

Brown adipose tissue sympathetic nerve activity is potentiated by activation of 5-hydroxytryptamine (5-HT)_{1A}/5-HT₇ receptors in the rat spinal cord

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Abstract

In urethane-chloralose anesthetized, neuromuscularly blocked, ventilated rats, microinjection of NMDA (12 pmol) into the right fourth thoracic segment (T4) spinal intermediolateral nucleus (IML) immediately increased ipsilateral brown adipose tissue (BAT) sympathetic nerve activity (SNA; peak +492% of control), expired CO₂ (+0.1%) heart rate (+48 beats min⁻¹) and arterial pressure (+8 mmHg). The increase in BAT SNA evoked by T4 IML microinjection of NMDA was potentiated when it was administered immediately following a T4 IML microinjection of 5-hydroxytryptamine (5-HT, 100 pmol) or the 5-HT_{1A}/5-HT₇ receptor agonist, 8-OH-DPAT (600 pmol), (area under the curve: 184%, and 259% of the NMDA-only response, respectively). In contrast, T4 IML microinjection of the 5-HT₂ receptor agonist, DOI (28 pmol) did not potentiate the NMDA-evoked increase in BAT SNA (101% of NMDA-only response). Microinjection into the T4 IML of the selective 5-HT_{1A} antagonist, WAY-100635 (500 pmol), plus the 5-HT₇ antagonist, SB-269970 (500 pmol), prevented the 5-HT-induced potentiation of the NMDA-evoked increase in BAT SNA. When administered separately, WAY-100635 (800 pmol) and SB-269970 (800 pmol) attenuated the 8-OH-DPAT-induced potentiation of the NMDA-evoked increase in BAT SNA through effects on the amplitude and duration of the response, respectively. The selective 5-HT₂ receptor antagonist, ketanserin (100 pmol), did not attenuate the potentiations of the NMDA-evoked increase in BAT SNA induced by either 5-HT or 8-OH-DPAT. These results demonstrate that activation of 5-HT_{1A}/5-HT₇ receptors can act synergistically with NMDA receptor activation within the IML to markedly increase BAT SNA.

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1. Introduction

5-Hydroxytryptamine (5-HT) receptor agonists produce changes in body temperature (Lipton and Clark, 1986) that depend on the receptor specificity of the agonist (Gudelsky et al., 1986) and on the route of administration. For example, systemic administration of 5-HT results in hypothermia whereas spinal administration of 5-HT results in hyperthermia (Clark and Lipton, 1986; Lopachin and Rudy, 1982). To elucidate the physiology and pharmacology underlying the changes in body temperature evoked by spinal administration of 5-HT

receptor agonists, the current study sought to determine the specific pharmacology of the serotonergic potentiation of glutamate agonist-evoked increases in the sympathetic outflow to brown adipose tissue (BAT), the principle effector of thermogenesis in small mammals (Cannon and Nedergaard, 2004).

Many serotonergic neurons of the rostral raphe pallidus area provide input to the intermediolateral cell column (IML) (Allen and Cechetto, 1994; Bowker et al., 1981; Helke et al., 1997) and are retrogradely labeled by injection of trans-synaptic viral tracers into BAT (Cano et al., 2003). We have recently demonstrated that activation of 5-HT receptors within the IML of the spinal cord can activate the sympathetic outflow to BAT and that concomitant activation of 5-HT and *N*-methyl-D-aspartate (NMDA) receptors within the IML elicits a robust activation

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of the sympathetic outflow to BAT and an enhanced BAT thermogenesis arising from a 5-HT-mediated potentiation of the BAT response to glutamate receptor stimulation in the IML (Madden and Morrison, 2006). However, several subtypes of 5-HT receptors, including 5-HT_{1A}/5-HT₇, 5-HT₂, and 5-HT_{5A} are located within the IML (Cornea-Hebert et al., 1999; Doly et al., 2004; Maeshima et al., 1998; Marlier et al., 1991; Thor et al., 1993) and the detailed neuropharmacology within the IML responsible for the powerful potentiating effect of 5-HT on BAT sympathetic nerve activity (SNA) is not clear. Therefore, in the present study we sought to determine the specific serotonin receptor subtype(s) involved in enhancing the spinally-evoked increase in BAT SNA.

2. Materials and methods

All procedures conform to the regulations detailed in the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committee of the Oregon Health and Science University. Male Sprague–Dawley rats (Charles River, Indianapolis, IN, $n = 32$) weighing 250–450 g were given ad libitum access to standard rat chow and water in a colony room maintained at 22–23 °C and kept on a 12:12-h light/dark cycle. All efforts were made to minimize animal suffering, and to limit the number of animals used. Rats were anesthetized with isoflurane (2–3% in oxygen) and instrumented with femoral arterial and venous catheters. Isoflurane anesthesia was gradually terminated over a ten minute period while urethane and chloralose anesthesia (750 mg/kg and 60 mg/kg i.v., respectively) was administered. All physiological variables were digitized (Micro 1401 MKII; Cambridge Electronic Design (CED), Cambridge, UK) and recorded onto a computer hard drive. Arterial blood pressure was recorded from the arterial catheter attached to a pressure transducer and heart rate (HR) was derived from the arterial pressure signal. The trachea was cannulated, and the animals were ventilated (tidal volume: ~1 ml per 100 g body weight, ~60 cycles per minute) with 100% oxygen. End-expiratory CO₂ was monitored using a capnometer (model 2200; Dynatech Electro-optics, Saline, MI, USA) and ventilation rate was adjusted to maintain resting end-expiratory CO₂ in the range of 3.5–5.0%. Neuromuscular blockade was achieved by administration of d-tubocurarine (0.5 mg i.v., supplemented with 0.1 mg as needed to suppress spontaneous contractions of the diaphragm). Adequacy of anesthesia was verified prior to initial and supplemental neuromuscular blockade by absence of a withdrawal reflex or pressor response to foot pinch as well as by absence of a corneal reflex. Colonic (core) temperature was monitored using a copper-constantan thermocouple inserted 6 cm into the rectum and was maintained between 36.5 and 37.5 °C with a water perfused heating/cooling blanket and a heat lamp. Animals were placed in a stereotaxic instrument with the incisor bar positioned 4 mm below the interaural line. To stabilize the spinal cord a clamp was placed on the caudal thoracic spinal vertebrae. The third thoracic vertebra was removed to provide access to the underlying fourth thoracic spinal segment (T4), see (Gelderd and Chopin, 1977).

2.1. Recording BAT SNA and temperature

The BAT temperature was monitored using a thermocouple meter (TC-1000, Sable Systems International, Las Vegas, NV, USA) with a copper-constantan thermocouple (Physitemp, Clifton, NJ, USA) inserted into the intact, left interscapular fat pad. Postganglionic BAT SNA was recorded under mineral oil with a bipolar hook electrode from the central cut end of a small diameter (~100 µm) nerve bundle isolated from the ventral surface of the right interscapular fat pad after dividing it along the midline and reflecting it laterally. Nerve activity was filtered (1–300 Hz) and amplified (10,000–50,000×) with a Cyberamp 380 (Axon Instruments, Union City, CA). Spike 2 software (CED) was used to obtain a continuous measure (4 s bins) of BAT SNA amplitude by calculating the root mean square (rms) amplitude of the BAT SNA (square root of the total power in the 0.1 to 20 Hz band) from the autospectra of sequential 4-s segments of BAT SNA. Control values of BAT

SNA were the averages of the BAT SNA amplitudes during the 32-s periods immediately prior to treatments. Peak BAT SNA was defined as the average value during the 32-s period of maximal change in BAT SNA evoked by a treatment. The onset of the evoked response was defined as the time point at which the measured variable first exceeded the maximal value recorded during the control period and remained elevated for at least 12 s. The offset was defined as the time point at which the measured variable returned to the maximal value recorded during the control period. To provide a measure of the BAT SNA response that included both its amplitude and duration, the area under the curve (AUC) was assessed using the area function of Spike 2 analysis software (CED) and is defined as the sum of the BAT SNA amplitudes (rms, 4 s bins) between the onset and offset of the response. For all paired comparisons between a control response and a test response following a treatment, the AUC for each response was calculated using the longer of the two response durations.

2.2. Microinjections into the spinal cord

Microinjection coordinates for the IML were 0.4–0.6 mm lateral to the midline (just medial to the area at which the dorsal roots enter the spinal cord), and 0.8 mm ventral to the dorsal surface of the spinal cord; and for the dorsal horn: 0.5 mm lateral to the midline and 0.15 mm ventral to the dorsal surface of the spinal cord. Glass micropipettes (outer tip diameter, 20–30 µm) were used for all microinjections which were given over a 5–20 s period using a pressure injection system (model IIe, Toohey Co., Fairfield, NJ, USA). The volume of the microinjection was determined using a reticule to watch the movement of the meniscus in the micropipette. To make multiple microinjections into the same IML coordinates the micropipette was retracted vertically, emptied, rinsed with distilled water or saline, refilled and then re-positioned into the IML.

The IML and dorsal horn microinjection sites were marked by pressure microinjection of fluorescent polystyrene microspheres (FluoSpheres, F8801 or F8803, Molecular Probes, Eugene, OR, USA) either included in the injectate (1:200 dilution of FluoSpheres in the injectate) or microinjected at the appropriate coordinates at the conclusion of the experiment. Rats were perfused (10% paraformaldehyde) transcardially, spinal cords were removed, postfixed (12–24 h) and sectioned (60 µm coronal sections) on a vibratome. Spinal cord sections were mounted on slides and microinjection sites were localized and photographed, as described previously (Madden and Morrison, 2005).

2.3. Drugs and solutions

All drugs were obtained from Sigma (St. Louis, MO, USA) except isoflurane, which was obtained from Abbott Laboratories (North Chicago, IL, USA) and SB-269970 hydrochloride which was obtained from Tocris Bioscience (Ellisville, MO, USA). All drugs were dissolved in saline, except for 5-HT which was dissolved in saline containing 0.1% ascorbic acid. The dose of 5-HT was chosen as the minimum dose capable of producing a significant potentiation of the NMDA-evoked increase in BAT SNA, based on our previous work (Madden and Morrison, 2006). Since 8-hydroxy-2-(di-*n*-propyl-amino)tetralin hydrobromide (8-OH-DPAT) has a binding affinity for the 5-HT_{1A} and 5-HT₇ receptors that is approximately equal to that of 5-HT (Stowe and Barnes, 1998; Zifa and Fillion, 1992), the dose of 8-OH-DPAT was chosen to be within the range of effective doses of 5-HT based on our previous work (Madden and Morrison, 2006). Since *R*(-)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (DOI) has a binding affinity for the 5-HT₂ receptor that is approximately five times that of 5-HT (Zifa and Fillion, 1992), the dose of DOI was chosen to be approximately one-fifth of the effective doses of 5-HT based on our previous work (Madden and Morrison, 2006). Doses of antagonists were chosen to exceed the agonist dose by at least five-fold according to the binding affinity at the receptor for which the antagonist has the highest affinity (Sharif et al., 2004; Stowe and Barnes, 1998; Zifa and Fillion, 1992; Tocris material data sheet).

2.4. Statistics

All statistics were performed using Systat software (version 10, Systat Software Inc., Richmond, CA, USA). Data are expressed as mean ± S.E. Statistical

significance was assessed using an ANOVA for repeated measures. Following a significant *F* value, post hoc testing was performed using layered Bonferroni analysis. Significance level was $p < 0.05$.

2.5. Protocols

The test for a potentiation of the spinal NMDA-evoked response of BAT SNA consisted of a protocol with three microinjections into the T4 IML: an initial (control) microinjection of NMDA, microinjection of a test compound, followed by a microinjection of NMDA. All rats received control microinjections of NMDA (12 pmol in 60 nl) into the right T4 IML (ipsilateral to the nerve recording). After the increased BAT SNA following the first microinjection of NMDA returned to control levels, one of the following drugs was microinjected into the same IML site: 5-HT (100 pmol in 60 nl, $n = 10$); the 5-HT_{1A}/5-HT₇ receptor agonist, 8-OH-DPAT (600 pmol in 60 nl, $n = 14$); the 5-HT₂ receptor agonist, DOI (28 pmol in 60 nl, $n = 5$) or the 5-HT_{1A} receptor antagonist, WAY-100635 (800 pmol in 80 nl, $n = 6$). Within 5 min of the microinjection of one of the preceding compounds a second microinjection of NMDA (12 pmol in 60 nl) was made at the same coordinates within the T4 IML.

To determine the 5-HT receptor subtype responsible for the potentiating effect of 5-HT on the NMDA-evoked response, the potentiation protocol was repeated, but immediately following the microinjection of 5-HT (100 pmol in 60 nl) a microinjection of either (1) the selective 5-HT_{1A} receptor antagonist, WAY-100635 (500 pmol) plus the selective 5-HT₇ antagonist, (2R)-1-[(3-hydroxyphenyl)sulfonyl]-2-[2-(4-methyl-1-piperidinyl)ethyl]pyrrolidine hydrochloride (SB-269970; 500 pmol (Thomas et al., 2000) in 100 nl ($n = 5$); or (2) the selective 5-HT₂ receptor antagonist, ketanserin tartrate (100 pmol in 100 nl, $n = 5$), was made into the T4 IML, followed by a subsequent microinjection of NMDA (12 pmol in 60 nl) into the same site. A similar protocol was used to evaluate the 5-HT receptor subtype responsible for the potentiating effect of 8-OH-DPAT with the exception that the three selective 5-HT receptor antagonists were administered in separate trials: WAY-100635 (800 pmol in 80 nl, $n = 5$), SB-269970 (800 pmol in 80 nl, $n = 5$), or ketanserin tartrate (80 pmol in 80 nl, $n = 5$) and the 5-HT receptor antagonists were administered prior to the microinjection of 8-OH-DPAT (600 pmol in 60 nl), followed by a subsequent microinjection of NMDA (12 pmol in 60 nl) into the same site.

To control for the possibility that the responses evoked by microinjection of serotonergic agents into the IML were mediated by diffusion to sites outside of the IML, a subset of rats ($n = 2$) also received a microinjection of NMDA into the IML followed by a microinjection of 8-OH-DPAT into the dorsal horn and a subsequent microinjection of NMDA into the IML. In addition, we have previously demonstrated that microinjection of NMDA into the dorsal horn does not increase BAT SNA and that microinjection of serotonin into the dorsal horn does not affect the NMDA-evoked increase in BAT SNA (Madden and Morrison, 2006).

3. Results

3.1. NMDA-evoked BAT SNA

Consistent with our previous report (Madden and Morrison, 2006), in untreated rats whose core temperature was maintained at 37.0 ± 0.5 °C, right BAT SNA exhibited only low-amplitude discharge and microinjection of NMDA into the right T4 IML (ipsilateral to the BAT sympathetic nerve recording) immediately increased BAT SNA, expired CO₂, arterial pressure and HR (Table 1), but not left BAT temperature which was recorded contralateral to the microinjections into the right T4 IML.

3.2. 5-HT potentiation of the NMDA-evoked BAT SNA is reversed by 5-HT_{1A}/5-HT₇ receptor antagonism but not by 5-HT₂ receptor antagonism

Microinjection of 5-HT into the T4 IML did not increase the basal level of BAT SNA (post-5-HT: $113 \pm 7\%$ of activity prior

Table 1

Effects on cardiovascular, metabolic and thermogenic variables elicited by microinjection of NMDA into the intermediolateral cell column at the fourth thoracic segment

	Control	NMDA into T4 IML
BAT SNA (% control)	100	+492 ± 52*
Expired CO ₂ (%)	4.6 ± 0.1	+0.1 ± 0.0*
HR (beats min ⁻¹)	391 ± 9	+48 ± 5*
MAP (mmHg)	102 ± 4	+8 ± 1*

Values are mean ± S.E. ($n = 32$) for physiological variables in the control condition and the peak changes within 10 min after the microinjection of NMDA into the intermediolateral cell column at the fourth thoracic segment on the right side (ipsilateral to the recorded nerve). * $p < 0.05$, compared to the control condition.

to 5-HT, $n = 10$), though there was an occasional small amplitude, brief increase in BAT SNA that returned to basal levels prior to any subsequent injections. The NMDA-evoked increase in BAT SNA was significantly potentiated following microinjection of 5-HT into the T4 IML (Fig. 1A and D). Following 5-HT, the area under the curve (AUC) of the BAT SNA response evoked by microinjection of NMDA into the T4 IML was nearly doubled to $184 \pm 10\%$ of the control NMDA response. The 5-HT-evoked potentiation of the increase in BAT SNA evoked by NMDA was manifested as a significant increase in the peak amplitude of the BAT SNA (NMDA-evoked peak increase in BAT SNA prior to 5-HT was $+484 \pm 75\%$ of control compared to $+561 \pm 66\%$ of control following 5-HT) and a significant lengthening of the duration of the response (NMDA-only: 109 ± 8 s compared to NMDA after 5-HT: 238 ± 23 s) (Fig. 1A). In six of these ten cases, a microinjection of a mixture of the 5-HT_{1A} receptor antagonist, WAY-100635 (500 pmol) and the 5-HT₇ antagonist, SB269970 (500 pmol), following the microinjection of 5-HT (100 pmol), prevented the potentiation of the NMDA response (Fig. 1B and D). In contrast, in the other four cases, a microinjection of the 5-HT₂ receptor antagonist, ketanserin (80 pmol) following the microinjection of 5-HT (100 pmol), did not attenuate the potentiation of the NMDA response (Fig. 1C and D).

3.3. 5-HT_{1A}/5-HT₇ but not 5-HT₂ receptor activation potentiates NMDA-evoked BAT SNA

The NMDA-evoked increase in BAT SNA was also potentiated following microinjection of the selective 5-HT_{1A}/5-HT₇ receptor agonist, 8-OH-DPAT, into the T4 IML (Fig. 2A and C), but not following microinjection of 8-OH-DPAT into the dorsal horn (data not shown). The example in Fig. 2A shows that the 8-OH-DPAT potentiation of the NMDA-evoked response in BAT SNA was manifested as increases in both the peak amplitude and the duration of the response. The peak increase in BAT SNA evoked by NMDA following 8-OH-DPAT was $+1815\%$ of control compared to $+1017\%$ of control prior to 8-OH-DPAT, representing a $+71\%$ increase in amplitude (group mean, $n = 14$: $+88 \pm 19\%$ increase in amplitude compared to NMDA-evoked responses prior to 8-OH-DPAT). In the example in Fig. 2A the response duration was 236 s following 8-OH-DPAT compared to 131 s prior to 8-OH-DPAT (for

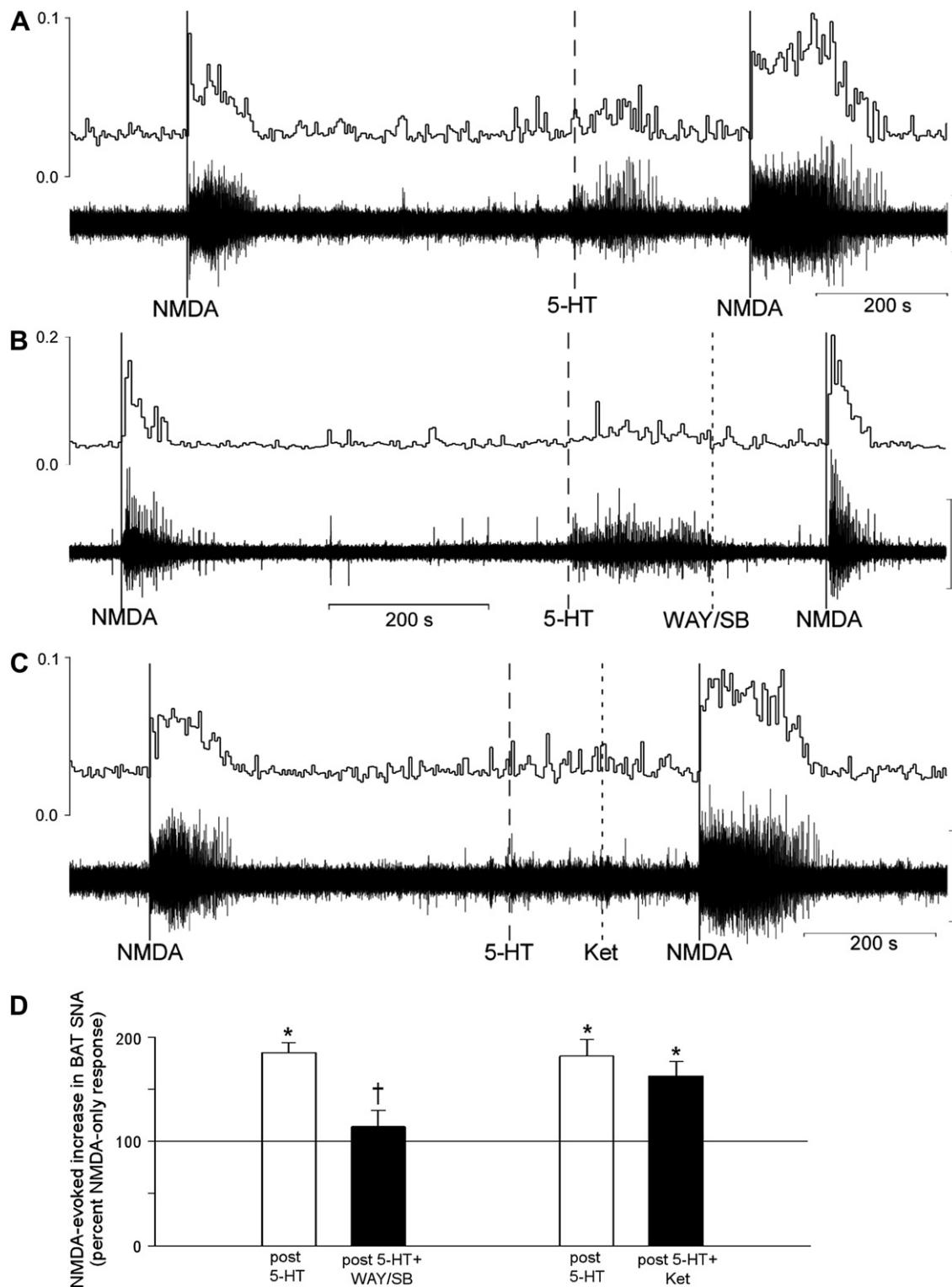


Fig. 1. Representative examples of BAT SNA during microinjections of NMDA (solid lines) into the intermediolateral nucleus at the fourth thoracic segment (T4 IML). (A) The NMDA-evoked increase in BAT SNA was potentiated by prior microinjection of 5-hydroxytryptamine (5-HT) into the T4 IML (dashed line). (B) This potentiation was prevented by microinjection of the 5-HT_{1A} receptor antagonist, WAY-100635 plus the 5-HT₇ receptor antagonist, SB-269970 into the T4 IML (dotted line). (C) Microinjection of the 5-HT_{2A} receptor antagonist, ketanserin into the T4 IML (dotted line) did not attenuate the 5-HT-evoked potentiation of the NMDA-evoked increase in BAT SNA. Vertical scale bar for BAT SNA represents 100 μ V in panels A, B, and C. (D) Magnitudes of the BAT SNA responses to NMDA were determined as the area under the curve (AUC) of the root mean square (rms) values of BAT SNA. For each treatment, the bar height represents the mean magnitude of the NMDA-evoked increases in BAT SNA expressed as a percent of the corresponding control response elicited by microinjection of NMDA alone (i.e., prior to 5-HT). Pre-treatment groups are: 5-HT (open bar, $n = 6$), 5-HT followed by WAY-100635 and SB-269970 (5-HT + WAY/SB, filled bar, $n = 6$), 5-HT (open bar, $n = 4$) and 5-HT followed by ketanserin (5-HT + Ket, filled bar, $n = 4$). * $p < 0.05$ repeated-measure comparison to the NMDA-only response. † $p < 0.05$ repeated-measure comparison to the NMDA after 5-HT response.

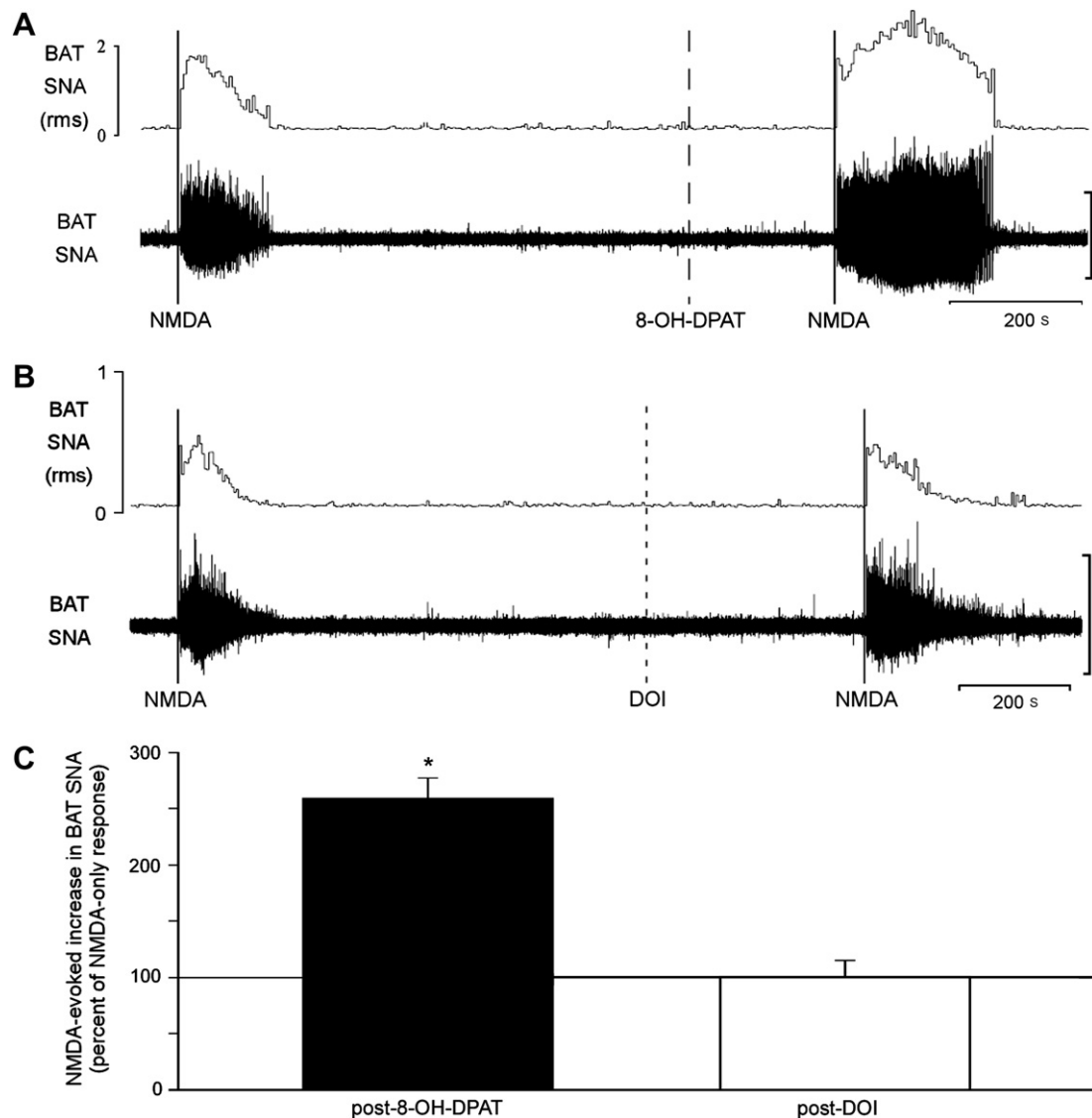


Fig. 2. The NMDA-evoked increase in BAT SNA is potentiated by activation of 5-HT_{1A}/5-HT₇ receptors but not by activation of 5-HT₂ receptors within the IML. (A) Representative example of BAT SNA during microinjections of NMDA (solid lines) into the intermediolateral nucleus at the fourth thoracic segment (T4 IML) before and after microinjection of the 5-HT_{1A}/5-HT₇ receptor agonist, 8-OH-DPAT into the T4 IML (dashed line). In this individual example, the NMDA response following 8-OH-DPAT was 278% of the NMDA response prior to 8-OH-DPAT. (B) Representative example of BAT SNA during microinjections of NMDA (solid lines) into the T4 IML before and after microinjection of the 5-HT_{2A} receptor agonist, DOI, into the T4 IML (dashed line). In this individual example, the NMDA response following DOI was 109% of the NMDA response prior to microinjection of DOI into the same site. (C) Group data for the NMDA-evoked increase in BAT SNA following microinjection of 8-OH-DPAT ($n = 14$) or DOI ($n = 5$) into the T4 IML (mean \pm S.E.M., expressed as a percentage of the paired NMDA-only response). $*p < 0.05$ compared to the NMDA-only response. Vertical scale bars for BAT SNA represent 40 μ V in A and 50 μ V in B.

group data see Table 2). In contrast to the effect of 8-OH-DPAT, the NMDA-evoked increase in BAT SNA following microinjection of the selective 5-HT₂ agonist, DOI into the T4 IML (Fig. 2B and C) did not differ from the increase evoked by NMDA alone (the AUC of the response following DOI was $101 \pm 14\%$ of the NMDA-only response; peak increase in BAT SNA: $+494 \pm 107\%$ of control for NMDA alone versus $+461 \pm 148\%$ of control following DOI; response duration: 161 ± 28 s for NMDA alone versus 170 ± 26 s following DOI; $n = 5$).

Microinjection of the selective 5-HT_{1A} receptor antagonist, WAY-100635, into the T4 IML prior to microinjection of

8-OH-DPAT attenuated the 8-OH-DPAT-mediated potentiation of the NMDA-evoked increase in BAT SNA (Fig. 3A and D, Fig. 4A and Table 2). Attenuation of the 8-OH-DPAT-mediated potentiation of the NMDA-evoked response by prior treatment with WAY-100635 was manifested as a reduction of both the enhancement of the peak amplitude and of the lengthening of the duration of the response (Figs. 3A and 4A, Table 2). Microinjection of WAY-100635 alone (without subsequent administration of 8-OH-DPAT) had no consistent effect on the NMDA-evoked changes in BAT SNA (the AUC of the NMDA response following WAY-100635 was $121 \pm 39\%$ of the control NMDA response, $n = 6$).

Table 2

Effects of 8-OH-DPAT and 5-hydroxytryptamine receptor antagonists on the peak magnitude and duration of brown adipose tissue sympathetic nerve activity (BAT SNA) evoked by microinjection of NMDA into the intermediolateral cell column

	NMDA only (<i>n</i> = 14)	NMDA after 8-OH-DPAT (<i>n</i> = 14)	NMDA after 8-OH-DPAT following		
			WAY-100635 (<i>n</i> = 5)	SB-269970 (<i>n</i> = 5)	Ketanserin (<i>n</i> = 4)
Peak BAT SNA (% control)	+479 ± 70	+935 ± 129*	+397 ± 139 [†]	+785 ± 153*	+889 ± 170*
BAT SNA response duration (s)	142 ± 10	241 ± 14*	161 ± 21 [†]	169 ± 23 [†]	188 ± 13*

Effects on brown adipose tissue sympathetic nerve activity (BAT SNA) of microinjection into the intermediolateral cell column at the fourth thoracic segment (T4 IML) of NMDA; NMDA after 8-OH-DPAT; or NMDA after 8-OH-DPAT following the 5-HT_{1A} antagonist, WAY-100635, or the 5-HT₇ antagonist, SB-269970, or the 5-HT₂ antagonist, ketanserin. Values are mean ± S.E.

**p* < 0.05, repeated-measure comparison to the NMDA only response.

[†]*p* < 0.05, repeated-measure comparison to the NMDA after 8-OH-DPAT response.

Microinjection of the selective 5-HT₇ receptor antagonist, SB-269970 into the T4 IML prior to microinjection of 8-OH-DPAT attenuated the 8-OH-DPAT potentiation of the NMDA-evoked increase in BAT SNA through an effect on the duration rather than the amplitude of the response (Fig. 3B and D, Fig. 4B, Table 2). Prior treatment with SB-269970 shortened the duration of the NMDA-evoked response following 8-OH-DPAT (190 s, Fig. 4B) compared to the response to NMDA after 8-OH-DPAT only (263 s, Fig. 4B; group data provided in Table 2). In contrast, the peak amplitude of the NMDA-evoked response following 8-OH-DPAT was not different from that following 8-OH-DPAT preceded by SB-269970 (Table 2). Microinjection of the 5-HT₂ antagonist, ketanserin, did not affect the 8-OH-DPAT potentiation of the NMDA-evoked increase in BAT SNA (Fig. 3C and D, Table 2).

3.4. Histological localization of microinjection sites

Representative examples illustrating the locations of the microinjection sites in the T4 IML and the dorsal horn are shown in Fig. 5. All microinjection sites targeting the T4 IML were found in the IML or just lateral to the IML in the white matter within 100 μm of the IML. Microinjection sites targeting the dorsal horn were found in lamina I or lamina II.

4. Discussion

The results of the present study demonstrate that microinjection of 5-HT, or the 5-HT_{1A}/5-HT₇ receptor agonist, 8-OH-DPAT, into the IML potentiates NMDA-evoked BAT SNA and that these potentiations are attenuated by 5-HT_{1A} and 5-HT₇ receptor antagonists. We conclude that activation of 5-HT_{1A}/5-HT₇ receptors can act synergistically with NMDA receptor activation within the IML to markedly potentiate the glutamate receptor-mediated increase in BAT SNA. In contrast, microinjection of the selective 5-HT₂ agonist, DOI, into the IML did not potentiate the NMDA-evoked increase in BAT SNA, nor did the selective 5-HT₂ antagonist, ketanserin, attenuate the 5-HT- or 8-OH-DPAT-evoked potentiations of the increase in BAT SNA evoked by NMDA. Thus, the current findings extend our previous observations by indicating a significant role for spinal 5-HT_{1A} and 5-HT₇ receptors, but

not those of the 5-HT₂ subtype, in mediating the 5-HT-evoked potentiation of NMDA-evoked increases in BAT SNA.

In the present study, spinal administration of the 5-HT_{1A}/5-HT₇ receptor agonist, 8-OH-DPAT, enhanced the increase in BAT SNA evoked by microinjection of NMDA into the T4 IML. We have previously demonstrated that the enhancement of BAT SNA following activation of 5-HT receptors within the spinal cord results in enhanced thermogenesis in that tissue (Madden and Morrison, 2006), therefore the present observation of an enhancement of BAT SNA by spinal administration of 8-OH-DPAT would seem to be at odds with the hypothermic effect, via activation of both 5-HT_{1A} and 5-HT₇ receptors, of systemically administered 8-OH-DPAT (Faure et al., 2006; Hedlund et al., 2004). This apparent discrepancy highlights the differing effects of 5-HT released at different sites in the central thermoregulatory network controlling body temperature. For example, while systemically administered 8-OH-DPAT may influence BAT thermogenesis and body temperature through actions in the spinal cord, it also has access to central sites such as the preoptic area, hypothalamus and medial medulla that play significant roles in thermoregulation (Morrison, 2004b; Romanovsky, 2007). Along these lines, microinjection of 8-OH-DPAT into the raphe pallidus area (RPa) inhibits BAT SNA and BAT thermogenesis (Morrison, 2004a) and indeed the anti-thermogenic effect of systemically administered 8-OH-DPAT is reversed by administration of the selective 5-HT_{1A} receptor antagonist, WAY-100635 into the RPa (S.F.M., unpublished observation). Additionally, the hypothermic effect of systemically administered 8-OH-DPAT is likely to be mediated, at least in part, via cutaneous vasodilation resulting in increased heat loss to the environment (Ootsuka and Blessing, 2003). In fact, the reduction in cutaneous vasoconstriction evoked by systemic administration of 8-OH-DPAT is also reversed by microinjection of WAY-100635 into the RPa (Ootsuka and Blessing, 2006a), further supporting the interpretation that the hypothermic effect of systemically administered 8-OH-DPAT is mediated by an action at sites other than the spinal cord.

In the present study microinjection of the 5-HT_{1A}/5-HT₇ agonist, 8-OH-DPAT, alone did not increase BAT SNA; however, it did elicit a potent facilitation of the NMDA-evoked increase in BAT SNA. Similarly, Lewis and Coote, recording from sympathetic preganglionic neurons (SPNs) in the upper thoracic spinal segments of rats demonstrated that application

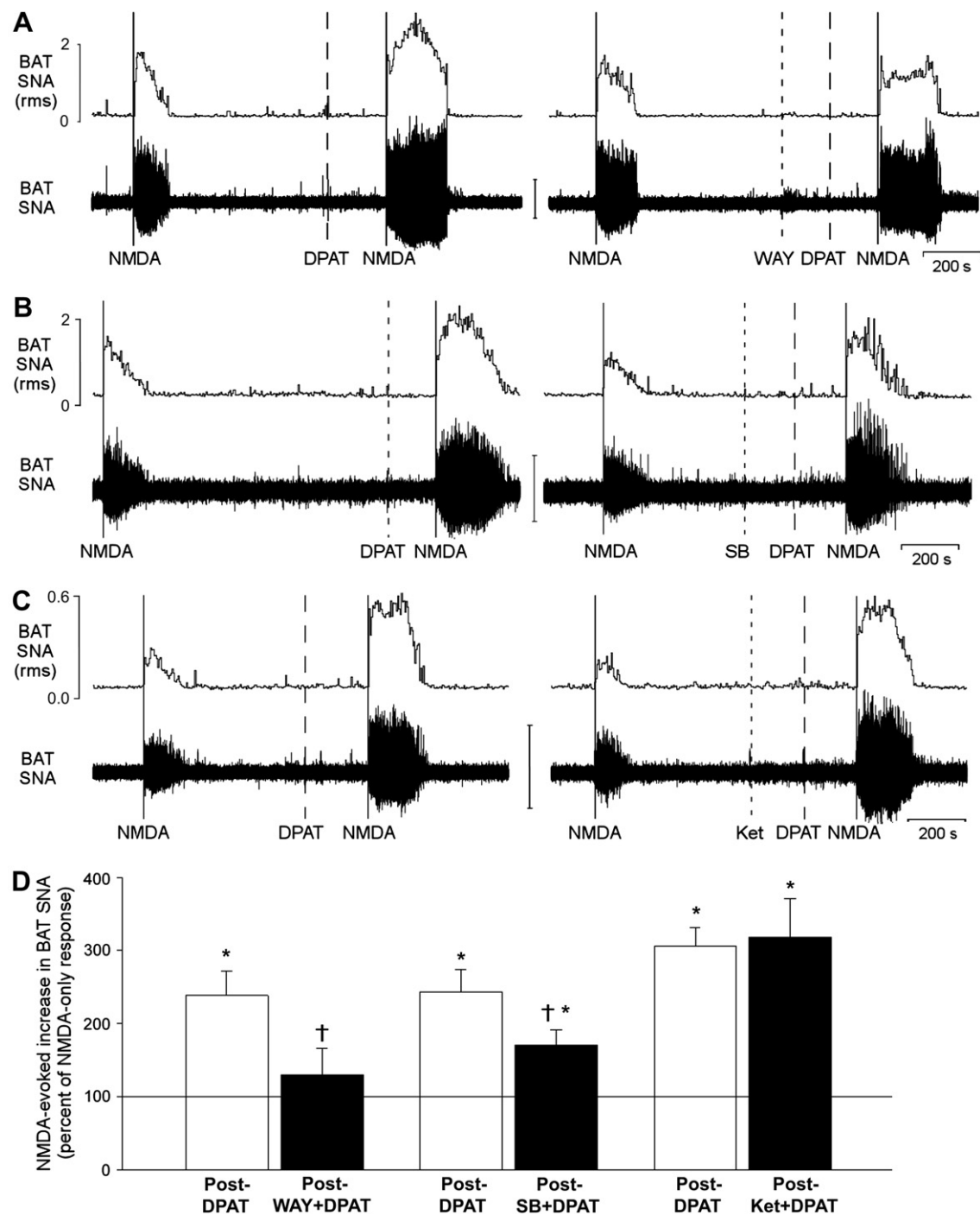


Fig. 3. Effect of selective 5-HT receptor antagonists on the ability of 8-OH-DPAT to potentiate the increase in BAT SNA evoked by microinjection of NMDA into the T4 IML. (A) Microinjection of the 5-HT_{1A} receptor antagonist, WAY-100635, prior to 8-OH-DPAT attenuated the 8-OH-DPAT potentiation of the NMDA response. (B) Prior microinjection of the 5-HT₇ receptor antagonist, SB-269970, attenuated the 8-OH-DPAT potentiation of the NMDA-evoked increase in BAT SNA. (C) The 5-HT_{2A} receptor antagonist, ketanserin, did not attenuate the 8-OH-DPAT potentiation of the NMDA-evoked increase in BAT SNA. Vertical scale bar for BAT SNA represents 50 μ V in panels A and C, and 100 μ V in panel B. (D) Magnitudes of the BAT SNA excitatory responses to NMDA were determined as the area under the curve (AUC) of the root mean square (rms) values of BAT SNA. For each treatment, the bar height represents the mean magnitude of the NMDA-evoked increases in BAT SNA expressed as a percent of the corresponding control response elicited by microinjection of NMDA alone (i.e., prior to 8-OH-DPAT). Pre-treatment groups are: 8-OH-DPAT (DPAT, open bar, $n = 5$), WAY-100635 followed by 8-OH-DPAT (WAY + DPAT, filled bar, $n = 5$), DPAT (open bar, $n = 5$), SB-269970 followed by 8-OH-DPAT (SB + DPAT, filled bar, $n = 5$), and DPAT (open bar, $n = 4$), ketanserin followed by 8-OH-DPAT (Ket + DPAT, filled bar $n = 4$). * $p < 0.05$ repeated-measure comparison to the NMDA-only response. † $p < 0.05$ repeated-measure comparison to the NMDA after 8-OH-DPAT response.

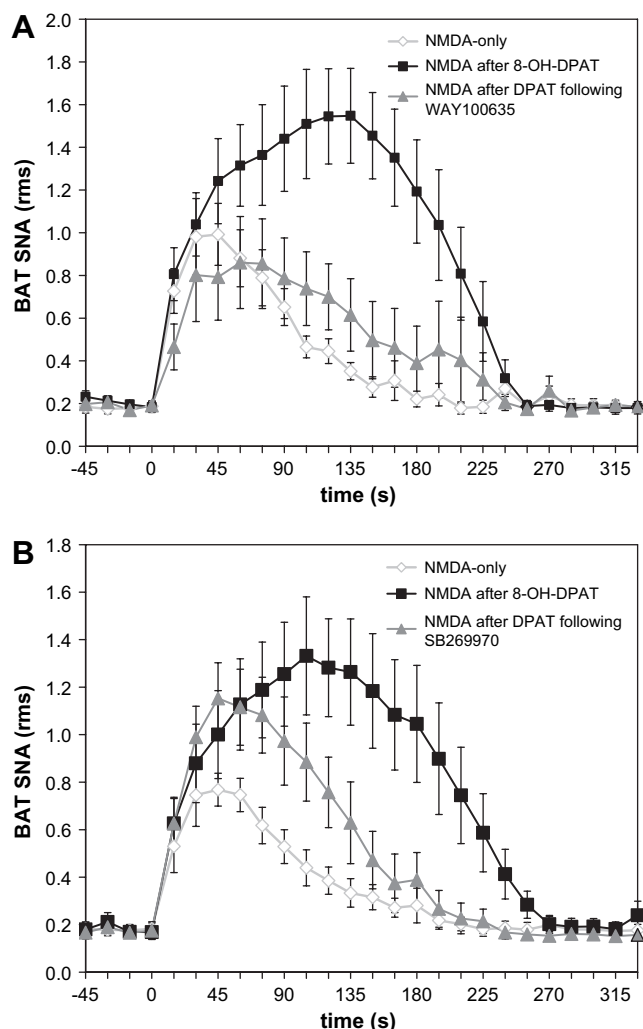


Fig. 4. The mean time courses of the effects of selective 5-HT receptor antagonists on the 8-OH-DPAT-mediated potentiation of the increase in BAT SNA evoked by microinjection of NMDA into the T4 IML. (A) The 5-HT_{1A} receptor antagonist, WAY-100635, attenuated both the amplitude and the duration of the 8-OH-DPAT potentiation. (B) The 5-HT₇ receptor antagonist, SB269970, attenuated the duration but not the amplitude of the 8-OH-DPAT potentiation. Data represent group means and S.E.M. for the BAT SNA (root mean square, rms) measured in 15-s bins over the time course of the NMDA-evoked responses to microinjection of NMDA-only into the T4 IML (open diamonds, $n = 5$), microinjection of NMDA into the T4 IML after microinjection of 8-OH-DPAT into the T4 IML (closed squares, $n = 5$), and microinjection of NMDA into the T4 IML following microinjection of 8-OH-DPAT into the T4 IML after microinjection of (A) WAY-100635 or (B) SB269970 into the T4 IML (closed triangles, $n = 5$).

of the 5-HT_{1A}/5-HT₇ agonist, 5-carboxamidotryptamine (5-CT) alone was not capable of exciting SPNs that had no spontaneous activity (Lewis and Coote, 1990). However, when a stable basal firing rate was maintained by exogenous application of excitatory amino acids, the majority of SPNs increased their firing rate in response to application of 5-CT (Lewis and Coote, 1990).

Systemic administration of a 5-HT_{2A} receptor agonist results in hyperthermia (Gudelsky et al., 1986), at least in part via an increase in sympathetic outflow to the cutaneous vasculature (Ootsuka et al., 2004) and possibly also via an increase in

BAT thermogenesis (Ootsuka and Blessing, 2006b). In the present study the inability of a microinjection of DOI into the T4 IML to affect BAT SNA suggests that the BAT SPNs are unlikely to be the site at which systemically administered DOI increases BAT thermogenesis, an interpretation that depends on the efficacy of the dose of DOI chosen in the current study. In this regard, it is important to note that the concentration of DOI used in the current study (0.28 mM) is equivalent to the concentration that, when administered systemically (1 ml/kg), increased BAT temperature (Ootsuka and Blessing, 2006b) and cutaneous sympathetic vasomotor outflow (Ootsuka et al., 2004). Systemically administered DOI may increase body temperature and BAT thermogenesis by acting at sites upstream of the SPNs, for example, areas of the hypothalamus that have been implicated in 5-HT₂ receptor-mediated hyperthermia (Chio et al., 2005). Alternatively, since DOI is hallucinogenic (Glennon et al., 1984), systemic administration of this agent in awake animals could result in a “stress”-induced thermogenesis that is secondary to its hallucinogenic properties.

Although the cellular mechanisms through which 5-HT_{1A}/5-HT₇ agonists potentiate the NMDA-evoked activation of BAT were not addressed in the present study, administration of the 5-HT_{1A} receptor antagonist, WAY-100635, or the 5-HT₇ receptor antagonist, SB-269970, was found to attenuate the 8-OH-DPAT-evoked increase in the NMDA-evoked BAT activation whereas the 5-HT₂ receptor antagonist, ketanserin, did not affect this potentiation. These data demonstrate that the potentiation evoked by 8-OH-DPAT is a specific receptor mediated effect and that the full potentiating effect of 8-OH-DPAT requires activation of both 5-HT_{1A} and 5-HT₇ receptors. 5-HT_{1A} and 5-HT₇ receptors are both G-protein coupled receptors; however their signal transduction systems differ significantly. 5-HT_{1A} receptors are negatively coupled to adenylyl cyclase (Barnes and Sharp, 1999; Zifa and Fillion, 1992). In addition, 5-HT_{1A} receptors can be coupled to K⁺ channels (with activation opening the channels) (Barnes and Sharp, 1999; Zifa and Fillion, 1992). These 5-HT_{1A} receptor mediated responses would tend to inhibit cellular activity. In contrast, 5-HT₇ receptors are positively coupled to adenylyl cyclase via G_s-proteins (Barnes and Sharp, 1999). Given the similarity in their ability to enhance the NMDA-evoked increase in BAT SNA, despite the opposing effects of 5-HT_{1A} and 5-HT₇ receptors on signal transduction mechanisms, it seems reasonable to speculate that these receptors are located on separate populations of neurons within the spinal cord. The simplest model to explain the current results would consist of a 5-HT_{1A} receptor-mediated inhibition of a GABAergic input to BAT SPNs coupled with a 5-HT₇ receptor-mediated increase in the excitability of SPNs. It will be of interest to determine the neurochemical phenotype of neurons within the IML that express the 5-HT_{1A} and 5-HT₇ receptor subtypes.

It is important to note that even though metabolism in BAT is not the principal contributor to thermogenesis in adult humans (Astrup et al., 1985), BAT is present in adult humans (Heaton, 1972) and several studies have begun to suggest that genetic variations in BAT effector systems (Clement et al., 1996) or expression levels of uncoupling protein 1

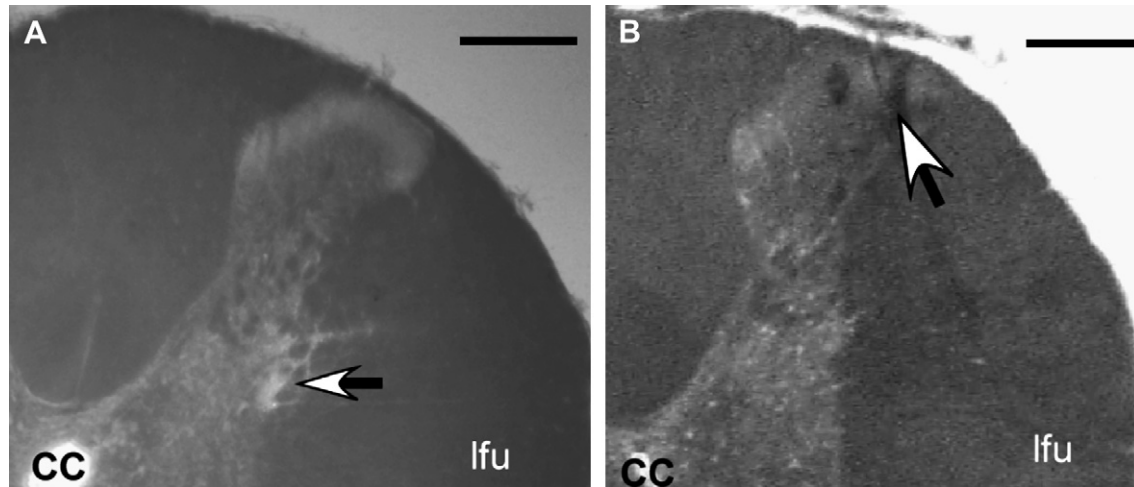


Fig. 5. Representative examples of the dorsal right quadrant of coronal sections through the spinal cord illustrating the histological localization of the position (arrow) of the micropipette tip targeting (A) the intermediolateral cell column and (B) the dorsal horn within the fourth thoracic segment. Scale bars = 250 μ m. cc, central canal; lfu, lateral funiculus.

(UCP1) in BAT (Oberkofler et al., 1997) can result in a propensity to gain weight in humans (for reviews see Avram et al., 2005; Del Mar Gonzalez-Barroso et al., 2000). Although serotonergic agents are known to affect metabolic regulation, remarkably little is known about the neural pathways through which 5-HT can influence energy expenditure. The current results provide a potential site at which serotonergic agents could affect metabolic regulation.

In conclusion, the findings of the present study demonstrate that combined activation of NMDA and 5-HT_{1A}/5-HT₇ receptors within the IML can act synergistically to markedly increase sympathetic activation of BAT. Further studies will be required to investigate the detailed neurocircuitry responsible for the interactions between excitatory amino acid and serotonergic mechanisms within the IML and to determine the specific physiological conditions under which these mechanisms play a role in regulating body temperature and metabolism.

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