BECAUSE the fetus and neonate are capable of sensing painful stimuli,1–3 and nociceptive-induced neuronal plasticity has long-term psychological and physiologic sequelae, including hyperalgesic states and worse perioperative morbidity and mortality,3–8 effective analgesia in the young is critical. In addition, neonatal pain processing pathways differ from mature systems,9,10 and therefore, one cannot assume that an analgesic in an adult model will be effective in younger age groups. Indeed, some anesthetics, e.g., nitrous oxide, seem to be ineffective because they require the participation of a dynamic range neurons within the intact spinal cord 19 tested.

Indeed, some anesthetics, e.g., nitrous oxide, seem to be ineffective because they require the participation of a pathway that is immature before the toddler stage.11 Xenon, a noble gas with anesthetic properties,12 exerts an analgesic effect in adult humans13,14 and animals,15,16 consistent with its profile as an N-methyl-D-aspartate (NMDA) antagonist.17,18 However the efficacy of xenon analgesia in younger age groups has not previously been tested.

Previous studies revealed that xenon suppressed wide dynamic range neurons within the intact spinal cord19 and was still effective in a spinal cord transection model,20 suggesting that xenon exerts an effect directly at the spinal cord, without requiring involvement of higher supraspinal centers. Therefore, we hypothesized that xenon could exert an antinociceptive effect in the presence of immature pain processing pathways that lack functional connectivity to supraspinal centers, as long as these express functional NMDA receptors.21,22 In this report, we investigate the efficacy of xenon- versus formalin-induced nociception as reflected by behavior and c-Fos expression (a marker of neuronal activation) in cohorts of rats at various ages.

Materials and Methods

The study protocol was approved by the Home Office (United Kingdom), and all efforts were made to minimize animal suffering and the number of animals used. Fischer rats were used for the entire study (B&K Universal, Grimston Aldbrough Hull, United Kingdom), which was conducted using a previously reported protocol.11 Experiments were performed on rat pups of 7, 19, and 28 days old and on adult rats (11–12 weeks old); these ages correlate with the human neonate, toddler, child, and adult, respectively.23

Within each age group, there were three cohorts (n = 3 or 4): air + formalin, xenon + formalin, and air + saline. Formalin groups were injected with 5% formalin subcutaneously into the plantar surface of their left hind paw; controls were injected with saline. The volume of formalin or saline injected was adjusted for each age group as previously reported11 and were as follows: 10 μl for 7 days old; 15 μl for 19 days old; 20 μl for 28 days old; 50 μl for adults. Xenon exposure consisted of 70% Xe–20% O2–10% N2 via a recirculating system. Formalin or saline was administered 15 min after gas exposure; thereafter, animals were exposed to the gas mixture for a further 90 min.

Immediately after injection of formalin, behavior was assessed for 60 min. Nociceptive behavior was assessed in the 7-day-old pups for the presence (1) or absence (0) of flexion, shaking, and whole body jerking per epoch of time and was calculated as

\[ \text{Nociceptive Score} = \frac{T}{300}, \]

where T is the duration (seconds) of nociceptive behavior exhibited during consecutive 300-s postinjection epochs.

Older rat pups were given scores across four categories of pain behavior: no pain (0); the injected paw was in continuous contact with floor, favoring (1; the injected paw rested lightly on the floor), lifting (2; the injected paw was elevated all the time), and licking (3; licking, biting, or shaking of the injected paw).24 These scores were calculated as

\[ \text{Nociceptive Score} = \frac{(T1 + [T2 \times 2] + [T3 \times 3])/300}, \]

where T1, T2, and T3 are the durations (seconds) spent in categories 1, 2, or 3 per 300-s epoch.

Ninety minutes after the formalin injection, animals were deeply anesthetized with pentobarbital (100 mg/kg, intraperitoneal) and perfused with 4% paraformaldehyde. The whole spinal cord was removed. The lumbar enlargement was sectioned transversely at 30 μm and then was stained for c-Fos as previously described.25

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Photomicrographs of three sections per animal were scored for c-Fos-positive neurons by an observer who was blinded to the experimental treatment. For the purpose of localizing the c-Fos-positive cells to functional regions of the spinal cord, each section was divided into A/B (laminae I–II or the superficial area), C (laminae II–IV or nucleus proprius area), D (laminae V–VI or the neck area), and E (laminae VII–X or the ventral area). The nociceptive intensity scoring against time in each animal was plotted, and the area under the curve (over a 60-min period) from each animal was calculated. The mean of c-Fos-positive neurons for three representative sections in each region as described above was the aggregate score for each animal. The results of nociceptive intensity or c-Fos-positive neurons are reported as mean ± SEM. The statistical analysis was performed by one-way analysis of variance, followed by Newman–Keuls test. A P value less than 0.05 was regarded as statistically significant.

Results

The time course of the nociceptive response of each cohort in each age category is presented in figure 1, and area under the curve data are shown in table 1. Saline injection caused minimal nociceptive behavior. Formalin injection in the presence of air caused a typical biphasic nociceptive response. During the preinjection period, rats exposed to air were awake and active. After injection with formalin, the 7-day-old animals exhibited intense nociceptive behavior for up to 50 min. Xenon exposure limited nociceptive behavior to the first 2 min, thereafter inducing immobility. Xenon significantly reduced the area under the curve compared with air (P < 0.001; table 1). Xenon also attenuated the formalin-induced nociceptive behavior at the other ages tested (19-day-old [P < 0.01], 28-day-old [P < 0.001], and adult ages [P < 0.001]; table 1) and also caused a reduced amount of movement relative to the other cohorts.

Formalin-induced c-Fos expression at the lumbar level of the spinal cord ipsilateral to the site of injection increased in all age groups in the presence of air. Exposure to xenon significantly suppressed c-Fos expression in all laminae in the spinal cord. In the 7-day-old pups, xenon exposure reduced c-Fos expression in response

Table 1. Areas under Curve, Calculated from Nociceptive Intensity Scoring Curves (Fig. 1)

<table>
<thead>
<tr>
<th></th>
<th>Air + Formalin</th>
<th>70% Xe + Formalin</th>
<th>Air + Saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-day-old</td>
<td>11 (0.4)</td>
<td>0.17 (0.04)†</td>
<td>0.15 (0.04)†</td>
</tr>
<tr>
<td>19-day-old</td>
<td>88 (8)</td>
<td>5 (0.9)†</td>
<td>4.6 (1.2)†</td>
</tr>
<tr>
<td>28-day-old</td>
<td>81 (7)</td>
<td>1.0 (0.3)†</td>
<td>5 (0.5)†</td>
</tr>
<tr>
<td>Adult</td>
<td>120 (0.7)</td>
<td>0.8 (0.1)†</td>
<td>0.4 (0.1)†</td>
</tr>
</tbody>
</table>

Data are presented as mean (SEM); n = 3 or 4.

* P < 0.01, † P < 0.001 relative to air + formalin group at the corresponding age group.
to formalin by 48% in laminae A/B ($P < 0.001$). In the 19-day-old rats, xenon suppressed mean c-Fos expression in response to xenon by 55% in laminae I–II ($P < 0.001$). In the 28-day-old rats, xenon depressed c-Fos expression in response to formalin by 34% in laminae I–II ($P < 0.001$). In adult rats, xenon inhibited c-Fos expression by 41% in laminae I–II ($P < 0.001$). Saline injection also caused c-Fos expression ipsilateral to the injection; however, this was much less intense than that induced by formalin injection (fig. 3).

To test whether xenon itself can cause c-Fos expression (as is the case with nitrous oxide), 27 naive animals...
Fig. 3. Number (mean ± SEM, n = 4) of c-Fos–positive cells at the lumbar level in response to formalin injection from the four age groups of animals receiving either air (black bars) or 70% Xe–20% O₂–10% N₂ (Xe) (dotted bars) or in response to saline injection from the four age groups receiving air (white bars). *P < 0.01, **P < 0.001 relative to air + formalin group at the corresponding region. +P < 0.01, ++P < 0.001 relative to xenon + formalin. The figures in the left column represent c-Fos expression ipsilaterally associated with injection, and those in the right column represent c-Fos expression contralaterally associated with injection. From 19 days old to adult, laminae I–II (superficial area), laminae II–IV (nucleus proprius area), laminae V–VI (neck area), and laminae VII–X (ventral area) in the spinal cord section are presented by A/B, C, D, and E, respectively, as equivalent to the five regions in 7-day-old pups.
were exposed to either air or the xenon mixture gas (70% Xe–20% O₂–10% N₂) for 90 min. The number of c-Fos–positive cells did not differ between these groups in any region of the spinal cord (data not shown).

Discussion

In the current study, we have demonstrated that xenon exerts an antinociceptive response versus formalin injection in Fischer rats at four developmental stages, i.e., at days 7, 19, and 28, as well as in adults. Xenon attenuated the formalin-induced pain response; however, the interpretation of behavioral data is confounded by the presence of sedation. Therefore, we also analyzed c-Fos expression immunohistochemically to objectively measure a surrogate marker of antinociception. As previously reported, 30 xenon attenuates both phases of the formalin test, unlike other NMDA antagonists, 28,29 which only inhibit phase 2. This may be because of a more pronounced sedative action of xenon relative to other anesthetics in this class, reflected by an awake minimum alveolar concentration of 33%; 30 alternatively, xenon may modulate nociception by mechanisms in addition to NMDA antagonism. However, the reduction in c-Fos immunoreactivity in the superficial laminae of the spinal cord at each age group shows that in the presence of xenon, nociceptive processing is attenuated with no discrimination for age.

These data are qualitatively different from those that we recently reported with nitrous oxide, 31 in which no antinociceptive effect (either behaviorally or immunohistochemically) was noted in animals younger than 23 days old, i.e., at ages when supraspinal centers have little influence on nociception. This is consistent with the observations of Miyazaki et al., 20 which showed little effect of nitrous oxide at the level of the spinal cord, unlike xenon. However, it should be stressed that we did not compare the effects of xenon and nitrous oxide directly at the different ages.

If these data can be extrapolated to the clinical setting, one would expect xenon to be an effective antinociceptive agent from a very early age in humans. The safety profile of xenon has yet to be examined in the very young, although it is a remarkably safe anesthetic in adults. 31 A major cause for concern in the clinical use of xenon, 25 Recently, a study involving neonatal rats suggested that widespread apoptosis occurred after the use of a combination of midazolam, nitrous oxide, and isoflurane resulting in deficits in hippocampal synaptic function and persistent memory-learning impairments. 34 Whether xenon has similar effects in neonates must be elucidated.

In summary, xenon suppresses both the behavioral and the immunohistochemical nociceptive responses to formalin even in very young animals. The antinociceptive effect of xenon does not seem to require functional connectivity between the supraspinal and spinal pain processing pathways. We suggest that xenon may be an effective analgesic in pediatric patients.

References