Posttraumatic syringomyelia is a disabling neurological condition that occurs between a few months and many years after a spinal cord injury. The symptoms of syringomyelia include pain, weakness, sensory deficits, and, in severe cases, respiratory compromise and death as a result of brainstem involvement. Despite favorable short-term results, surgical treatment for this condition remains unsatisfactory; long-term failure rates are as high as 80%. Better treatments are unlikely to be developed until the mechanisms for the formation and expansion of posttraumatic syringomyelia are more clearly understood.

Many theories regarding the pathogenesis of posttraumatic syringomyelia have been proposed, but a theory that is consistent with all available evidence remains elusive. Most theories have been based on clinical and radiological observations without experimental validation. Experimental validation can generally be performed only by using computational, mechanical, or animal models.

Computational modeling is often based on data obtained from human MRI studies and has focused mainly on CSF dynamics within the spinal subarachnoid space, spinal cord, and syrinx cavity. In recent years, more complex 2- and 3-dimensional computational models that account for the elastic properties of the spinal cord and...
syringomyelia. The ideal posttraumatic syrinx model is yet to be developed, but its characteristics should include the following: 1) excellent reproducibility and reliability of expanding syrinx production; 2) a mechanism of initial injury similar to that of human spinal cord injuries; 3) samples that histologically resemble postmortem human samples; 4) an extracanalicular location of syrinx cavities; and 5) progressive enlargement and neurological deficits similar to those in the human condition. A model that produces a small initial cavity with mild neurological deficits would be preferable to differentiate the effects of myelomalacia caused by spinal cord injury and subsequent syrinx enlargement.

Previous animal models of posttraumatic syringomyelia used different mechanisms of spinal cord injury with or without the induction of arachnoiditis. From early experimental work on a weight-drop model of spinal cord injury, researchers reported the formation of extracanalicular syrinx cavities. However, only a small proportion of the animals developed cavities, and some of these cavities resolved over time. A combined weight-drop and arachnoiditis model was developed by Cho et al. to produce extracanalicular syringomyelia in rabbits. Syrinx cavities developed in 55% of the animals, and the lack of reproducibility made this model unsuitable for further physiological investigations. Compression with aneurysm clips in combination with subarachnoid kaolin was used in posttraumatic syrinx models developed independently by Mizuno et al. and Seki and Fehlings. Josephson et al. developed a rodent thecal sac constriction model, in which a silk ligature was applied to obliterate the spinal subarachnoid space. Significant paralysis in all the animals was noted in all 3 studies, and the overall syrinx cavity rate was between 83% and 100%. A concern with both the compression and constriction methods is the severity of the neurological deficits and the difficulty in differentiating whether the resultant cystic cavities represented myelomalacia or posttraumatic syringomyelia.

An alternative posttraumatic syrinx model was developed by Yezierski et al., Yang et al., and Brodbelt et al. using intraparenchymal injections of quisqualic acid combined with a subarachnoid kaolin injection. Quisqualic acid produces selective neuronal cell death, inflammation, and extracanalicular cavities. Brodbelt et al. reported syrinx formation at 6 weeks in all the animals that received multiple doses of quisqualic acid, and cavities were larger and more spinal levels were involved when arachnoiditis was induced. The advantages of this model are the reliability in syrinx production, histological similarities to human specimens, and the absence of significant neurological deficits. A major criticism of this technique is that the mechanism of injury is not the compressive and contusional type of trauma sustained during human spinal cord injury.

Computer-controlled motorized spinal cord impactors that are capable of delivering more consistent impacts than the weight-drop or clip compression methods were recently developed. Spinal cord impactors have been demonstrated to yield more reproducible spinal cord contusion injuries than the traditional weight-drop method. Many studies have investigated the validity of these devices in studying spinal cord injury. The objective of this study was to develop an animal model of posttraumatic syringomyelia using a computer-controlled motorized spinal cord impactor and subarachnoid kaolin to reliably produce initial mild spinal cord injury and enlarging cystic cavities that resemble human posttraumatic syringomyelia.

Methods
After ethical approval from the Macquarie University Animal Ethics Committee, 70 male Sprague-Dawley rats that weighed 300–600 g were used. Two animals were killed early because of severe neurological deficits suffered from complications during the application of the stabilizing clamps, and they were excluded from the remainder of the study.

The study consisted of 2 parts. The first experiment (n = 20 animals) was performed to determine the optimal force required to induce an initial cavity without causing significant neurological deficits. Using the optimal force from the first experiment, the second experiment (n = 48 animals) aimed to characterize the temporal progression of the cystic cavities with and those without concurrent arachnoiditis.

Experiment 1: Determining the Optimal Force Setting
The Infinite Horizon spinal cord impactor (Precision Systems and Instrumentation, LLC) was used for this study. Previous studies using this impactor produced paraplegia with forces greater than 150 kDyn at midthoracic levels. To determine the force required to induce an initial cavity in the cervical cord without producing permanent neurological deficits, 20 animals were allocated to 1 of 5 groups (4 animals per group) with a force setting of 50, 75, 100, 125, or 150 kDyn. The dwell time for each impact was set at 0 seconds. The delivered force and displacement of the impacts were recorded. Neurological outcome was assessed using the Tarlov scale, and histology was performed 3 weeks after the initial operation.
Experiment 2: Temporal Progression of Cystic Cavities

The second experiment was performed to compare the progression of the cystic cavities after spinal cord impact in animals with \( n = 24 \) animals) and those without \( n = 24 \) animals) kaolin-induced arachnoiditis at 4 time points (1, 3, 6, and 12 weeks; 6 animals per time point per group). Each animal underwent a spinal cord impact at the optimal force determined in Experiment 1. The outcome measures were impact force, impactor tip displacement, impact velocity, neurological function score, and cavity dimensions.

Operative Procedure

Each procedure was performed in a sterile field with the animal in a state of general anesthesia. Anesthesia was induced with 4% isoflurane and maintained with 2%–2.5% isoflurane via a nose cone. The rat was positioned prone on the operating stage, and the skin was shaved and prepared with povidone-iodine. Under magnification with an operating microscope, the C6–T2 spinous processes, laminae, and lateral masses were exposed via a midline incision. A C7–T1 laminectomy was performed with rongeurs. Stabilizing clamps were attached to the lateral masses of C-6 and transverse process of T-2. The operating stage was then positioned such that the impactor tip was aligned over the midline of the spinal cord at the C-8 level. The desired force was programmed and delivered according to the computer software provided (Infinite Horizon spinal cord impactor v.5.0.4, Precision Systems and Instrumentation).

To induce arachnoiditis in rats in the kaolin groups, a 10-μl injection of 250 mg/ml kaolin (Sigma-Aldrich) in 0.9% saline solution was administered slowly into the subarachnoid space with a 0.5-ml 30-gauge insulin syringe (Ultra-Fine II, Becton Dickinson and Co.) after the spinal cord impact.

A layered closure was performed with resorbable sutures to fascia and skin. The animals were allowed to recover with analgesia and antibiotic coverage as required and access to food ad libitum. They were monitored for neurological deficits, excessive weight loss, and signs of distress.

Perfusion and Fixation

At the completion of the experiment, each animal was anesthetized with 4% isoflurane. Heparin (5000 IU) was injected via an intracardiac injection, and the left ventricle was cannulated to allow influx of fixative. The right atrium was incised to permit the efflux of blood and fixative. Each animal was perfused with 400 ml of 4% paraformaldehyde (Lancaster Synthesis Ltd) in 0.1 M phosphate-buffered saline (pH 7.4).

Histology

The brain and spinal cord were dissected out and post-fixed in 4% paraformaldehyde solution overnight. The spinal cord was divided into individual segments according to the spinal cord level from C-2 to T-4. The specimens were processed in paraffin over a 4-hour cycle in an automated tissue processor (ASP200S, Leica Biosystems) and then embedded in paraffin wax blocks. Transverse 5-μm sections were cut with a microtome and mounted on glass slides. The slides were allowed to air dry for at least 4 hours before staining.

H & E staining was carried out using the Lillie-Mayer protocol. After graded ethanol baths and xylene washes, the slides were coverslipped with DPX mounting medium (Scharlau Chemie SA) and allowed to dry in a 37°C oven for 3 days.

Image Acquisition and Processing

The sections were studied using light microscopy with an Axio Imager Z1 microscope (Carl Zeiss Microimaging GmbH). Photographs of the H & E–stained sections were taken using the AxioVision 4.8.1 program (Carl Zeiss Microimaging GmbH) at ×25 and ×50 magnifications. The dimensions and areas of the cavities and spinal cords were calculated using the “measure–length” and “measure–outline” functions, respectively, of the AxioVision program. The dimensions measured were the maximum anteroposterior and lateral diameters of the cavities, and the area of each cavity was measured and expressed as a ratio of the total area of the spinal cord section.

Statistical Analysis

Data are expressed as the means ± SD for the impact force, displacement, neurological outcome, dimensions, and area of the cavity. The Pearson correlation coefficient \( r \) was calculated to evaluate the relationship between impact force and displacement. Cavity areas and transverse cavity dimensions were compared between the different impact groups using 1-way ANOVA with Tukey’s posthoc multiple comparisons test. In Experiment 2, the 2 groups were compared over all time points using 2-way ANOVA with posthoc Bonferroni correction to adjust for multiple comparisons. A \( p \) value of < 0.05 was considered statistically significant. Software used included Excel 2007 (Microsoft Corp), Prism 6 (GraphPad Software), and SPSS 19 (IBM Corp).

Results

Experiment 1: Determining the Optimal Force Setting

The impact forces and displacements at the different programmed force settings are summarized in Table 1. Overall, all the rats underwent an impact force within 10 kDyn of the programmed force (Fig. 1 left), with the exception of 1 animal in the 150-kDyn group, which was killed according to the animal ethics protocol 24 hours after the operation because of complete paraplegia. The mean impactor tip displacement increased in a linear fashion with the programmed force (Pearson correlation coefficient \( r = 0.89 \)) (Fig. 1 right).

Higher programmed forces were associated with a greater degree of neurological deficit and a longer duration of recovery to normal muscle power. The mean Tarlov scores for hindlimb function over the first 5 postoperative days are shown in Fig. 2. With the exception of the animal in the 150-kDyn group that was killed, all the other rats recovered normal neurological function within 3 days of the operation.
The overall rate of cavity formation, mean cavity area, and mean transverse cavity dimensions were measured from the H & E–stained histological sections (Table 1). Cavities were present in all the animals except for 1 animal in the 50-kDyn group. There were no significant differences in the maximum anteroposterior cavity diameters or lateral diameters between animals in the different groups. The mean cavity area was greater in the 100-kDyn group than in the 50-kDyn group ($p = 0.046$, 1-way ANOVA).

Histological sections revealed a midline cystic cavity involving only the dorsal columns in the 50-kDyn group (Fig. 3A). At 75 kDyn, the cavity was adjacent to the central canal and did not disrupt the ependymal lining (Fig. 3B). At higher forces, the cavity involved the central canal, dorsal horns, and medial portions of the ventral gray matter (Fig. 3C–E). It was determined that 75 kDyn would be used as the optimal force setting for Experiment 2, because this force resulted in a 100% cavity-formation rate, no central canal involvement, and complete return to normal neurological function.

### Experiment 2: Temporal Progression of Cystic Cavity Dimensions

The results of Experiment 2 are summarized in Table 2. There were no significant differences in the impact forces between the kaolin and nonkaolin groups ($p > 0.99$, 2-way ANOVA). However, there was more variability in the displacement measurement, as demonstrated in Fig. 4, at the 12-week time point ($p = 0.014$, 2-way ANOVA). The overall cavity-formation rate in all the animals was 92%. In the 3-week kaolin and 12-week nonkaolin groups, the cavity-formation rate was 83%, and in the 12-week kaolin group, the cavity-formation rate was 66%. The remainder of the animals all had cavities.

Neurological function was similar between the groups ($p = 0.65$, 2-way ANOVA), with all the animals suffering transient weakness and recovering to normal function within 2 days postoperatively.

Cavity areas were significantly greater in the kaolin groups than in the nonkaolin groups ($p = 0.047$, 2-way ANOVA). At each time point, the kaolin groups had larger cavities in transverse dimensions when compared with those in the nonkaolin cohorts, although statistical significance was not reached (Fig. 5).

There were differences in cavity size at different time points within the groups. In the kaolin groups, the C-8 lateral dimensions were smaller at the 12-week time point than at the 6-week ($p = 0.039$, 2-way ANOVA) and 1-week ($p = 0.0026$, 2-way ANOVA) time points. Cavity areas were also smaller at 12 weeks than at 1 week ($p = 0.0048$, 2-way ANOVA) and 6 weeks ($p = 0.028$, 2-way ANOVA). In the nonkaolin groups, the lateral dimensions at C-8 were greater at the 1- and 6-week time points than at the 12-week time point ($p = 0.0045$ and 0.0060, respectively, 2-way ANOVA). Cavities were smaller at the 12-week time point than at the 1-week time point ($p = 0.0089$, 2-way ANOVA).

In the histological sections, there were larger, multiloculated cystic cavities and more dilated perivascular spaces.
and microcysts in the kaolin groups, particularly at the 6- and 12-week time points. There was also evidence of an ongoing chronic inflammatory response within the cystic cavities in the kaolin group at these time points (Figs. 6 and 7).

Discussion

The results of this study demonstrate the feasibility of using a controlled contusion injury combined with kaolin-induced arachnoiditis to model posttraumatic syringomyelia in rodents. Syrinx cavities were produced reliably, were histologically similar to those observed in humans, and were separate from the central canal. An impact force that produces a spinal cord cavity without significant neurological deficits was determined.

In this study, we used a computer-controlled motorized spinal cord impactor to deliver a contusional spinal cord injury at the C-7/T-1 level, the same location as in the quisqualic acid model.2,45,46 Lesions at the cervicothoracic region are preferable because human posttraumatic syringomyelia commonly affects the cervical cord. The benefits of the impact model are the more precise and accurate delivery of a designated force without previous contact with the spinal cord and the ability to measure the force and displacement of the impact using sensors within the impactor tip.30 The impact model produces a contusion injury that is thought to be the most appropriate simulation of human spinal cord injury pathology.11,19,24 In Experiment 1, the impact force was correlated with the displacement of the impactor tip and the duration of neurological deficits.

Previous studies that used the Infinite Horizon spinal cord impactor on rodents produced paraplegia with forces from 150 to 250 kDyn.33 The results of our Experiment 1 indicate that, in rodents that underwent a spinal cord impact between 50 and 125 kDyn of force, transient neurological deficits developed, and with the exception of 1 animal, recovery to a normal neurological state occurred within 3 days. The duration of neurological recovery correlated with the location of the cavities at the C-8 level. In the 50- and 75-kDyn groups, the cavities involved the dorsal columns but did not affect the central canal, whereas in the 100-, 125-, and 150-kDyn groups, the cavities involved the dorsal columns, central canal, and anterior horn of the spinal cord. To the best of our knowledge, this is the first study to characterize the neurological and histological outcomes of mild-to-moderate spinal cord injury using a rodent spinal cord impactor injury model.

Based on the results from Experiment 1, an optimal impact force of 75 kDyn was selected for the following reasons: 1) the overall initial cavity-formation rate was 100%; 2) the central canal was not involved, which is a hallmark of posttraumatic syringomyelia based on human autopsy studies; and 3) the neurological deficits were mild and transient. Not all the animals developed a cavity when an impact force of 50 kDyn was used, whereas in the groups that underwent impacts with a force of 100 kDyn or more, the central canal was involved.

In Experiment 2, although the overall cavity-formation rate was 92%, it was 100% in most groups except the 3-week-postinjury kaolin group (cavity-formation rate 83%) and the 12-week-postinjury group, in which cavity-formation rates of 67% and 83% were observed in animals that received kaolin and those that did not receive kaolin, respectively. Despite the demonstrated reproducibility of displacements in Experiment 1, the 12-week-postinjury group of animals showed a wider range of displacements, which could explain the lower cavity-formation rate within this group, and a cavity size that was smaller than those in the 6-week-postinjury group. The other explanation for this observation might be related to tissue processing and sectioning. In humans, syrinx cavities have been observed intraoperatively to be tense and expansile. However, in postmortem animal specimens, cavities tend to collapse after paraffin embedding and tissue processing and might...
give the appearance of a smaller cyst within an atrophic spinal cord. Another concern is the difficulty in differentiating cavities from myelomalacia and syringomyelia. Cho et al. defined a posttraumatic syrinx in a rabbit model as a cavity that extends at least 2 cm away from the site of the initial injury. In contrast, Seki and Fehlings, Brodbelt et al., and Radojicic et al. did not define the criteria for a syrinx cavity.

The rates of cavity formation reported in this study are comparable to those described in other posttraumatic syr-

TABLE 2. Experiment 2: Impact force parameters and resulting cavity-formation rates and cavity sizes in animals with and those without a subarachnoid injection of kaolin

<table>
<thead>
<tr>
<th>Time Point &amp; Kaolin Group</th>
<th>Delivered Force (kDyn)*</th>
<th>Displacement (μm)*</th>
<th>Overall Cavity-Formation Rate (%)</th>
<th>C-8 AP Dimension (μm)*</th>
<th>C-8 Lateral Dimension (μm)*</th>
<th>Area of Spinal Cord (mean %)</th>
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</thead>
<tbody>
<tr>
<td>1 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No kaolin</td>
<td>79 ± 3.13</td>
<td>676 ± 102.63</td>
<td>100</td>
<td>1160 ± 269.14</td>
<td>1371 ± 463.92</td>
<td>19</td>
</tr>
<tr>
<td>Kaolin</td>
<td>78 ± 2.86</td>
<td>656 ± 112.42</td>
<td>100</td>
<td>1204 ± 300.01</td>
<td>1722 ± 571.75</td>
<td>21</td>
</tr>
<tr>
<td>3 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No kaolin</td>
<td>79 ± 3.62</td>
<td>626 ± 209.61</td>
<td>100</td>
<td>819 ± 229.61</td>
<td>785 ± 457.01</td>
<td>11</td>
</tr>
<tr>
<td>Kaolin</td>
<td>83 ± 6.23</td>
<td>673 ± 233.26</td>
<td>83</td>
<td>835 ± 435.75</td>
<td>1189 ± 620.10</td>
<td>16</td>
</tr>
<tr>
<td>6 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No kaolin</td>
<td>80 ± 2.19</td>
<td>638 ± 96.81</td>
<td>100</td>
<td>797 ± 246.62</td>
<td>1374 ± 650.10</td>
<td>11</td>
</tr>
<tr>
<td>Kaolin</td>
<td>77 ± 3.25</td>
<td>767 ± 129.06</td>
<td>100</td>
<td>1075 ± 243.44</td>
<td>1449 ± 399.04</td>
<td>18</td>
</tr>
<tr>
<td>12 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No kaolin</td>
<td>80 ± 2.80</td>
<td>843 ± 204.83</td>
<td>83</td>
<td>459 ± 251.74</td>
<td>409 ± 290.19</td>
<td>4.9</td>
</tr>
<tr>
<td>Kaolin</td>
<td>79 ± 4.69</td>
<td>546 ± 169.49</td>
<td>66</td>
<td>467 ± 283.93</td>
<td>629 ± 533.19</td>
<td>6.2</td>
</tr>
<tr>
<td>Overall</td>
<td>79 ± 3.89</td>
<td>678 ± 174.78</td>
<td>92</td>
<td></td>
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</tbody>
</table>

* Values are means ± SD.

FIG. 4. Actual forces and displacements across groups in Experiment 2.

FIG. 5. Size of the syrinx at different time points. Left: Mean C-8 cavity lateral dimensions. Right: Percentage area of cord. Results shown are the mean ± SD.
At each corresponding time point, there was a trend toward animals with arachnoiditis having larger cavities, although it was significant only for syrinx area at 6 weeks. These results support observations made in previous studies, in which syrinx cavities were larger and involved more spinal levels when combined with arachnoiditis.6,27,38 On histological sections, the cavities were irregular in shape without an ependymal lining and surrounded by a chronic inflammatory infiltrate containing macrophages and microglia, which is consistent with the microscopic appearance of human posttraumatic syrinx spinal cord specimens.5,6,25 In addition, animals with arachnoiditis had more multiloculated cysts, an increased number of vacuolations within the spinal cord parenchyma, and more enlarged perivascular spaces than animals without arachnoiditis. In a feline model of cervical arachnoiditis, Klekamp et al.18 also observed enlarged perivascular spaces and proposed that they might lead to increased parenchymal edema. Coalescence of vacuolations into multiloculated cysts was postulated to eventually form syrinx cavities. Progression of the cavities from simple lesions in the earlier time points to multiloculated cystic cavities with more dilated perivascular spaces and microcysts at the later time points and the larger syrinx cavities observed in the kaolin group demonstrate that the cavities formed are not simply the result of myelomalacia but instead represent subsequent syrinx enlargement. It is likely that this enlargement is a result of CSF obstruction in the subarachnoid space.

An association between syringomyelia, arachnoiditis, and spinal cord trauma was described by Hallopeau in 1871 and Joffroy in 1887 based on necropsy studies.39 More recently, Bilston et al.4 demonstrated that arachnoiditis might cause increased perivascular flow through CSF–arterial pulsation decoupling. Similarly, Cheng et al.8 used a computational model to show that CSF flow dynamics can be altered with various degrees of obstruction within the spinal subarachnoid space as a result of arachnoiditis. However, the exact role of arachnoiditis in the pathophysiology of posttraumatic syringomyelia is still unknown.

Conclusions

Future investigations on the mechanisms of syrinx formation and expansion will require reproducible and reliable models that replicate the human condition. Physiological and molecular studies can be performed only on animal models. Human spinal cord injury consists of a complex combination of biomechanical mechanisms including contusion, compression, transection, and secondary chemical changes from excitotoxic amino acid release or ischemia.30 The spinal cord impactor injury does not replicate the entire spectrum of changes after a human spinal cord injury; however, it provides a reproducible and reliable model of contusional spinal cord injury that overcomes many of the limitations of previous models. The results of this study show that this model, which combines a contusion injury with arachnoiditis, would be suitable for further studies and may provide further insight into the pathophysiology of posttraumatic syringomyelia.

Acknowledgments

This project was funded by the Column of Hope Chiari & Syringomyelia Research Foundation and the National Health and Medical Research Council of Australia (project grant 604008). Dr. Wong was supported by scholarships from the Royal Australasian College of Surgeons.
and the Neurosurgical Society of Australasia. Prof. Bilston is supported by a senior research fellowship from the National Health and Medical Research Council of Australia.

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Disclosures
The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author Contributions
Conception and design: Wong, Bilston, Stoodley. Acquisition of data: Wong, Song. Analysis and interpretation of data: Wong, Song. Drafting the article: Wong. Critically revising the article: Hemley, Bilston, Cheng, Stoodley. Reviewed submitted version of manuscript: all authors. Statistical analysis: Hemley, Wong, Bilston. Study supervision: Stoodley.

Supplemental Information
Previous Presentations
Portions of this work were presented at the Neurosurgical Society of Australasia Annual Scientific Meeting held in Nadi, Fiji, on September 21–24, 2011; the ACSR Spinal Research Symposium IX held in Adelaide, Australia, on August 9–11, 2011; and the Syringomyelia 2010 International Symposium held in Berlin, Germany, on December 9–11, 2010.

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