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Activation of *N*-methyl-D-aspartate receptors in the feline retrorubral nucleus elicits orofacial dyskinesia

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Abstract

Stimulation of dopamine receptors within a circumscribed subregion of the feline caudate nucleus, that is the anterodorsal part of this nucleus, with dopamine or the dopamine receptor agonist (3,4-dihydroxyphenylimino)-2-imidazoline (DPI) elicits orofacial dyskinesia. Orofacial dyskinesia is a syndrome of tic-like contractions of the facial muscles which ends with a tongue protrusion. Afferent fibres of the anterodorsal part of the caudate nucleus are known to emanate from the retrorubral nucleus, including the dopaminergic A8 cell group. The present study was undertaken to investigate whether excitation of A8 cells can mediate and/or modulate orofacial dyskinesia. For this purpose, the activity of the retrorubral nucleus was manipulated with local injections of *N*-methyl-D-aspartate (NMDA). These local injections into the retrorubral nucleus were subsequently combined with manipulations of the dopamine transmission in the anterodorsal part of the caudate nucleus with local injections of DPI. The present study shows that injections of NMDA into the retrorubral nucleus elicits orofacial dyskinesia. This effect is dose-dependent, NMDA-specific, and inhibited by intra-caudate injections of DPI.

Keywords: Retrorubral nucleus; Caudate nucleus; (Cat); Orofacial dyskinesia; Dopamine; NMDA (*N*-methyl-D-aspartate)

1. Introduction

Orofacial dyskinesia in man is a syndrome of involuntary movements of the tongue, lips and facial muscles. Its manifestation include protrusions of the tongue and abnormal movements of the tongue in the oral cavity (Jeste and Caligiuri, 1993; Sigwald et al., 1959). This syndrome is one of the most severe side-effects of chronic treatment with neuroleptics (dopamine receptor antagonists) or L-DOPA (dopamine precursor), although it appears in unmedicated elderly people as well (for review, see the report of Waddington, 1989). It has been hypothesized that orofacial dyskinesia is due, at least in part, to alterations in dopaminergic processes (Klawans et al., 1980), although the simple notion that it merely reflects supersensitivity of dopamine receptors is no longer tenable (Cools, 1983; Ellison and See, 1989; Jenner and Marsden, 1986; Waddington, 1990).

The symptoms of orofacial dyskinesia, especially tongue protrusions, can be elicited acutely by local injections of dopamine or the dopamine receptor agonist (3,4-dihydroxyphenylimino)-2-imidazoline (DPI; Cools et al., 1976; Spooren et al., 1991a) into a circumscribed subregion of the feline caudate nucleus, that is the anterodorsal part of this nucleus. DPI is a potent receptor agonist of the so-called DAi (IPSP-inducing) dopamine receptor subtype (Cools et al., 1976; Struyker Boudier et al., 1975), according to the original dopamine receptor subtype classification of Cools and van Rossum (1976). For similarities and dissimilarities between receptors of this receptor classification and the more recently discovered dopamine receptor subtypes, see the report of Cools et al. (1989b). As described elsewhere (Cools et al., 1978), the anterodorsal part of the caudate nucleus is, amongst others, characterized by the presents of DAi receptors. A full-blown orofacial dyskinesia attack, evoked from the anterodorsal part of the feline caudate nucleus, consists of successive dyskinetic movements of the ear-, eye- and cheek muscles. An attack always ends with an abnormal tongue protrusion. Spooren et al. (1991a,b, 1993) have demonstrated that

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orofacial dyskinesia elicited from the anterodorsal part of the caudate nucleus is funnelled via the subcommissural part of the globus pallidus/extended amygdala and subthalamic nucleus/lateral hypothalamic area to lower order output stations. In addition to this efferent pathway, Spooren et al. (1991a) have also demonstrated that afferent fibres of the anterodorsal part of the caudate nucleus emanate from the retrorubral nucleus, including the A8 cell group. On the basis of these findings, it was hypothesized that the dopaminergic retrorubral A8 cell group forms part of the circuitry that directs and/or modulates orofacial dyskinesia. Indeed, a recent pharmacobehavioural study has shown that inhibition of $GABA_A$ receptors in the retrorubral nucleus enhances orofacial dyskinesia elicited by the dopamine DAi receptor agonist DPI injected into the anterodorsal part of the caudate nucleus (Arts et al., 1998).

The present study was undertaken to investigate whether excitation of A8 cells can mediate orofacial dyskinetic behaviour. For this purpose, the activity of the retrorubral nucleus was manipulated with local injections of *N*-methyl-D-aspartate (NMDA). NMDA receptor activation was chosen for two reasons. First, it has been shown in the raccoon that the A8 cell group in the retrorubral nucleus receives a glutamatergic projection from the supplementary motor cortex (Sakai, 1988). Second, NMDA receptor activation is known to enhance the release of dopamine from nerve terminals of nigro-striatal dopaminergic neurons (Kornhuber et al., 1984; Westerink et al., 1992). In order to investigate to what extent the effects that are elicited by activation of NMDA-receptors in the A8 cell region are related to dopaminergic processes in the terminal areas of A8 fibres in the caudate nucleus, local injections of NMDA into the retrorubral nucleus were combined with DPI injections into the anterodorsal part of the caudate nucleus. The susceptibility to dopamine and DPI is known to vary across the year: it is high during the months of March, April, August and September, and low during the remaining part of the year. It has been suggested that susceptibility further decreases as the release of dopamine increases (Cools et al., 1978). As discussed elsewhere in detail (Cools et al., 1987), a high synaptic release results not only in a low susceptibility of postsynaptic receptors, but also in a high susceptibility of presynaptic receptors. Because injections of NMDA into the retrorubral nucleus where expected to enhance the release of dopamine in the anterodorsal part of the caudate nucleus, we hypothesized that stimulation of the dopaminergic presynaptic receptors in the anterodorsal part of the caudate nucleus would inhibit the behavioural effects of NMDA, when given during periods marked by a high synaptic release of dopamine.

The present study shows that local injections of NMDA into the retrorubral nucleus elicits orofacial dyskinetic behaviour. This effect is dose-dependent, NMDA-specific, and inhibited by intracaudate injections of DPI.

2. Materials and methods

2.1. Subjects and apparatus

Intact male cats ($n = 8$, Central Animal Laboratory, University of Nijmegen), weighing 4.2–5.1 kg, and 13–16 months of age were used during the experiment. They were housed together in a room ($2 \times 3 \times 2$ m) with a 12-h day/night cycle (lights on at 0630–1830 h). Food (Hope Farm) and water were available ad libitum.

The experiments were performed in a soundproof observation cage ($90 \times 60 \times 60$ cm), with a transparent front panel to allow visual analysis. The cage was equipped with two ventilators which produced a constant background noise.

All experiments were performed according to international, national, and institutional guidelines for animal experimentation.

2.2. Surgery

Cats were anaesthetized with a mixture of O_2, N_2O and 3–3.5% Ethrane® (Abbot, the Netherlands) and subsequently tracheally intubated to maintain anaesthetic levels of gasses during the operation. Atropini sulfas (0.5 mg; Pharmachemie, Haarlem, the Netherlands), which stabilizes cardiac rhythm and prevents abundant secretion of saliva, and the antibiotic albipen (150 mg ampicillin; Mycofarm, De Bilt, the Netherlands) were injected i.m. and s.c., respectively.

Stainless steel guide cannulas (o.d. = 0.80 mm) were bilaterally implanted into the anterodorsal part of the caudate nucleus (coordinates: anterior = 18.0, lateral = 5.0, ventral = 5.3 mm, with respect to the interaural point; according to Arts et al., 1998; Snider and Niemer, 1964) and into the retrorubral nucleus (coordinates: anterior = 2.0 and 10°, tip pointing caudally, lateral = 3.0, ventral = (−)5.75 mm, with respect to the interaural point; according to Arts et al., 1998; Snider and Niemer, 1964). To avoid tissue damage, the tip of the cannulas were placed 0.5–1.0 mm above the anterodorsal part of the caudate nucleus and 1.5–2.0 mm above the retrorubral nucleus. Cannulas were fixed onto the skull with dental acrylic cement (Palladur powder and liquid; Kulzer, Wehrheim) and supportive stainless screws.

The incisions were disinfected with chlortetracycline HCl (AUV, Cuijk, the Netherlands). During recovery from the operation the wounds were treated with Acederm (Ace Veterinary Products, Maarssen, the Netherlands).

2.3. Habituation and experimental set-up

2.3.1. Habituation

After at least 1 week of recovery from the operation, the animals were habituated to the observation cage during

three 60-min sessions on consecutive days. On the second habituation day, the inner cannulas were removed and replaced after 30 min ($t = 30$). On the third habituation day, each cat received bilateral sham injections into the target areas, according to the procedure described below.

2.3.2. Experimental set-up

Cats were tested once a week in a fixed order, according to the procedure described earlier (Arts et al., 1998). The inner cannulas were removed, and the cat was placed in the observation cage ($t = 0$). Both the pre-injection period (preceding the two sets of bilateral injections), and the post-injection period (following the two sets of bilateral injections) lasted 30 min. Intra-caudate injections ($t =$ 30 min) were followed by local injections into the retrorubral nucleus ($t = 40$ min). Injections were given by means of a 5-μl Hamilton syringe (needle o.d.: 0.45 mm, blunt tip). The substances were injected at a speed of 0.5 μl/10 s. After each injection the needle was kept in situ for another 10 s. Thereafter, the inner cannulas were replaced.

Behaviour, recorded via a closed television-circuit, was analyzed with the help of The Observer® (Noldus Information Technology, Wageningen, the Netherlands).

2.4. Dependent variables

In case a full-blown orofacial dyskinesia attack was elicited it consisted of sudden attacks of tic-like contractions involving the ear-, eye- and cheek muscles, which were accompanied by tongue protrusions. Two variables which indicate the start and end of an orofacial dyskinesia attack, namely dyskinetic behaviour of ear muscles and tongue protrusions, respectively, were chosen from a standardised ethogram in order to evaluate quantitatively the elicited orofacial dyskinesia. This ethogram was based on previous findings in which the orofacial dyskinesia was studied in detail (Cools et al., 1989a; Spooren et al., 1991b).

2.4.1. Tongue protrusions

2.4.1.1. Overall number of tongue protrusions. Since each attack has been shown to end with a tongue protrusion (see Section 1; Cools et al., 1976; Spooren et al., 1989), the overall number of tongue protrusions was used to quantify orofacial dyskinesia. Tongue protrusions aimed at an object or the subject itself were not incorporated in the analysis. The overall number of tongue protrusions were subdivided into normal and abnormal tongue protrusions where appropriate.

Normal. A normal tongue protrusion consists of a protrusion of the flat tongue.

Abnormal. An abnormal tongue protrusion implies a variety of movements: a curling upwards of the lateral sides of the tongue and then protruding the curled tongue via the left or right corner of the mouth; a curling upwards and inwards of the tip of the tongue against the palatum and then protruding; and pressing the tongue against the inner side of the cheek and then protruding.

2.4.2. Ear twitch

An ear twitch consists of a burst of tic-like contractions of the ear; the ear wiggles as if tickling or irritation takes place. The movements are independent of an auditory stimulus or contact with an object. The number of ear twitches were analyzed where appropriate.

2.5. Drugs

The following drugs and combination of drugs were injected: (1) NMDA receptor agonist NMDA (dose: 25, 100, 250 or 500 ng; RBI, Natick, USA), or the NMDA receptor antagonist AP-5 (dose: 50 ng; RBI) into the retrorubral nucleus in order to investigate the potency of glutamatergic agents to elicit orofacial dyskinesia. (2) NMDA (250 ng) and AP-5 (50 ng) in a cocktail injected into the A8 cell group in order to investigate the specificity of the drug induced effects. (3) DAi receptor agonist DPI (dose: 10 μg/5 μl; Boehringer Ingelheim, Germany) into the anterodorsal part of the caudate nucleus in order to elicit orofacial dyskinesia. (4) Intracaudate injections of DPI (10 μg/5 μl) in combination with NMDA (25 or 500 ng/0.5 μl) injections into the retrorubral nucleus in order to investigate the possible modulatory role of the retrorubral nucleus on the dopamine activity in the anterodorsal part of the caudate nucleus. The injections into the A8 cell group (experiments 1 and 2) were always combined with injections of the solvent of DPI, namely distilled water, into the anterodorsal part of the caudate nucleus in order to provide adequate controls for experiment 4; likewise, the injections into the anterodorsal part of the caudate nucleus were combined with the solvent of NMDA, namely distilled water, into the retrorubral nucleus. The experiment started with the injections of the vehicle of the drugs. The intracaudate injection volume was 5 μl per site, whereas the volume injected into the retrorubral nucleus was 0.5 μl per site. The dosage of DPI was chosen in view of the fact that it has been shown to be the most potent dosage for inducing orofacial dyskinesia when injected into the anterodorsal part of the caudate nucleus during the months of March, April, August and September (Cools et al., 1976; Spooren et al., 1991a). The present experiment was performed in a period of relative insusceptibility to DPI (November and December). The solutions were stored at 4°C. At least 30 min prior to injection, the solutions were adjusted to room temperature.

2.6. Histological verification

After the final experiment, the cats were injected with pentobarbital (Narcovet, 60 mg/kg i.p. and subsequently,

15 mg/kg i.v.; Apharma, Arnhem, the Netherlands). Thereafter, the animals were transcardially perfused with saline followed by a fixative containing 0.05% glutaraldehyde and 4% paraformaldehyde dissolved in 0.1 M phosphate buffer (PB; pH 7.4). The dissected brains were kept on a 30% sucrose solution in PB for at least 48 h and

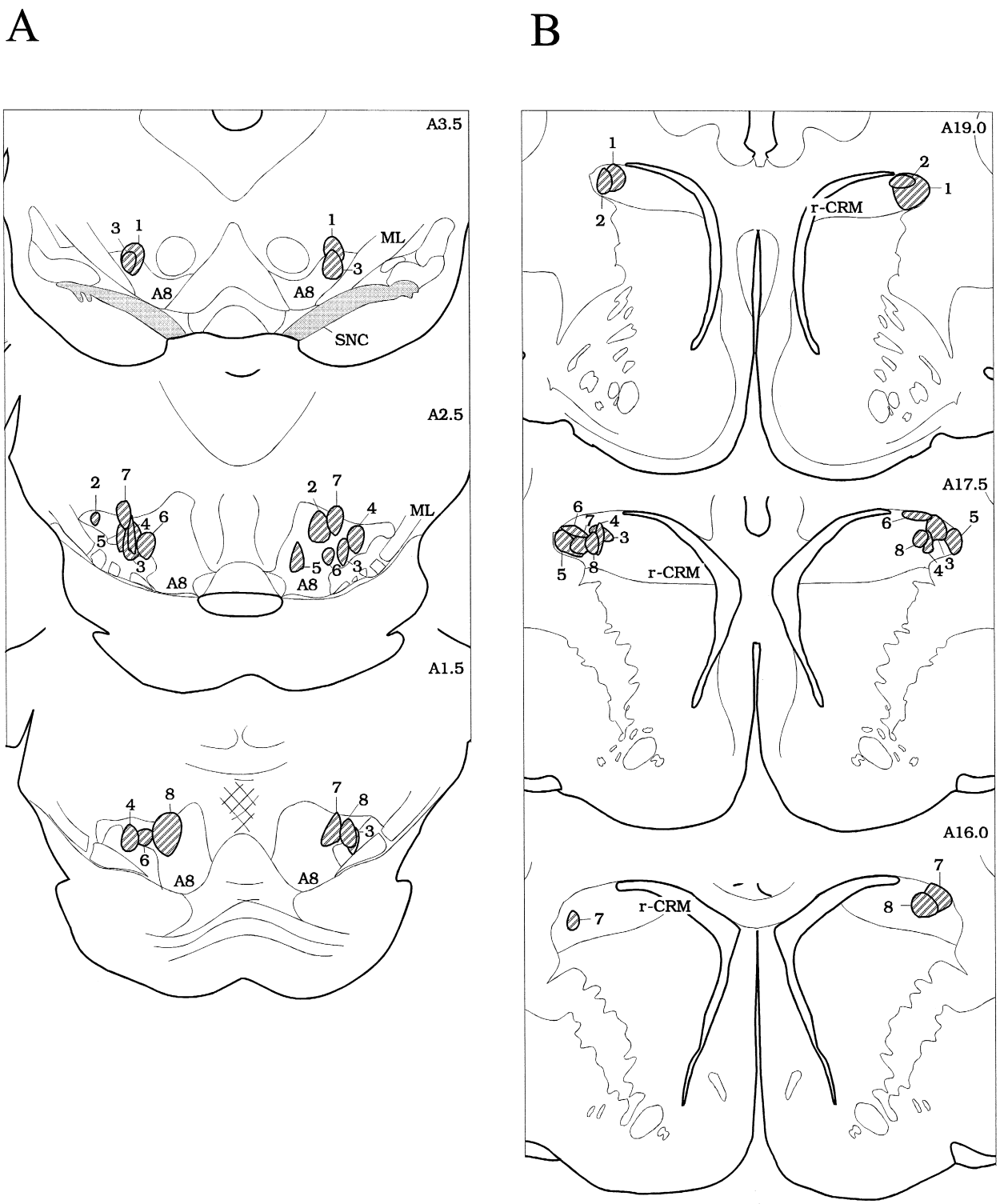

Fig. 1. The schematic drawings of transverse sections, ordered from rostral (top) to caudal (bottom), showing the bilateral injection sites in individual cats ($n = 8$) in the retrorubral nucleus (A) and the anterodorsal part of the caudate nucleus (B). Abbreviations: A8, A8 cell group; ML, medial lemniscus; r-CRM, anterodorsal part of the caudate nucleus; SNC, substantia nigra pars compacta.

thereafter cut on a freezing microtome into 70-μm sections. Sections were mounted and subsequently stained with cresyl violet.

2.7. Statistical analysis

Given the large inter-individual variation in orofacial behaviours, delta values were calculated by subtracting the number of scored tongue protrusions (or ear twitches) in the 30 min pre-injection period from the number of scored tongue protrusions (or ear twitches) in the 30-min post-injection period. These delta values, which are normally distributed, were statistically analyzed, using the paired *t*-test. A probability level of $P < 0.05$ was considered statistically significant. Dose-response effects were analyzed with the help of the GLM-repeated measures analysis of variance (ANOVA; SPSS), followed by the post-hoc comparison using the paired *t*-test, which was adjusted for multiple testing with the Bonferroni technique. Therefore, only a probability level of $P < 0.0125$ was considered statistically significant.

3. Results

3.1. Histological verification

Histological verification of the injection sites revealed that all injections were placed within the dorsal region of the retrorubral nucleus or within the anterodorsal part of the caudate nucleus. The distribution of the injection sites is schematically illustrated in Fig. 1A and B. An example of a representative injection site in the retrorubral nucleus and anterodorsal part of the caudate nucleus is provided in Fig. 2.

3.2. Dyskinetic behaviours

Tongue protrusions consisted of both normal and abnormal tongue movements. Occasionally ear twitches were seen prior to an abnormal tongue protrusion.

In case a full-blown orofacial dyskinesia attack was elicited, it consisted of tic-like contractions of one ear,

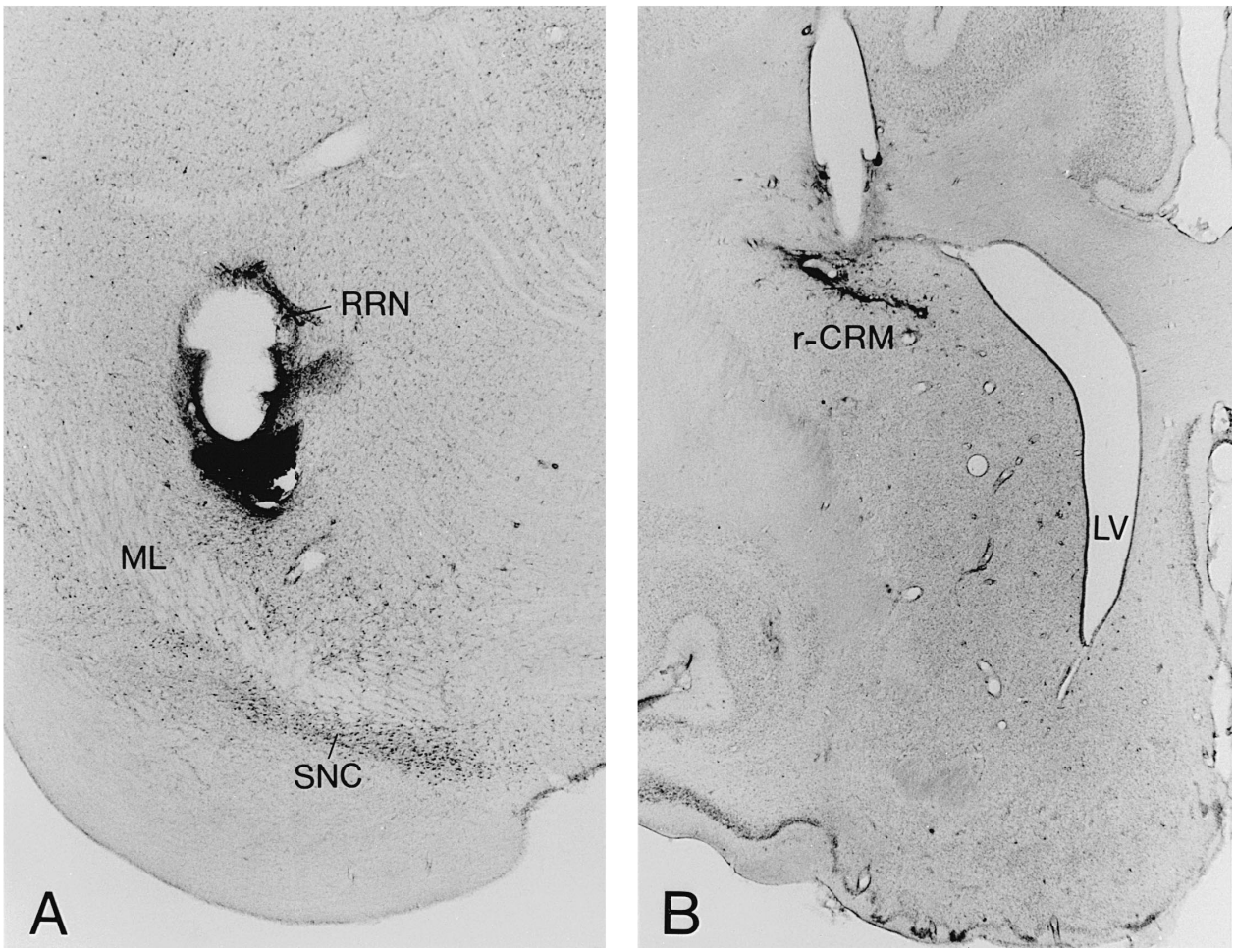

Fig. 2. Photomicrograph of (A) a representative location of an injection site in the retrorubral nucleus, and (B) a representative location of an injection site in the anterodorsal part of the caudate nucleus, including the cannula-tip directed to this region. Abbreviations: A8, A8 cell group; ML, medial lemniscus; r-CRM, anterodorsal part of the caudate nucleus; SNC, substantia nigra pars compacta.

which was moved back and forth with high frequency, and subsequently of the ipsilateral eye, which was quickly opened and closed or kept half closed, whereas the cheek displayed fast contractions in various degrees of intensity. An attack always ended with an abnormal tongue protrusion.

3.3. Drug-induced effects

3.3.1. Overall number of tongue protrusions

NMDA (25, 100, 250 and 500 ng) bilaterally injected into the retrorubral nucleus, increased the number of tongue protrusions (ANOVA: $F(4,28) = 4.86$; $P < 0.005$). As shown in Fig. 3A, a bell-shaped curve was found, with effects of NMDA (250 ng) and NMDA (500 ng) injections greater than control injections (solvent/solvent vs. solvent/NMDA; 250 ng: $t(1,7) = 4.82$; $P < 0.005$, 500 ng: $t(1,7) = 4.02$; $P < 0.005$). AP-5, which per se was ineffective in the dose tested, significantly decreased the NMDA-induced increase in the number of tongue protrusions (saline/NMDA (250 ng) and AP-5 (50 ng) vs. saline/NMDA (250 ng): $t(1,7) = -2.84$; $P < 0.05$).

Bilateral injections of DPI into the anterodorsal part of the caudate nucleus significantly increased the number of

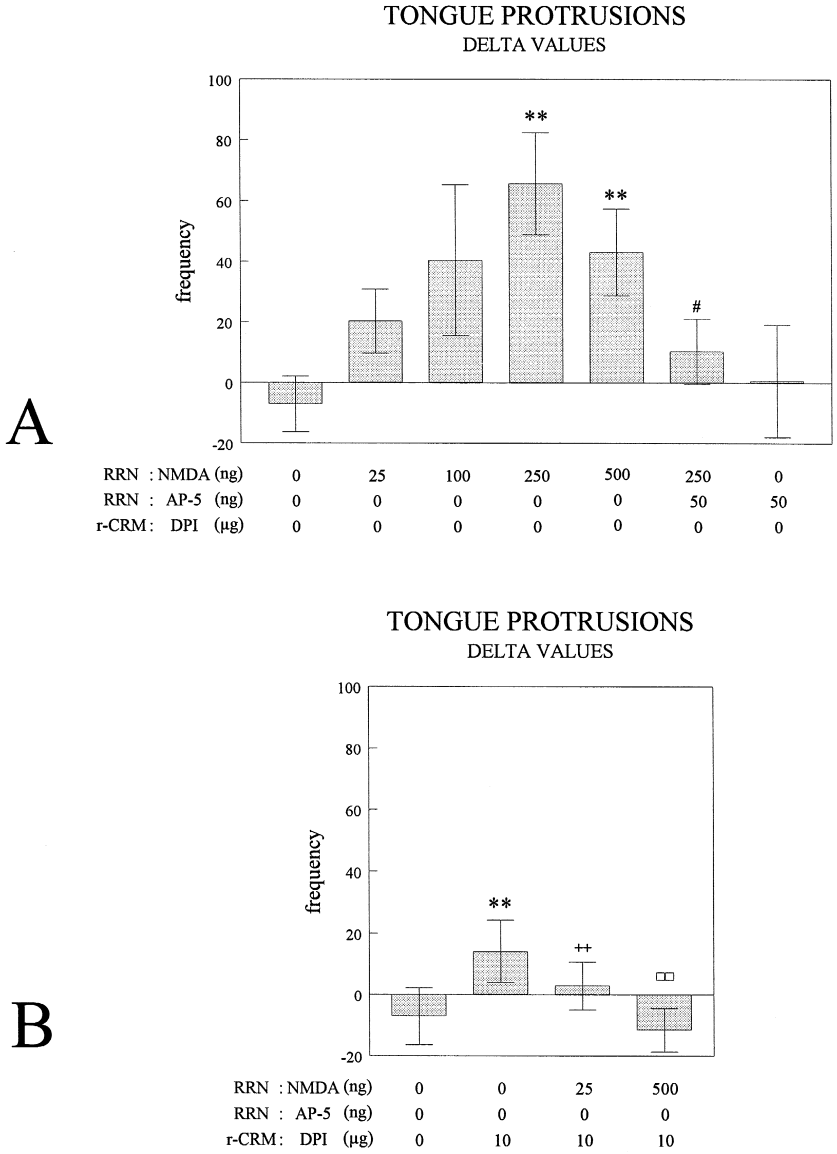

Fig. 3. Delta values of the number of tongue protrusions 30 min after (A) local injections of solvent, NMDA (25, 100, 250 and 500 ng), AP-5 (50 ng) and a cocktail of 250 ng NMDA and 50 ng AP-5 into the retrorubral nucleus in combination with solvent into the anterodorsal part of the caudate nucleus, and (B) solvent (25 and 500 ng) NMDA into the retrorubral nucleus in combination with DPI into the anterodorsal part of the caudate nucleus. Represented are mean values and standard errors of the mean. Abbreviations: r-CRM, anterodorsal part of the caudate nucleus; RRN, retrorubral nucleus. Differences indicated: ** ($P < 0.005$) as compared to solvent/solvent; # ($P < 0.05$) as compared to solvent/NMDA (250 ng); ++ ($P < 0.005$) as compared to solvent/NMDA (25 ng); □□ ($P < 0.005$) as compared to solvent/NMDA (500 ng).

tongue protrusions (Fig. 3B; DPI/solvent vs. solvent/solvent: $t(1,7) = 5.10$; $P < 0.005$).

Bilateral injections of DPI into the anterodorsal part of the caudate nucleus significantly attenuated the effect induced by retrorubral injections of NMDA (25 ng and 500 ng; solvent/NMDA (25 ng) vs. DPI/NMDA (25 ng): $t(1,7) = 4.39$; $P < 0.005$; solvent/NMDA (500 ng) vs. DPI/NMDA (500 ng): $t(1,7) = -4.71$; $P < 0.005$).

3.3.2. Normal and abnormal tongue protrusions

Bilateral injections of NMDA (250 ng) into the retrorubral nucleus increased the number of normal and abnormal tongue protrusions when compared to their corresponding control values (Fig. 4; solvent/solvent vs. solvent/NMDA (250 ng); normal: $t(1,7) = 5.19$; $P < 0.005$; abnormal: $t(1,7) = -4.15$; $P < 0.005$). Furthermore, the NMDA receptor antagonist AP-5 significantly reduced the number of abnormal tongue protrusions elicited by NMDA (250 ng), whereas the number of normal tongue protrusions showed a non-significant tendency in this respect (Fig. 4; solvent/NMDA (250 ng) vs. solvent/NMDA (250 ng) and AP-5 (50 ng); abnormal: $t(1,7) = -2.83$; $P < 0.05$; normal: *t*-test, n.s.).

Injections of the dopaminergic receptor agonist DPI into the anterodorsal part of the caudate nucleus significantly increased the number of normal tongue protrusions, whereas these did not alter the number of abnormal tongue protrusions (Fig. 4; solvent/solvent vs. DPI/solvent; abnormal: *t*-test, n.s.; normal: $t(1,7) = 3.51$; $P < 0.05$).

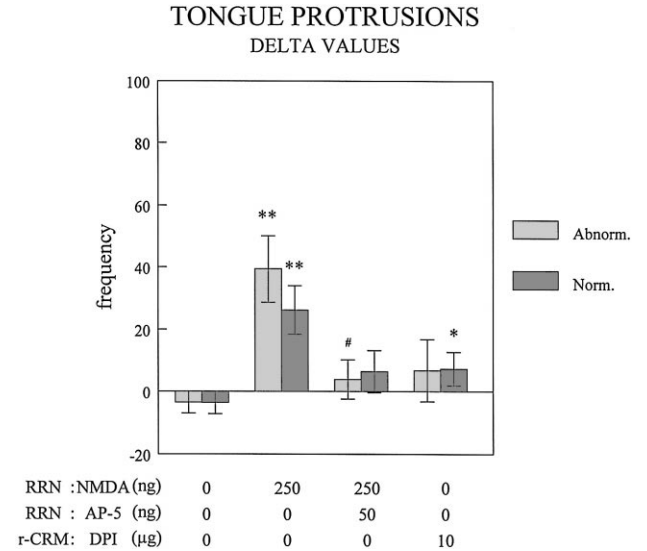

Fig. 4. Delta values of the number of normal and abnormal tongue protrusions 30 min after local injections of solvent, NMDA (250 ng) and the cocktail of NMDA (250 ng) and AP-5 (50 ng) into the retrorubral nucleus in combination with solvent or DPI into the anterodorsal part of the caudate nucleus. Represented are mean values and standard errors of the mean. Abbreviations: r-CRM, anterodorsal part of the caudate nucleus; RRN, retrorubral nucleus. Differences indicated: $*$ ($P < 0.05$) and $**$ ($P < 0.005$) as compared to solvent/solvent; # ($P < 0.05$) as compared to solvent/NMDA (250 ng).

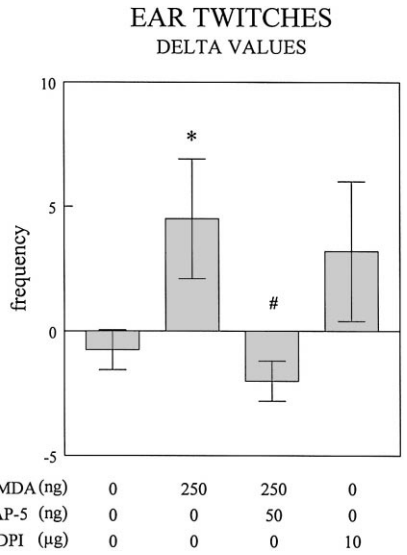

Fig. 5. Delta values of the number of ear twitches 30 min after local injections of solvent, NMDA (250 ng) and the cocktail of NMDA (250 ng) and AP-5 (50 ng) into the retrorubral nucleus in combination with solvent or DPI into the anterodorsal part of the caudate nucleus. Represented are mean values and standard errors of the mean. Abbreviations: r-CRM, anterodorsal part of the caudate nucleus; RRN, retrorubral nucleus. Differences indicated: $*$ ($P < 0.05$) as compared to solvent/solvent; # ($P < 0.05$) as compared to solvent/NMDA (250 ng).

3.3.3. Ear twitches

Local injections of 250 ng NMDA into the retrorubral nucleus significantly increased the number of ear twitches (Fig. 5; solvent/solvent vs. solvent/NMDA (250 ng): $t(1,7) = 2.38$; $P < 0.05$). This effect of 250 ng NMDA was significantly antagonized by 50 ng AP-5. DPI was ineffective in increasing the number of ear twitches (*t*-test: n.s.).

4. Discussion

The present study shows that NMDA receptor stimulation in the feline retrorubral nucleus, including the A8 cell group, results in dyskinetic orofacial movements: the number of ear twitches and number of abnormal tongue protrusions are enhanced. These effects are NMDA-specific. In contrast to these NMDA induced dyskinetic movements, intracaudate administration of DPI increased the number of normal tongue protrusions. Finally, DPI attenuated the NMDA-elicited increase in the number of tongue protrusions.

4.1. Effect of NMDA and AP-5 injections into the retrorubral nucleus

The results from the present study unambiguously show the involvement of the retrorubral nucleus in oral behaviour. These data fits in with our previously reported conclusion that both the retrorubral nucleus and its

dopaminergic, terminal region in the caudate nucleus play a role in the control of orofacial dyskinesia: this conclusion was based on the finding that injections of the GABA receptor antagonist bicuculline into the retrorubral nucleus are able to potentiate the orofacial dyskinesia that is elicited by intra-caudate injections of DPI (Arts et al., 1998). Since the retrorubral nucleus gives rise to dopaminergic A8 fibres which terminate in the anterodorsal part of the caudate nucleus (Jiménez-Castellanos and Graybiel, 1987; Spooren et al., 1991a), the present findings suggest that orofacial dyskinesia is elicited either directly or indirectly, by an excitatory effect of NMDA on A8 cells projecting to the anterodorsal part of the caudate nucleus. The effects of NMDA on orofacial dyskinesia were dose-dependent and NMDA-specific. These finding are in line with the fact that glutamate stimulates dopamine release from neurons projecting to the striatum in in vitro experiments (Chadieu et al., 1994; Grace and Bunny, 1984; Kornhuber et al., 1984; Westerink et al., 1992). Taken together, these data show that activation of NMDA receptors in the feline retrorubral nucleus elicits orofacial dyskinesia.

4.2. Effect of DPI injections into the anterodorsal part of the caudate nucleus

The present study was performed in a period marked by both a relative insusceptibility of the postsynaptic receptors to dopamine and the dopamine DAi receptor agonist DPI and a relatively high susceptibility of presynaptic receptors to dopamine and DPI (November and December; see Section 1). In this case, DPI will diminish the release of striatal dopamine. Indeed, DPI was ineffective in inducing orofacial dyskinesia attacks: only the number of normal tongue protrusions was affected, whereas the number of abnormal tongue protrusions and the number of ear twitches remained unaffected.

4.3. Effect of DPI on NMDA induced effect

Local injections of DPI into the anterodorsal part of the caudate nucleus completely abolished the number of tongue protrusions elicited by local injections of NMDA into the retrorubral nucleus. Given the relatively high susceptibility of presynaptic receptors for dopamine and its receptor agonist DPI in the chosen test period (Cools et al., 1978; Spooren et al., 1991a), DPI is suggested to have decreased the release of dopamine and, accordingly, diminished the capacity of NMDA to enhance the release of striatal dopamine via activation of NMDA-receptors in the A8 cell group. This hypothesis is being investigated in our ongoing study, in which (a) similar experiments are performed during the annual period, in which dopamine and DPI are primarily acting at the postsynaptic level, and (b) the effects of a postsynaptic, dopaminergic receptor antagonist on the NMDA-effects under study are compared with those of DPI in the annual period used in the present study.

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