Changes in Striatal N-methyl-D-aspartate (NMDA) Stimulation of Dopamine Release and Receptor Subunit Expression During Expression of and Recovery from MPTP-Induced Parkinsonism

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Abstract

Normal and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine(MPTP)-treated cats were used to examine changes in N-methyl-D-aspartate (NMDA) receptor function. In vivo microdialysis studies showed that NMDA-stimulated dopamine (DA) release was similar in the normal dorso-lateral and ventro-medial caudate nucleus. In symptomatic animals, NMDA-stimulated DA release was significantly decreased in both striatal regions. In symptomatic animals, NMDA-stimulated dopamine release was significantly decreased in both striatal regions. In recovered animals, the dorsal striatum and ventral striatum demonstrated an upregulation in NMDA-stimulated dopamine release compared to symptomatic animals. Receptor autoradiography showed no significant differences in NMDA receptor binding between normal, symptomatic, and recovered animals in the dorso-lateral caudate. NMDA receptor binding was, however, upregulated in the ventro-medial caudate of recovered animals. With Western analysis, NR1 and NR2A subunit levels in the dorso-lateral caudate were shown to decrease significantly in symptomatic animals compared to normal and then increase in recovered animals compared to symptomatic animals. In the ventro-medial caudate, NR1 and NR2A levels in the symptomatic group were significantly increased compared to normal and recovered groups. These data suggest that there may be recovery-induced changes in the functional regulation of the NMDA receptors in the striatum contributing to the behavioral recovery seen in this model.

Theme: Disorders of the nervous system

Topic: Degenerative disease: Parkinson's

Keywords: NMDA receptor; NR1; NR2A; cat; autoradiography; protein; striatum; microdialysis; dopamine

1. Introduction

Idiopathic Parkinson's disease is a progressive neurological disorder characterized by akinesia, bradykinesia, resting tremor, and muscular rigidity. Symptoms appear when 85% or more of the dopaminergic projections from the substantia nigra pars compacta (SNc) to the striatum are lost [6]. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-hydroxydopamine (6-OHDA) lesioned animals are commonly used experimental models for the study of Parkinson's disease. However unlike the human disease, these experimental models can show various degrees of spontaneous functional recovery [29, 18 23]. One model in particular that shows reliable spontaneous functional recovery is the MPTP-treated cat [23]. Neurochemical deficits in symptomatic MPTP-treated cats include decreased tissue dopamine levels in the dorso-lateral (DL) and ventro-medial (VM) caudate [20]. In recovered animals tissue dopamine levels in the DL caudate remain depleted while extra-cellular fluid (ECF) dopamine levels significantly recover [21]. In the VM caudate, both tissue and ECF dopamine levels significantly recover [20]. Volume transmission of dopamine from the ventral caudate to the dorsal caudate [22] promoted by decreased clearance of dopamine in the caudate [19] have been proposed as mechanisms contributing to recovery of ECF dopamine in the DL caudate.

Other neurochemical compensatory mechanisms may contribute to recovery of dopamine neurotransmission in the DL caudate. The glutamatergic system may act in a dopamine denervated striatum to help restore ECF dopamine levels. Glutamatergic fibers from the cortex and thalamus [5, 9, 7] as well as dopaminergic fibers from the ventral

mescencephalic nuclei [25] project to the striatum. Loss of striatal dopamine innervation in uni-lateral 6-OHDA lesioned rats alters glutamate receptor binding [26, 16, 28] and glutamate receptor subunit expression [4, 3]. Infusion of glutamate agonists, such as N-methyl-D-aspartate (NMDA), into the striatum of rats and cats stimulates dopamine release (for review see [15]). In uni-lateral 6-OHDA lesioned rats, NMDA-stimulated dopamine release has been shown to increase the availability of ECF dopamine [1]. The present study was designed to assess the extent to which such mechanisms may be operative in the MPTP-lesioned cat striatum and the extent to which they may contribute to spontaneous functional recovery. These studies, specifically, examine changes in striatal NMDA-stimulated dopamine release, NMDA receptor binding, and NMDA subunit expression in normal, symptomatic, and recovered MPTP-treated cats to determine if NMDA neurotransmission contributes to recovery of ECF dopamine levels in the striatum and recovery of sensorimotor function.

2. Materials and Methods

2.1. Surgical procedures. Four adult male cats (3.0 to 5.0 kg in weight) were each implanted with guide cannulae (Plastics One, Inc.) for the insertion of microdialysis probes into the DL and VM caudate of both brain hemispheres. Surgeries were performed under general anesthesia (3% isoflurane). Cannulae above the DL caudate were placed at stereotaxic coordinates AP +16, L +6.5, and D +20.5, at a 6^{o} angle from vertical; cannulae above the VM caudate at stereotaxic coordinates AP +16, L +3, and D +18.5 [2].

Cannulae were fixed in place with dental cement, and the scalp was sutured around a protective barrier surrounding the cannulae. Dummy inserts (Plastics One, Inc.) were placed into the guide cannulae to ensure they remained patent. All procedures were performed in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Thomas Jefferson University Institutional Animal Care and Use Committee.

2.2. MPTP administration. Eight cats to be used in microdialysis experiments were administered MPTP. Twelve cats to be used in receptor binding and protein analysis studies were also administered MPTP. MPTP-HCl (5mg/kg, i.m.; Sigma-Aldrich) was given for 7-10 consecutive days to produce a severe parkinsonian syndrome, according to previously defined criteria and using a rating scale developed in this lab [19]. Control animals (n = 4 for microdialysis and n = 6 for receptor binding and protein analysis) received saline injections for the same period. S*ymptomatic* cats were euthanized 7-10 days after the last MPTP injection and while still parkinsonian. *Recovered* cats were euthanized 6 weeks after the last MPTP injection when no gross motor signs of parkinsonism were evident. Animals in each group were euthanized by lethal injection of sodium pentobarbital (150 mg/kg, i.v.). Brains were removed, rinsed in 0.1M phosphate buffered saline, flash frozen on dry ice, and stored at –80^{O}C.

2.3. Microdialysis. Microdialysis probes were constructed with 3 mm long cellulose membranes (MW cutoff 13 kDa; Spectrum) as described previously [17]. Probes were perfused with artificial cerebrospinal fluid (ACSF, 4 mM KCl, 147 mM NaCl, 2 mM $CaCl_2$) at 1.5 μl/min using a syringe pump (CMA). Each probe was calibrated in vitro at 37^{o}C to determine dopamine recovery levels. Four cats from each condition (i.e. normal, symptomatic, and recovered) were used to determine dopamine-glutamate interactions and effects on extracellular dopamine levels in the dorso-lateral and ventro-medial caudates. Cats were administered alpha-chloralose as a chemical restraint (50mg/kg, i.v.; Sigma-Aldrich) prior to the start of each study. Animals were continuously hydrated with lactated Ringer solution and body temperature was maintained at 37^{o}C with a homeothermic blanket. Microdialysis samples were collected every 15 min. and 1 ng isoproterenol was added as internal standard. Microdialysis probes were inserted into either the DL or VM caudate and allowed to perfuse the tissue until stable basal dopamine levels were obtained. Once a stable baseline (i.e. 3 consecutive collections with equal dopamine quantities) was established, 2.5 mM NMDA, 5 mM NMDA, or 100 mM KCl were perfused through the probe for 30 min. The dose-dependent effects of 2.5 and 5 mM NMDA were chosen since lower concentrations of NMDA did not cause dopamine release. When 100 mM KCl was used, a decreased concentration of NaCl was added to the aCSF to maintain the chloride ion concentration. Raw data from each experiment were corrected for probe recovery, and are reported as percent increases from baseline. To test the specificity of NMDA-stimulated striatal dopamine release, 2.5 mM MK-801, an NMDA receptor antagonist, was administered to *normal*, *symptomatic*, and *recovered* cats prior to administering NMDA. Probe placement

was confirmed by sectioning post-mortem tissue that was then submersion fixed in 4% paraformaldehyde, and stained with Cresyl violet. Probes were visible as small stab wounds in the striatal tissue. A comparison of KCl or NMDA data across region or group was performed using analysis of variance (ANOVA) with post-hoc comparisons (Fisher's LSD). A comparison of 100 mM KCl and NMDA-stimulated dopamine release data (i.e. percent increases) for the same region and group was performed using a Student's t-Test.

Analysis of dialysate samples was done using microbore high pressure liquid chromatography (HPLC) and electrochemical detection (EC). The HPLC-EC system utilized a reverse phase microbore column (BAS Inc., 3μm particle size). The electrochemical detector was set at +0.7 V with a 0.1 Hz filter. The mobile phase contained 0.18 M NaH_2PO_4, 2.6 mM sodium octane sulfonate, 1 mM EDTA, and 11% methanol. Data were recorded on-line using EZCHROM v6.7 (Scientific Software, Inc.). The HPLC-EC system was calibrated at the beginning of each experiment by injecting artificial cerebrospinal fluid ACSF containing 0.4 ng of dopamine-HCl. The detection limit for dopamine was 0.1 pg/μl. All chemicals were obtained from Sigma-Aldrich.

2.4. NMDA receptor binding. Quantitative receptor autoradiography was performed using coronal sections of the caudate nucleus at approximately AP +16 [2]. Brains from 18 cats not used in microdialysis (6 *normal*, 6 *symptomatic*, and 6 *recovered*) were cut on a cryostat into 20 μm sections and thaw-mounted onto gelatin coated slides. Sections were pre-incubated in 50mM Tris-acetate buffer (pH 7.4) for 30 min. at 0^{o}C, and for 20 min. at 30^{o}C, and then incubated with [^{3}H]-glutamate (100nM; 40 Ci/mmol;

NEN) in Tris-acetate buffer containing 1μM kainate, 5μM alpha-amino-3-hydroxy-5-methyl-isoxazole-4-propionate, and 100μM 4-acetamide-4'-isothiocyan-stilbene-2,2'-disulfonic acid (SITS) at 4^{o}C for 10 min. Non-specific binding was determined in the presence of 200 μM NMDA. Slides were rinsed for 4 x 10 sec. in Tris-acetate buffer at 4^{o}C, and were dried at room temperature. Slides were loaded into X-ray cassettes with plastic tritium standards (American Radiochemicals, Inc.) and apposed to tritium sensitive film (Amersham Hyperfilm) for 21 days. The film was developed in Kodak D19 developer and fixed. Densitometric analysis of receptor binding in the DL and VM regions was performed using a computer-driven analysis system (Brain v3.0, Drexel University). Four sections for total and 2 sections for non-specific binding of radioligand were used. Gray values were converted to fmoles/mg protein bound. Non-specific binding values were subtracted from total binding to determine specific binding. Statistical significance between groups was determined by ANOVA with post-hoc comparisons (Fisher's LSD).

2.5. Receptor protein quantification. Membrane protein was isolated from the DL and VM caudate regions of 12 cats also used in receptor binding (4 *normal*, 4 *symptomatic*, and 4 *recovered*). Tissue was homogenized in ice-cold sucrose phosphate buffer (0.01 M Na_2HPO_4, 0.32 M Sucrose) with proteinase inhibitor. The homogenate was centrifuged at 20,000 g at 4^{o}C to pellet the membrane fraction. The cytosolic supernatant was removed and the membrane pellet was resuspended in ice-cold sucrose phosphate buffer. Combined membrane protein from whole caudates from normal cats (i.e. pooled protein) was prepared in the same manner, and was used for generating a

standard curve for each experiment. SDS-polyacrylamide gel electrophoresis and transfer of proteins to polyvinylidene difluoride (PVDF) was performed as described previously [27]. Protein concentrations were determined using a standard protein assay kit (Biorad, Inc.). Six percent polyacrylamide gels were used for protein separation, and 1 μg/mL of primary antibody (rat NR1 and NR2A raised in rabbit, Upstate) was used for immunoblotting. Bands were visualized by chemiluminescence (Pierce, Inc.) and exposure of the blot to film (Kodak Bio-max). Band optical densities were quantified using a densitometric imaging system (Alpha-Innotech, Inc.). Protein expression in the experimental samples was quantitated in respect to the standard curve [27]. Groups were compared by ANOVA with post-hoc comparisons (Fisher's LSD).

3. Results

3.1. Behavior. Symptomatic cats had significant sensorimotor and motor impairments as compared to normal and recovered animals as shown in Figure 1 (*$P<0.01$). Specifically, cats showed decreased tactile, auditory, and visual response times. Cats displayed very little spontaneous locomotion and splayed posture. Step down times were significantly increased.

3.2. Microdialysis. There were significant differences between groups for 5 mM NMDA-stimulated ($F(5,27)=44.66$; $p<0.0001$) and 100 mM KCl-stimulated ($F(5,29)=19.32$; $p<0.0001$) increases in dopamine release (Figure 2). In the DL caudate

of normal animals, 5 mM NMDA-stimulated and KCl-stimulated increases in ECF dopamine release were not significantly different. In the VM caudate of normal animals, NMDA-stimulated increases in ECF dopamine release were 2-fold greater than KCl-stimulated increases in release ($p<0.01$).

In both the DL and VM caudate of symptomatic animals, 5 mM NMDA-stimulated and KCl-stimulated increases in ECF dopamine release were significantly less than seen in normal animals ($t=10.44$, $p<0.01$ for NMDA DL; $t=9.29$, $p<0.01$ for NMDA VM; $t=7.69$, $p<0.01$ for KCl DL; $t=3.45$, $p<0.01$ for KCl VM), but were not significantly different from each other.

In the DL caudate, 5 mM NMDA-stimulated increases in ECF dopamine release were significantly greater in recovered animals compared to symptomatic animals ($t=-2.63$, $p<0.05$), although significantly less than that of normal animals ($t=8.28$, $p<0.01$). 100 mM KCl-stimulated increases in ECF dopamine release were not significantly altered in the DL caudate of recovered animals compared to symptomatic animals. Therefore in the DL caudate, NMDA-stimulated increases in dopamine release recovered while KCl-stimulated increases in release did not, resulting in a significant difference between NMDA and KCl stimulated release ($p<0.01$). In the VM caudate, both KCl-stimulated ($t=-3.52$, $p<0.01$) and NMDA-stimulated ($t=-2.17$, $p<0.05$) increases in dopamine release were significantly greater than seen in symptomatic animals, but were not significantly different from each other.

There were similar significant differences between the 2.5 mM NMDA-stimulated groups in the DL caudate ($F(5,29)=2.54$, $p=0.05$; Figure 2). 2.5 mM NMDA-stimulated increases in ECF dopamine release in the DL caudate of symptomatic animals were significantly less than in normal animals ($t=2.54$, $p<0.05$). Furthermore, the 2.5 mM NMDA-stimulated increases in the DL caudate of recovered animals were also significantly greater compared to symptomatic animals ($t=-2.18$, $p<0.05$), but showed no significant differences compared to normal animals.

Across all conditions and regions, MK-801 had an inhibitory effect on NMDA-stimulated dopamine release confirming the specificity of the receptor effect (Figure 3). Five millimolar NMDA-stimulated dopamine release in the presence of 2.5 mM MK-801 was reduced 72 ± 8% from 5 mM NMDA stimulated-dopamine release in the absence of MK-801.

Representative chromatograms of the HPLC-EC analysis of dialysate samples are shown in Figure 4, and composite drawings of all probe placements across conditions are shown in Figure 5.

3.3. NMDA receptor binding. No significant differences in receptor binding were observed between the normal, symptomatic, and recovered groups in the DL caudate (Figure 6). However in the VM caudate, the recovered group was significantly increased from the symptomatic group ($F(2, 16)=4.16$, $p=0.038$; Fisher's post-hoc, $t=-2.88$, $p<0.05$).

3.4. Receptor protein quantification. The NR1 subunit was examined because it is widely expressed in the brain and is suggested to be part of all functional NMDA receptors [30]. NR2A was examined because it is specifically expressed in the entire striatum [8, 24].

Group comparisons of the NR1 ($F(2,10)=26.50$, $p=0.0003$) and NR2A ($F(2,10)=19.24$, $p=0.0009$) expression data showed significant differences in the DL caudate. In the VM caudate, group comparisons of the NR1 ($F(2,11)=6.95$, $p=0.01$) and NR2A ($F(2,11)=15.47$, $p=0.0012$) expression data also showed significant differences.

In the DL caudate (Figures 7 & 8), there were decreases in NR1 and NR2A protein levels compared to normal in symptomatic (NR1, 75 ± 2% decrease, $t=6.80$, $p<0.01$; NR2A, 88 ± 3 % decrease, $t=6.09$, $p<0.01$) and recovered animals (NR1, 55 ± 4% decrease, $t=5.44$, $p<0.01$; NR2A, 27 ± 10% non-significant decrease). There was an increase in NR1 (78 ± 15% increase) and a significant increase in NR2A (501 ± 87% increase, $t=-3.87$, $p<0.01$) protein levels in recovered animals compared to symptomatic animals in the DL caudate. In the VM caudate (Figures 7 & 8), NR1 and NR2A protein levels in symptomatic animals were significantly increased compared to normal (NR1, 32 ± 7%, $t=-2.62$, $p< 0.01$; NR2A, 38 ± 5 %, $t=-3.54$, $p<0.01$), and recovered animals (NR1, 44 ± 10%, $t=3.60$, $p<0.01$; NR2A, 59 ± 3%, $t=5.48$, $p<0.01$). No significant differences in NR1 and NR2A levels were observed between normal and recovered animals in the VM caudate.

4. Discussion

These results suggest that, in a behaviorally recovered animal receiving a parkinsonian producing dose of MPTP, NMDA-stimulated release of dopamine in the DL and VM caudate can be up-regulated. Glutamatergic mechanism in the DL and VM caudate may contribute to recovery of ECF dopamine [21] by increasing dopamine release. Utilizing a uni-lateral 6-OHDA rat lesion model for parkinsonism, Andres et al. [1] similarly observed that striatal, NMDA-stimulated ECF dopamine release was upregulated per terminal in rats with >95% striatal dopamine depletion.

KCl-stimulated dopamine release was significantly decreased in both the DL and VM caudate of symptomatic animals. However, KCl-stimulated dopamine release returned in the VM caudate, but not in the DL caudate of recovered animals. These studies confirm previous post-mortem HPLC analyses of dopamine tissue levels that the DL and VM striatum are equally depleted of terminal dopamine stores in symptomatic animals, but that in recovered animals there is no significant return of terminal dopamine stores in the DL caudate [20].

The NMDA receptor binding data suggests that ligand binding may not be sensitive enough to predict changes in NMDA receptor subunit expression accompanying the upregulation of NMDA-mediated ECF dopamine release. Meoni et al. have similarly

shown that changes in NMDA receptor binding do not coincide with changes in NR1 subunit expression across regions of the rat and human brain [13], and across conditions in the pre-frontal cortex of Parkinsonian patients compared to controls [12]. Differences between in vivo glutamate receptor binding and single subunit expression data may be a result of ligand binding that changes with expression and receptor assembly of not one but multiple different subunits. Using in vitro glutamate receptor expression systems, Laurie et al. [11] have shown that multiple different NMDA subunits (e.g. NR2A, NR2B, NR2C, NR2D) can alter receptor binding.

NR1 and NR2A subunit expression appear to change in parallel. Since NR1 and NR2A subunits can exclusively combine to form functional NMDA receptors [10, 14], NR2A may be following the expression trends of NR1 in normal, symptomatic, and recovered cats. In the striatum of 6-OHDA lesioned rats, Dunah et al. have similarly suggested simultaneous decreases in NR1 and NR2B expression due to decreased expression of the NR1/NR2B receptor [3].

This study suggests that there are behavioral recovery associated changes in NMDA-stimulated dopamine release and NMDA subunit expression in the striatum of the MPTP-treated cat. Such changes in NMDA-mediated neurotransmission may permit the recovery of ECF dopamine levels in the lesioned cat striatum accompanying behavioral recovery. Future in vitro studies examining the dopamine releasing ability of neurons expressing various combinations of NMDA subunits as receptors may be

necessary to further clarify roles of NMDA subunits in altering dopamine release and thereby potentially contributing to behavioral recovery seen in the MPTP-treated cat.

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6. References

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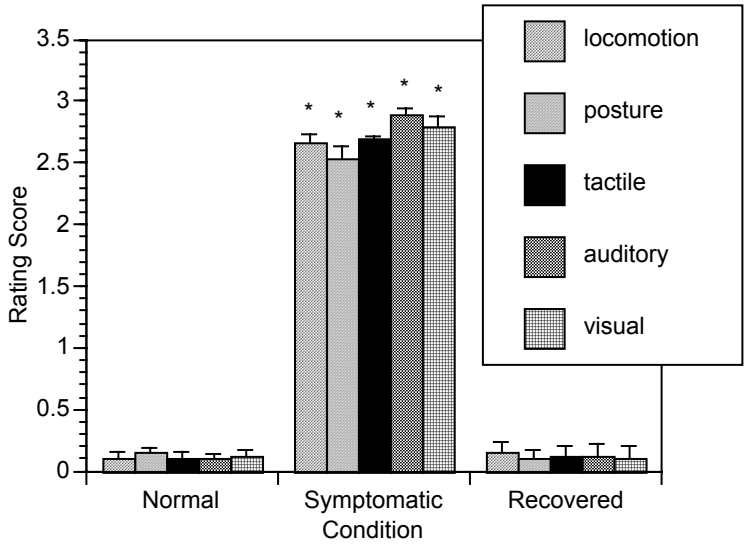
(A)



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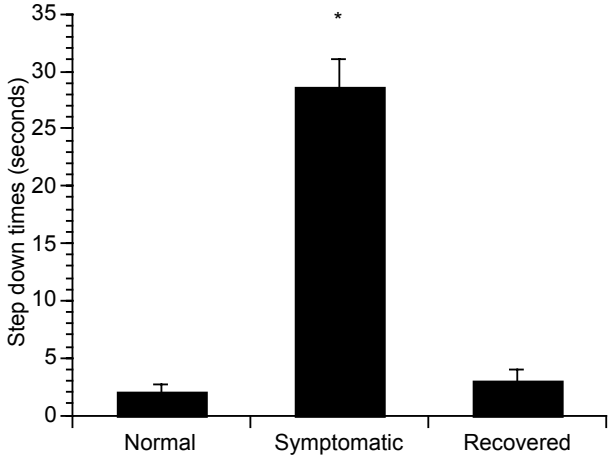


Figure 1: (A) Average behavioral ratings of cats in each condition. Normal and recovered cats show similar sensorimotor function whereas the symptomatic animals are grossly impaired. No significant differences were noticed between normal and recovered groups. (B) Average step down times in each condition. Symptomatic cats are significantly more akinetic. (*P<0.01; ANOVA with Fisher's post-hoc analysis).

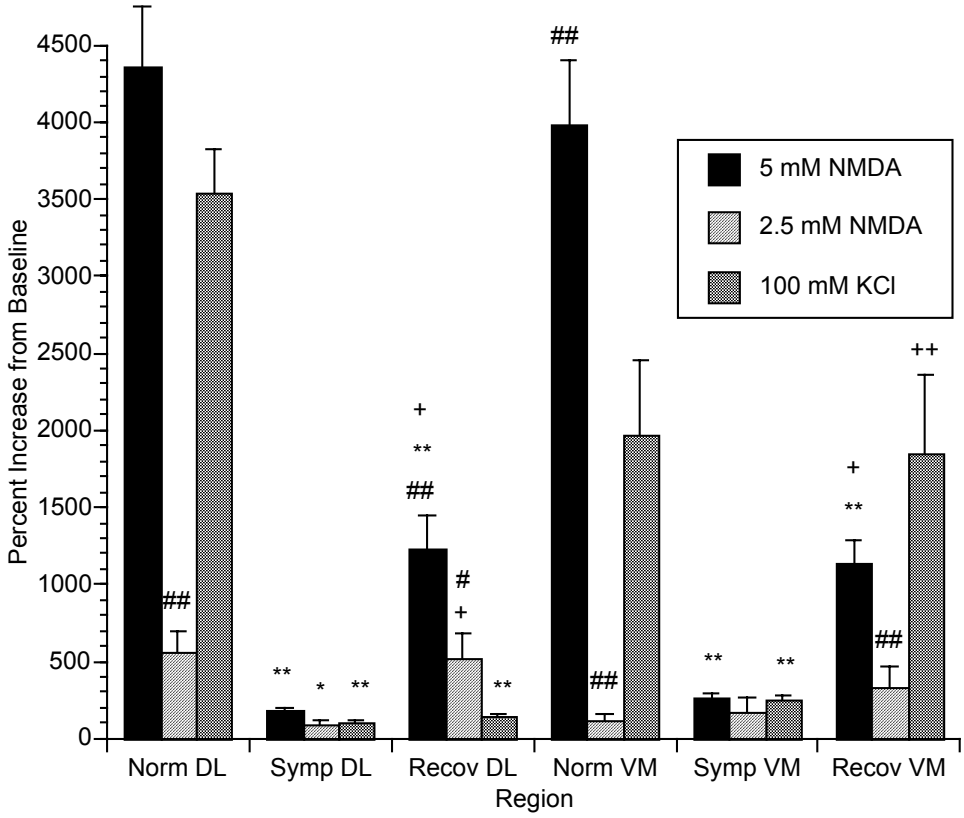
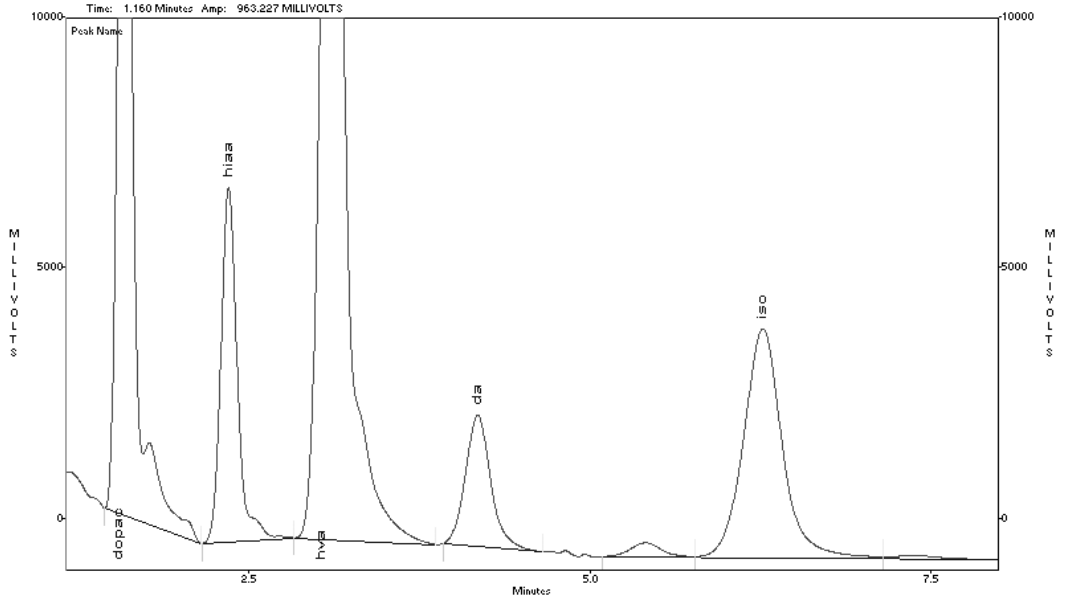


Figure 2: Stimulated increases in dopamine release after perfusion of the dorso-lateral and ventro-medial caudate with 5mM NMDA, 2.5 mM NMDA, and 100 mM KCl. DL = dorso-lateral caudate; VM = ventro-medial caudate; Norm = normal; Symp = symptomatic; Recov = recovered. N = 4 for Norm, Symp, and Recov. Student's t-Test performed to compare NMDA vs. KCl in each condition and region; #P<0.05; ##P <0.01. ANOVA with Fisher's post-hoc analysis performed individually for NMDA and KCl stimulated release to compare against other conditions within a region; *P<0.05 versus Norm in the same region; **P<0.01 versus Norm in the same region; +P<0.05 versus Symp in the same region; ++P<0.01 versus Symp in the same region.

(A) 5 mM NMDA without MK-801



(B) 5mM NMDA with MK-801

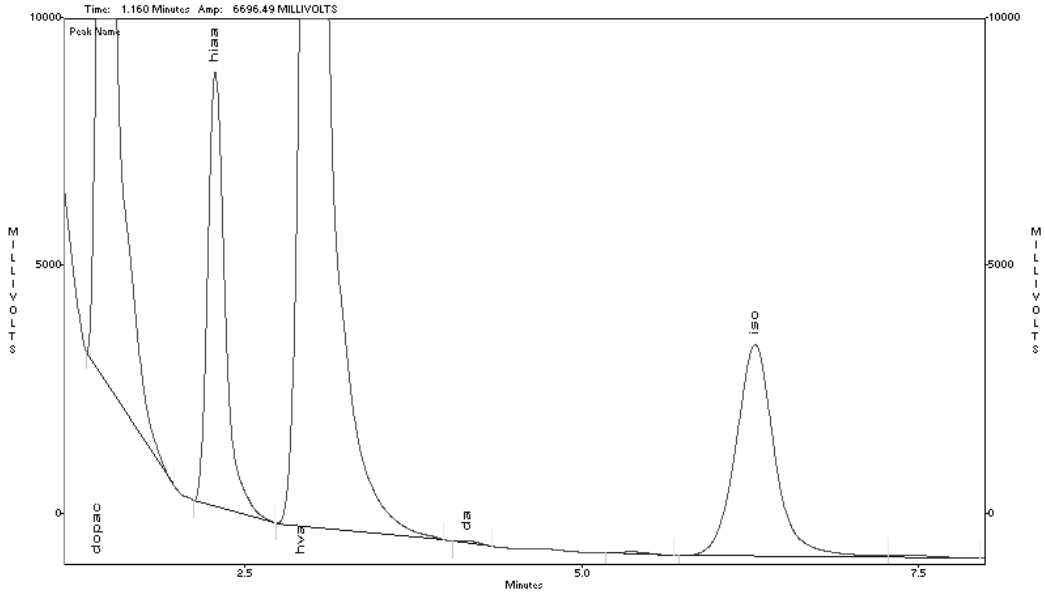
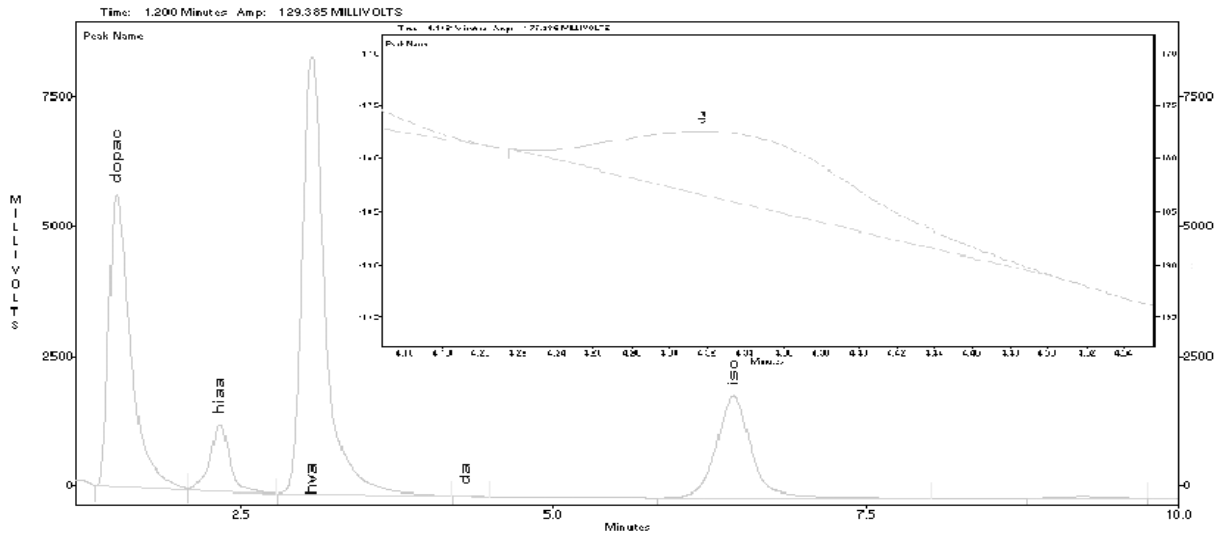


Figure 3: Perfusion of 2.5 mM MK-801 inhibited NMDA-mediated stimulation of dopamine release. (A) Perfusion with 5 mM NMDA. (B) Perfusion with 5 mM NMDA is performed in the presence of 2.5 mM MK-801. da = dopamine. Peaks for homovanillic acid (hva; degradative product of dopamine), 5-hydroxyindoleacetic acid (hiaa; degradative product of serotonin), and the internal standard (i.e. isoproterenol = iso) are also shown.

(A) baseline



(B) stimulated

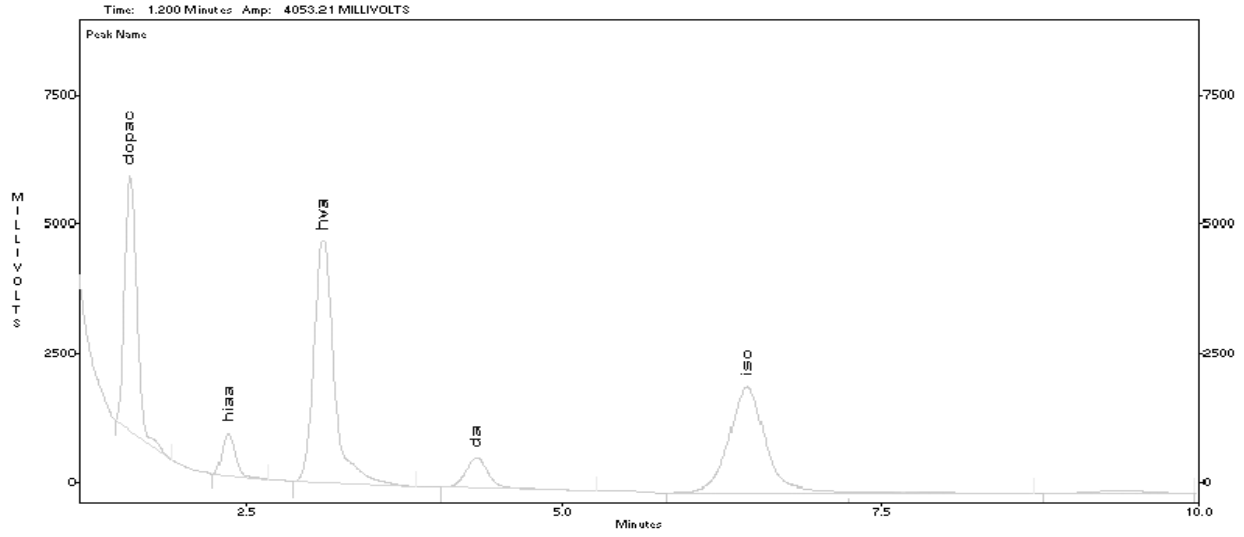
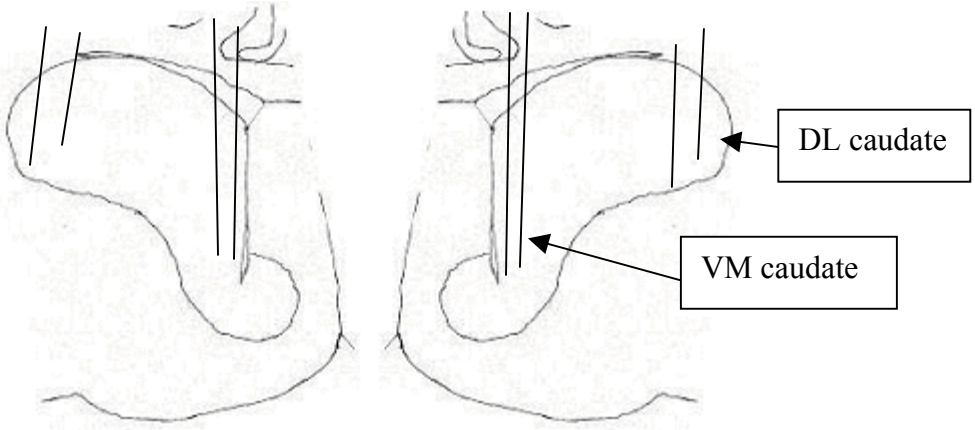
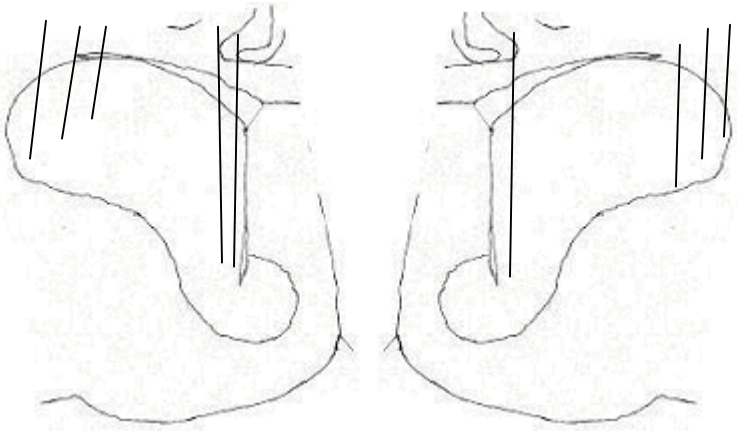


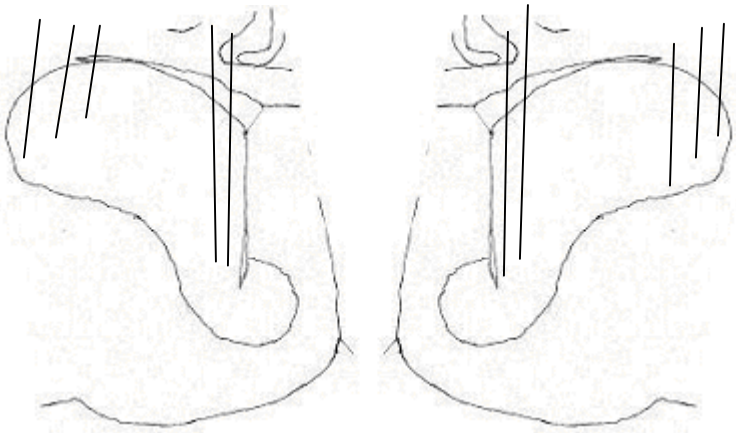
Figure 4: Sample chromatograms of (A) baseline dopamine levels and (B) 5 mM NMDA stimulated dopamine levels from the DL caudate. da = dopamine. Peaks for homovanillic acid (hva; degradative product of dopamine), 5-hydroxyindoleacetic acid (hiaa; degradative product of serotonin), and the internal standard (i.e. isoproterenol = iso) are also shown. Inset in (A) is a magnification of the dopamine peak.



Normal



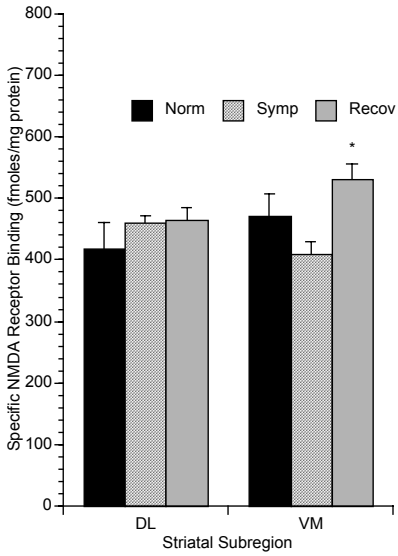
Symptomatic



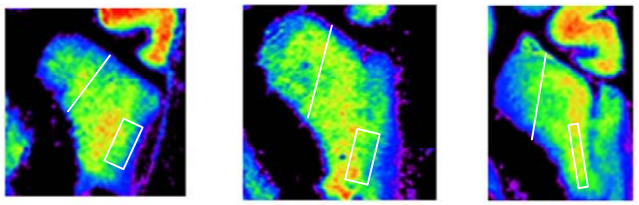
Recovered

Figure 5: Representative diagrams of microdialysis probe insertions in the dorso-lateral and ventro-medial caudate of normal, symptomatic, and recovered animals. Lines represent probe tracts in both hemispheres. DL = dorso-lateral; VM = ventro-medial

(A)



(B) **Normal** **Symptomatic** **Recovered**



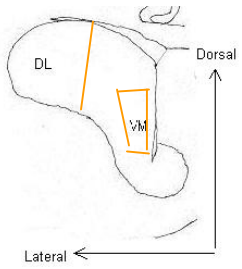


Figure 6: (A) NMDA receptor binding in the cat caudate across conditions. Receptor binding is expressed as fmoles/mg protein. Norm = normal; Symp = symptomatic; Recov = recovered; DL = dorso-lateral caudate; VM = ventro-medial caudate; N = 6 for Norm, Symp, and Recov. Receptor binding increased significantly in recovered versus symptomatic animals in the VM caudate. ANOVA performed with Fisher's post-hoc analysis; *P<0.05 versus Symp. (B) Pseudocolor autoradiograms of total NMDA receptor binding in the caudate nucleus in normal, symptomatic, and recovered animals. White lines designate boundaries of the DL and VM regions on these representative autoradiograms.

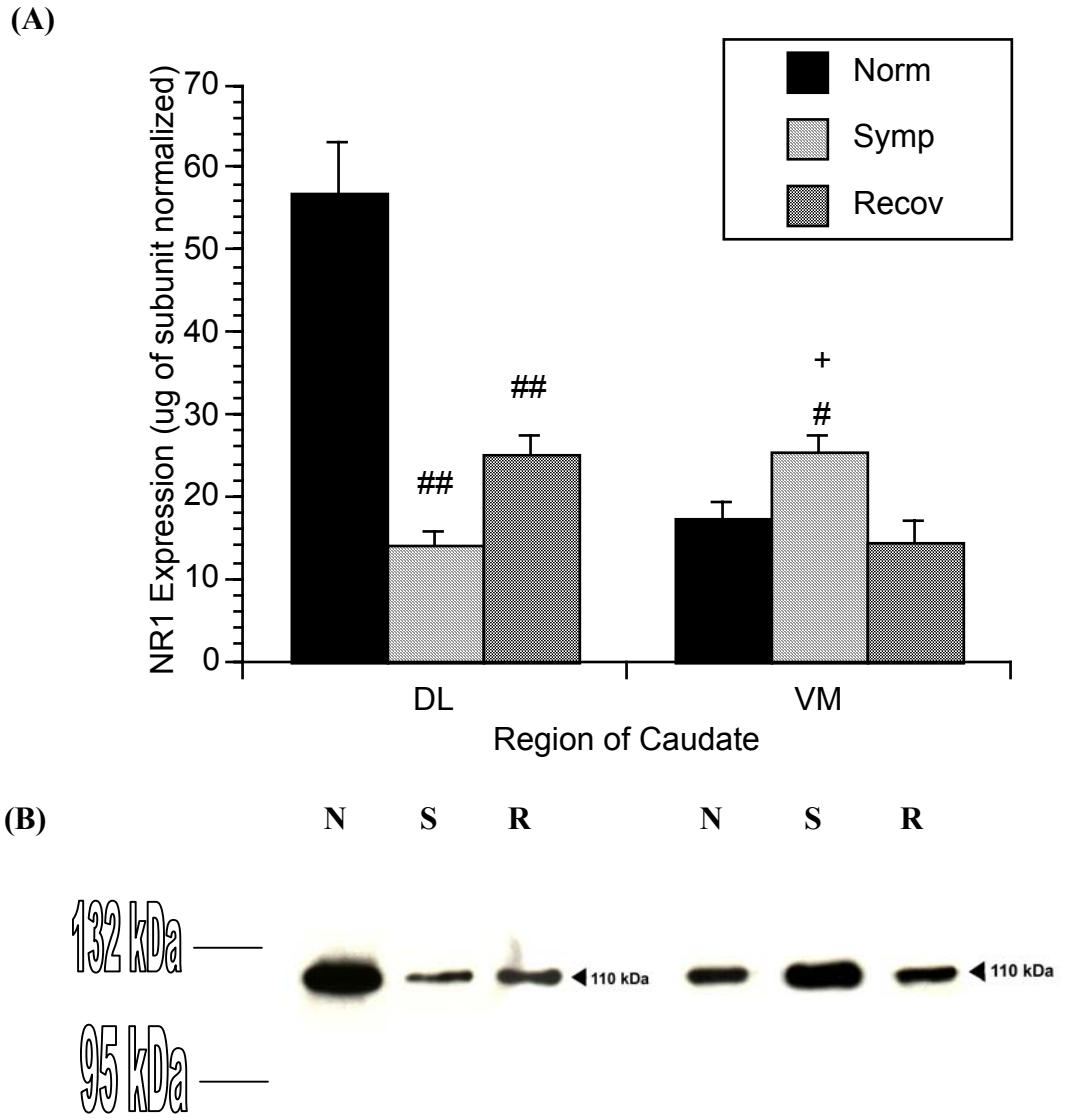


Figure 7: (A) Protein expression of NR1 subunit of the NMDA receptor in the cat caudate across conditions. DL = dorso-lateral caudate; VM = ventro-medial caudate; Norm = normal; Symp = symptomatic; Recov = recovered; N = 4 for Norm, Symp, and Recov. ANOVA performed with Fisher's post-hoc analysis; ##P < 0.01 versus Norm in the same region; #P < 0.05 versus Norm in the same region; +P<0.05 versus Recov in the same region; NR1~ 110 kDa. In the DL caudate, NR1 decreased significantly in symptomatic and recovered animals from normal. In the VM caudate, NR1 increased significantly for symptomatic versus normal and recovered animals. (B) Western blot of NR1 subunit of the NMDA receptor in dorso-lateral and ventro-medial caudate for all three conditions. Bands are at 110 kDa; N = Normal; S = Symptomatic; R = Recovered. Molecular weight markers are shown on left. Lanes coincide with bar graph for each region and condition.

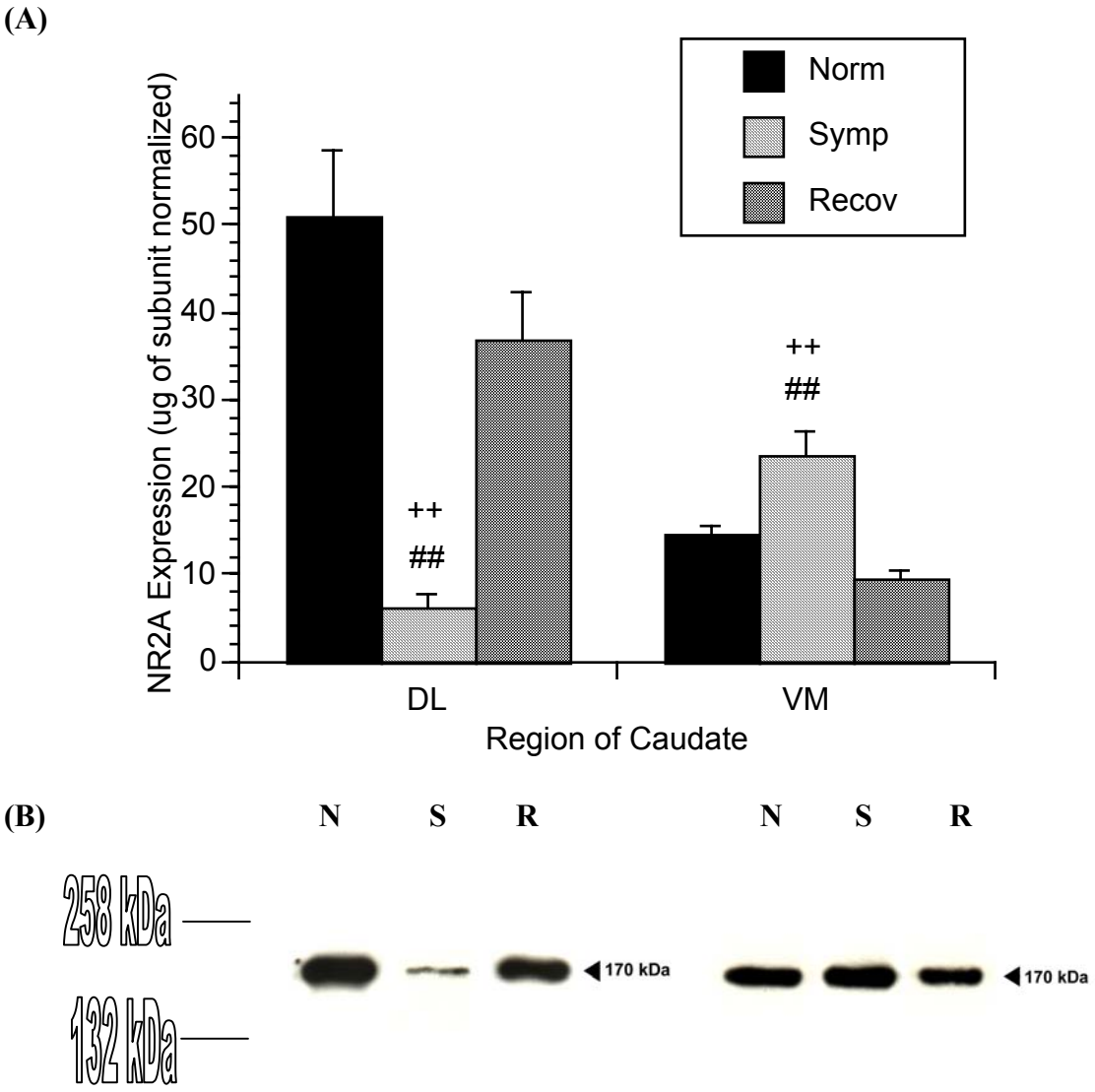


Figure 8: (A) Protein expression of NR2A subunit of the NMDA receptor in the cat caudate across conditions. DL = dorso-lateral caudate; VM = ventro-medial caudate; Norm = normal; Symp = symptomatic; Recov = recovered; N = 4 for Norm, Symp, and Recov. ANOVA performed with Fisher's post-hoc analysis; ##P < 0.01 versus Norm in the same region; ++P<0.01 versus Recov in the same region; **P<0.01 versus the same condition in the DL region. In the DL caudate, NR2A decreased significantly in symptomatic animals versus normal animals and increased significantly in recovered animals from symptomatic animals. In the VM caudate, NR2A increased significantly for symptomatic versus normal and recovered animals. (B) Western blot of NR2A subunit of the NMDA receptor in dorso-lateral and ventro-medial caudate for all three conditions. Bands are at 170 kDa; N = Normal; S = Symptomatic; R = Recovered. Molecular weight markers are shown on left. Lanes coincide with bar graph for each region and condition.