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Antisense oligodeoxynucleotide-induced suppression of basal forebrain NMDA-NR1 subunits selectively impairs visual attentional performance in rats

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Abstract

It is generally agreed that basal forebrain neuronal circuits contribute to the mediation of the ability to detect, select and discriminate signals, to suppress the processing of irrelevant information, and to allocate processing resources to competing tasks. Rats were trained in a task designed to assess sustained attention, or in a cued discrimination task that did not tax attentional processes. Animals were equipped with guide cannula to infuse bilaterally antisense oligodeoxynucleotides (ODNs) against the *N*-methyl-D-aspartate (NMDA) NR1 subunits, or missense ODNs, into the substantia innominata of the basal forebrain. Infusions of antisense or missense ODNs did not affect cued visual discrimination performance. Infusions of antisense ODNs dose-dependently impaired sustained attention performance by selectively decreasing the animals' ability to detect signals while their ability to reject nonsignal trials remained unchanged. The detrimental attentional effects of antisense infusions were maximal 24 h after the third and final infusion, and performance returned to baseline 24 h later. Missense infusions did not affect attentional performance. Separate experiments demonstrated extensive suppression of NR1 subunit immunoreactivity in the substantia innominata. Furthermore, infusions of antisense did not produce neurotoxic effects in that region as demonstrated by the Fluoro-Jade method. The present data support the hypothesis that NMDA receptor (NMDAR) stimulation in the basal forebrain, largely via glutamatergic inputs originating in the prefrontal cortex, represents a necessary mechanism to activate the basal forebrain corticopetal system for mediation of attentional performance.

Introduction

The cognitive functions mediated via corticopetal projections arising in the basal forebrain include a wide spectrum of attentional functions ranging from the detection, selection and discrimination of stimuli to the allocation of processing resources to competing tasks (Voytko, 1996; Everitt & Robbins, 1997; Sarter & Bruno, 1997). The attentional functions of the cholinergic component of this system have been inferred from studies demonstrating the attentional consequences of selective lesions of this projection (e.g. Chiba *et al*., 1995; McGaughy *et al*., 1996; Turchi & Sarter, 1997), the attentional performance-associated increases in cortical acetylcholine (ACh) release (Himmelheber *et al*., 2000), ACh-mediated changes in cortical neuronal activity (Gill *et al*., 2000), and the attentional consequences of intrabasalis manipulations of the activity of basal forebrain corticopetal projections (Sarter & Bruno, 1994; Holley *et al*., 1995).

Telencephalic information reaches the basal forebrain primarily via glutamatergic afferents (Záborszky *et al*., 1991, 1997). Intrabasalis infusions of glutamate stimulated basal cortical ACh efflux (Kurosawa *et al*., 1989; Casamenti *et al*., 1986). Infusions of NMDA did not affect basal ACh efflux but augmented increases in cortical ACh efflux produced by an activating stimulus (Fadel *et al*., 2001). These data indicated that the effects of manipulations of basal forebrain NMDA receptor (NMDAR) depend on sufficient basal forebrain activation to remove the NMDAR Mg^{2+} blockade.

Antisense oligodeoxynucleotides (ODNs) targeting brain NMDAR subunits have been employed previously in experiments involving *in vivo* intraparenchymal infusions (Matthies *et al*., 1995; Standaert *et al*., 1996; Kammesheidt *et al*., 1997; Dean *et al*., 1998; Roberts *et al*., 1998; Garry *et al*., 2000). The present experiment assessed the attentional and nonattentional consequences of antisense ODN-induced decreases in the density of basal forebrain NR1 subunits and concomitant diminution of functional hetero-oligomeric NMDA receptors. Given the necessity of the NR1 subunit presence for functional hetero-oligomeric NMDA receptors (Sprengel & Seeburg, 1993), the NR1 subunit was targeted for antisense ODN-induced disruption of NMDAR function. As NMDAR modulation of cortical ACh was hypothesized to be critically dependent on the attentional demand-associated status of the basal forebrain, effects on performance were not anticipated following antisense-induced suppression of NR1 subunit synthesis in rats trained in the cued visual discrimination task, which did not involve taxation of attentional processes. In contrast, basal forebrain suppression of NMDAR expression was expected to impair sustained attention performance, and the pattern of the impairment was predicted to resemble that observed following lesions of the corticopetal cholinergic system (McGaughy *et al*.,

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TABLE 1. Experimental design

Effects of antisense (1 or 8 nmol) or missense (8 nmol) on sustained attention performance
Day 1: behavioural test session, followed by first infusion (at noon)
Day 1: second infusion 12 h later at midnight
Day 2: behavioural test 12 h after second infusion (at noon), followed by third infusion
Day 3: behavioural test 24 h after third infusion (at noon)
Day 4: behavioural test 48 h after third infusion (at noon)
Effects of antisense (8 nmol) or missense (8 nmol) on cued discrimination performance
Day 1: behavioural test session, followed by first infusion (at noon)
Day 1: second infusion at midnight
Day 2: behavioural test 12 h after second infusion (at noon), followed by third infusion
Day 3: behavioural test 24 h after third infusion (at noon)
Effects of antisense (8 nmol) or missense (8 nmol) on NMDA-R1 immunoreactivity*
Day 1: first infusion at noon
Day 1: second infusion at midnight
Day 2: third infusion at noon
Day 3: perfusion 24 h after the third infusion†

*Unilateral infusions, with saline infused into the contralateral hemisphere; parallel sections were processed for Fluoro-Jade staining. †This time-point matches the time after three infusions at which maximal attentional impairments were observed.

1996). Experimental controls included the infusion of missense, the assessment of potential neurotoxicity of the ODNs by using the Fluoro-Jade method (Schmued *et al*., 1997), and the immunohistochemical demonstration of antisense ODN-induced NR1 suppression.

Materials and methods

Animals

Male BNNia/F344 rats (Harlan Sprague–Dawley, Indianapolis, IN, USA), weighing approximately 200 g at the inception of the study, were used to examine the effects of intrabasalis infusions of different doses of NR1 antisense ODNs, as well as missense ODNs, upon sustained attention performance (n = 7). Two groups of animals were used to assess the effects of intrabasalis infusion of antisense ODNs and missense ODNs in animals performing a control procedure (n = 6 per group). A third group (n = 10) was employed for immunohistochemical and histochemical studies (see Table 1). Rats were housed individually in a temperature- and humidity-controlled vivarium with a 12-h light : 12-h dark schedule (lights on at 01.00 h and off at 13.00 h). Animals were moderately water deprived (maintained at 90% predeprivation body weight) and food was available *ad libitum*. Experiments were conducted in AAALAC-accredited facilities and in accordance with the US Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training.

Apparatus

Subjects were trained in an operant system (MedAssociates, East Fairfield, VT, USA) that consisted of eight operant boxes; each was enclosed in a sound-attenuating chamber and equipped with two retractable levers, three panel lights (2.8 W each, located above the levers), a houselight (2.8 W), and water dispenser. The houselight was positioned high on the wall opposite the levers (for luminance values, see Holley *et al*., 1995). The water reinforcement was available on the same wall as the intelligence panel. Signal presentation, lever operation and water dispensing were controlled by an IBM clone computer using MED-PC software (MedAssociates).

Behavioural training

After training to bar press for water reinforcement (40 μL), the rats designated for the sustained attention task were trained initially to discriminate between signals (central panel light illumination: 1 s) and nonsignals (no illumination), without the houselight illumination (see Fig. 1 for a schematic depiction of the task). For a behavioural session, animals were placed in the operant chambers and once the 3-min adaptation period had concluded, the two levers were extended into the chamber 2 s following the commencement of a trial and remained active for 4 s. Left lever depression following signal events was recorded as a hit and rewarded, whereas depression of the right lever was not reinforced and was counted as a miss. Nonsignal events that elicited right lever depression were rewarded and recorded as correct rejections. Conversely, left lever presses for nonsignal trials constituted false alarms and were not reinforced. For this shaping stage, misses and false alarms resulted in correction trials (repetition of the previous stimulus type). Failure to respond correctly after three correction trials resulted in a 'forced trial' during which the same stimulus (signal or nonsignal) was repeated and only the correct response lever was made available. Should a response not have been made within 4 s of the trial event (signal or nonsignal), the levers were retracted and the lack of response was recorded as an omission. At this training stage, one session was comprised of 162 trials, with 81 trials of each stimulus type (signal or nonsignal) pseudorandomly presented, and intertrial intervals of 9 ± 3 s.

Once animals were able to respond correctly to 70% of signal and nonsignal trials, they progressed to the final task, wherein the correction and forced trials were no longer present, and the signal duration was of variable length (500, 50 or 25 ms). Within each 162 trial session, 27 trials of each of the three signal lengths were presented and 81 of the trials were of the nonsignal type. These signal and nonsignal trials were pseudorandomly presented. Performance measures included percentage hits, percentage correct rejections, errors of omission, and indices of vigilance and side bias. Animals were trained to asymptotic performance at the following minimum criteria: >65% hits for 500-ms signals, >65% correct rejections, and <25 errors of omission. Upon attainment of this performance criterion, animals were placed in the final task, wherein the parameters mentioned earlier (total trial number, signal lengths and event rate) were maintained as described but the houselight was illuminated throughout the session. Once animals regained performance criteria (> 65% hits for 500-ms signals, >65% correct rejections, and <25 errors of omission), they were subjected to surgical and/or pharmacological manipulations indicated as described later. Animals required 4–6 months of training to reach the criterion in this task.

Animals designated for the cued visual discrimination task, a task that did not explicitly tax attentional processing as only the correct lever was cued by the visual stimulus placement (Himmelheber *et al*., 1997), were similarly shaped to bar press for water reinforcement (40 μL); these animals were then trained to press the lever under one of two possible illuminated panel lights (left or right). Levers were extended throughout the session following task onset, and light stimuli were 3 s in duration. A response of left lever pressing for left light illumination resulted in water reinforcement; likewise, right lever presses were rewarded following right light illumination (see Fig. 1 for task schematic). This represented the final version of the task, which was comprised of 48 min of such visual stimuli,

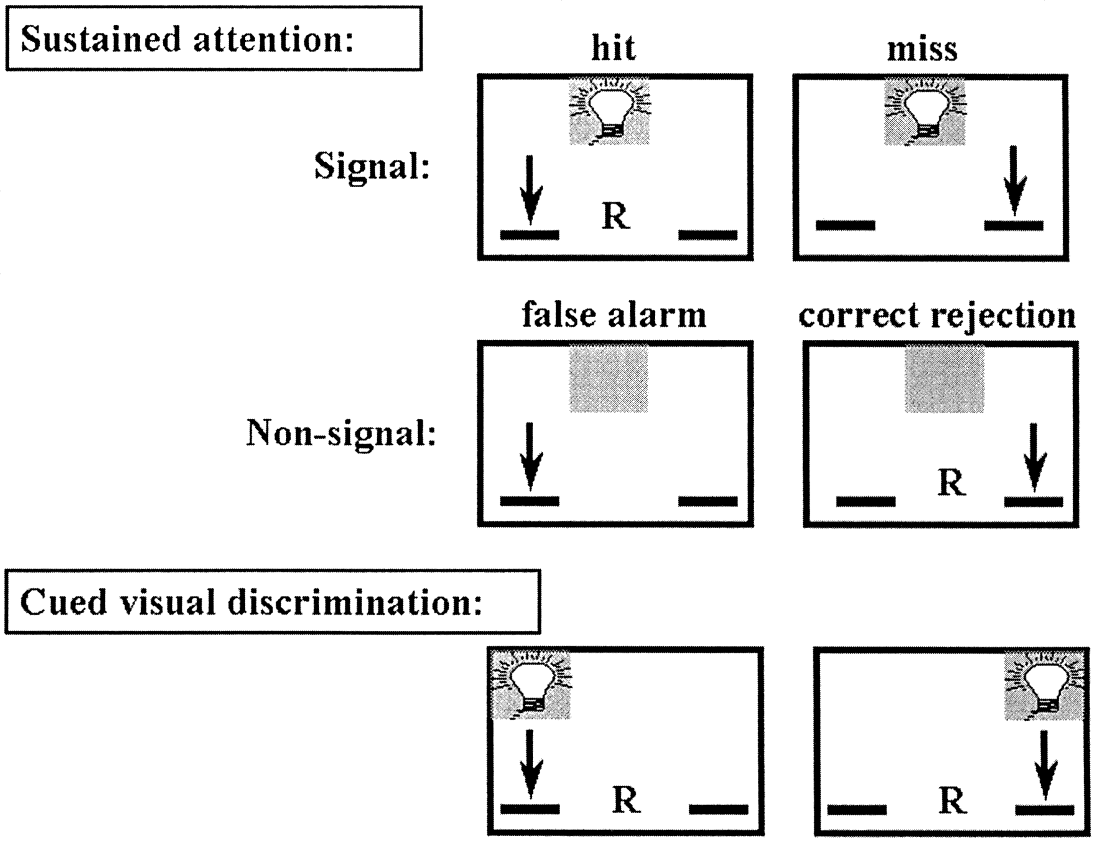

FIG. 1. Schematic illustration of the main operant rules governing the sustained attention task and the cued visual discrimination control task. In the sustained attention task, rats were trained to discriminate between signals (centre light illuminated for 500, 50 or 25 ms) and nonsignals for 162 trials per training session. Two seconds following either stimulus, the levers were extended and remained active for 4 s. Responses of left lever presses after signal trials were rewarded as hits and right lever responses after nonsignal trials were rewarded as correct rejections. Converse responses (right bar press for signal and left bar press for nonsignal) were not rewarded and constituted misses and false alarms, respectively (intertrial interval: 9 ± 3 s; for data supporting the construct validity of this task see McGaughy & Sarter, 1995). The cued visual discrimination task was designed to involve comparable operant rules without taxing attentional processes. The animals were required to press the lever below a light illuminated for 3 s. Animals rapidly achieved almost perfect response accuracy in this task (see Results).

pseudorandomly presented over the left or right lever. Animals required an average of 4 weeks of training to gain stable performance in this task. The relative numbers of both correct responses and omissions were recorded and analysed for these experiments.

Measures of performance

Performance measures generated by the cued visual discrimination task were the relative number of correct responses and omissions, and these measures were recorded over four blocks of equal duration (12 min), as well as collapsed across all trials in the session. Records of the number of hits, misses, correct rejections, false alarms and omissions were obtained for each behavioural session of the sustained attention task (see Fig. 1). Each session, composed of 162 trials, was further analysed over three blocks of 54 trials (27 trials each of signal and nonsignal events) in order to determine performance over time and to identify possible decrements in performance (termed 'vigilance decrement'). Calculation of the relative number of hits [hits/(hits + misses)] was made for each signal length per 54 trial block of the task. Likewise, calculation of the relative number of correct rejections [correct rejections/(correct rejections + false alarms)] per block was made. A vigilance index (*VI*) was computed by collapsing values for the relative number of hits (h) and false alarms (f) associated with each signal length according to the following formula, modified from an index of signal sensitivity: $VI = (h - f)/[2(h + f) - (h + f)^2$ (Frey & Colliver, 1973). Values for *VI* approaching 1.0 indicate optimal discrimination of signal vs. nonsignal events for the animal performing the sustained attention task, whereas *VI* values approaching 0.0 indicate substantive impairments in the ability of the animal to distinguish these two types of trial event. Additionally, a measure of side bias (*SB*), indicating the proclivity of an animal to respond on a single side was included for each block of 54 trials [$SB = (h + f)$/(total responses)]. An *SB* value of 0.5 represents the absence of a side bias; namely, neither lever is preferentially manipulated during the task session, while a value less than 0.5 indicates a bias for the right (correct rejection/miss) lever. Conversely, an *SB*-value of greater than 0.5 reveals a bias to the left (hit/false alarm) lever. Most animals attain a stable performance criterion of at least 70% correct responses to the 500 ms stimulus, as well as correct rejection trial events; however, the relative number of hits to 50- and 25-ms stimuli are often lower (50–60% and 40–45%, respectively). Thus, more responses tend to be generated on the right lever, yielding *SB* values of 0.33–0.44 for baseline performance.

Surgical protocols

After attaining a stable baseline performance in the final level of either the sustained attention task or the cued visual discrimination task, the rats were implanted surgically with chronic guide cannula that targeted the dorsomedial substantia innominata (for a represen-

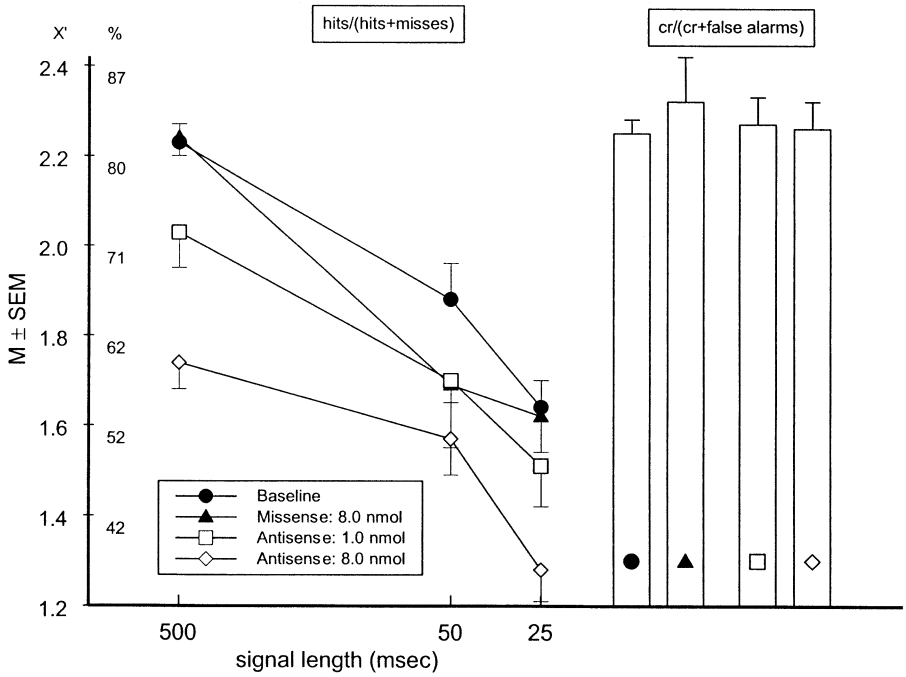

FIG. 2. Attentional performance assessed 24 h following the third intrabasalis infusion of antisense and missense. The ordinate depicts the transformed (X′) relative number of hits or correct rejections used for statistical analysis; to enhance the readability of this figure, percentage values are annotated (%). The left part of the graph illustrates the effects on the relative number of hits across the three signal lengths (500, 50 and 25 ms). The right part of the figure (bars) depicts the effects on the relative number of correct rejections (i.e. the animals' ability to reject nonsignal trials). Infusions of missense failed to produce significant effects on performance. Infusions of antisense resulted in a decrease in the animals' ability to detect signals, while their ability to reject nonsignal trials remained unaffected.

tative placement see Fig. 5A). These same surgical procedures were followed for the rats involved in the immunohistochemistry and neurotoxicity evaluations (see later descriptions). Rats were anaesthetized with ketamine (90 mg/kg, i.p.) and xylazine (6 mg/kg, i.p.) and all surgical operations occurred under aseptic conditions. Following placement in a stereotaxic instrument (David Kopf, Tujunga, CA, USA) guide cannula (26-gauge, o.d. 0.46 mm; Plastics One, Roanoke, VA, USA) were implanted into both hemispheres according the following coordinates relative to bregma: AP, –0.8 mm; ML, ± 2.5 mm; DV, 7.2 mm below dura (Paxinos & Watson, 1997). Posterior to the cannula, two stainless steel screws were drilled into the skull and the entire assembly was covered in dental acrylic. Dummy cannula were inserted in order to prevent clogging of the cannula shafts. After surgery, animals were returned to their home cages and allowed to recover for 7 days with free access to food and water. Animals were then trained until stable, asymptotic performance levels were re-established.

Antisense and missense oligodeoxynucleotides

Consisting of short chains of nucleic acids (15–25 nucleotides), antisense ODNs are targeted to an mRNA sequence of complementary bases. Inhibition of the targeted mRNA translation is achieved when the nucleotide bases of the antisense ODN form hydrogen bonds with the complementary nucleotide bases of the targeted mRNA, thereby blocking the mechanisms of translation (Stein & Cohen, 1988; Walder & Walder, 1988). Another possible mechanism of action of ODNs is functioning as a substrate for enzymatic degradation by RNase H (Walder & Walder, 1988; Toulmé, 1992; Wahlestedt, 1994). The primary means of ODN uptake has been proposed as pinocytosis (Stein *et al*., 1993) or receptor-mediated endocytosis (Loke *et al*., 1989; Yakubov *et al*., 1989; Krieg, 1993), although other mechanisms may be involved. Antisense ODNs provide a highly specific (when accompanied by adequate controls) and reversible means for inhibition of particular gene expression. While modification of phosphodiester (PDE) ODNs can increase cellular uptake and efficacy (Krieg, 1993), phosphorothioate analogues of unmodified antisense ODNs, wherein sulphur is substituted for one of the nonbridging internucleotide oxygen molecules, may have enhanced uptake relative to PDE-ODNs. Further, they have demonstrable resistance to degradation following intracerebroventricular administration and have been infused, without apparent toxic effects, at continuous micromolar levels for 1 week (Whitesell *et al*., 1993). The work upon which selection of both the current sequence for the antisense oligomer, as well as dose information was derived, employed fully phosphorothioate-substituted ODNs (Standaert *et al*., 1996). In an effort to avoid potential neurotoxicity associated with such fully substituted oligomers, the missense and antisense sequences selected for this experiment were 'second-generation chimeras', having less total sulphur substitutions along the phosphate backbone, and included 2′-*O*-methyl substitutions.

'Optimized oligomers', processed through level 1 purification (HPLC), were purchased from Oligos Etc., Inc. (Wilsonville, OR, USA). A 20-polymer ODN with the following sequence, which is complementary to a region including the putative translation initiation site for the NR1 subunit, was used as the NR1 antisense ODN: 5′-G-CAG-GTG-CAT-GGT-GCT-CAT-G-3′. The underlined portion represents the region of the oligodeoxynucleotide that was complementary to the translation initiation codon. The sequence for the missense control was 5′-G-CAG-GTG-CCT-GGT-GAT-CTA-G-3′. The missense sequence selected was in accord with suggestions to establish adequately stringent controls (Wagner, 1994; Wahlestedt, 1994); this sequence was a mismatched analogue of the antisense

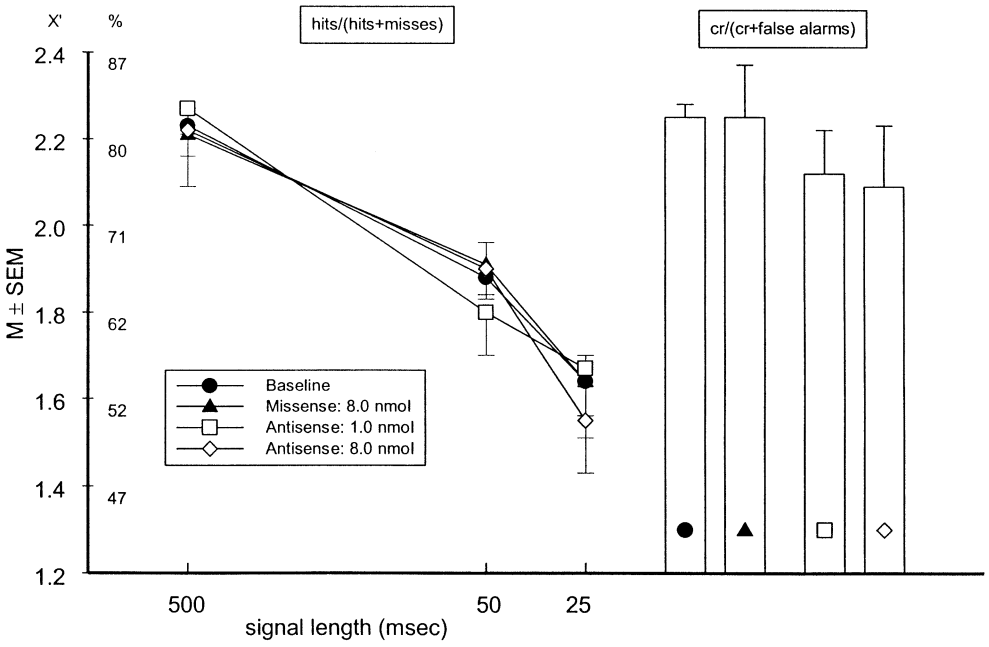

FIG. 3. Attentional performance assessed 48 h following the third intrabasalis infusion of antisense and missense. For details concerning the construction of the axis, see the legend for Fig. 2. At this time-point following the third infusion, performance effects were no longer observed, indicating the complete reversibility of the effects of antisense infusions observed 24 h earlier.

sequence that contained the same base pair proportions and a minimal number of mismatches in order to preclude specific hybridization to the target mRNA (see also Nicot & Pfaff, 1997). Once received, the oligos were dissolved in fresh sterile saline, lacking alcohol-based bacteriostatic agents, and multiple aliquots of each dose of missense and antisense were made in gas-sterilized, certified RNase- and DNase-free microcentrifuge tubes. These aliquots were then lyophilized and maintained in pellet form in a –80 °C freezer until resuspension prior to infusions, as the dissolved oligos were only viable for 1 week.

Intracranial infusions

Dummy cannula were removed and polyethylene tubing was attached several times throughout the postsurgery training period to habituate the animals to intracranial infusion procedures. A Hamilton microsyringe, attached to polyethylene tubing and connected to internal cannula, was used to make the infusions (33 gauge, i.d. 0.10 mm; Plastics One). Internal cannula did not project beyond the tip of the guide cannula. The same rate of infusion (0.5 μL/min, using a BAS infusion pump model MD1001, West Lafayette, IN, USA) was employed for all administrations, and internal cannula were allowed to remain in place for 2 min following all infusions to ensure adequate perfusion of the respective solution. Doses and volumes were selected on the basis of previous studies using NR1 antisense ODNs and related missense (Matthies *et al*., 1995; Sun & Faden, 1995; Standaert *et al*., 1996).

Once animals regained baseline performance, they commenced ODN infusion regimens (see Table 1 for details). For the sustained attention group, this schedule was comprised of bilateral infusions into the basal forebrain, with each animal receiving a total of three separate regimens of three infusions each of NR1 ODNs (1.0 or 8.0 nmol) or missense ODNs (8.0 nmol). All infusions within a regimen were administered 12 h apart. The three regimens were administered in a counterbalanced order. Animals in the cued visual discrimination task received a total of two regimens, composed of three infusions each of missense (8.0 nmol) or antisense (8.0 nmol). The doses selected for testing were similar to those that had been successfully employed in several other studies involving NR1 antisense ODN infusions and represented an effort to elicit behavioural effects while avoiding substantial cellular pathology (Matthies *et al*., 1995; Sun & Faden, 1995; Standaert *et al*., 1996). As the turnover rate of NR1 subunits has been characterized as relatively rapid (hippocampal CA1 region; Soltesz *et al*., 1994), in order to successfully inhibit synthesis of a receptor component, at least 2 days of antisense oligodeoxynucleotide treatment may be required (Wahlestedt, 1994). The time-point selected for primary behavioural data collection reflected observations by Standaert *et al*. (1996), which indicated maximal expression of behavioural changes associated with NR1 antisense ODN infusions occur 24 h after a single, larger-volume antisense administration. Data were also collected from the behavioural sessions 12 h following the second and 48 h following the third infusion for all animals. Each of the three (sustained attention) or two (cued discrimination) treatment regimens were separated by at least two behavioural sessions per day following the final test session, or by as many as were required for the animal to regain baseline performance. While the total number of infusions to which each behaving animal was subjected (nine) exceeded limitations originally suggested (five) by Routtenberg (1972), all doses (8.0 nmol missense, 1.0 nmol antisense, 8.0 nmol antisense) were administered in counterbalanced order, and baseline performance values had to be regained prior to any subsequent infusion. As will be shown later, the performance of animals tested in the sustained attention task returned to baseline 48 h following the third antisense infusion, indicating the viability of this design.

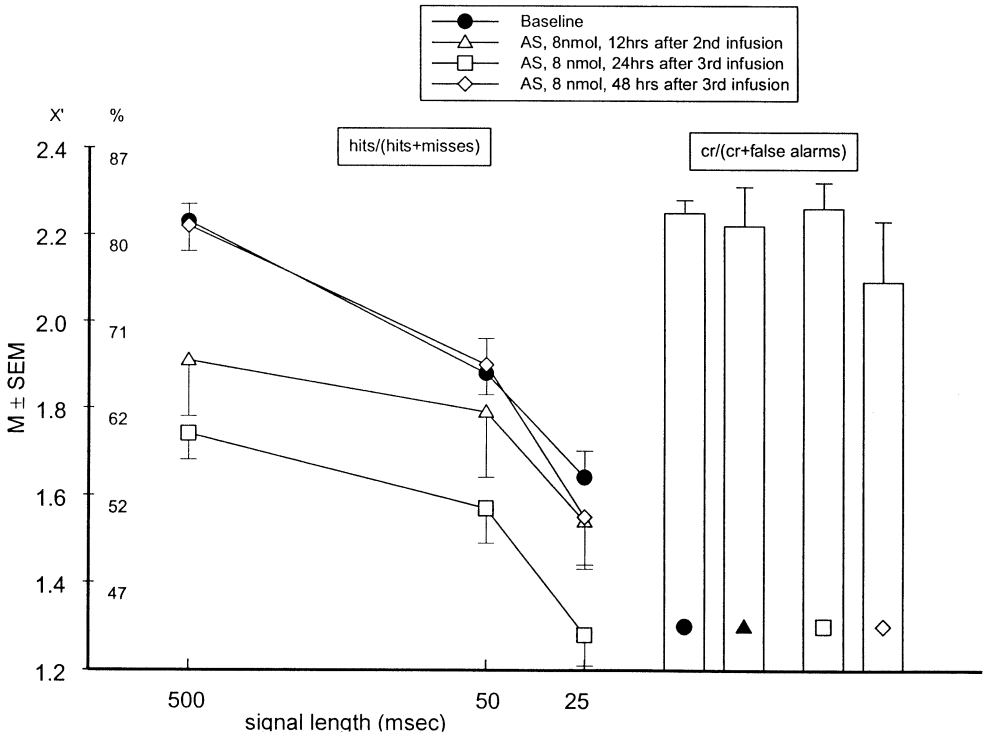

FIG. 4. Attentional effects of intrabasalis infusions of the highest dose of antisense assessed across three test points. The figure illustrates the increasing impairment in the detection of hits following the second and third infusion of antisense, and the return to baseline performance 48 h following the third infusion of antisense. These infusions did not significantly alter the relative number of correct rejections. AS, antisense.

Histological assessments, immunohistochemistry, neurotoxicity and microscopy

Following completion of the NR1 antisense ODN testing schedule, animals performing in either behavioural task were deeply anaesthetized and transcardially perfused with heparinized saline followed by 10% formalin. The brains were removed and postfixed in 10% formalin for 1 day prior to placement in 30% sucrose-phosphate buffer. Sections (40 μm) were Nissl stained to verify cannula placement.

It was anticipated that, whereas infusions of missense ODNs would not affect the expression of NR1 subunits, antisense ODNs targeting NR1 would inhibit the expression of NMDA-NR1 subunits. Two groups of animals were implanted (as described in the surgical protocols) with chronic guide cannula using the coordinates listed earlier. After a postsurgery recovery period, one group of animals was subjected to three unilateral infusions, each spaced 12 h apart (identical to the administration regimen employed in the main experiment), of antisense ODNs for the NR1 subunit (8.0 nmol, 0.5 μL per infusion; 0.5 μL/min), and the other group received three infusions of missense ODNs (8.0 nmol, 0.5 μL per infusion; 0.5 μL/min) following the same schedule. All animals received infusions of the vehicle (sterile saline, 0.5 μL) into the basal forebrain of one hemisphere and antisense, or missense, into the contralateral hemisphere. All animals were anaesthetized deeply and perfused transcardially with heparinized saline followed by 10% formalin, 24 h after the final ODN infusion; that is at the point in time when maximal effects on performance were observed in antisense-infused animals. The brains were placed in 10% formalin postfix for 24 h and subsequently transferred to 20% sucrose in 0.1 M phosphate buffer (PB). One set of sections per animal were subjected to immunohistochemical processing, while adjacent sections were processed for Fluoro-Jade staining (as described later).

Processing for NR1 immunohistochemistry used a polyclonal antibody directed against an intracellular loop of the NMDA-NR1 subunit (anti-NR1-CT, lot #17580; Upstate Biotechnology, Lake Placid, NY, USA); the specificity of this antibody for NR1 splice variant forms A, B, C and F has been confirmed by immunoblot and immunoprecipitation analysis. The antibody arrived as a frozen solution on dry ice, and aliquots were made and kept at –20 °C, as recommended. Sections (38 μm thick) were collected using an electric stage coupled freezing microtome ('MG', model #PS10AD, and serial #5812, Spencer Lens Co., Buffalo, NY, respectively) into 0.1 M PB (pH 7.4). The sections were first rinsed extensively in phosphate-buffered saline (PBS, pH 7.4) and then mounted onto clean slides. These slides were placed in a Coplin jar containing 10 mM citrate acid solution; this jar was then placed in a water-filled Pyrex chamber for successive high-temperature antigen retrieval treatments (four successively repeated sessions of microwaving for 1.5 min on high in a 600-W oven, and cooling for 3 min). The sections were cooled in the Coplin jar for 20 min, removed from the slides, placed into staining nets and rinsed in distilled water. They were then placed in an 0.5% H_2O_2 solution for 10 min, followed by extensive rinses in PBS. The next incubation was for 60 min in 1% bovine serum albumin (BSA)/PBS solution. Subsequently, a final blocking incubation (3% normal serum in 1% BSA/PBS) took place for 30 min. Primary antibody incubation (1.0 μg/mL of anti-NR1) was conducted at room temperature for 12 h and involved continuous agitation of the sections. After the primary antiserum incubation, sections were rinsed extensively in 0.1 M PBS. The secondary biotinylated goat antimouse IgG (1 : 400, Vector Laboratories,

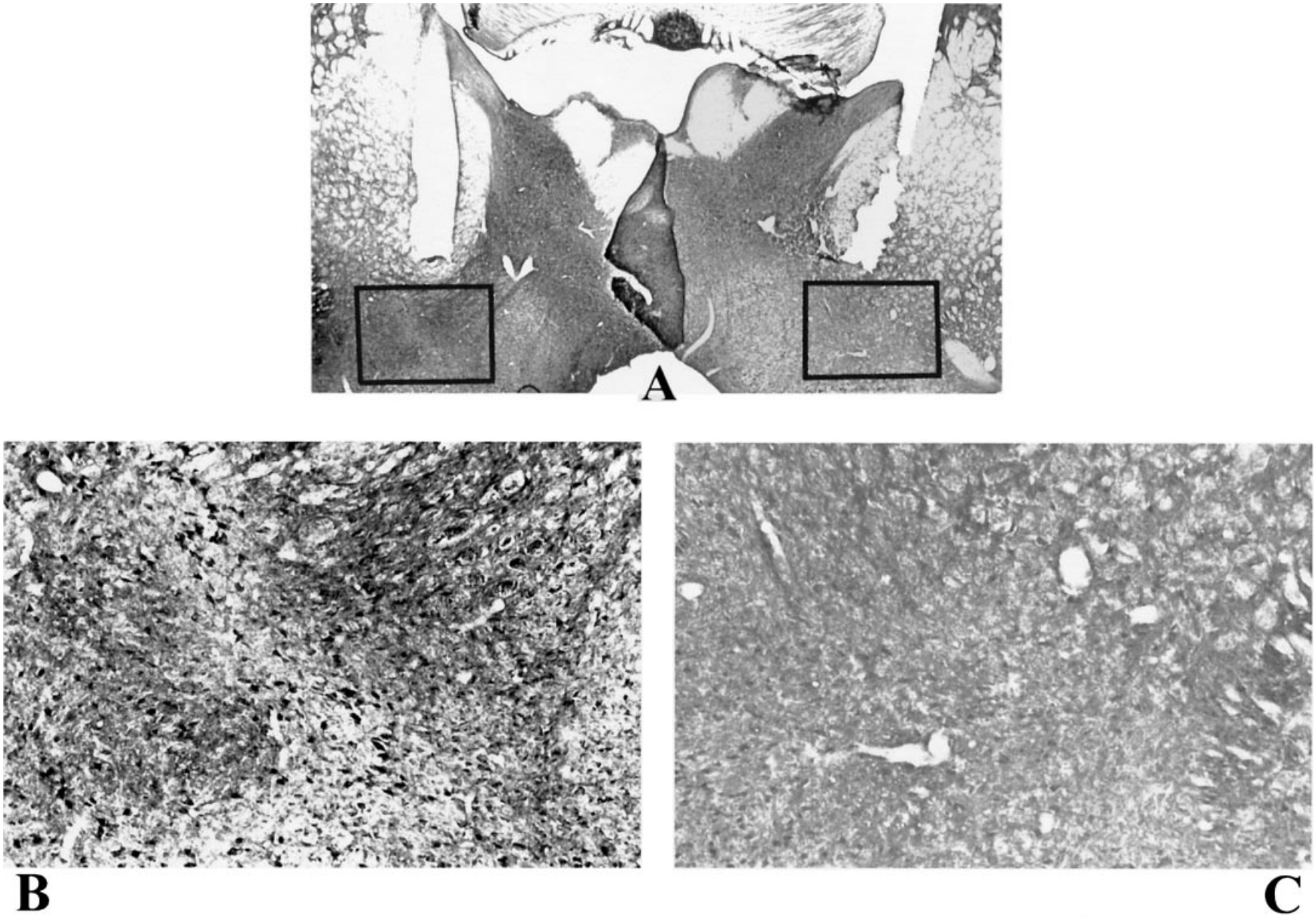

FIG. 5. Effects of three intrabasalis infusions of antisense (8.0 nmol) on NR1-IR determined 24 h following the final infusion. (A)The bilateral tracks produced by the guide cannula (magnification, 3.75 ×). Antisense was infused into the right hemisphere while saline was infused into the left hemisphere. The left and right inserts depict the regions of the substantia innominata magnified in B and C, respectively. In B, the neurons showing NR1-IR (appearing in black) are visible while NR1-IR is suppressed extensively in C, the site of antisense infusions (magnification of B and C, 18.75 ×).

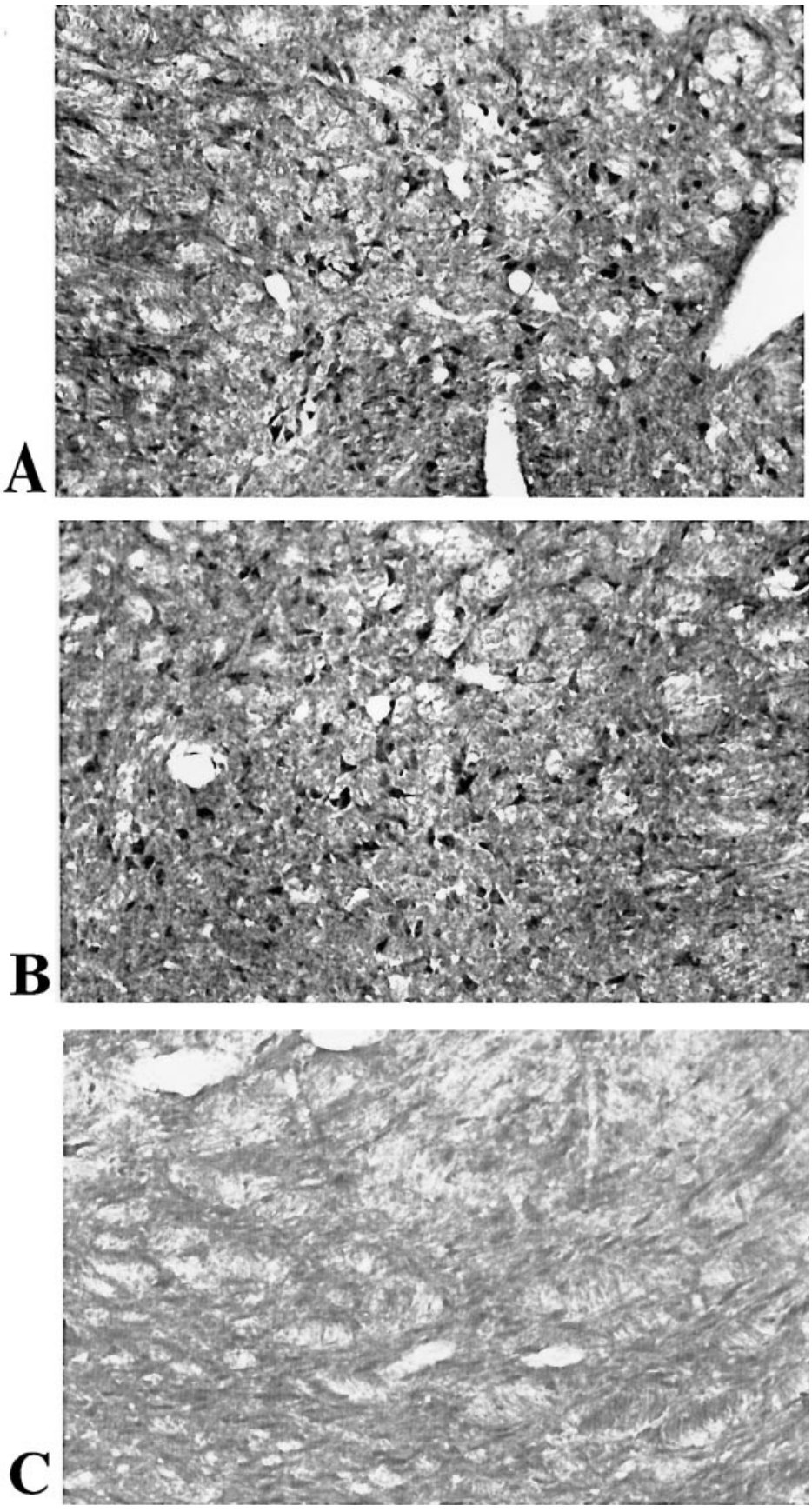

FIG. 6. Comparison between the effects of intrabasalis infusions of (saline), missense (8.0 nmol) or antisense (8.0 nmol) upon NR1-IR in the substantia innominata. The areas represented in (A–C) (magnification: 28.125 ×) correspond with the regions indicated by the inserts in Fig. 5A. (A) NR1-IR neurons (appearing in black) following the infusions of saline. (B) NR1-IR neurons following the infusion of missense. (C) The significant antisense-induced suppression of NR1-IR in the substantia innominata.

Burlingame, CA, USA) was subsequently incubated for 90 min. Following rinses in PBS, sections were then placed in avidin-biotin horseradish peroxidase complex (ABC Elite Kit; Vector Laboratories) for 90 min. Sections were rinsed extensively in

FIG. 7. Effects of intrabasalis infusions on the integrity of neurons as revealed by Fluoro-Jade (FJ) staining. All infusions, irrespective of the material infused, produced FJ staining around the tip of the cannula. The FJ label possibly reflected the consequences of mechanically induced damage as a result of infusion-associated movements of the tip of the guide. In both A (infused with saline) and B (infused with missense; magnification: 18.75 ×), the location of the tip of the guide is clearly visible at the top of the photographs. The FJ label remained restricted to the immediate vicinity of the tip, maximally extending 100–200 μm from the tip. These findings support the assumption that the missense or antisense used in the present experiment did not produce neurotoxic effects, as was also indicated by the finding that the attentional effects of antisense infusions were fully reversible (see Fig. 3).

0.1 M PB, and next processed for 6 min in 3-3′ diaminobenzidine tetrahydrochloride (100 mg, in 100 mL of 50 mM PB) with 125 μL of 3% $CoCl_2$ and 12.5 μL of 30% H_2O_2. Final rinses in 50 mM PB followed by 0.1 M PB preceded mounting of the sections on gelatine-coated slides, and subsequent dehydration and coverslipping. Additional sections were processed as described earlier, save exposure to the primary antibody, for control measures; as expected, these sections did not display NR1 positive immunoreactivity.

Sections adjacent to those processed for immunohistochemistry were processed for Fluoro-Jade staining, with positive Fluoro-Jade label indicating neurodegeneration. The exact mechanism of Fluoro-Jade staining of degenerating neurons has remained poorly characterized, but has been posited to reflect binding of the acidic Fluoro-Jade dye with a yet undefined basic component associated with degenerating neurons (Schmued *et al*., 1997; Eisch *et al*., 1998; Hopkins *et al*., 2000; Savaskan *et al*., 2000). First, the sections were mounted onto gelatine-coated slides and allowed to air dry for 12 h. They were then placed in 100% EtOH for 3 min incubation followed by successive rinses of 1 min each in 70% EtOH and in distilled water. Sections were then maintained on constant agitation, via orbital shaker, immersed in 0.06% $KMnO_4$ for 15 min. Following a 1-min rinse in distilled water, sections remained in a solution comprised of 10% Fluoro-Jade (Histo-Chem, Jefferson, AR, USA) and 0.09% glacial acetic acid for 30 min. Upon the conclusion of this final incubation, sections were placed in three successive 1-min rinses in distilled water and then air dried before dehydrating through ethanol, defatting in xylenes and coverslipping.

Microscopic inspection of NR1-IR and Fluoro-Jade fluorescence was performed using an Olympus Provis AX microscope (Olympus America, Melville, NY, USA). Microphotographs were obtained using an Olympus Magnafire Digital CCD camera (Model S99806) equipped with a 2/3 inch chip, attached to the microscope by an Olympus-USPT coupler (Olympus America). Optical magnifications are indicated in the figure legends.

Statistical analyses

Statistical analyses of the behavioural data collected were conducted separately for each task type. For all experimental data, percentage values were normalized using an arcsine transformation ($X' = 2$ arcsine $x^{1/2}$; Zar, 1974) and statistical analyses were performed only on these transformed values. The data collected from the cued visual discrimination task were examined concerning differences between presurgical vs. preinfusion values for the relative numbers of correct responses and of omissions with a repeated-measures analysis of variance (ANOVA), having two factors for each of those measures, respectively: [surgery (two levels: presurgery and preinfusion) and block (four levels)]. Likewise, analysis of either missense (8.0 nmol) or antisense ODN (8.0 nmol) effects on the relative number of correct responses, or of omissions, were conducted via repeated-measures ANOVA, having two factors for each of those measures, respectively: [dose (four levels) and block (four levels)]; these analyses were conducted separately for each of the three different time-points following the infusion regimen (12 h after the second infusion, 24 h after the third infusion and 48 h after the third infusion). Additional analysis of data from the cued visual discrimination task was performed utilizing repeated-measures ANOVA for the relative number of correct responses and relative number of omissions per block of 12 min [correct responses, having two factors: dose (three levels), block (four levels); and omissions, with two factors: dose (three levels), block (four levels)].

For the sustained attention task, a repeated-measures ANOVA was conducted to assess any differences in presurgical vs. preinfusion baselines with respect to the *VI* [within subject factors: surgery (two levels, pre vs. postop) and signal length (three levels)]. Pre-infusion baseline *VI*s were compared with those following the ODN infusions by repeated-measures ANOVA, *VI* having two factors [dose (four levels, including baseline, missense, antisense low dose, antisense high dose) and signal length (three levels)]; this same analysis was conducted three separate times for each of the three time-points at which the data had been collected (12 h after the second infusion, 24 h after the third infusion and 48 h after the third infusion). Likewise, three separate analyses for the effects of missense, and both doses of antisense, were conducted for all of the behavioural measures collected as follows. Analyses of *VI* and of the relative number of hits had two factors [dose (four levels), and signal length (three levels)]. Effects on the relative number of correct rejections, as well as *SB* and omissions, involved only one factor: dose (four levels). Further, the effects of time on task upon sustained attention performance measures were assessed by repeated-measures ANOVAs of *VI*, having three factors [dose (four levels, including baseline and antisense, or missense, ODN concentrations), block (three levels), signal length (three levels)], and *SB*, having two factors [dose (four levels) and block (three levels)]. The relative numbers of hits (*h*) and of correct rejections (*cr*) were also subjected to repeated-measures ANOVA in order to clarify any significant effects found for *VI* [*h*, three factors (dose × block × signal length); *cr*, two factors (dose × block)].

Possible violations of the assumption of sphericity were countered by evaluating repeated-measures ANOVA with greater than two levels for any factor analysed using Huynh–Feldt ε-corrected degrees of freedom (Vasey & Thayer, 1987); Huynh–Feldt corrected degrees of freedom, as well as *P*-values, are documented. Further analysis of significant *F*-values was obtained by conducting one-way ANOVAs of relevant factors and Tukey's honestly significant difference (HSD) *post hoc* tests ($\alpha = 0.05$). Statistical analyses were performed using the SPSS/PC+ 10.0.0 version (SPSS, Chicago, IL, USA).

Results

Cued visual discrimination task: baseline performance

Animals in the cued visual discrimination task did not exhibit any effect of the cannula implantation surgery upon either the relative number of correct responses or the relative number of omissions; this comparison involved the collapsed means of three criterion performance days preceding surgery and the collapsed preinfusion baselines for all ODN infusions (correct responses, $F_{2,10} = 0.51$, $P = 0.61$; omissions, $F_{1.94,9.70} = 3.81$, $P = 0.06$). The animals' performance was characterized by high response accuracy and low omission rates, averaging 95.3 ± 1.1% (mean ± SEM) correct responses and 15.9 ± 1.3% omissions per session. There was an effect of block upon the relative number of omissions ($F_{3,15} = 8.20$, $P = 0.002$), reflecting the fact the relative number of omissions was significantly higher in block 4 than in each of the other blocks (Tukey's HSD: 0.002 for block 1, <0.001 for block 2 and 0.03 for block 3. Omissions: block 1, 16 ± 1%; block 2, 15 ± 1%; block 3, 19 ± 3%; and block 4, 28 ± 3%).

Cued visual discrimination task: oligodeoxynucleotide effects

Infusions of neither antisense nor missense affected the performance of animals tested in the cued visual discrimination task. These negative findings were observed for all time-points of data collection;

for the behavioural session conducted 12 h following the second infusion (correct responses, $F_{1.44,7.18} = 1.97$, $P = 0.21$; omissions, $F_{1.74,8.68} = 1.93$, $P = 0.20$), for the session 24 h following the third infusion (correct responses, $F_{2,10} = 0.86$, $P = 0.45$; omissions, $F_{1.27,6.36} = 0.07$, $P = 0.86$), and for the session 48 h following the third infusion (correct responses, $F_{2.00,9.98} = 0.12$, $P = 0.89$; omissions, $F_{1.57,7.86} = 2.01$, $P = 0.20$).

Sustained attention task: baseline analysis

Analysis of the potential effects of the cannula implantation upon the animals' ability to discriminate signal and nonsignal events, as measured by *VI*, yielded negative results; this test involved comparison of the collapsed *VI* values from three criterion days preceding surgery and the *VI* values from the preinfusion baseline sessions prior to each administration regimen ($F_{2.55,\ 15.31} = 1.24$, $P = 0.32$). As expected, an effect of signal length was observed upon vigilance performance (*VI*, $F_{1.39,8.34} = 75.00$, $P < 0.001$). *Post hoc* analysis revealed decreases of *VI* values corresponding to decreases in signal length ($VI_{500\ ms}$ vs. $VI_{50\ ms}$, $t_{41} = 8.80$, $P < 0.001$; $VI_{50\ ms}$ vs. $VI_{25\ ms}$, $t_{41} = 5.94$, $P < 0.001$; $VI_{500\ ms}$, 0.60 ± 0.01; $VI_{50\ ms}$, 0.43 ± 0.02; $VI_{25\ ms}$, 0.33 ± 0.02). However, no effect of time on task upon *VI* was observed (block, $F_{2,12} = 0.12$, $P = 0.89$), nor were there any interactions among the effects of condition and block, block and stimulus length, or condition by block by stimulus length (*VI*, presurgery vs. preinfusion, $P \geqslant 0.44$ for all). Analysis of *VI* values from the three baselines taken prior to each administration regimen did not indicate any difference in performance ($P > 0.44$ for all). As is shown in Figs 2–4, the animals' baseline performance was characterized by stable levels of hits (500 ms, 80.1 ± 1.4%; 50 ms, 64.5 ± 3.6%; and 25 ms, 53.2 ± 2.98%) and correct rejections (81.0 ± 1.2%). The relative number of hits expectedly varied with signal length ($F_{1.37,8.20} = 33.15$, $P < 0.001$).

Sustained attention task: effects of intrabasalis oligodeoxynucleotide infusions

12 h following the second infusion

Neither the infusions of missense nor antisense were found to affect overall sustained attention performance, as indicated by *VI* in the session conducted 12 h following the second infusion ($F_{3,18} = 2.34$, $P = 0.11$). While an effect of signal length upon *VI* was present ($F_{2,12} = 43.05$, $P < 0.001$; $VI_{500\ ms}$ vs. $VI_{50\ ms}$, $t_{41} = 6.58$, $P < 0.001$; $VI_{50\ ms}$ vs. $VI_{25\ ms}$, $t_{41} = 5.38$, $P < 0.001$; $VI_{500\ ms}$, 0.57 ± 0.02, $VI_{50\ ms}$, 0.43 ± 0.02, $VI_{25\ ms}$, 0.34 ± 0.02), there was no interaction between the effects of dose and signal length upon *VI* for this time-point. Likewise, no effects of dose were found on the relative number of hits, the relative number of correct rejections, *SB*, nor omissions for this time-point ($P > 0.27$ for all). Interactions between the effects of dose and block, as well as block and signal length, were noted (block, $F_{1.21,7.24} = 2.74$, $P = 0.19$; dose × block, $F_{6,36} = 3.19$, $P = 0.01$; block × stim, $F_{2.41,24} = 3.59$, $P = 0.02$). *Post hoc* analyses revealed the interaction of the effects of dose and block was indicative of decreased *VI* solely within the first block of trials following administration of the low dose of antisense as compared with values at either baseline or following missense infusions ($VI_{block\ 1}$: baseline vs. antisense ODN, 1 nmol, HSD = 0.05; missense vs. antisense ODN, 1 nmol, HSD = 0.01); the interaction between the effects of block and stimulus length reflected a decrease in *VI* from block 2 to block 3 selectively for the 25 ms signal ($VI_{25\ ms}$, block 1 vs. block 2, HSD = 0.033). Further analysis indicated that the effects on *VI* were due to both an effect of block on the relative number of hits ($F_{2,12} = 5.00$, $P = 0.03$; although *post hoc* analysis failed to reveal the locus of this effect; HSD ⩾ 0.18 for all) and a significant interaction between the effects of block and signal length upon this measure (*h*: $F_{3.83,22.96} = 3.37$, $P = 0.03$; 50 ms signal, block 1 vs. block 2, Tukey's HSD = 0.04; block 1, 55.5 ± 3.2%; block 2, 66.4 ± 3.5%; all other HSDs ⩾ 0.09). Additionally, these effects upon the relative number of hits occurred in the absence of any such effect of time on task, or interaction of the effects of dose and block for all other performance measures (the relative number of correct rejections, *SB* or number of omissions, $P \geqslant 0.19$ for all).

24 h following the third infusion

In contrast to the data collected for the 12 h time-point, a main effect of dose on *VI* was observed for the behavioural data collected 24 h following the third infusion ($F_{2.07,12.45} = 5.14$, $P = 0.02$). *Post hoc* analysis of this effect revealed a strong trend solely for the effects of infusions of the high dose of the antisense ODN at this time-point (8.0 nmol, Tukey's HSD = 0.05. *VI*: baseline: 0.48 ± 0.03; missense, 0.49 ± 0.05; 1.0 nmol antisense, 0.43 ± 0.03; 8.0 nmol antisense, 0.33 ± 0.03). Further examination of this data was conducted by analysis of the relative number of hits and correct rejections. A main effect of dose was found on the relative number of hits ($F_{3,18} = 10.89$, $P < 0.001$) in the absence of any effect upon the relative number of correct rejections ($F_{1.65,9.87} = 0.50$, $P = 0.58$; see Fig. 2).

Additional analysis indicated the effect of dose upon the relative number of hits was exclusive to the 8.0 nmol antisense ODN infusion, which induced a decrease in the relative number of hits (*h*) as compared with both baseline and missense infusion values (baseline comparison, Tukey's HSD = 0.001; missense comparison, HSD = 0.026; baseline (*h*), 65 ± 2%; missense, 62 ± 3%; antisense, 1.0 nmol, 58 ± 3%; antisense, 8.0 nmol, 48 ± 3%). An effect of signal length upon the relative number of hits was also noted ($F_{1.87,11.26} = 27.76$, $P < 0.001$), reflecting decreases in the relative number of hits corresponding to decreases in signal length 9for $h = 500$ ms vs. $h = 50$ ms: $t_{41} = 6.49$, $P < 0.001$; for $h = 50$ ms vs. $h = 25$ ms: $t_{41} = 5.25$, $P < 0.001$; *h* (500 ms), 72.5 ± 2.0%; *h* (50 ms), 56.2 ± 3.7%, *h* (25 ms), 47.0 ± 3.2%). An effect of dose on *SB* was found for this time-point ($F_{3,18} = 9.79$, $P < 0.001$), reflecting an increased tendency for animals to respond to the right lever following the 8.0 nmol antisense administration (Tukey's HSD < 0.001. *SB*: baseline, 0.42 ± 0.007; 8.0 nmol antisense, 0.33 ± 0.01). In the absence of any significant dose effect upon the relative number of correct rejections (cr: baseline, 81 ± 1%; 8.0 nmol antisense, 81.5 ± 2%), it would appear that the dose effect upon the relative number of hits reflected a suppression of the animals' ability to discriminate the signal events following infusion of the highest dose of antisense ODN. No interaction between the effects of dose and signal length upon the relative number of hits was observed for this time-point ($F_{5.05,30.29} = 1.49$, $P = 0.22$). Likewise, there was no effect of dose upon the number of omissions ($F_{3,18} = 0.59$, $P = 0.63$).

While no effect of time on task upon *VI* was present for this collection period, nor any interaction observed between the effects of dose and block on *VI* ($P > 0.70$), there was a strong trend for an interaction between the effects of block and signal length ($F_{3.06,18.35} = 3.01$, $P = 0.06$) that could not be further defined on the basis of multiple comparisons. A significant interaction between the effects of dose, block and signal length on *VI* was found ($F_{9.61,57.65} = 2.65$, $P = 0.01$). *Post hoc* analysis again failed to determine the locus of the block component for this interaction (HSD ⩾ 0.22 for all). Inspection of the data indicated a decrease in

$VI_{500\ ms}$ between blocks 2 and 3 following the high dose of antisense (block 2, 0.56 ± 0.04; block 3, 0.28 ± 0.08) and an increase in $VI_{25\ ms}$ for these same blocks at this dose (block 2, –0.01 ± 0.15; block 3, 0.32 ± 0.08). Additional analysis of the effects of block upon performance measures revealed both a significant interaction between the effects of block and signal length upon the relative number of hits (*h*: $F_{3.06,18.36} = 3.67$, $P = 0.03$; 25 ms signal: block 1 vs. block 2, Tukey's HSD = 0.07; block 1, 51.1 ± 3.6%; block 2, 41.2 ± 3.5%; all other HSDs ⩾ 0.131), as well as a significant interaction among the effects of dose, block and signal length on this measure ($F_{9.71,58.26} = 2.09$, $P = 0.04$). This latter interaction reflected a block-associated decrease in hits to 25 ms signals following antisense infusions (25 ms signal, block 1 vs. block 2, Tukey's HSD = 0.035; missense, block 1, 63.3 ± 7.8%; missense, block 2, 44.9 ± 6.5%; 8 nmol antisense, block 1, 35.8 ± 7.9%; 8 nmol antisense, block 2, 22.5 ± 6.9%). These effects of time on task upon the relative number of hits occurred in the absence of any findings associated with effects of block upon the relative number of correct rejections (cr, $P \geqslant 0.07$ for all), and were likewise noted without accompanying effects of block upon the measure of *SB* ($P \geqslant 0.69$ for all). Finally, while there was no interaction present at this time-point between the effect of dose and block upon the number of omissions ($P = 0.62$), an effect of time on task was found on the number of omissions ($F_{2,12} = 7.86$, $P = 0.007$), reflecting a tendency for the animal to omit a slightly greater number of trials in the final block of the task as compared with the first block of the task, though *post hoc* analysis did not substantiate this finding (HSD ⩾ 0.28 for all; block 1, 4.08 ± 0.54; block 3, 5.51 ± 0.76).

48 h following the third infusion

Analysis of the sustained attention performance data collected 48 h following the third infusion of ODNs indicated no effect of dose upon any of the behavioural measures recorded (*VI*, relative numbers of hits and correct rejections, *SB* and omissions, $P > 0.40$ for all; see Fig. 3). The characteristic effect of signal length upon *VI* was again observed at this time-point ($F_{1.45,8.70} = 37.23$, $P < 0.001$), with *VI* values decreasing concomitantly with decreases in signal length ($VI_{500\ ms}$, 0.59 ± 0.01; $VI_{50\ ms}$, 0.45 ± 0.02; $VI_{25\ ms}$, 0.34 ± 0.02). Data collected for this period exhibited no effects either of time on task upon sustained attention performance, or interactions among the effects of dose and block, block and signal length or dose, block and signal length (*VI*: $P \geqslant 0.49$ for all). The analysis conducted at this time-point indicates the transient nature of the effects of antisense infusions (see Fig. 4).

Histological analyses

NMDA-NR1 immunohistochemistry

Immunohistochemical analyses of the effects of missense as compared with antisense ODN infusions revealed extensive suppression of NR1-IR exclusively in response to antisense infusions (all animals exhibiting > 85% suppression of NR1-IR following antisense ODN administration; see Figs 5 and 6). An almost complete loss of positively labelled cells was particularly apparent in the substantia innominata, with sections depicting the substantia innominata ventral to the infusion with complete loss of positive label. The decrease in NR1-IR extended into the less densely labelled population of cells in the globus pallidus lateral to the infusion and, less prominently, into the horizontal nucleus of the diagonal band. The extent of loss of IR along the anterior–posterior gradient was rather circumscribed and the spatial extent of antisense-induced suppression of NR1-positive label was akin to areas of maximal functional reactivity that have been described in Peciña & Berridge (2000), a study addressing a different compound and infusion site, but involving similar infusion volumes (0.5 μL) and the same rate of infusion (0.5 μL/min). These observations support the efficacy of the infusions of antisense, and they correspond with the hypothesis that the attentional effects of antisense infusions were due to loss of NMDAR functions in the substantia innominata.

Fluoro-Jade fluorescence

Fluoro-Jade staining did not indicate neurotoxic damage in the substantia innominata following the infusion of antisense, missense or saline. As illustrated in Fig. 7, Fluoro-Jade fluorescence was present in a restricted region immediately proximate to the tip of the guide cannula in all hemispheres irrespective of treatment, and this label did not extend into the region of the substantia innominata where the antisense-induced suppression of NR1-IR was observed. As the internal cannula were flush with the guide cannula length, the presence of this positive label suggested modest degeneration was induced by aspects of the infusion process itself (e.g. cannula movement, perfusion of the solution) and may reflect blood–brain barrier disruption associated with cannula insertion that may persist for up to 28 days (Groothuis *et al*., 1998).

Discussion

The present experiment demonstrated that infusions of antisense ODNs into the basal forebrain resulted in a selective decrease in the relative number of hits in animals performing a sustained attention task. The performance of animals engaged in a cued discrimination task that did not involve implicit demands on attentional processes was unaffected by antisense infusions, and intrabasalis infusions of missense remained without effect. The effects of antisense ODN infusions reached maximum levels, as suggested by previous studies (Standaert *et al*., 1996), 24 h following the third infusion, and these effects were fully attenuated 48 h following this third, final infusion. Separate experiments demonstrated antisense ODN-induced suppression of NR1-IR in the substantia innominata corresponding to the time-point when attentional performance was impaired. Missense infusions did not suppress NR1-IR, and neither antisense nor missense infusions produced neurotoxic effects in the substantia innominata as indicated by the Fluoro-Jade method. The discussion later focuses on the nature of the performance impairment and integrates the present data into a larger literature on the role of basal forebrain glutamatergic afferents in the regulation of attentional performance and cortical ACh release.

The fact that infusions of antisense ODNs resulted in impairments in animals performing the sustained attention task but not the cued discrimination task clearly indicates that only the former depended on the integrity of basal forebrain NMDA receptors. This dissociation corresponds with earlier studies assessing the effects of GABAergic manipulations in the basal forebrain or task-associated cortical ACh release (see earlier references). Moreover, infusions of the NMDA receptor antagonist APV into the basal forebrain was recently observed to impair sustained attention performance (Turchi & Sarter, 2001). If the effects of antisense ODNs were due to a general disruption of operant performance, including motivational processes, motor or perceptual functions, the effects of the antisense infusions should have been manifested in animals performing either task; however, this was clearly not the case. Both tasks required the animals to press levers and to monitor visual stimuli; the sustained attention task differed from the cued discrimination task by requiring

the detection of variable and brief signals, the discrimination between signals and nonsignals, and the processing of the propositional rules governing the selection of the lever following a signal and a blank (for an examination of the construct validity of these variables see McGaughy & Sarter, 1995). The present data suggest that such demands on attentional processes specifically activated basal forebrain NMDAR, further supporting the hypothesis that basal forebrain circuits mediate attentional processes (Sarter & Bruno, 1997; Sarter *et al*., 2001).

The finding that infusions of antisense ODNs selectively impaired the animals' ability to detect signals but not the rejection of nonsignals suggests that the effects of antisense ODN infusions cannot be attributed to an impairment in the processing of the task rules. Furthermore, the detrimental effects of antisense ODN infusions interacted with the effects of time on task (or block), adding support for the hypothesis that the effects reflected impairments in sustained attentional processing. Although the present data cannot be specifically attributed to the suppression of NMDAR-mediated activation of corticopetal cholinergic neurons, the pattern of the behavioural effects corresponds very closely with that following the loss of cortical cholinergic inputs (McGaughy *et al*., 1996). The selective effects of antisense infusions on the detection of signals agrees with the extensively documented role of cortical ACh in the selection and amplification of cortical sensory stimulus processing (e.g. Tremblay *et al*., 1990; Murphy & Sillito, 1991; Metherate & Ashe, 1993). Speculation that the present results were due mainly to suppression of NMDAR-mediated signalling in basal forebrain cholinergic neurons is also supported by recent findings indicating that loss of noncholinergic, parvalbumin-positive basal forebrain neurons spares the animals' ability to detect hits but increases their false alarm rates (Burk & Sarter, 2001).

The results from the immunohistochemical portion of the experiment were based on the same concentration and volume as that found to produce attentional impairments, and the time-point selected for perfusion corresponded with the time at which these impairments were observed. The results indicate the selectivity of antisense-induced suppression of NR1-IR. Furthermore, they suggest that the maximum suppression of NR1-IR occurred in the substantia innominata, where predominantly prefrontal glutamatergic afferents have been shown to terminate and contact neurons that innervate primarily isocortical regions (Carnes *et al*., 1990; Gaykema *et al*., 1991; Woolf, 1991; Záborszky *et al*., 1997). As infusions of NMDAR antagonists into this area block increases in cortical ACh release (e.g. Rasmusson *et al*., 1996), it is conceivable that antisense ODN-induced suppression of NMDAR synthesis profoundly reduced cortical cholinergic output.

As it is hypothesized that the suppression of NR1 subunits, and consequently functional NMDAR synthesis, in the substantia innominata markedly attenuated the ability of prefrontal glutamatergic inputs to modulate basal forebrain neurons via NMDAR, the specific component of the attentional processing mediated via these inputs deserves speculation. The selective processing of task-relevant stimuli in attention-demanding situations depends on top-down functions, the efficacy of which appears to depend crucially on prefrontal functioning (e.g. Knight *et al*., 1995; Smith & Jonides, 1999; Fuster, 2000); these top-down functions serve to propagate the processing of relevant inputs in sensory and sensory-associational areas, and suppress activity in areas processing other modalities (e.g. Desimone & Duncan, 1995; Treue & Maunsell, 1996; Shulman *et al*., 1997; Hopfinger *et al*., 2000; Kastner & Ungerleider, 2000; Reynolds *et al*., 2000). Furthermore, such top-down functions organize the allocation of processing resources to competing inputs and tasks (e.g. Iidaka *et al*., 2000). The basal forebrain corticopetal projection system has been hypothesized to be activated via glutamatergic projections of the prefrontal cortex to the substantia innominata, to mediate crucial aspects of such top-down processes, including the amplification of relevant sensory input processing (references cited earlier), as well as the regulation of processing resources (Turchi & Sarter, 1997, 2000). This hypothesis corresponds with the finding that performance of a sustained attention task under increased demands on attentional processing, produced by the presentation of a distracter, is associated with increases in cortical ACh release (Himmelheber *et al*., 2000) and with ACh-mediated neuronal activity in the prefrontal cortex (Gill *et al*., 2000). The hypothesis is further substantiated if, as suggested by Záborszky, *et al*. (1997), the prefrontal innervation of basal forebrain neurons is topographically precise, perhaps even involving different signalling mechanisms connecting prefrontal inputs to separate groups of corticopetal projections, because such circuits could directly modulate cortical information processing in a modality- and task-specific fashion. Based on this conceptual framework, the present results suggest that suppression of the expression of basal forebrain NMDAR impairs the function of basal forebrain corticopetal projections to mediate the task-specific processing of relevant stimuli and associations in the cortex.

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Abbreviations

ACh, acetylcholine; ANOVA, analysis of variance; BSA, bovine serum albumin; cr, correct rejection; FJ, Fluoro-Jade; IR, immunoreactivity; h, hit; NMDA, *N*-methyl-D-aspartate; ODN, oligodeoxynucleotide; PB, phosphate buffer; PBS, phosphate-buffered saline; PDE, phosphodiester; *SB*, side bias; Tukey's HSD, Tukey's highly significant difference; *VI*, vigilance index.

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