The Effects of Aromatic Anesthetics on Dorsal Horn Neuronal Responses to Noxious Stimulation

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BACKGROUND: Gamma-aminobutyric acid type A receptor potentiation and/or *N*-methyl-D-aspartate (NMDA) receptor inhibition might explain the anesthetic properties of fluorinated aromatic compounds. We hypothesized that depression of dorsal horn neuronal responses to noxious stimulation would correlate with the magnitude of effect of benzene (BNZ), *o*-difluorobenzene, and hexafluorobenzene (HFB) on NMDA receptors.

METHODS: Rats were anesthetized with desflurane. A T13-L1 laminectomy allowed extracellular recording of neuronal activity from the lumbar spinal cord. After discontinuing desflurane administration, MAC for each aromatic anesthetic was determined. A 5-s noxious mechanical stimulus was then applied to the hindpaw receptive field of nociceptive dorsal horn neurons, and single-neuron responses were recorded at 0.8 and 1.2 MAC. These responses were also recorded in decerebrate rats receiving BNZ and HFB at 0–1.2 MAC.

RESULTS: In intact rats, depression of responses of dorsal horn neurons to noxious stimulation by peri-MAC increases in BZN, *o*-difluorobenzene, and HFB correlated directly with their *in vitro* capacity to block NMDA receptors. In decerebrate rats, 1.2 MAC BNZ depressed nociceptive responses by 60%, with a further percentage decrease continuing from 0.8 to 1.2 MAC approximately equal to that found in intact rats. In decerebrate rats, HFB caused a progressive dose-related decrease in MAC (maximum 25%), but in intact rats, an increase from 0.8 to 1.2 neuronal response caused an (insignificant) increase in neuronal response.

CONCLUSIONS: The findings in intact rats suggest that NMDA blockade contributes to the depression of dorsal horn neurons to nociceptive stimulation by fluorinated aromatic anesthetics. These results, combined with the additional findings in decerebrate rats, suggest that supraspinal effects (perhaps on γ-aminobutyric acid type A receptors) may have a supraspinal facilitatory effect on nociception for HFB.

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Fluorinated benzene (BNZ) compounds have anesthetic properties.[1] At MAC, they inhibit the *N*-methyl-D-aspartate (NMDA) receptor[2] by an amount that varies inversely with their capacity to enhance the action of γ-aminobutyric acid (GABA) on the $GABA_A$ receptor.[3] These differences provide an opportunity to test the hypothesis that differential effects on NMDA and $GABA_A$ receptors determine spinal nociceptive responses. We studied *o*-difluorobenzene (ODFB), hexafluorobenzene (HFB), and BNZ because BNZ and ODFB versus HFB are at opposite ends of the spectrum with regard to $GABA_A$ receptor enhancement and NMDA receptor depression. BNZ and ODFB have predominantly NMDA receptor-depressive effects and HFB has predominantly $GABA_A$ receptor-enhancing effects.[3]

METHODS

The University of California, Davis Animal Care and Use Committee approved this study. Adult male rats (weight ≈ 500 g) were anesthetized with desflurane, a tracheostomy tube was placed, and mechanical ventilation was initiated (end-tidal CO_2 30–35 mm Hg) with oxygen as the carrier gas. Rectal temperature was maintained at ≈37 ± 1°C. Catheters were placed into a carotid artery (to measure mean arterial blood pressure) and jugular vein (for fluid and drug administration). A laminectomy was performed at approximately the L5–6 spinal segment.

After discontinuing desflurane administration, we determined the minimum alveolar concentration (MAC) of ODFB, BNZ, and HFB (each administered from a halothane vaporizer) in three groups of rats. End-tidal samples (10 mL) were taken after at least 30

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min of equilibration. The gas sample was mixed with an equal volume of isoflurane (1%) that acted as an internal standard. Aromatic anesthetic concentrations were determined using gas chromatography (Varian model 3900, Walnut Creek, CA). The ratio of the peak of the aromatic anesthetic to the isoflurane peak was compared with peaks resulting from injection of standards (correlation = 0.99). In four experiments, we used a Gow-Mac gas chromatograph machine (Bethlehem, PA).[4] After anesthetic equilibration >45 min, a 10-inch hemostat was applied to the tail and oscillated for 60 s or until movement occurred. Positive movement was defined as movement of the head or pawing or running motions of the limbs. Stiffening of the legs or body was not considered positive. If the animal moved, we increased the anesthetic concentration about 30%; if the animal did not move we decreased the anesthetic concentration 30%. After equilibration for 20 min, the tail clamp was applied. This process continued until concentrations were found that just prevented, and just permitted, movement. The average of these concentrations was MAC. We noted that changes in end-tidal concentrations of these anesthetics, in particular BNZ, occurred slowly, presumably because of high blood gas solubilities (~11 for BNZ, 9 for ODFB and 2.5 for HFB).[1] Thus, when increasing the concentrations we "over-pressurized," and when decreasing these anesthetics we turned off the vaporizer, for 5–10 min to compensate for the slow kinetics. The 20-min equilibration period included the 5–10 min compensation period.

In pilot studies, we observed cyanosis and pulmonary edema after 2–3 h of exposure to HFB, precluding determination of MAC and neuronal responses in the same animals. Pilot studies of BNZ required prolonged assessment. Thus, MACs for HFB and BNZ were determined in separate groups of rats (n = 4 for HFB and n = 6 for BNZ) and these population MACs were used for the electrophysiological studies. In HFB animals, we obtained arterial blood at the end of the electrophysiological studies to document that hypoxemia was not present. In the ODFB group, after determining MAC, we used the animals for the electrophysiological studies described below, and we used each animal's individual ODFB MAC.

We placed the rat into a stereotaxic frame using two vertebral clamps attached to the vertebrae rostral and caudal to the laminectomy. The head was secured to the frame using an incisor bar and ear bars. The dura was removed and a tungsten electrode was advanced into the dorsal horn of the lumbar spinal cord (≈L5–6 segment) to identify neurons with mechanical receptive fields on the plantar hindpaw. Extracellular action potentials were amplified (10,000×; Tucker-Davis, Inc, Alachua, FL), bandpass filtered (300–3000Hz), and relayed to a computer (Chart5, ADInstruments, CO Springs, CO). Cells were classified as wide-dynamic range if they developed an increased response to increasing stimulus intensity. If they responded only to pinching in the noxious range, they were classified as nociceptive specific.

We then administered 0.8× or 1.2× MAC, the order alternating from animal to animal. A noxious mechanical pinch stimulus was applied using a curved forceps with a force transducer (SensoTec, Columbus, OH) between the arms of the forceps.[5] Output from the force transducer was routed to the computer and recorded on-line. The pinch stimulus was applied to the receptive field for 5 s. The force of the pinch stimulus ranged from 30 to 50 N (force constant in any individual animal) depending on the response properties of the neuron studied. Three to six stimuli were delivered at each anesthetic concentration. The anesthetic was then increased or decreased (depending on the concentration studied first) and after a 30 min equilibration period the series of noxious stimuli was repeated. For most animals in each experimental group, the neuronal response was retested at the original anesthetic concentration and the responses were averaged with the initial responses at the same anesthetic concentration to control for any time effects.

A second aim of this study was to determine supraspinal effects, if any, of BNZ and HFB on nociceptive responses in decerebrate rats. Decerebration permitted determination of baseline spontaneous activity and nociceptive responses in the absence of anesthesia versus when BNZ and HFB were present. Rats (n = 12 for BNZ and n = 7 for HFB) were anesthetized with halothane and surgical procedures (tracheostomy, IV/arterial catheterization, and laminectomy) were performed as described above. In addition, we made a large craniotomy. Dexamethasone (1–4 mg/kg) was administered IV to minimize swelling. In four rats, we determined that 4 mg/kg dexamethasone did not alter neuronal nociceptive responses over 2 h after administration. A precollicular brain transection and aspiration of brain anterior to the transection[6] was performed 60–90 min after administration of dexamethasone. Halothane administration was discontinued for 60 min or more. Rats were excluded from further study if they did not move vigorously in response to tail clamping. Neuronal responses were recorded at 0, 0.8, and 1.2 MAC BNZ or HFB as described above, using the MAC values for intact rats. In most rats, we also redetermined the response at 0 MAC after discontinuation of aromatic anesthetic administration.

In another group of decerebrate rats (n = 7), we determined nociceptive responses in the absence of anesthesia over a period of 4–6 h after decerebration, thereby matching the average time needed to obtain responses in the animals receiving BNZ or HFB. This control group demonstrated that any changes in nociceptive responses were not due to repeated application of the noxious pinch or to decerebration itself.

Action potentials were counted for the 30-s period before stimulation (spontaneous activity), and for the 5-s period during stimulation (neuronal response.)

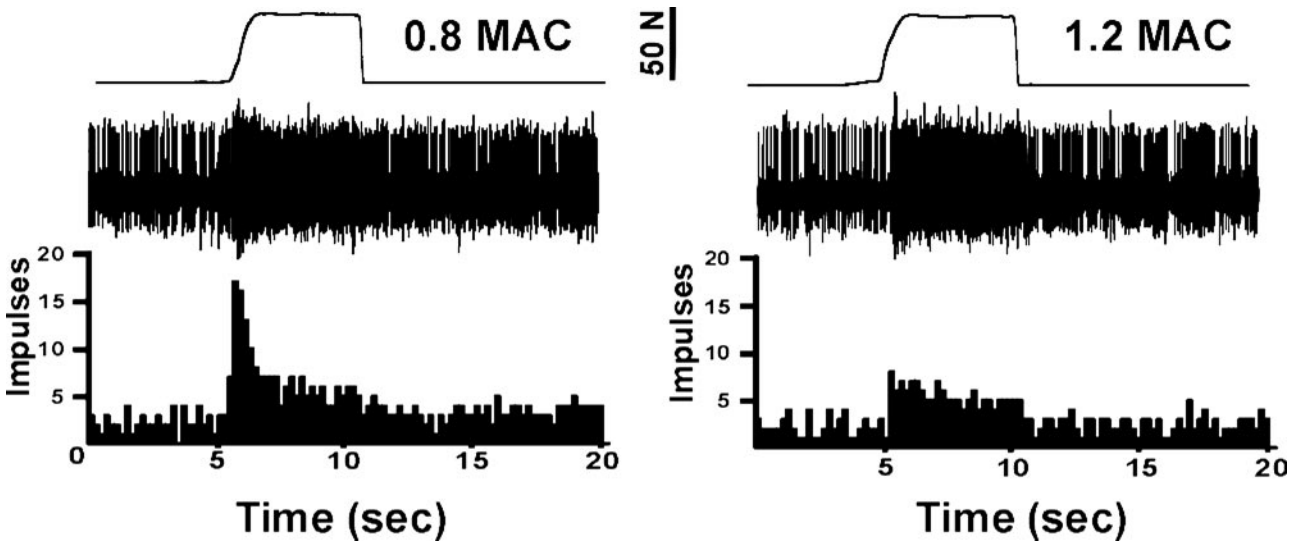

Figure 1. Shown are tracings of action potentials recorded from a neuron in an animal anesthetized with benzene. The force tracing above each example shows application of the noxious mechanical stimulus, whereas the peristimulus histogram is below each tracing (binwidth = 200 ms). At 0.8 minimum alveolar concentration, (MAC) application of a noxious mechanical stimulus to the receptive field on the hindpaw evoked a neuronal response. Application of the mechanical stimulus at 1.2 MAC benzene evoked fewer action potentials, when compared with the response at 0.8 MAC. Also, note the significant spontaneous activity.

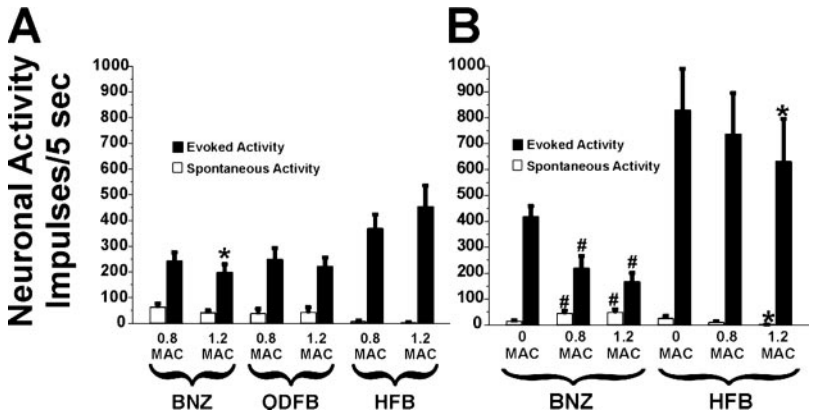

Figure 2. Evoked neuronal responses to noxious stimulation and spontaneous neuronal activity (impulses/5 s) in rats receiving benzene (BNZ), *o*-difluorobenzene (ODFB), or hexafluorobenzene (HFB) (mean, SE); minimum alveolar concentration = MAC. (A) Data from intact rats; $*P < 0.05$ compared with 0.8 MAC response. (B) Data from decerebrate rats; $*P < 0.05$ compared with 0 MAC response, $\#P < 0.01$ compared with 0 MAC response. Evoked responses (black bars) are number of action potentials in the 5 s period of pinching minus the spontaneous activity (gray bars) preceding the stimulation. Spontaneous activity was determined from the number of action potentials in the 30-s period preceding stimulation and is expressed as impulses/5 s. The total response during pinching would be the evoked response plus the spontaneous activity.

Neuronal responses at each anesthetic concentration were averaged. In the intact rats (no decerebration), the responses at 0.8 and 1.2 MAC and spontaneous activity were compared using a paired *t*-test. In the decerebrate rats, we used repeated measures analysis of variance (followed by the Student–Newman–Keuls test) to compare responses at 0, 0.8, and 1.2 MAC and to compare spontaneous activity. Spontaneous activity among anesthetics was compared using analysis of variance followed by the Student–Newman–Keuls test. Data are presented as mean and standard error. A P value <0.05 was assumed to be significant.

RESULTS

MACs (expressed as volume %; mean, standard error) for BNZ, ODFB, and HFB were 1.43 ± 0.10%, 0.64 ± 0.03%, and 1.31 ± 0.11%, respectively, in intact rats (i.e., without decerebration). In these rats, we studied 33 neurons (10 neurons with BNZ, 11 neurons with ODFB, and 12 neurons with HFB); 30 of the 33 neurons were wide-dynamic range type, and 3 were nociceptive specific. In intact rats groups did not differ significantly with respect to spinal recording depth (665 ± 35 μm for all three groups combined). Figure 1 shows an example of the spontaneous activity often occurring during BNZ anesthesia and the depression of the evoked response when increasing BNZ from 0.8 to 1.2 MAC.

Summary data are shown in Figure 2. The transition from 0.8 to 1.2 MAC decreased evoked neuronal responses during BNZ by 19% (Fig. 2A, $P < 0.05$). ODFB produced an average decrease of 11%, but this

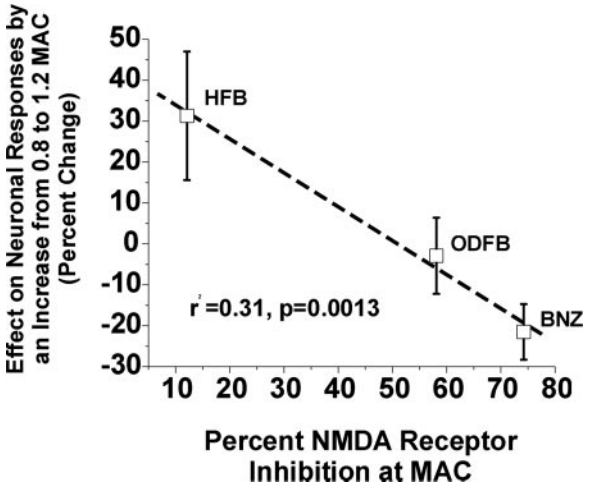

Figure 3. Effect on neuronal response compared with *N*-methyl-D-aspartate (NMDA) receptor inhibition by hexafluorobenzene (HFB), *o*-difluorobenzene (ODFB), and benzene (BNZ). The receptor inhibition data are from Solt et al.[2] Note that there is a significant correlation between the effect on neuronal responses of increasing anesthetic concentration from 0.8 to 1.2 minimum alveolar concentration (MAC) and the percent of NMDA receptor inhibition.

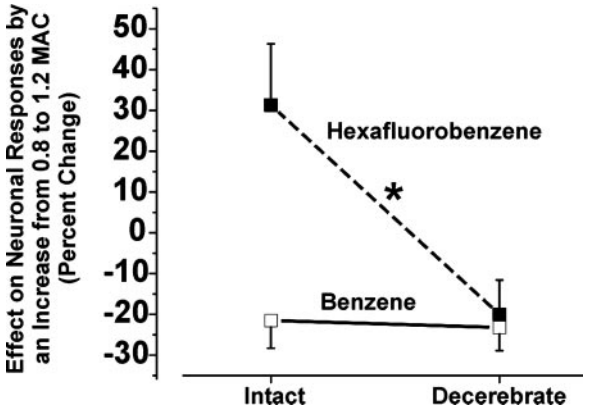

Figure 4. Effect on neuronal responses of increasing anesthetic concentration from 0.8 to 1.2 minimum alveolar concentration (MAC) in intact and decerebrate rats. Hexafluorobenzene resulted in an enhancement of responses in intact rats but depression in decerebrate rats (*$P < 0.05$, responses in intact rats versus responses in decerebrate rats).

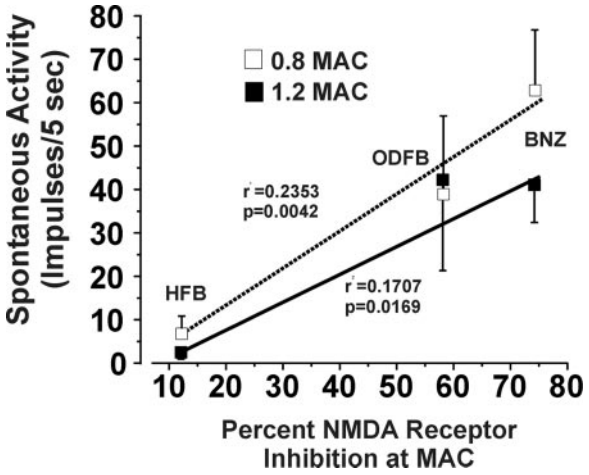

Figure 5. Effect on spontaneous neuronal activity compared with *N*-methyl-D-aspartate (NMDA) receptor inhibition by hexafluorobenzene (HFB), *o*-difluorobenzene (ODFB), and benzene (BNZ). The receptor inhibition data are from Solt et al.[2] Note that there is a significant correlation between the effect on spontaneous neuronal activity of increasing anesthetic concentration from 0.8 to 1.2 minimum alveolar concentration (MAC) and the percent of NMDA receptor inhibition.

was not significant. HFB produced an average increase of approximately 30%, but this also was not significant (Fig. 2A). There was a linear correlation between the percent change in evoked response and the percent inhibition of the NMDA receptor at MAC (Fig. 3; NMDA receptor data from Solt et al.[2]).

At the average neuronal recording depth of 744 ± 45 μm, 0.8 MAC BNZ depressed evoked neuronal responses nearly 50% in decerebrate animals (Fig. 2B), with further depression occurring between 0.8 and 1.2 MAC. The further depression approximated that found in intact rats having the same 0.8–1.2 MAC transition (Fig. 2A).

HFB at 0.8 MAC depressed evoked neuronal responses in decerebrate rats, and the increase from 0.8 to 1.2 MAC caused a further 15% depression (Fig. 2B). However, increasing HFB from 0.8 MAC to 1.2 MAC had an opposite effect in intact rats, increasing evoked neuronal responses by nearly 30% (Fig. 2A). This effect of HFB in intact rats and decerebrate rats was significant (Fig. 4). Recovery responses at 0 MAC did not differ from the initial control responses for this less soluble anesthetic.

In intact rats, spontaneous activity was not significantly affected by increasing anesthetic concentration from 0.8 to 1.2 MAC for any of the anesthetics. In decerebrate rats, however, 0.8 MAC BNZ increased spontaneous activity (from 14 ± 4 at 0 MAC to 45 ± 11 impulses/5 s at 0.8 MAC, $P < 0.05$; Fig. 2B), but further significant changes were not seen when BNZ was increased from 0.8 MAC to 1.2 MAC. In addition, HFB at 1.2 MAC decreased spontaneous activity compared to activity at 0 MAC (from 25 ± 9 at 0 MAC to 2 ± 1 impulses/5 s at 1.2 MAC, $P < 0.05$). There was a significant correlation between spontaneous activity and the percent inhibition of the NMDA receptor at MAC (Fig. 5; NMDA receptor data from Solt et al.[2]).

Only partial recovery occurred in BNZ animals after 60 min of washout, but this could be explained by residual (i.e., 0.3 MAC) BNZ. In five rats at 0.4 MAC, we found 50% of the neuronal depression seen at 0.8 MAC BNZ (data not shown).

In decerebrate animals in the absence of anesthesia, evoked activity averaged 462 ± 131, 484 ± 82, and 453 ± 101 impulses/5 s at approximately 3, 4.5, and 6 h after decerebration, thus indicating no change over time or with repeated application of the noxious stimulus.

Both intact and decerebrate animals anesthetized with BNZ had spontaneous muscle twitching, with

brief and rapid movements of the limbs, and twitching of the whiskers.

DISCUSSION

Our MAC values for BNZ, ODFB, and HFB were 1.43%, 0.64%, and 1.31%, whereas Eger et al. found respective MAC values of 0.78%, 0.46%, and 1.74%.[7] Differences may have arisen because of differences in analytical techniques. Eger et al. did not use an internal standard and did not inject directly into the carrier stream, instead using a gas sampling loop.[7] In addition, they calculated MAC from separate measures of the ratio of inspired and arterial aromatic partial pressures, whereas we directly measured end-tidal concentrations. In the end, however, these differences should not have affected our results and conclusions, since results for the neuronal determinations were made at concentrations equivalent to those made in the estimate of MAC (i.e., are internally consistent).

Our main findings in intact rats (Fig. 2A) were the following. First, changes in neuronal responses to noxious stimulation produced by 0.8–1.2 MAC increases in BZN, ODFB, and HFB correlated inversely with the *in vitro* capacity of these aromatic compounds to block NMDA receptors (Fig. 3). Second, decerebration shifted these relationships downward only slightly for BZN; a change from 0.8 to 1.2 MAC caused a 25% decrease rather than the 19% decrease seen in intact rats (Fig. 2B), but shifted them markedly for HFB, changing the response from a 30% increase to an approximate 15% decrease (Fig. 4). Third, in decerebrate rats, a 0–1.2 MAC change in BNZ produced a much larger (60%) decrease in evoked responses than did HFB (24%). In intact rats, we studied the 0.8–1.2 MAC range because this is the range in which all animals would be expected to have movement (in response to noxious stimulation) abolished, which represents a critical pharmacological and clinical effect.

The importance of the $GABA_A$ receptor to immobility depends on the specific anesthetic. Immobility produced by etomidate and propofol crucially depends on an action at the beta3 subunit of the $GABA_A$, and no other, receptor.[8] However, mutation of this subunit minimally (if at all) affects the immobilizing potency of inhaled anesthetics.[8,9] Likewise, mutation of the α subunit, to eliminate the enhancement of the $GABA_A$ receptor to GABA, does not affect the immobilizing properties of isoflurane in intact mutant mice.[10] Zhang et al. concluded that the $GABA_A$ receptor does not mediate the immobility produced by inhaled anesthetics[11] because administration of the $GABA_A$ antagonist picrotoxin equally increased the MAC of anesthetics that minimally (cyclopropane) or considerably (isoflurane, halothane) enhance the *in vitro* response of $GABA_A$ receptors to GABA (implying that blockade of the $GABA_A$ receptor increases MAC through a nonspecific increase in neuronal excitability, as opposed to reversal of an anesthetic action at the $GABA_A$ receptor.) These investigators also reported that picrotoxin reversed the immobilizing effect of propofol much more than ketamine or isoflurane,[12] consistent with propofol (but not ketamine or isoflurane) acting at the $GABA_A$ receptor.

Anesthetic depression of the NMDA receptor supplies a possible mechanism by which immobility is produced.[2] Nitrous oxide, ketamine, and xenon all significantly depress the NMDA receptor at concentrations that produce immobility.[13,14] Volatile anesthetics such as isoflurane and halothane depress the NMDA receptor much less than nitrous oxide, ketamine, and xenon.[2,13] Consistent with the presently observed depression of spinal nociceptive responses by BZN, blockade of the NMDA receptor produces analgesia.[15] Because HFB did not depress the dorsal horn in the 0.8–1.2 MAC range in intact rats, the present data suggest that HFB might exert its immobilizing effect at more ventral neurons. Our studies of decerebrate rats suggest that HFB does have some (albeit less than BZN) capacity to affect dorsal horn neurons (Fig. 2B). Combined with the data of Kim et al.,[16] the present data suggest that anesthetics with a predominantly NMDA-depressive action might depress nociceptive responses of both dorsal and ventral horn neurons, whereas anesthetics with a predominantly GABAergic affect might depress ventral horn neurons more than dorsal horn neurons.

Our discussion has focused on differential effects of aromatic compounds on $GABA_A$ versus NMDA receptors. However, we cannot exclude the possibility that these compounds may act through some other neurotransmitter system (i.e., glycine) or at ion channels, such as potassium or sodium channels. A further limitation of our study was the small number of animals in some groups, which thereby restricted our ability to observe other possible effects of these compounds. Also, we did not test MAC in decerebrate rats, although in our experience decerebration does not have major effects on anesthetic requirements, and this has been reported by others.[17] Further to the issue of the anatomic sites at which inhaled anesthetics produce immobility, using a selective cerebral perfusion system in goats, we found that HFB produced immobility via a predominant spinal action, whereas ODFB produced immobility via actions on both spinal cord and brain.[18] We speculated that these anesthetics differed because of differences in their effects on $GABA_A$ versus NMDA receptors. Although we did not study BNZ using our selective perfusion model, the present data suggest that BNZ has predominant actions in the spinal cord, because decerebration did not appreciably alter the extent of depression of nociceptive responses between 0.8 and 1.2 MAC. In contrast, HFB appears to have a supraspinal facilitatory effect on dorsal horn neurons, in that nociceptive

responses diverged between intact rats (a slight numerical enhancement, Fig. 2A) and decerebrate rats (a depressive effect, Fig. 2B). Such a facilitatory action has been reported with isoflurane. In a selective cranial bypass study, we found that anesthetic requirements to produce immobility were decreased when isoflurane concentrations delivered to the head were decreased.[19] Kingery et al. reported that isoflurane acting at supraspinal sites facilitated nociceptive responses in addition to having supraspinal and spinal depressive effects.[20] Thus, some volatile anesthetics may have complex and opposing supraspinal and spinal effects on nociception and their capacities to produce immobility.

As observed previously,[1] BNZ causes spontaneous muscle twitching. Furthermore, nitrous oxide, a strong blocker of the NMDA receptor, also causes twitching, albeit limited to the whiskers.[21] This twitching persists even after decerebration, suggesting that it originates in the brainstem or spinal cord rather than the cerebral cortex. Because twitching was not observed after administration of pancuronium, a direct muscle effect was excluded. The cause of the twitching is unclear.

Our data and discussion concern the sites and mechanisms of action of fluorinated aromatic compounds. One might hope that these considerations apply broadly to all inhaled anesthetics. This may not be the case. Eger et al. found that blockade of NMDA receptors with MK-801 decreased the MAC of such compounds (including BZN, ODFB, and HFB) in inverse proportion to the *in vitro* capacity of these compounds to block NMDA receptors at MAC.[7] These findings are consistent with our results. However, the same report found no correlation between the effect of MK-801 on the MAC of conventional anesthetics (e.g., nitrous oxide, xenon, isoflurane, and halothane) and their capacity to block NMDA receptors at MAC. We did not test such anesthetics, and thus cannot say that our results apply to all inhaled anesthetics.

In summary, we found that fluorinated aromatic compounds depress dorsal horn neuronal nociceptive responses in proportion to their capacity to depress NMDA receptors. That is, the degree to which aromatic anesthetics inhibit the NMDA receptor may play a role in how these anesthetics modulate responses of dorsal horn neurons to noxious stimulation. In addition, HFB appears to have a supraspinal facilitatory effect on nociceptive responses in lumbar dorsal horn neurons.

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