr light/dark cycle) and given food and water *ad libitum*. The experimental protocols were approved by the Ethical Committee of the University of Ferrara and and the Italian Ministry of University (licenses 94-2007-B and 194-2008-B). Adequate measures were taken to minimize animal pain and discomfort, and to limit the number of animals used.

6-OHDA lesion

Unilateral lesion of dopamine (DA) neurons was induced in isoflurane-anesthetized rats as previously described [23, 32]. Eight micrograms of 6-OHDA (in 4 µl of saline containing 0.02% ascorbic acid) were stereotaxically injected according to the following coordinates from bregma: anteroposterior (AP) -4.4 mm; mediolateral (ML) -1.2 mm; ventrodorsal (VD) -7.8 mm below dura [33]. The rotational model [34] was used to select the rats that had been successfully lesioned. Two weeks after surgery, rats were injected with amphetamine (5 mg/kg, i.p., dissolved in saline) and only those rats performing more than seven ipsilateral turns per minute were enrolled in the study. This behavior has been associated with >95 % loss of striatal DA terminals [35] and extracellular DA levels [32]. Experiments were performed 6-8 weeks after lesion.

Microdialysis experiments

Dual probe microdialysis was performed as previously described [27, 32]. Two probes of concentric design were stereotaxically implanted under isoflurane anaesthesia in the right DLS (3 mm dialysing membrane, AN69, Gambro Industries, Meyzieu, France) and ipsilateral SNr (1 mm) or GP (1.5 mm) according to the following coordinates from bregma and the dural surface [33]: DLS; AP +1.0, ML -3.5, VD –6; GP, AP –1.3, ML -3.3, VD –6.5; SNr, AP -5.5, ML -2.2, VD –8.3. Forty-eight hours after surgery, probes were perfused with a modified Ringer solution ($CaCl_2$ 1.2 mM; KCl 2.7 mM; NaCl 148 mM; $MgCl_2$ 0.85 mM) at a 3 µl/min flow rate. After 6 h rinsing, samples were collected every 10 min. At least four baseline samples were collected before NMDA perfusion

(0.01-100 µM; 10 min) through the probe implanted in DLS. At the end of the experiments, animals were sacrificed and the correct placement of the probes was verified histologically.

Amino acid analysis

Endogenous GLU and GABA levels were measured by high-performance liquid chromatography (HPLC) coupled to fluorometric detection according to [23, 24]. Briefly, 30 µl sample were pipetted into glass microvials and placed in a thermostated (4°C) Triathlon autosampler (Spark Holland, Emmen, The Netherlands). Forty microliters of o-phthaldialdehyde/boric acid solution were added to each sample, and 60 µl of the solution injected onto a 5-C18 Chromsep analytical column (3 mm inner diameter, 10 cm length; Chrompack, Middelburg, Netherlands) perfused at a flow rate of 0.48 ml/min (Beckman 125 pump; Beckman Instruments, Fullerton, CA, USA) with a mobile phase containing 0.1 M sodium acetate, 10% methanol and 2.2% tetrahydrofuran (pH 6.5). GLU and GABA were detected by means of a fluorescence spectrophotometer FP-2020 Plus (Jasco, Tokyo, Japan) with the excitation and the emission wavelengths set at 370 and 450 nm respectively. GLU and GABA retention time were ~3.5 and ~17.5 min, respectively. The limits of detection for GLU and GABA were ~1 and ~0.5 nM, respectively.

Data presentation and statistical analysis

Data (mean ± SEM) from microdialysis experiments are reported as percentage of basal value (calculated as the mean of two samples before treatment). Mean neurotransmitter levels in the dialysate (in nM; not corrected for in vitro probe recovery) have been reported in text and Figure legends. Statistical analysis was performed on area-under-the curve (AUC) values by one-way ANOVA followed by the Newman-Keuls test for multiple comparisons. P values <0.05 were considered to be statistically significant.

Materials

6-OHDA hydrobromide, D-amphetamine sulfate, and NMDA (free acid; molecular weight 147.13) were purchased from Tocris (Bristol, UK). 6-OHDA was dissolved in saline containing 0.02 %

ascorbic acid, D-amphetamine in saline and NMDA in Ringer (final pH adjusted to 7.3 with NaOH).

Results

Intrastriatal NMDA perfusion in naïve rats

To investigate whether striatal NMDA receptors regulate striato-pallidal and striato-nigral MSNs, reverse dialysis of NMDA was performed in the DLS of naïve rats, and GABA and GLU release monitored in DLS and its projection areas, namely the ipsilateral GP and SNr.

DLS. Basal GABA and GLU levels in DLS were 15.1±2.5 nM (n=48) and 191.6±17.9 nM (n=47), respectively. ANOVA revealed that intrastriatal perfusion with NMDA elevated both GABA ($F_{4,48}$=3.87, p=0.0084) and GLU ($F_{4,47}$=8.56, p<0.0001) levels in the dialysate (Fig. 1A-B). Posthoc analysis showed that NMDA stimulated GABA levels at 10 and 100 µM, and GLU levels in the 1-100 µM concentration range.

GP. Basal GABA and GLU levels in GP were 15.1±1.4 nM (n=37) and 134.6±19.0 nM (n=40), respectively. ANOVA revealed that intrastriatal perfusion with NMDA evoked both GABA ($F_{4,42}$=7.37, p<0.0001) and GLU ($F_{4,44}$=3.75, p=0.0105) release in GP (Fig. 2A-B). Posthoc analysis revealed an effect of 10 µM and 100 µM NMDA on pallidal GABA release. Conversely, the effect of NMDA on pallidal GLU release was limited to the 1 µM concentration.

SNr. Basal GABA and GLU levels in SNr were 13.9±0.8 nM (n=43) and 138.2±11.7 nM (n=48), respectively. ANOVA revealed that intrastriatal perfusion with NMDA evoked GABA ($F_{4,43}$=5.74, p=0.0010) and GLU ($F_{4,46}$=9.52, p<0.0001) release in SNr (Fig. 3A-B). NMDA evoked nigral GABA release in the 1-100 µM concentration range, whereas it evoked nigral GLU release at 1 and 10 µM, the 100 µM concentration being ineffective.

Intrastriatal NMDA perfusion in hemiparkinsonian rats

To investigate whether removal of endogenous DA could affect the response to exogenous NMDA, we performed reverse dialysis of NMDA in the DA-depleted striatum of hemiparkinsonian rats.

DLS. Basal GABA levels in the DA-depleted DLS of hemiparkinsonian rats (12.1 ± 1.2 nM) were not significantly different from naïve animals. Conversely, GLU levels were significantly reduced by 65% (t=4.61,df=67, p<0001). ANOVA revealed that NMDA elevated both GABA ($F_{3,25}$=6.72, p=0.0018) and GLU ($F_{3,27}$=4.27, p=0.0136) levels (Fig. 4A-B). However, the effect of NMDA on GABA release was evident at 0.1 and 1 µM, whereas that on GLU release only at 0.1 µM. In a few animals (n=4), 0.01 µM NMDA was also perfused. At this concentration, NMDA failed to affect local amino acid levels (not shown).

GP. Basal GABA (9.4±1.8 nM; n=32) and GLU (86.7±19.8 nM; n=30) levels in the GP of hemiparkinsonian rats were not significantly different from naïve animals. ANOVA revealed that NMDA elevated both GABA ($F_{3,21}$=8.87, p=0.0005) and GLU ($F_{3,24}$=5.02, p=0.0076) pallidal levels (Fig. 5A-B). The effect of NMDA on both amino acids was significant at 0.1 and 1 µM, but not at higher concentrations. The lower (0.01 µM) NMDA concentration was also ineffective (n=4; not shown).

SNr. Basal GABA (11.2±1.1 nM; n=32) and GLU (103.1±28.6 nM; n=31) levels in the SNr of hemiparkinsonian rats were not significantly different from naïve animals. ANOVA revealed that intrastriatal NMDA elevated both GABA ($F_{3,23}$=10.81, p=0.0001) and GLU ($F_{3,23}$=5.39, p=0.0059) nigral levels (Fig. 6A-B). The effect of NMDA on both amino acids was significant at 0.1 but not higher or lower (0.01 µM; n=4; not shown) concentrations.

Discussion

The present study shows that stimulation of striatal NMDA receptors leads to the increase of GABA release in both SNr and GP, suggesting that NMDA receptors can activate both striatal output pathways [24]. Nonetheless, drawing a complete NMDA concentration-response curve revealed that,

under normal conditions, NMDA evoked GABA release more potently in SNr than GP (Tab. 1), suggesting a preferential control over striato-nigral MSNs.

Cortico-striatal and thalamo-striatal glutamatergic afferents synapse onto both populations of striatal MSNs [21, 22]. However, not only striatal MSNs but also striatal interneurons express the NMDA receptor [7, 8]. As a matter of fact, being tonically active, interneurons might be the first target of NMDA since membrane depolarization would relieve NMDA channel from Mg^{++} block and favour its opening upon binding of NMDA to its receptor. Since striatal interneurons powerfully regulate the excitability of striatal output pathways [36-38], the greater responsitivity of nigral vs pallidal GABA to intrastriatal NMDA might rely both on the intrinsic characteristics of the two populations of striatal MSNs and the striatal microcircuitry. Indeed, although striato-pallidal MSNs are thought to be more excitable than the striato-nigral ones [39], cortico-striatal responses, and their NMDA (APV-sensitive) components, are larger and more prolonged in striato-nigral MSNs [40, 41]. In addition, striatal output neurons differently respond to inputs originating from GABA interneurones. In fact, GABA inputs drive both excitation and inhibition to striato-nigral MSNs, but only inhibition to the striato-pallidal ones [40]. A greater effect of NMDA along the striato-nigral pathway would predict a predominantly stimulatory impact of NMDA receptor activation on motor function. Indeed, microinjection of NMDA in the dorsal striatum of naïve rats evoked contraversive rotation [42], a behavioural index of direct pathway activation [17].

The ability of intrastriatal NMDA to stimulate nigral and pallidal GABA release was dramatically altered by removal of endogenous DA, as in the 6-OHDA hemilesioned rat model of PD. In particular, DA depletion enhanced the potency of NMDA and cancelled the preferential effect on nigral GABA, since NMDA evoked GABA release yet at 0.1 μM in all structures analysed (Tab. 2). Considering the voltage-dependence of the opening of the NMDA channel, such increase in potency might be explained on the basis of the increased excitability of striatal MSNs which has been consistently observed after DA depletion [43-48]. This increase of excitability is thought to compensate for the dramatic pruning of cortico-striatal and thalamo-striatal contacts which follows DA loss [49-52],

although whether striato-pallidal MSNs only [52] or both striato-pallidal and striato-nigral MSNs are affected by pruning in the 6-OHDA rat model remains controversial [51]. In addition, other factors might contribute to the changes in neurons excitability observed in striatal MSNs after DA denervation, such as an imbalance in cortical inputs [53], striatal interneuron modulation [53-55], enhanced GLU release and responsiveness to GLU inputs [48] or altered synaptic plasticity at cortico-striatal afferents [56, 57]. Not last, it is well known that DA denervation results in the loss of a D2 inhibitory influence on GLU inputs at striato-pallidal MSNs, and of a D1 facilitatory influence on GLU inputs at striato-nigral MSNs [58-61].

In vivo [53] and in vitro [41] electrophysiology on identified MSNs has clarified that only striato-pallidal MSNs exhibit an increase in responsiveness to cortical inputs following DA loss, since striato-nigral MSNs show opposite changes. Our in vivo data are in line with these findings, showing that intrastriatal NMDA perfusion in the DA-denervated striatum evoked GABA release in GP and DLS in a wider range of concentrations (0.1-1 μM) than in SNr (0.1 μM), possibly indicating that DA loss has compromised the ability of NMDA to stimulate striato-nigral MSNs. DA denervation was accompanied by a significant reduction of basal striatal GLU levels. This is consistent with previous studies in 6-OHDA hemilesioned rats [62], although at variance with the majority of microdialysis studies showing either increase [63-65] or no change [32, 66-70] of GLU levels in the DA-denervated striatum. Nonetheless, time-dependent changes in dialysate GLU levels, i.e. increase at 1 month and reduction at 3 months after DA-denervation, have been reported in 6-OHDA hemilesioned rats [71]. These changes have been inversely correlated to GLU content in nerve terminals, although the underlying mechanism remains elusive [71].

Increases in GABA release were usually associated with increases in GLU release in the three areas examined, with two exceptions: i) NMDA (1 μM) elevated GLU but not GABA in DLS; ii) NMDA (100 μM) elevated GABA without affecting GLU in both GP and SNr. Although GABA and GLU can be co-released in some areas of the CNS (for a review see [72]), no such evidence has been yet provided for GABA afferents in DLS, GP or SNr. Therefore, other factors should be called into

play. For instance, as far as the local effects of NMDA in DLS are concerned, indirect modulation might be involved. Indeed, we failed in demonstrating the presence of NMDA receptors facilitating GLU release on striatal glutamatergic afferents (i.e. NMDA autoreceptors) [67]. Conversely, we showed that the ability of intrastriatal NMDA to elevate striatal GLU was attenuated by blockade of striatal D1 and NK1 receptors, or action potential propagation, suggesting that the increase of striatal GLU was due to activation of intra- or extrastriatal loops [67]. The present study points to the activation of the direct striato-nigral pathway at the basal ganglia-thalamo-cortico-basal ganglia loop, since NMDA (1 µM) elevated striatal GLU concurrently with nigral GABA (and nigral GLU). Also the increase in GLU release in GP might be explained on the basis of the activation of extrastriatal loops. Indeed, stimulation of striato-pallidal neurons would cause inhibition of pallido-subthalamic neurons and disinhibition of the subthalamo-pallidal glutamatergic projection, leading to a feedback increase in GLU release in GP [15]. Consistently, blockade of $GABA_A$ receptors in STN prevented the increase in pallidal GLU induced by intrastriatal neurotensin administration [73]. Therefore, the enhanced pallidal response to intrastriatal NMDA observed in hemiparkinsonian rats is consistent with an enhancement of the NMDA-induced activity along the indirect pathway[66]. The reason why intrastriatal perfusion of 100 µM NMDA stimulated GABA but not GLU release in GP remains a matter of conjecture. It is possible that higher NMDA concentrations cause a higher increase in the firing rate of striato-pallidal MSNs, with greater release of GABA and co-transmitters (enkephalins), leading to inhibition of pallidal GLU release. Indeed, enkephalins are the endogenous ligands of delta opioid receptor, and delta opioid receptor stimulation can reduce GLU levels [74, 75].

The changes in the GABA responses in SNr and GP to intrastriatal perfusion of NMDA adds to previous microdialysis studies, and strengthen the evidence of a change in the NMDA receptor mediated control of striatal output pathways in the parkinsonian brain. Reverse dialysis of (R)-[(S)-1-(4-bromo-phenyl)-ethylamino]-(2,3-dioxo-1,2,3,4-tetrahydroquinoxalin-5-yl)-methyl]-phosphonic acid (NVP-AAM077) [76], a preferential GluN2A antagonists, or (R-(R*,S*)-α-(4-hydroxyphenyl)-β-

methyl-4-(phenylmethyl)-1-piperidinepropanol (Ro 25-6981) [77], a selective GluN2B antagonist, in the DLS of naïve rats caused inhibition of GABA release in GP and SNr, respectively [24]. Moreover, reverse dialysis of Ro 25-6981 in the DA-depleted striatum failed to reduce GABA release in SNr, whereas intrastriatal NVP-AAM077 became more potent in inhibiting GABA release in GP [23, 25]. Based on these microdialysis data, we proposed that GluN2A and GluN2B receptors preferentially regulate the indirect and direct pathway, respectively [23, 24]. Indeed, these data are consistent with the opposite changes in responsiveness of striato-nigral and striato-pallidal MSNs to cortical inputs after DA loss (hypo- and hyperesponsitivity, respectively) [41, 53]. Moreover, they parallel morphological studies showing that DA loss results in the loss of synaptic GluN2B receptors and in the increase in the GluN2A/GluN2B ratio [78-80].

Concluding remarks

Dual-probe microdialysis shows that reverse dialysis of NMDA in striatum evokes GABA release more potently in SNr than GP. This preferential control is regulated by endogenous DA since striatal DA denervation causes an increase in NMDA potency along with selective attenuation of the nigral response. Overall, it appears that striatal NMDA receptors preferentially regulate the striato-nigral pathway under physiological conditions, and that this control is unbalanced in favour of the striato-pallidal pathway in the absence of endogenous DA. Abnormal function of striatal NMDA receptors has been found to be associated with symptoms of PD (for reviews see [3, 81, 82]). The present study suggests that changes in the NMDA receptor mediated control of striatal output pathways might contribute to these motor disturbances. Therapeutic strategies aimed at correcting such imbalance might therefore consider opposite interventions, e.g, via negative and positive allosteric modulators [83], at striatal GluN2A and GluN2B receptors regulating striato-pallidal and striato-nigral MSNs [23, 25], respectively.

Figure legends

Fig. 1. Effect of NMDA on striatal GABA and GLU release in naïve rats. Effect of reverse dialysis of NMDA (1-100 µmol/L; 10 min, black bar) in the dorsolateral striatum (DLS) on local GABA and GLU extracellular levels. Data are expressed as percentages ± SEM of basal pre-treatment levels (calculated as mean of the two samples before the treatment) and are means of 6-8 rats per group. Basal GABA and GLU levels (nmol/L), calculated as the mean of all rats enrolled, were 15.1±2.5 (n=48) and 191.6±17.9 (n=47), respectively. Statistical analysis was performed on the area-under-the-curve values (AUC; arbitrary units, Panel B) using 1-way ANOVA followed by the Newman-Keuls test. *$p<0.05$, **$p<0.01$ different from Ringer

Fig 2. Effect of NMDA on pallidal GABA and GLU release in naïve rats. Effect of reverse dialysis of NMDA (1-100 µmol/L; 10 min, open bar) in the dorsolateral striatum (DLS) on GABA and GLU extracellular levels in ipsilateral globus pallidus (GP). Data are expressed as percentages ± SEM of basal pre-treatment levels (calculated as mean of the two samples before the treatment) and are means of 6-8 rats per group. Basal GABA and GLU levels (nmol/L), calculated as the mean of all rats enrolled, were 15.1±1.4 nmol/L and 134.6±19.0, respectively. Statistical analysis was performed on the area-under-the-curve values (AUC; arbitrary units, Panel B) using 1-way ANOVA followed by the Newman-Keuls test. *$p<0.05$, **$p<0.01$ different from Ringer

Fig 3. Effect of NMDA on nigral GABA and GLU release in naïve rats. Effect of reverse dialysis of NMDA (1-100 µmol/L; 10 min, open bar) in the dorsolateral striatum (DLS) on GABA and GLU extracellular levels in ipsilateral substantia nigra reticulata (SNr). Data are expressed as percentages ± SEM of basal pre-treatment levels (calculated as mean of the two samples before the treatment) and are means of 6-8 rats per group. Basal GABA and GLU levels (nmol/L), calculated as the mean of all rats enrolled, were 13.9±0.8 (n=43) and 138.2±11.7 (n=48), respectively. Statistical analysis

was performed on the area-under-the-curve values (AUC; arbitrary units, Panel B) using 1-way ANOVA followed by the Newman-Keuls test. *p<0.05, **p<0.01 different from Ringer

Fig. 4. Effect of NMDA on striatal GABA and GLU release in hemiparkinsonian rats. Effect of reverse dialysis of NMDA (1-100 µmol/L; 10 min, black bar) in the dopamine-depleted dorsolateral striatum (DLS) of 6-OHDA hemilesioned rats on local GABA and GLU extracellular levels. Data are expressed as percentages ± SEM of basal pre-treatment levels (calculated as mean of the two samples before the treatment) and are means of 6-8 rats per group. Basal GABA and GLU levels (nmol/L), calculated as the mean of all rats enrolled, were 12.1 ± 1.2 and 67.9±8.0 (n=22 each), respectively. Statistical analysis was performed on the area-under-the-curve values (AUC; arbitrary units, Panel B) using 1-way ANOVA followed by the Newman-Keuls test. *p<0.05, **p<0.01 different from Ringer

Fig. 5. Effect of NMDA on pallidal GABA and GLU release in hemiparkinsonian rats. Effect of reverse dialysis of NMDA (1-100 µmol/L; 10 min, open bar) in the dopamine-depleted dorsolateral striatum (DLS) of 6-OHDA hemilesioned rats on GABA and GLU extracellular levels in ipsilateral globus pallidus (GP). Data are expressed as percentages ± SEM of basal pre-treatment levels (calculated as mean of the two samples before the treatment) and are means of 6-8 rats per group. Basal GABA and GLU levels (nmol/L), calculated as the mean of all rats enrolled, were 9.4±0.8 (n=32) and 86.7±19.8 nmol/L (n=30), respectively. Statistical analysis was performed on the area-under-the-curve values (AUC; arbitrary units, Panel B) using 1-way ANOVA followed by the Newman-Keuls test. *p<0.05, **p<0.01 different from Ringer

Fig. 6. Effect of NMDA on nigral GABA and GLU release in hemiparkinsonian rats. Effect of reverse dialysis of NMDA (1-100 µmol/L; 10 min, open bar) in the dopamine-depleted dorsolateral striatum (DLS) of 6-OHDA hemilesioned rats on GABA and GLU extracellular levels in ipsilateral

substantia nigra reticulate (SNr). Data are expressed as percentages ± SEM of basal pre-treatment levels (calculated as mean of the two samples before the treatment) and are means of 6-8 rats per group. Basal GABA and GLU levels (nmol/L), calculated as the mean of all rats enrolled, were 11.2±1.1 (n=32) and 103.1±28.6 (n=31), respectively. Statistical analysis was performed on the area-under-the-curve values (AUC; arbitrary units, Panel B) using 1-way ANOVA followed by the Newman-Keuls test. *$p<0.05$, **$p<0.01$ different from Ringer

Table 1. Comparative effects induced by reverse dialysis of NMDA (0.1-100 µM, 10 min) in the dorsolateral striatum (DLS) of naive rats on GABA and glutamate levels in DLS, ipsilateral globus pallidus (GP) and substantia nigra reticulata (SNr).

NMDA (µM)		**DLS**			**GP**			**SNr**	
		GABA	**glutamate**		**GABA**	**glutamate**		**GABA**	**glutamate**
0.1		ne	ne		ne	ne		ne	ne
1		ne	+		ne	ne		+	+
10		+	+		+	+		+	+
100		+	+		+	ne		+	ne

+ = increase
ne = not effective

Table 2. Comparative effects induced by reverse dialysis of NMDA (0.1-100 µM, 10 min) in the dorsolateral striatum (DLS) of 6-OHDA hemilesioned rats on GABA and glutamate levels in DLS, ipsilateral globus pallidus (GP) and substantia nigra reticulata (SNr).

NMDA (µM)		**DLS**			**GP**			**SNr**	
		GABA	**glutamate**		**GABA**	**glutamate**		**GABA**	**glutamate**
0.01		ne	ne		ne	ne		ne	ne
0.1		+	+		+	+		+	+
1		+	ne		+	+		ne	ne
10		ne	ne		ne	ne		ne	ne
100		nt	nt		nt	nt		nt	nt

+ = increase
ne = not effective
nt = not tested

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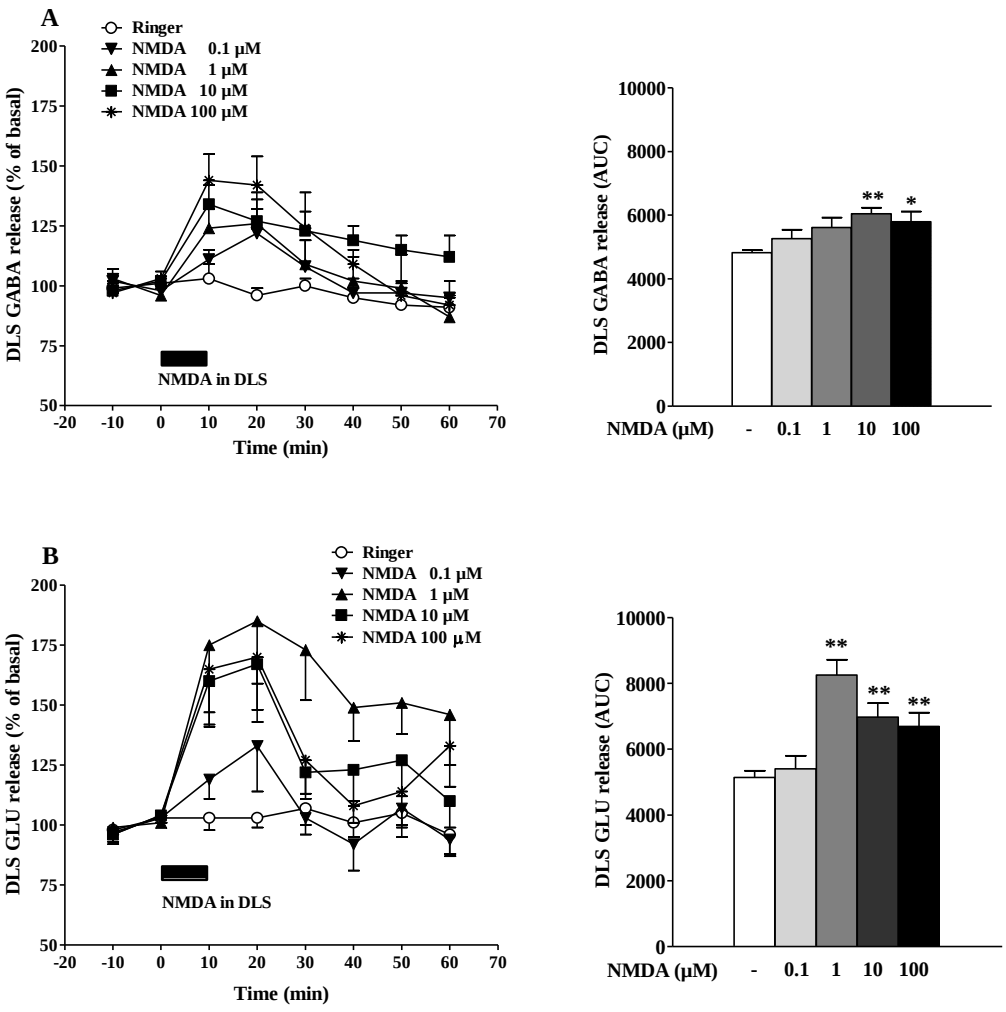

A
Ringer
NMDA 0.1 µM
NMDA 1 µM
NMDA 10 µM
NMDA 100 µM
DLS GABA release (% of basal)
NMDA in DLS
Time (min)
DLS GABA release (AUC)
NMDA (µM)
B
Ringer
NMDA 0.1 µM
NMDA 1 µM
NMDA 10 µM
NMDA 100 µM
DLS GLU release (% of basal)
NMDA in DLS
Time (min)
DLS GLU release (AUC)
NMDA (µM)

Figure 1

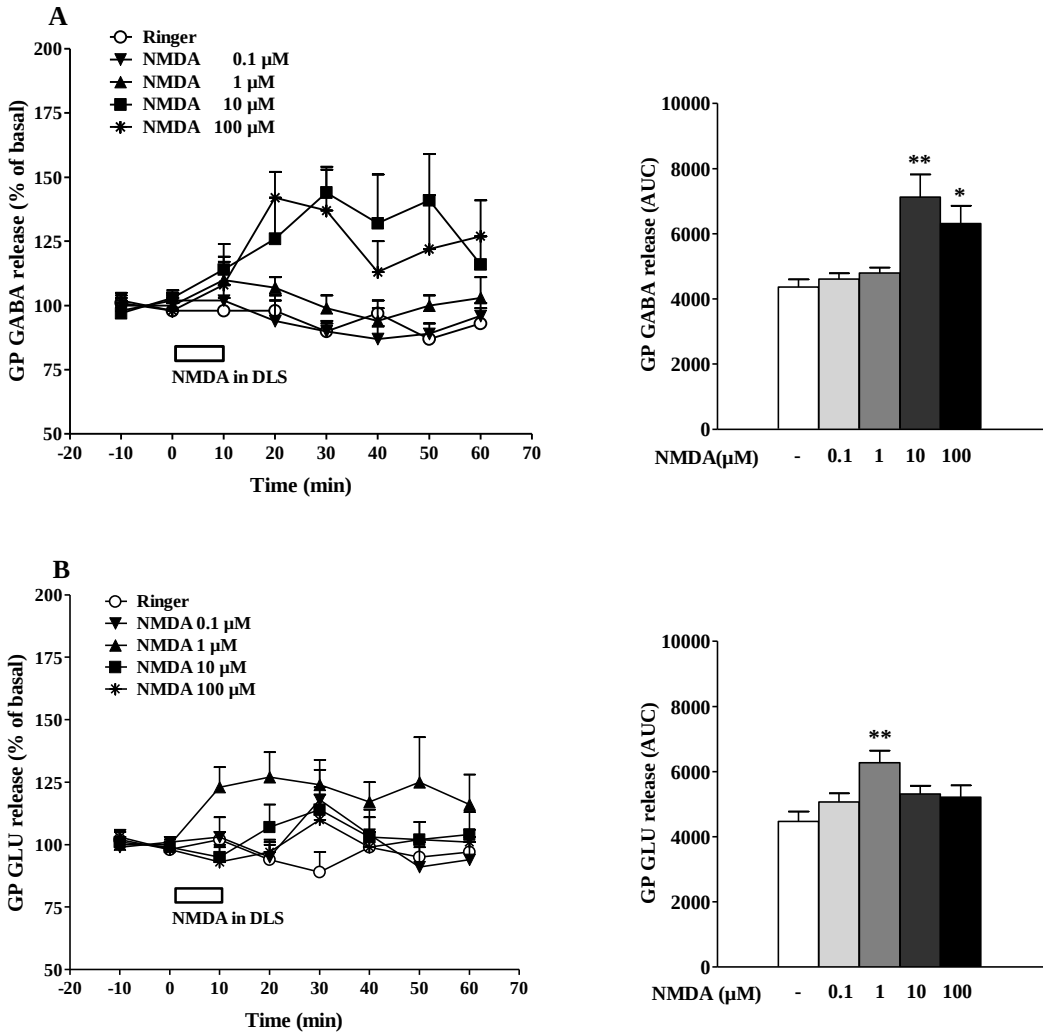
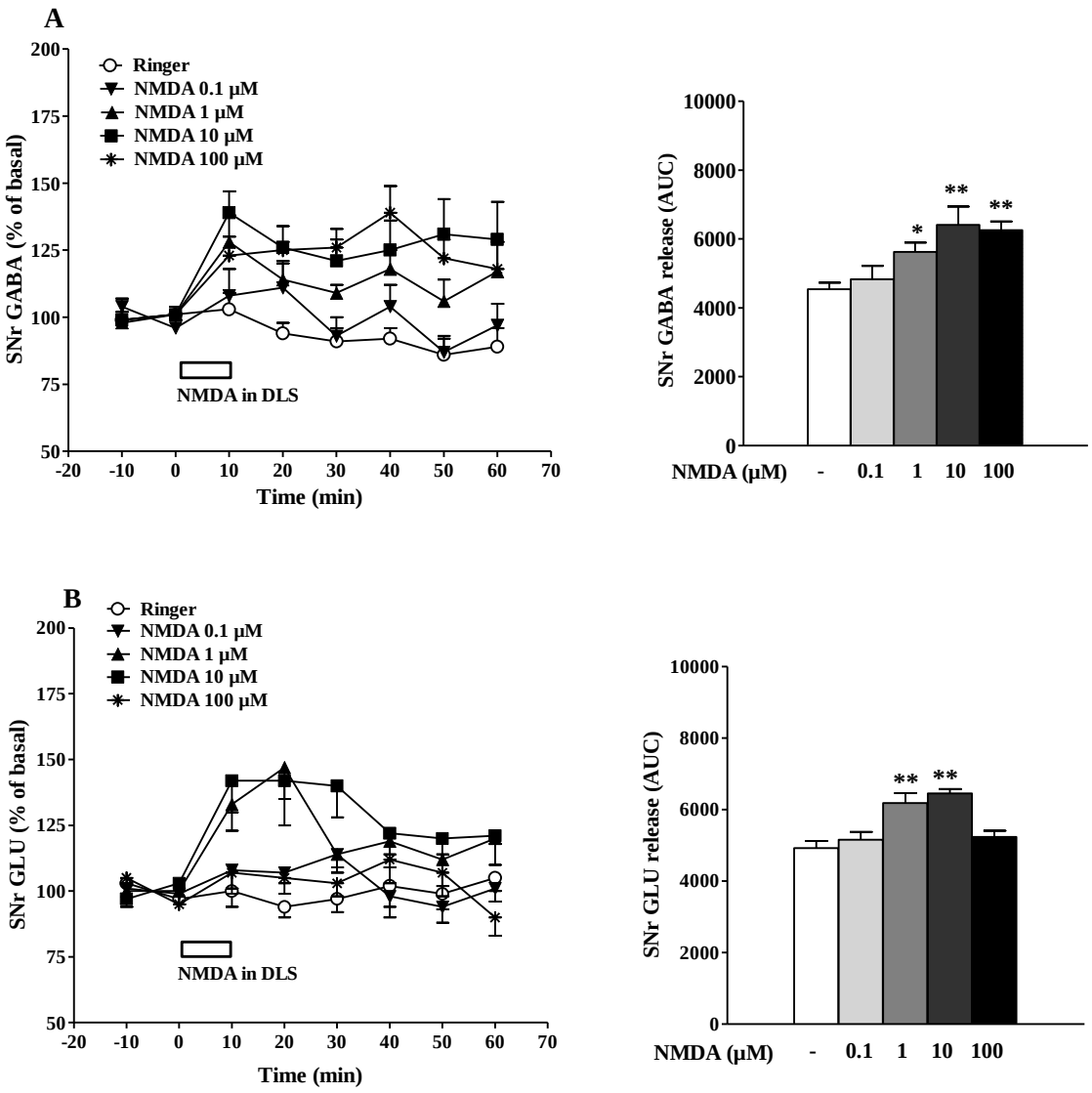

A
Ringer
NMDA 0.1 µM
NMDA 1 µM
NMDA 10 µM
NMDA 100 µM
GP GABA release (% of basal)
NMDA in DLS
Time (min)
GP GABA release (AUC)
NMDA(µM) - 0.1 1 10 100
B
Ringer
NMDA 0.1 µM
NMDA 1 µM
NMDA 10 µM
NMDA 100 µM
GP GLU release (% of basal)
NMDA in DLS
Time (min)
GP GLU release (AUC)
NMDA (µM) - 0.1 1 10 100

Figure 2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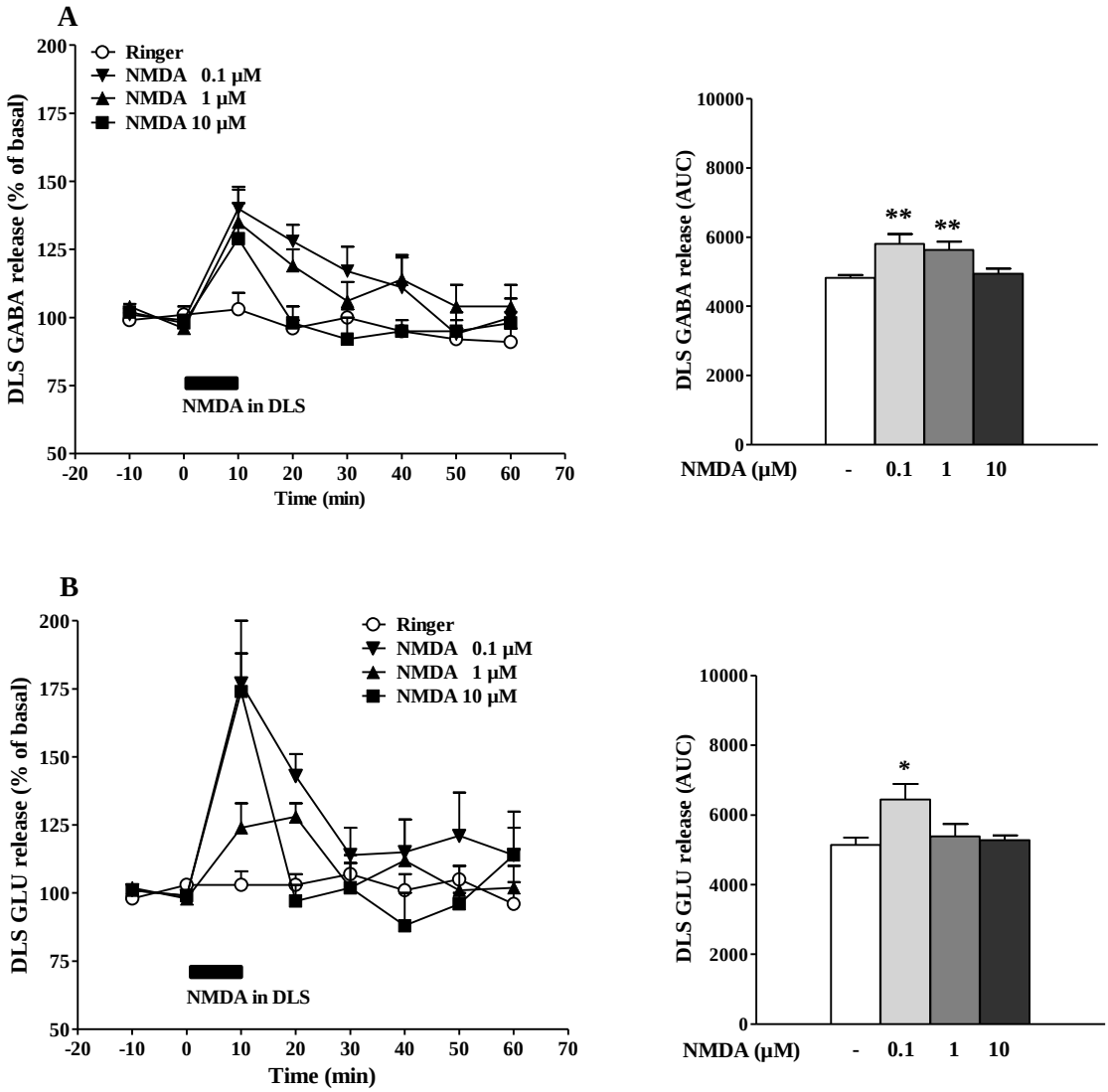
NMDA 1 µM
NMDA 10 µM
NMDA 100 µM
SNr GABA (% of basal)
NMDA in DLS
Time (min)
SNr GABA release (AUC)
NMDA (µM) - 0.1 1 10 100
B
Ringer
NMDA 0.1 µM
NMDA 1 µM
NMDA 10 µM
NMDA 100 µM
SNr GLU (% of basal)
NMDA in DLS
Time (min)
SNr GLU release (AUC)
NMDA (µM) - 0.1 1 10 100

Figure 3


A
Ringer
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NMDA 1 µM
NMDA 10 µM
DLS GABA release (% of basal)
NMDA in DLS
Time (min)
DLS GABA release (AUC)
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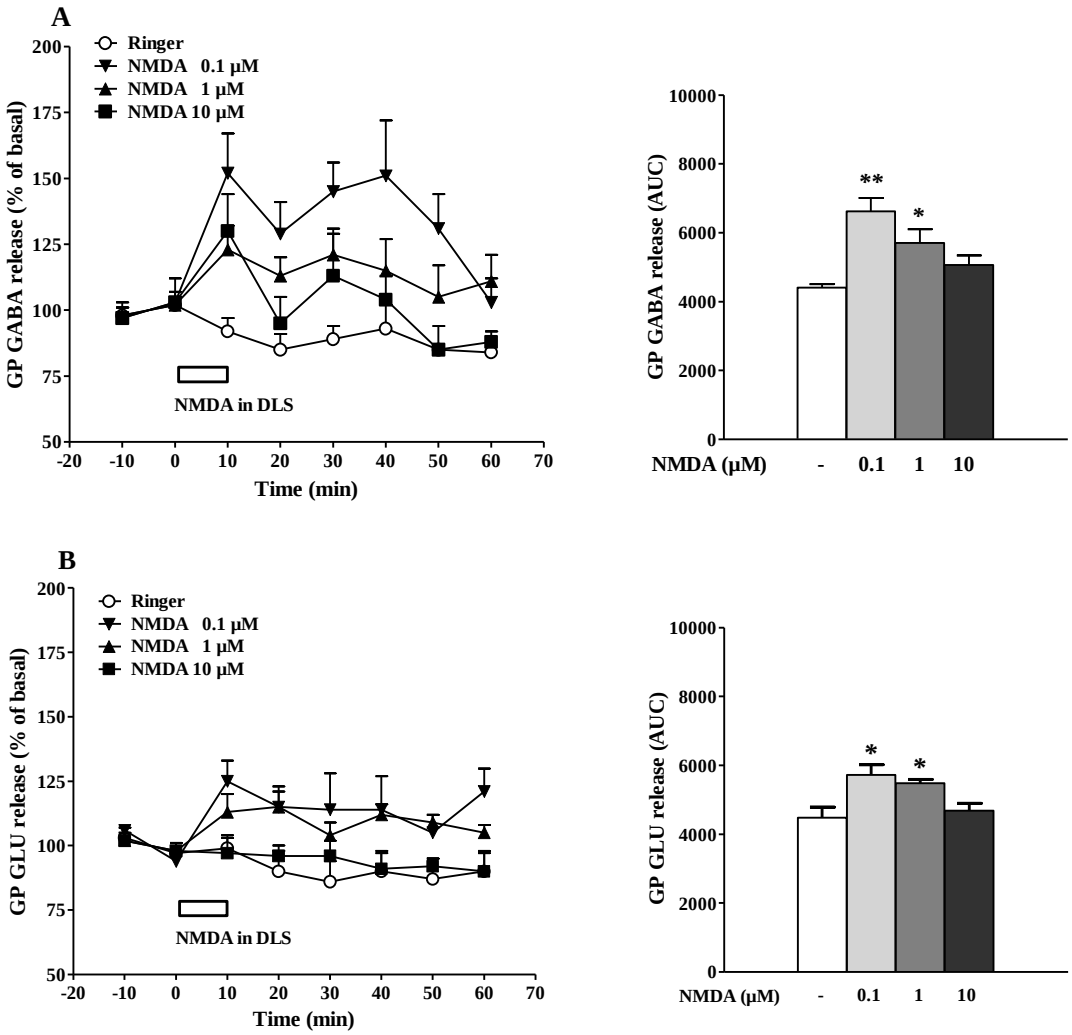
**
NMDA (µM) - 0.1 1 10
B
Ringer
NMDA 0.1 µM
NMDA 1 µM
NMDA 10 µM
DLS GLU release (% of basal)
NMDA in DLS
Time (min)
DLS GLU release (AUC)
*
NMDA (µM) - 0.1 1 10

Figure 4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Ringer
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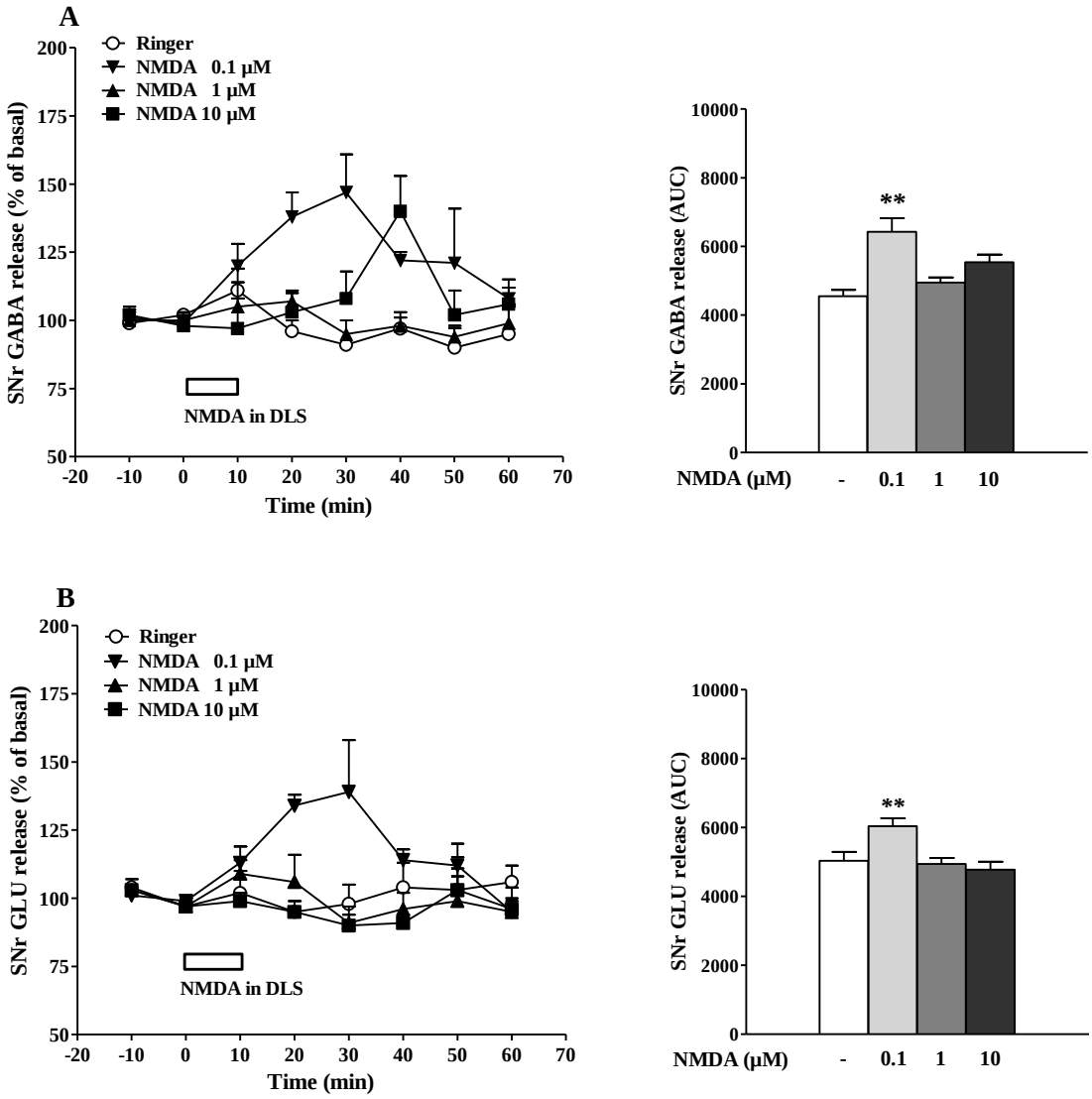
NMDA 1 µM
NMDA 10 µM
GP GABA release (% of basal)
NMDA in DLS
Time (min)
GP GABA release (AUC)
NMDA (µM)
B
GP GLU release (% of basal)
GP GLU release (AUC)

Figure 5


A
Ringer
NMDA 0.1 µM
NMDA 1 µM
NMDA 10 µM
SNr GABA release (% of basal)
NMDA in DLS
Time (min)
SNr GABA release (AUC)
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NMDA (µM)
-
0.1
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B
Ringer
NMDA 0.1 µM
NMDA 1 µM
NMDA 10 µM
SNr GLU release (% of basal)
NMDA in DLS
Time (min)
SNr GLU release (AUC)
**
NMDA (µM)
-
0.1
1
10

Figure 6