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CHARACTERISING EFFECTS OF IMPACT VELOCITY ON BRAIN AND BEHAVIOUR IN A MODEL OF DIFFUSE TRAUMATIC AXONAL INJURY

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Abstract—The velocity of impact between an object and the human head is a critical factor influencing brain injury outcomes but has not been explored in any detail in animal models. Here we provide a comprehensive overview of the interplay between impact velocity and injury severity in a well-established weight-drop impact acceleration (WDIA) model of diffuse brain injury in rodents. We modified the standard WDIA model to produce impact velocities of 5.4, 5.85 and 6.15 m/s while keeping constant the weight and the drop height. Gradations in impact velocity produced progressive degrees of injury severity measured behaviourally, electrophysiologically and anatomically, with the former two methods showing greater sensitivity to changes in impact velocity. There were impact velocity-dependent reductions in sensorimotor performance and in cortical depth-related depression of sensory cortex responses; however axonal injury (demonstrated by immunohistochemistry for β-amyloid precursor protein and neurofilament heavy-chain) was discernible only at the highest impact velocity. We conclude that the WDIA model is capable of producing graded axonal injury in a repeatable manner, and as such will prove useful in the study of the biomechanics, pathophysiology and potential treatment of diffuse axonal injury.

Key words: traumatic brain injury, impact velocity, diffuse axonal injury, behaviour deficit, electrophysiology, sensory cortex.

INTRODUCTION

Traumatic brain injury (TBI) is one of the most common injuries encountered in motor vehicle accidents and many contact sports. In all these impact injury-inducing activities, an important factor known to determine injury outcomes is the impact kinematics. Thus changes in car velocity very well describe the risk and degree of injury for passengers in front-end car–car and car-barrier accidents (Dischinger et al., 1998; Nance et al., 2006; Kleiven, 2007; Ydenius, 2010) and for pedestrians in car–pedestrian accidents (Wood et al., 2005; Zhao et al., 2010); for car side-impact accidents, a major predictor of pelvic and thoracic injuries is the velocity of the door impacting on the crash test dummy (Tencer et al., 2005); and both in models of and in real car–pedestrian accidents the single most important factor in reducing pedestrian injury is the impact velocity (Zhao et al., 2010; Han et al., 2012).

There are several experimental models of TBI (viz. Gennarelli, 1994; Gilchrist, 2004; Cernak, 2005; Morales et al., 2005; Xiong et al., 2013), all of which share a common aim to reproduce either focal or diffuse brain damage as is encountered clinically (Morganti-Kossmann et al., 2010). Some models of focal TBI allow for variations in impact kinematics (e.g., in fluid pressure in the lateral fluid percussion (LFP) method – Thompson et al., 2005; Alder et al., 2011; Xiong et al., 2013), and newer models for creation of TBI recognise the importance of the injury kinematics (Gilchrist, 2004; Viano et al., 2009; Davidsson and Risling, 2011). However, there have been few studies on how variations in impact kinematics affect diffuse brain injury (the most common form of TBI), which is characterised by axonal swelling and bulb formations without focal lesion (Xiong et al., 2013). In previous studies (Hellewell et al., 2010; Yan et al., 2011; Alwis et al., 2012) we have used the well-established weight drop impact acceleration (WDIA) method (Maramou et al., 1994) to produce diffuse axonal injury with a fixed impact velocity generated by dropping a 450 g weight from 2 m height, the standard and most common parameters used for this method (Maramou et al., 1994). Potentially the WDIA method allows for variations in impact kinematics, but no single study has provided a link between anatomy, physiology and behavioural abnormalities following varying injury severity in this model. In the original report of the model, Marmarou et al. (1994) presented some data on the relationship between impact acceleration and injury. In a later study they further characterised the use of the model by altering the height at which the weight was dropped and in some instances varying animal weight (Beaumont et al., 1999), to find an increase in the degree of behavioural deficit when the weight was dropped from a greater

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Abbreviations: ANOVA, analysis of variance; CC, corpus callosum; LFP, lateral fluid percussion; NF-H, neurofilament heavy-chain; PFR, peak firing rate; PSTH, peristimulus time histograms; PW, principal whisker; SVZ, subventricular zone; TBI, traumatic brain injury; WDIA, weight drop impact acceleration; β-APP, β-amyloid precursor protein.

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height; however no anatomical or electrophysiological parameters were measured. Engelborghs et al. (1998) documented changes in intracranial pressure, skull fracture and an increase in mortality when drop heights were increased but no biological or behavioural indices were reported. Li et al. (2011) showed increased signs of axonal injury in the corpus callosum (CC) (as determined by β-amyloid precursor protein (β-APP) staining) with increasing drop height, but no other biological index or behaviour change was presented. Tsenter et al. (2008) used different drop heights to produce graded injury severity in mice (as determined by extensive behavioural testing and MRI assessment), but no functional neuronal changes were measured. A comparison of the WDIA model and the fluid percussion model by Hallam et al. (2004) studied behaviour and neuro-pathology but the WDIA method used there involved the additional complication of hypoxia, known to exacerbate diffuse brain injury (Hellewell et al., 2010; Yan et al., 2011; Alwis et al., 2012). Therefore, none of these studies allow overarching conclusions about the effect of different weight drop parameters (e.g., El Sayed et al., 2008; Li et al., 2011) on diffuse brain injury outcomes.

We have conducted a detailed study in rodents of behavioural, anatomical and electrophysiological changes associated with different degrees of injury produced by changes in impact velocity in the WDIA injury model. Diffuse TBI is the most common form of TBI (Helps et al., 2008; Myburgh et al., 2008; Faul et al., 2010) and knowledge of how impact kinematics affects the production of different facets of diffuse TBI is of interest in understanding this form of brain damage and critical in aiding the design of experiments that require graded levels of injury.

EXPERIMENTAL PROCEDURES

Induction of trauma

Animal experiments were conducted in accordance with the Code of Practice for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council, Australia), and received approval from the Monash University Standing Committee on Ethics in Animal Experimentation (Approval Nos.: MARP/2012/047). Adult male Sprague–Dawley rats were housed in a 12-h light/dark cycle with food and water *ad libitum*. Rats aged ∼12 weeks and weighing ∼350 g (range: 300–390 g) received a diffuse traumatic axonal injury using the weight drop impact acceleration method (Marmarou et al., 1994), modified as described previously (Yan et al., 2011). We have described our methods in detail elsewhere (Yan et al., 2011) and only a brief summary is presented here.

Rats were anaesthetised with 5% isoflurane in 22% O_2/78% N_2, intubated, and mechanically ventilated with a maintenance dose of 3% isoflurane in 22% O_2/78% N_2, at 75 breaths/min. The ventilation did not prevent spontaneous breathing and was essential to maintain oxygenation when animals were subsequently disconnected from the ventilator for trauma induction. A steel disc (10 mm in diameter and 3-mm thickness) was attached to the skull between bregma and lambda suture, using dental acrylic. The animals were then briefly disconnected from the ventilator and moved onto a foam mattress (Type E polyurethane foam, Foam2Size, VA, USA). A weight of 450 g was then dropped from 2 m within a vertical tube positioned directly over the steel disc on the animal's head. The weight was allowed to make only one impact on the disc; as it bounced back up into the tube, the tube was swivelled away so that any subsequent impacts due to weight bounce were prevented. Then the animals were quickly reconnected to the ventilator with isoflurane withdrawn from the ventilation gas so that they were ventilated in 22% O_2/78% N_2, again at 75 breaths/min. In the WDIA model, apnoea can commonly develop, over the period 5–10 min post-impact, and ventilation is therefore essential for survival. Animals were regularly checked during this period of ventilation for resumption of spontaneous breathing. Once breathing was re-established, animals were disconnected from the ventilator and time elapsed since trauma was recorded. Rats were housed in individual cages after surgery and placed on heat pads (37 °C) for 24 h to maintain normal body temperature during the recovery period with access to water and soft jelly products.

Sham surgery controls went through the same processes except that the weight was not dropped onto their heads.

Weight drop velocity and animal grouping

As in the Li et al. (2011) study, impact velocity was indexed by measuring the travelling speed of the weight at a point just above the rat's head, using videography with an ultrahigh speed camera at a rate of 2000 frames/s. To modulate the impact velocity we applied three modifications to obtain speeds, of 5.4, 5.85, and 6.15 m/s. The basic system was as described by Marmarou et al. (1994), with a brass weight of 450 g held in position in a perspex tube at a drop height of 2 m, using a pin that was removed manually to release the weight, consistently producing an impact velocity of 6.15 m/s. This basic system was modified to restrict the air flow from the top of the tube into the perspex tube while the weight was falling. The reduction in air flow decreased the weight drop velocity, but ensured that the height and weight remained constant. These modifications produced lower impact velocities of 5.4 and 5.85 m/s respectively. Except for velocity, every other component was kept constant between groups, i.e., weight, drop height, and experimental protocol. The number of animals in each test group was: 5.4 m/s impact velocity, $n = 11$, 5.85 m/s impact, $n = 9$, and 6.15 m/s impact, $n = 14$; six animals were tested as Sham surgery controls.

The effect of the different weight drop impact velocities was quantified in terms of mortality and, for surviving animals, sensorimotor function was examined at 24 h post-treatment followed by electrophysiological as well as histological examination with immunohistochemistry for two biomarkers for diffuse injury. Animals were