

that consonants are more difficult to produce than vowels. More importantly, this fact and the fact that error performance depends neither on the sonority value of individual phonemes nor on their feature properties suggest that consonants and vowels are categorically distinct objects at some level of representation even though they are not categorically distinguishable at a phonetic level. This conclusion is consistent with recent experimental work in speech production with neurologically intact speakers, which has shown that phonological encoding operates over segments (consonants and vowels) and not features¹⁴. Evidence consistent with the possibility that consonants and vowels are represented categorically in perception is provided by the results of a study that stimulated the left superior temporal gyrus of patients with implanted subdural electrode arrays¹⁵. Stimulation impaired discrimination of consonants but not vowels. Importantly, the disruptive effect of the stimulation was equal across all consonants tested, independently of their sonority.

The conclusion that consonants and vowels are represented autonomously does not imply that sonority does not play a role in speech production. The sonority gradient plays a crucial role in determining consonant ordering within the onset and coda of a syllable and in determining syllable boundary. Furthermore, sonority has been used to explain various patterns of speech errors in aphasia^{16–18} and it has been shown to play a direct role in the distribution of consonant errors produced by a non-fluent aphasic patient with frontal lobe damage¹³. The contrasting sets of results suggest that sonority and C/V structure information are used at different levels of the speech production process.

Finally, we considered the possible functional motivation for representing consonants and vowels independently and categorically. The distinction between consonants and vowels plays a crucial role in determining the prosodic structure of speech and in the organization of syllables^{16,17}. It has been proposed that syllable structure is not stored directly with our knowledge of the sounds of words but is computed 'on-line' during speech^{19,20}. This on-line process is necessary because the domain of syllabification is not the lexical word (where syllable structure might be represented) but the phonological word²¹. As a consequence syllable boundaries often straddle word boundaries—parts of one word are syllabified with parts of an adjacent word. For example, the phrase 'understand it' is syllabified as un-der-stan-dit and not as un-der-stand-it²¹. The independent representation of C/V structure could serve as the basis for this syllabification process by using this information to assign segments to nucleus and non-nucleus positions in a syllable. □

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Correspondence and requests for materials should be addressed to A.C. (e-mail: caram@wjh.harvard.edu) or G.M. (e-mail: g.miceli@mclink.it).

Noradrenaline in the ventral forebrain is critical for opiate withdrawal-induced aversion

J. M. Delfs, Y. Zhu, J. P. Druhan & G. Aston-Jones

University of Pennsylvania School of Medicine, Department of Psychiatry, VA Medical Center (151), University & Woodland Avenues, Philadelphia, Pennsylvania 19104, USA

Cessation of drug use in chronic opiate abusers produces a severe withdrawal syndrome that is highly aversive, and avoidance of withdrawal or associated stimuli is a major factor contributing to opiate abuse^{1,2}. Increased noradrenaline in the brain has long been implicated in opiate withdrawal³, but it has not been clear which noradrenergic systems are involved. Here we show that micro-injection of β -noradrenergic-receptor antagonists, or of an α 2-receptor agonist, into the bed nucleus of the stria terminalis (BNST) in rats markedly attenuates opiate-withdrawal-induced conditioned place aversion. Immunohistochemical studies revealed that numerous BNST-projecting cells in the A1 and A2 noradrenergic cell groups of the caudal medulla were activated during withdrawal. Lesion of these ascending medullary projections also greatly reduced opiate-withdrawal-induced place aversion, whereas lesion of locus coeruleus noradrenergic projections had no effect on opiate-withdrawal behaviour. We conclude that noradrenergic inputs to the BNST from the caudal medulla are critically involved in the aversiveness of opiate withdrawal.

Opiate withdrawal results in marked hyperactivity of central noradrenergic neurons^{3,4}. There is evidence that increased noradrenaline is involved in various aspects of the withdrawal response³, but it has not been determined where and how increased noradrenaline release contributes to the opiate withdrawal syndrome. The bed nucleus of the stria terminalis (BNST), a component of the extended amygdala⁵, has the highest density of noradrenergic inputs in the brain⁶, and is anatomically connected with other brain areas implicated in drug abuse⁵. We therefore hypothesized that the BNST may be an important site for the actions of noradrenaline during opiate withdrawal.

To determine whether noradrenaline afferents to the BNST are stimulated by opiate withdrawal, we injected a retrograde tracer into the BNST and used triple labelling for the tracer, for tyrosine hydroxylase (a marker for noradrenergic neurons), and for Fos-related antigens (FRAs; a marker of cellular activation). Consistent with previous studies⁷, we found numerous retrogradely

labelled tyrosine hydroxylase-immunoreactive neurons in the A1 and A2 cell groups of the caudal medulla (Fig. 1), with fewer also present in the locus coeruleus. Furthermore, as previously reported^{8,9}, we observed a marked increase in FRA expression in tyrosine hydroxylase-containing neurons of these structures following opiate withdrawal.

Quantification of triple-labelled (tracer, tyrosine hydroxylase and FRA) and double-labelled (tracer and tyrosine hydroxylase) neurons in the A1 and A2 regions of morphine-dependent animals confirmed that most of the tyrosine hydroxylase-immunoreactive cells projecting to the BNST expressed FRAs during withdrawal. In morphine-dependent rats that were given naltrexone ($n = 5$), $81.2 \pm 2.5\%$ of the tyrosine hydroxylase-immunoreactive neurons retrogradely labelled from the BNST in the A1 region were FRA-positive (4.9 ± 0.4 triple-labelled neurons per section), whereas, in non-dependent control animals given naltrexone ($n = 5$), only $13.8 \pm 3.0\%$ of the tyrosine hydroxylase-positive BNST afferents were FRA-immunoreactive (0.6 ± 0.1 triple-labelled neurons per section). In the A2 region of morphine-dependent animals, $76.8 \pm 3.5\%$ of the tyrosine hydroxylase-containing neurons retrogradely labelled from the BNST were FRA-positive after naltrexone-precipitated withdrawal (6.0 ± 1.6 triple-labelled neurons per section), whereas, in non-dependent control animals given naltrexone, only $15.8 \pm 2.0\%$ of such cells were FRA-stained (0.7 ± 0.1 triple-labelled neurons per section). Analysis of variance revealed that both the percentages and raw numbers of FRA-positive, noradrenergic BNST afferent neurons were significantly greater in morphine-dependent than in non-dependent animals ($P < 0.001$ each). Most of the brainstem noradrenergic neurons of the A1 and A2 cell groups that project to the BNST are therefore stimulated by opiate withdrawal. Although many tyrosine hydroxylase-positive neurons in the C1 and C2 medullary regions also become FRA-positive after withdrawal^{8,9}, very few neurons in these regions were retrogradely labelled from the BNST.

As well as measuring the classic somatic (physical) signs of withdrawal, we also assessed opiate withdrawal by using a two-chamber place-conditioning procedure to determine aversion induced by withdrawal. Rats avoid an environment previously paired with precipitated opiate withdrawal¹⁰, which provides a

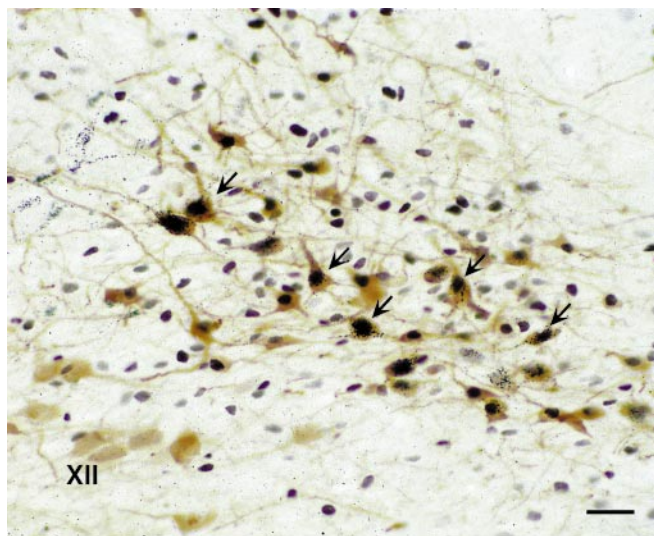


Figure 1 Noradrenergic afferents to the BNST are stimulated by opiate withdrawal. Triple labelling for WGA-gold (small black particles in cell body), FRAs (dark purple-black nuclei) and tyrosine hydroxylase (light brown) in the A2 region of the NTS. Similar results were observed in the A1 and locus coeruleus noradrenergic cell groups, although fewer cells were retrogradely labelled in those areas. Minimal FRA expression was seen in similar sections from control animals (non-dependent saline- and/or naltrexone-injected animals). XII, hypoglossal nucleus. Scale bar, $40 \mu\text{m}$.

measure of the negative affective consequences of withdrawal that contribute to aversively motivated drug seeking².

Ascending axons of the A1 and A2 noradrenergic neurons combine to form the ventral noradrenergic bundle (VNAB), whereas those of the locus coeruleus form the dorsal noradrenergic bundle (DNAB). Because many withdrawal-stimulated, retrogradely labelled noradrenergic neurons projecting to the BNST were found in the A1 and A2 cell groups, we tested whether lesions of the VNAB, produced with 6-hydroxydopamine (6-OHDA), would affect the expression of opiate withdrawal behaviours. VNAB lesions markedly reduced withdrawal-induced place aversion in morphine-dependent rats (Fig. 2a), whereas somatic withdrawal symptoms were not significantly affected (Fig. 2b).

To ensure that VNAB lesions did not impair learning ability, we trained a separate group of drug-naïve animals with VNAB lesions in a conditioned place-aversion task by using footshock rather than opiate withdrawal. In contrast to withdrawal-conditioned animals, VNAB-lesioned rats conditioned with footshock had similar aversion scores to unlesioned controls (control, -376.5 ± 56 s; lesion, -371.5 ± 36 s; $P = 0.94$, Student's *t*-test, $n = 8$ per group). Thus, the VNAB lesion did not generally impair either place learning or aversion, but specifically disrupted withdrawal-induced aversion.

In contrast to the effect of VNAB lesions, destruction of the DNAB with 6-OHDA (Table 1) did not reduce aversive (Fig. 2c) or somatic signs of withdrawal (Fig. 2d). Similarly, lesions of the locus coeruleus noradrenergic system using DSP-4 (50 mg per kg; intraperitoneal; Table 1)¹¹ had no significant effect on withdrawal-induced place aversion (control, -226.4 ± 25 s; lesion, -173.2 ± 38 s; $P = 0.27$, Student's *t*-test, $n = 8$ per group) or somatic signs (data not shown). Thus, consistent with converging lines of evidence^{12,13}, noradrenergic neurons of the locus coeruleus did not seem to be required for aversive or somatic opiate withdrawal symptoms. Instead, our findings indicate that noradrenergic projections from the A1 and A2 cell groups in the medulla are critically

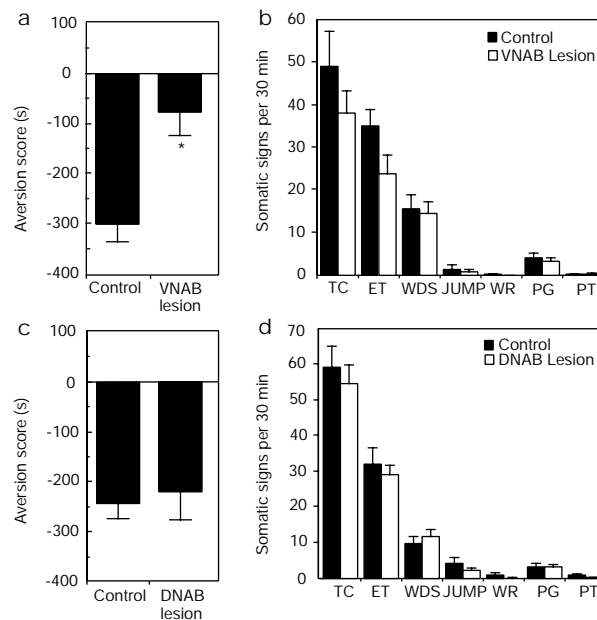


Figure 2 Effects of dorsal (DNAB) and ventral (VNAB) noradrenergic bundle lesions on aversive and somatic signs of opiate withdrawal. **a, c**, Aversion scores are time in the naltrexone-paired side on the test day minus that on the preconditioning day. **b, d**, Number of somatic counts in 30 min. TC, teeth chatter; ET, eye twitch; WDS, wet dog shakes; JUMP, jumping; WR, writhing; PG, penile grooming; PT, paw tremor. Non-opiate-dependent lesioned animals exhibited neither aversion nor somatic signs following naltrexone injection (data not shown). All data are mean \pm s.e.m. ($n = 6-8$ control animals, $n = 10-11$ lesioned animals per group). $*P < 0.05$, analysis of variance followed by Fisher's PLSD test for multiple comparisons.

involved in the development of withdrawal-induced place aversion.

We tested the possibility that the BNST is an important site of noradrenaline action during withdrawal by microinjecting noradrenergic drugs directly into it before withdrawal was precipitated. Microinjection of selective β_1 (betaxolol) and β_2 (ICI 118,551) noradrenergic antagonists into the BNST 5 min before naltrrexone-precipitated withdrawal and place conditioning markedly attenuated conditioned place aversion at 0.1 nmol, and completely eliminated conditioned aversion at 1.0 nmol (Fig. 3a). These effects of the β antagonists were unlikely to result from positive reinforcing effects, as they were administered in both chambers of the conditioning environment. Also, the β -antagonist propranolol has no valence when given alone to morphine-dependent rats¹⁰. Two somatic signs, teeth chattering and eye twitching, were reduced by injection of the β -antagonist cocktail into the BNST (Fig. 3b). Control injections of betaxolol and ICI 118,551 (1.0 nmol) into the overlying striatum (Fig. 4) had no significant effect on aversive (artificial cerebrospinal fluid (ACSF), -220.3 ± 38.5 s; β antagonist, -229.3 ± 44.3 s, $P = 0.88$ Student's t -test, $n = 8$ per group) or somatic signs of withdrawal (data not shown).

To test a second β -receptor antagonist, and to control for a possible local anaesthetic affect of β antagonists, we used isomers of the mixed β_1/β_2 receptor antagonist propranolol. Both isomers have anaesthetic properties, but only the *S* isomer is a β -adrenoceptor antagonist. Infusion of *S*-propranolol (5 nmol) in the BNST eliminated withdrawal-induced conditioned place aversion, whereas the same dose of *R*-propranolol had no effect (Fig. 3c). Similarly, *S*-propranolol significantly reduced teeth chattering and eye twitching, whereas *R*-propranolol had no significant effect on any somatic withdrawal sign (Fig. 3d).

Like β antagonists, the α_2 adrenergic agonist clonidine reduces some symptoms of opiate withdrawal in animals and humans^{14–17}. To determine whether α_2 agonists act within the BNST to reduce

opiate withdrawal, we injected a polar analogue of clonidine, ST-91, into this nucleus. Low doses of ST-91 (0.025–0.25 nmol) significantly attenuated conditioned place aversion compared with ACSF-injected controls (Fig. 3e). Clonidine lacks positive motivational effects when given alone to dependent animals¹⁴, indicating that α_2 agonists do not counteract aversion by producing rewarding effects.

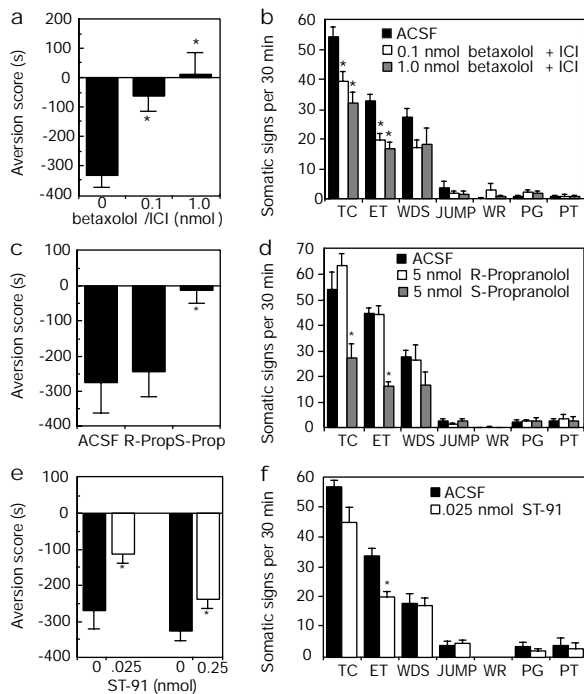


Figure 3 Effects of infusing noradrenergic drugs into the BNST on conditioned place aversion and somatic signs of opiate withdrawal. **a–d**, Effects of the β -antagonist cocktail betaxolol/ICI 118,551 (**a**, **b**) or propranolol isomers (**c**, **d**) on place aversion and somatic signs. **e**, **f**, Effects of ST-91 on place aversion and somatic signs. See Fig. 2 legend for details and abbreviations. All data are expressed as mean \pm s.e.m. ($n = 6–8$ animals per dose). For **a–d**, $*P < 0.05$, analysis of variance followed by Fisher's PLSD for multiple comparisons. For **e**, **f**, $*P < 0.05$, Student's t -test.

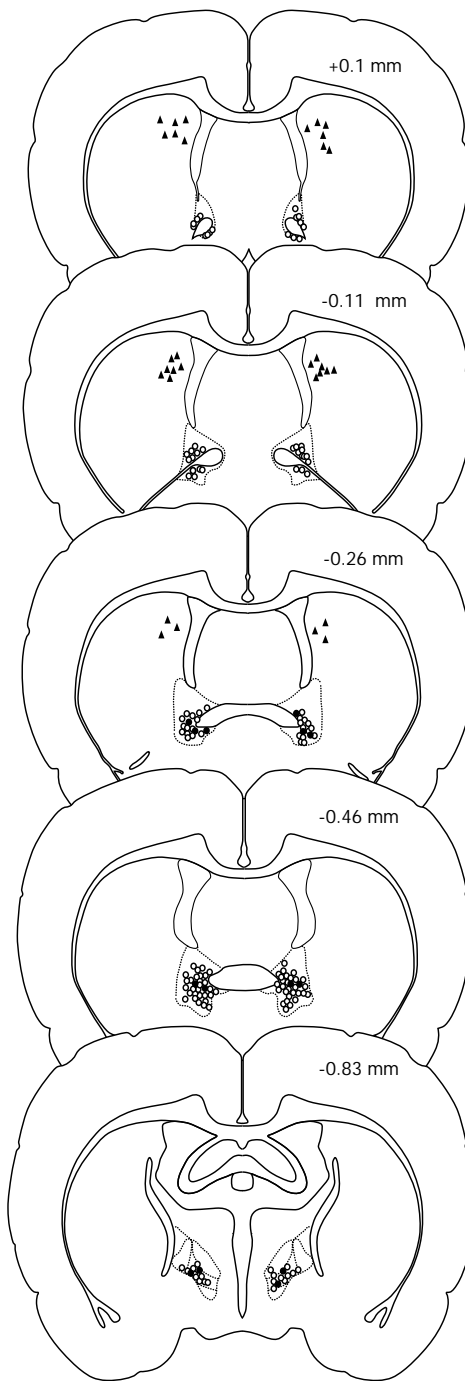


Figure 4 Locations of injection sites. Numbers refer to the distance from the bregma. Effective injections of betaxolol/ICI 118,551, *S*-propranolol and ST-91 are shown by open circles. The combined aversion scores for these compounds at different levels were, from +0.1 mm to -0.83 mm (mean \pm s.e.m. (n)): -136.4 ± 29 (8); -91.2 ± 90.0 (6); -166.7 ± 25.3 (11); 82.6 ± 40.8 (5); -17.0 ± 68.8 (7). These scores were not significantly different ($P > 0.09$; analysis of variance). The trend for greater effectiveness in mid-caudal BNST may indicate an important role for this sub-area. Ineffective injections are denoted by filled triangles (dorsal injections of betaxolol/ICI 118,551) and filled circles (*R*-propranolol). Each symbol represents one cannula tip (0.35 mm diameter). The BNST is denoted by the dotted line.

Table 1 Effect of chemical lesions on ³H-nisoxetine labeled noradrenaline uptake sites in various brain regions

Lesion	Chronic treatment	Binding (%)			
		Cortex	Hippocampus	BNST	Hypothalamus
DNAB (6-OHDA)	Morphine	8.2 ± 2.9*	5.4 ± 1.4*	88.7 ± 8.9	54.0 ± 7.3*
DNAB (6-OHDA)	Placebo	1.7 ± 1.0*	6.1 ± 1.8*	72.9 ± 0.3	57.0 ± 9.4*
LC-NE (DSP-4)	Morphine	55.2 ± 17.0†	33.6 ± 13.9†	91.3 ± 6.1	70.1 ± 9.2†
VNAB (6-OHDA)	Morphine	83.5 ± 13.2	54.7 ± 7.1*	12.2 ± 6.4*	10.7 ± 3.4*
VNAB (6-OHDA)	Placebo	75.1 ± 11.4	84.5 ± 10.7	2.9 ± 1.4*	9.7 ± 2.7*

Data represent binding in non-lesioned animals ± s.e.m. All animals were killed 24 h after the final behavioural test. Note that 6-OHDA lesions of the VNAB markedly reduced noradrenaline uptake sites in the BNST and hypothalamus, with lesser effect on the cortex and hippocampus. DNAB and DSP-4 lesions caused significant reductions in noradrenaline uptake sites in the cortex and hippocampus, with minimal effects on the BNST. LC-NE, locus coeruleus.

**P* < 0.05, analysis of variance followed by Fisher's PLSD test for multiple comparisons (*n* = 6–8 control, *n* = 10–11 lesioned animals per group). †*P* < 0.05, unpaired *t*-test (*n* = 8 per group).

Reductions in eye twitching and non-significant decreases in teeth chattering were also observed (Fig. 3f).

These results indicate that noradrenergic afferents to BNST are critically involved in the aversive properties of opiate withdrawal. Our anatomical and lesion studies show that these afferents originate in the A2 and A1 cell groups and project to the BNST through the VNAB. Injections of noradrenergic compounds into the BNST indicate that noradrenaline in the BNST promotes withdrawal-induced aversion by stimulating β-adrenergic receptors. This finding reveals a possible site of action for systemic β antagonists that attenuate aversive signs of opiate withdrawal¹⁰. Microinjection of the α2-autoreceptor agonist ST-91 into the BNST, which reduces the release of noradrenaline, also reduced withdrawal aversion, which is consistent with the idea that noradrenaline release in the BNST during withdrawal is important for withdrawal aversion. Moreover, this result indicates that the BNST is a possible locus for the therapeutic effects of α2-adrenergic agonists such as clonidine^{14,15}. Our findings also support other evidence that noradrenaline from the locus coeruleus, long believed to be critical for opiate withdrawal³, is not necessary for the associated somatic or aversive responses^{12,13,18,19}.

It is possible that some of the effects reported here resulted from the diffusion of microinjected compounds into neighbouring structures. However, a regional analysis of cannula placements revealed that rostral or caudal injections of adrenergic drugs in the BNST produced no larger effect on place aversion than did centrally located injections (Fig. 4 legend). Also, injections of β antagonists dorsal and equally close to the lateral ventricle (Fig. 4) had no effect on somatic or aversive signs of withdrawal. These results indicate that the BNST is the likely site of action of compounds injected in this study. However, additional experiments are needed to determine whether neighbouring structures, or other parts of the extended amygdala³, are also possible sites of noradrenaline action during withdrawal.

The small reduction in teeth chattering and eye twitching following injections of β antagonists into the BNST indicate that noradrenergic projections to the BNST may participate in a subset of somatic withdrawals behaviours. As other somatic signs are attenuated when β antagonists, or α2 agonists, are administered systematically^{3,10}, it is likely that many of the somatic symptoms of withdrawal involve targets of noradrenaline other than the BNST.

The differential effects of VNAB lesions and BNST infusions on aversive versus somatic symptoms have implications for the relation between these two indices of withdrawal. It has often been assumed that withdrawal aversion is secondary to activation of visceral afferents associated with somatic withdrawal responses. Indeed, evidence that A1 and A2 neurons mediate certain organismic responses to visceral stimuli²⁰ indicates that aversive and somatic signs of withdrawal may depend on similar brainstem processes. However, microinjections of opiate antagonists into the nucleus accumbens (NAC) or amygdala of morphine-dependent rats produce aversive but not somatic signs of withdrawal²¹. Similarly, aversive withdrawal signs can be elicited by doses of naloxone that do not evoke somatic signs²², and they are unaffected by treatments

that completely block the expression of somatic signs¹⁰. These results, together with our findings, indicate that distinct circuits may be responsible for somatic and aversive signs of withdrawal²³, and that the marked reduction in aversion observed after interfering with noradrenaline in the BNST is not dependent on the small decrease in somatic symptoms produced by these manipulations.

The BNST has reciprocal connections with a variety of limbic (NAC and amygdala) and autonomic (periaqueductal gray, hypothalamus and nucleus tractus solitarius structures⁵ that may be involved in withdrawal behaviours¹². Increased noradrenaline during opiate withdrawal has also been linked to decreased dopamine in the NAC^{24,25}, which is important in the opiate withdrawal response²⁶. The location for this apparent interaction between noradrenaline and dopamine during withdrawal is unknown. However, the present findings indicate that the BNST, may be involved through its noradrenaline input and its direct projections to the NAC and midbrain dopaminergic neurons.

From a clinical perspective, avoidance of the aversive aspects of opiate withdrawal is a significant motivating factor for drug seeking in addicts². Our results therefore indicate that the BNST may be an important component of the brain systems involved in the maintenance of drug taking and addictive behaviour. □

Methods

Animal subjects and drug administration

Male Sprague-Dawley rats (Taconic) weighing 250–300 g were used. Morphine dependence was induced by two 75-mg morphine pellets (NIDA) implanted subcutaneously 4–6 days before behavioural testing. Rats were housed in accordance to NIH guidelines on a 12-h light/dark cycle with food and water available ad libitum. All procedures were approved by the institutional animal care and use committee.

Triple immunohistochemistry protocol

Microinjections of a retrograde tracer (wheatgerm agglutinin-*apo*HRP gold (WGA-gold) or cholera toxin b subunit (CTb)) into the BNST were made as described^{27,28}. Injection sites were centred on the ventral subcommissural division of the BNST with minimal spread into neighbouring structures. Rats were made dependent on morphine, and withdrawal was precipitated 5 days later with naltrexone (1 mg per kg, intraperitoneal). Rats were perfused 2 h after the naltrexone injection and brains were processed for visualization of WGA-gold or CTb, FRAs and tyrosine hydroxylase in the same tissue sections using previously published techniques^{9,27,28}. Quantification was performed by counting the cells within the A1 and A2 regions that were both positive for tyrosine hydroxylase and retrogradely labelled. The number of such double-labelled cells that also contained FRA-positive nuclei was then determined. Cells were counted from every third section to avoid double counting.

6-hydroxydopamine lesions

Rats were anaesthetized with Nembutal (50 mg per kg, intraperitoneal), and bilateral infusion cannulae (30 gauge) were aimed at the dorsal or ventral noradrenergic bundles²⁹. Infusions of 2 μl of the selective neurotoxin 6-hydroxydopamine or vehicle (DNAB, 2 μg μl⁻¹; VNAB, 3 μg μl⁻¹; in 0.1% ascorbic acid 0.9% saline) were made over 8 min. Ten days after the surgery, animals were implanted with morphine or placebo pellets and testing began 4 days later. Animals were killed by decapitation 24 h after the final behavioural test and brains were sectioned. All lesions were quantified using ³H-nisoxetine autoradiography for noradrenaline uptake sites³⁰. Similar lesions have been shown to have no significant effect on dopamine levels in the caudate-putamen²⁹.

Intracerebral microinjections

Under Nembutal anaesthesia, bilateral indwelling guide cannulae (22 gauge) were implanted into the BNST. Cannulae were angled to avoid the lateral ventricle and were

fixed to the skull using dental acrylic. About a week after surgery, animals were implanted with morphine pellets and behavioural testing began 4 days later. Infusions of 0.5 µl per side were made through 28-gauge injector cannulae over 1 min, and cannulae were left in place for 1 min. All drugs were made fresh each day and were dissolved in ACSF. Dye was injected after the experiment to mark the injection site in all animals.

Conditioned place-aversion procedure

A balanced place-conditioning procedure was used to measure aversion in a chamber with two distinct sides¹⁰. On the first day (preconditioning day), rats were allowed free access to both sides of the chamber for 15 min. Animals that spent more than 80% of the time on one side were eliminated. On the next two days (pairing days), the animals were given an intraperitoneal injection of naltrexone (1 mg per kg) or saline, and were confined to one side for 30 min. Animals given naltrexone on pairing day 1 were given saline on pairing day 2 and confined to the opposite side, and vice versa. All adrenergic drugs were micro-injected on each of the two pairing days 5 min before naltrexone or saline; controls were similarly injected with ACSF. During pairing, an observer scored each occurrence of somatic withdrawal signs. On day 4 (test day), animals were given no drug injections and were returned to the test apparatus for 15 min with free access to both compartments, and the time spent in each compartment was measured. For shock training, place conditioning was carried out in drug-naïve animals as above, except that, on pairing day 1, animals received a 0.8 mA foot shock (randomly given for 1 s every 3 min through the chamber floor) over the course of the 30-min session; on pairing day 2, they received no foot shock and were confined to the opposite side.

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Correspondence and requests for materials should be addressed to G.A.-J. (e-mail: gaj@mail.med.upenn.edu).

Nogo-A is a myelin-associated neurite outgrowth inhibitor and an antigen for monoclonal antibody IN-1

Maio S. Chen*, Andrea B. Huber*, Marjan E. van der Haar*, Marcus Frank, Lisa Schnell, Adrian A. Spillmann, Franziska Christ & Martin E. Schwab

Brain Research Institute, Department of Neuromorphology, University of Zurich and Swiss Federal Institute of Technology Zurich, 8057 Zurich, Switzerland

* These authors contributed equally to this work

The capacity of the adult brain and spinal cord to repair lesions by axonal regeneration or compensatory fibre growth is extremely limited. A monoclonal antibody (IN-1) raised against NI-220/250, a myelin protein that is a potent inhibitor of neurite growth, promoted axonal regeneration and compensatory plasticity following lesions of the central nervous system (CNS) in adult rats^{1–4}. Here we report the cloning of *nogo A*, the rat complementary DNA encoding NI-220/250. The *nogo* gene encodes at least three major protein products (Nogo-A, -B and -C). Recombinant Nogo-A is recognized by monoclonal antibody IN-1, and it inhibits neurite outgrowth from dorsal root ganglia and spreading of 3T3 fibroblasts in an IN-1-sensitive manner. Antibodies against Nogo-A stain CNS myelin and oligodendrocytes and allow dorsal root ganglion neurites to grow on CNS myelin and into optic nerve explants. These data show that Nogo-A is a potent inhibitor of neurite growth and an IN-1 antigen produced by oligodendrocytes, and may allow the generation of new reagents to enhance CNS regeneration and plasticity.

We have purified the bovine homologue of rat NI-250, bNI220, a membrane protein of high relative molecular mass (M_r) with potent neurite outgrowth inhibitory activity that can be neutralized by monoclonal antibody (mAb) IN-1 (ref. 5). Microsequencing resulted in six peptide sequences that were used to screen cDNA libraries and databases. We isolated several cDNA clones, and a rat expressed sequence tag (EST) clone (EST111410; ref. 6) showed high homology to the bNI220 peptide sequences 2, 3 and 5. DNA sequence analysis and northern blotting indicated that three different transcripts (A, B and C) originate from one gene, resulting from both alternative promoter usage and alternative splicing (Fig. 1b and data not shown). This gene was designated *nogo* (Fig. 1a). Its protein products are Nogo-A (1,163 amino acids; database accession number AJ242961), Nogo-B (360 amino acids; AJ242962) and Nogo-C (199 amino acids; AJ242963). As Nogo-A contains all six peptide sequences obtained from purified bNI220 (Fig. 1a), it probably corresponds to rat NI-250. Nogo-A, -B and -C have a common carboxy terminus of 188 amino acids (the common