Invited Review

Lateral Fluid Percussion Brain Injury:
A 15-Year Review and Evaluation

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You can’t always get what you want
But if you try sometimes,
You just might find
You get what you need!

—Mick Jagger and Keith Richards
The Rolling Stones

ABSTRACT

This article comprehensively reviews the lateral fluid percussion (LFP) model of traumatic brain injury (TBI) in small animal species with particular emphasis on its validity, clinical relevance and reliability. The LFP model, initially described in 1989, has become the most extensively utilized animal model of TBI (to date, 232 PubMed citations), producing both focal and diffuse (mixed) brain injury. Despite subtle variations in injury parameters between laboratories, universal findings are evident across studies, including histological, physiological, metabolic, and behavioral changes that serve to increase the reliability of the model. Moreover, demonstrable histological damage and severity-dependent behavioral deficits, which partially recover over time, validate LFP as a clinically-relevant model of human TBI. The LFP model, also has been used extensively to evaluate potential therapeutic interventions, including resuscitation, pharmacologic therapies, transplantation, and other neuroprotective and neuroregenerative strategies. Although a number of positive studies have identified promising therapies for moderate TBI, the predictive validity of the model may be compromised when findings are

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translated to severely injured patients. Recently, the clinical relevance of LFP has been enhanced by combining the injury with secondary insults, as well as broadening studies to incorporate issues of gender and age to better approximate the range of human TBI within study design. We conclude that the LFP brain injury model is an appropriate tool to study the cellular and mechanistic aspects of human TBI that cannot be addressed in the clinical setting, as well as for the development and characterization of novel therapeutic interventions. Continued translation of pre-clinical findings to human TBI will enhance the predictive validity of the LFP model, and allow novel neuroprotective and neuroregenerative treatment strategies developed in the laboratory to reach the appropriate TBI patients.

Key words: animal models; head injury; therapeutic interventions; reliability; validity

INTRODUCTION

Initially developed for use in cat and rabbit (Hayes et al., 1987; Stalhammar et al., 1987), the midline fluid percussion model of brain injury was adapted to the rat (Dixon et al., 1987; McIntosh et al., 1987) and then modified to produce the injury over a single hemisphere in the rodent (McIntosh et al., 1989a). The lateral fluid percussion (LFP) model has since become the most extensively used and well characterized model of experimental TBI (Laurer et al., 2002). After exposure of the skull and trephination, injury is produced by the rapid impact of a fluid bolus against the intact dural surface (Fig. 1). Reproducible levels of injury severity can be achieved by adjusting the pendulum height, which defines the force of the fluid pressure pulse transmitted through the saline reservoir. Variations on the injury device have included physical changes, such as the substitution of a high pressure pump for the pendulum and saline reservoir (Toulmond et al., 1993a).

Although the initial aim for developing the LFP model was to generate coup-contrecoup injury in a small animal model (T.K. McIntosh, personal notes), the resulting pathology has been identified, rather, as a mixed model of TBI, with both focal and diffuse injury characteristics (subdural hematoma, subarachnoid hemorrhage, white matter tears) (Cortez et al., 1989; Hicks et al., 1996; Bramlett et al., 1997b; Graham et al., 2000b). Over the years, LFP has been used primarily to identify TBI-induced cellular and molecular changes and evaluate potential therapies which have established it as a valid and reliable model to study the pathophysiology of human TBI.

Intra- and Inter-Laboratory Reliability of the LFP Model of TBI

The LFP brain injury model has been widely adapted as a standard experimental model of TBI. The reliability of this model within each laboratory can be maintained through periodic examination of cognitive, neuromotor,

FIG. 1. Representative placement and site of originally described lateral (parasaggital) fluid percussion brain injury (inset) and schematic of a fluid percussion brain injury device. A pendulum from a known height impacts the piston of a saline-filled reservoir, forcing a brief fluid bolus into the sealed cranial cavity.
and histological injury-induced alterations and comparison to historical data; however systematic evaluation of model reliability across institutions remains difficult to achieve. Since publication of the original model paper, investigators purposefully or inadvertently have varied the craniectomy location (Yoshino et al., 1991; Dietrich et al., 1994b), the choice of anesthesia (Toulmond et al., 1993b; Dietrich et al., 1994b; Saatman et al., 1997; Hamm, 2001; Marklund et al., 2002), the size of craniectomy (Sato et al., 2001; D’Ambrosio et al., 2004), and the attachment method of the animal to the injury device via the implantation of a plastic cap (Toulmond et al., 1993b; Sato et al., 2001). Variations in attachment methods include the use of caps of different internal diameters, the placement of caps on top of versus inside the craniectomy, and the use of straight, 90° angle or long extension tubes, all of which markedly change the resistance delivered by the fluid pulse and alter the forces delivered to the brain. In addition to the above factors, the actual configuration of the fluid percussion device varies between laboratories, confounding the interpretation of injury severity. Although common presentation of atmospheric pressure as a measure of injury severity indicates the pressure produced by the apparatus (not what the animal received), the return of reflexes or mortality may better serve as biological indicators of injury severity. Movement of the craniectomy location or incident angle of injury can change dramatically the cellular characteristics of the injury [for reviews see (Povlishock et al., 1994; Laurer et al., 2002)].

As originally described for the rat, LFP involves a 4.8-mm craniectomy centered over the top of the parietal bone, 4.0 mm lateral to the sagittal suture (McIntosh et al., 1989a). The neuropathological sequelae evolve as a cortical contusion lateral to the actual impact site and cell death in the hippocampus, thalamus, and cortex, with limited involvement of the brainstem and contralateral hemisphere (Hicks et al., 1996; Smith et al., 1997a). Dietrich et al. (1994) modified the model to include a craniectomy centered over the right parietal bone, 2.0 mm more medial and 0.2 mm more rostral than originally described, resulting in ipsilateral neuronal cell loss in the hippocampus, thalamus, and cortex. However, neuronal loss in the CA2/CA3 region of the ipsilateral hippocampus was not reported in a subsequent study using this model (Bramlett et al., 1997a).

To accommodate transgenic technology and the associated molecular and genetic tools, Carbonell et al. (1998) adapted LFP to the mouse. As expected, the incident impact angle, craniotomy size, craniotomy location and anesthesia were varied to accommodate smaller body weight, skull size, and skull thickness. Despite the subtle variations in the injury induction, similar patterns of pathobiology (neuronal and glial) and behavioral deficits to the rat were observed (Carbonell et al., 1998, 1999). Additionally, LFP in the mouse results in uniform, non-progressive neuronal loss from all hippocampal subregions ipsilateral, but not contralateral, to the injury (Witgen et al., 2003; Tran et al., 2004), where an initial impairment of anterograde hippocampal-dependent cognitive function recovers by one month post-injury (Lifshitz et al., 2004).

Two more recent studies (Vink et al., 2001; Floyd et al., 2002) have emphasized the influence of craniectomy position in LFP injury. At craniectomy positions less than 3.5 mm away from the sagittal suture, both ipsilateral and contralateral cortical damage could be identified using magnetic resonance imaging (MRI) and conventional histological analysis, where the increased size of ipsilateral lesions were associated with greater motor deficits (Vink et al., 2001). When craniectomy positions were moved to a location greater than 3.5 mm from the sagittal suture, no contralateral cortical lesion developed (Vink et al., 2001). In contrast, the extent of hippocampal damage did not appear to correlate with craniotomy location. Larger rostral, caudal, medial and lateral shifts in craniectomy location within the boundaries of lambda, bregma, the sagittal suture and the temporal ridge also affected cognitive and histological outcome (Floyd et al., 2002). The extreme medial and rostral shifts in craniectomy location blunted the injury-induced cognitive deficit, and rostral shifts lessened the injury-induced hippocampal pathology. Thus, careful attention to craniectomy position on the part of the surgeon can enhance reproducibility and reliability of the model.

The use of different anesthesia paradigms may alter the extent of injury via neuroprotective or vascular effects (Salzman et al., 1993; Warner et al., 1996; Statler et al., 2000), thereby complicating the interpretation of data across laboratories. However, therapeutic evaluation of anesthetics alone has yet to be evaluated after LFP. Despite these variations in the mechanics of producing LFP brain injury, numerous reports across several different groups present a close association between injury severity and extent of injury (Perri et al., 1997; Saatman et al., 1998; Dhillon et al., 1999; Sanders et al., 1999; Vink et al., 2001), defining the reproducibility of the model across laboratories.

Histology/Pathophysiology

Time course and regional degeneration. It is generally accepted that LFP reproduces the histopathology associated with multiple types of human traumatic brain injury. Depending on the severity of injury, the predominant histopathological feature of LFP is a focal contusion in
the cortex with accompanying petechial or intraparenchymal hemorrhage (Cortez et al., 1989; McIntosh et al., 1989a; Bramlett et al., 1997b), similar to that observed after human closed head injury (Graham et al., 2002). Histological markers of degeneration used to document the regional and temporal pattern of cell death and dysfunction following LFP include: loss of Nissl substance, degeneration as seen in silver stained preparation and by acid fucsin, gene activation, and immunohistochemical detection of kinases, phosphatases, and structural proteins. Acutely after injury, contused tissue is evident in the cortex beneath the injury site, which enlarges over weeks to become a cavity lined with glia (glia limi- tans) that progressively expands due to ongoing cell death up to one year post-injury (Smith et al., 1997a; Pierce et al., 1998; Bramlett et al., 2002). Over days to months, progressive degenerative cascades persist in additional, selectively vulnerable brain regions, including the hippocampus (Cortez et al., 1989; Smith et al., 1991; Lowenstein et al., 1992; Hicks et al., 1993, 1996; Conti et al., 1998; Carbonell et al., 1999; Dhillon et al., 1999), thalamus (Hicks et al., 1996; Conti et al., 1998; Pierce et al., 1998; Saatman et al., 1998; Carbonell et al., 1998; Sato et al., 2001; Raghupathi et al., 2003), medial septum (Sinson et al., 1997; Smith et al., 1997a), striatum (Hicks et al., 1996; Pierce et al., 1996; Hallam et al., 2004), and amygdala (Hallam et al., 2004). Due to the lateralization of the injury, however, few reports have demonstrated pathology extending into the brainstem or contralateral hemisphere (Lowenstein et al., 1992; Pierce et al., 1998; Grady et al., 2003), where hippocampal atrophy and hilar neuronal loss have been demonstrated. Yet, the lateralization of the pathology may allow for qualitative comparisons between the directly injured hemisphere and the contralateral hemisphere, which may or may not exhibit damage depending on the outcome measure and specific configuration of the injury. The time course and extent of degeneration demonstrate that LFP is a mixed model of brain injury (both focal and diffuse), which progresses through time and across brain regions. Although variations in the model can affect the extent and progression of degeneration observed after LFP, cortical damage without brainstem compression is a characteristic feature of LFP.

In the human condition, moderate to severe TBI is often associated with skull fracture and surface contusions across multiple gyri (Graham et al., 2005). Despite these clinical features that cannot be modeled by LFP, intracranial hemorrhage, brain swelling and progressive gray matter damage are hallmarks of the pathophysiology of both TBI and LFP (Graham et al., 2000a). Although the primary mechanical injuries differ, the craniectomy after severe human TBI allows the ensuing secondary damage to occur under similar conditions in both animal and man. Subdural and intraparenchymal hematomas increase intracranial pressure and brain swelling, which may result in the progressive distortion and herniation of brain tissue.

Secondary cellular damage and loss. Within different brain regions, all cell types are susceptible to the mechanical forces of TBI. The primary injury consists of rapid deformation of the brain, leading to rupture of cell membranes, escape of intracellular contents, and disruption of blood flow (McIntosh, 1994), resulting in primarily necrotic cell death (Dietrich et al., 1994b; Rink et al., 1995). The delayed or secondary injury, however, is a complex series of biochemical, structural and molecular changes that, in combination, can lead to cellular damage and loss resembling both apoptosis and necrosis (Rink et al., 1995; Conti et al., 1998; Raghupathi et al., 2002). Pathologic release of excitatory amino acid (EAA) neurotransmitters (glutamate, aspartate) and subsequent activation of glutamate receptors, results in the influx of Na+, efflux of K+, and subsequent Ca2+ influx into the cell (Faden et al., 1989; Katayama et al., 1990), causing cellular swelling (cytotoxic edema) and the excitotoxic destruction of cells through direct or indirect pathways. Although, the initial rise in extracellular potassium, cellular depolarization and uncontrolled release of excitatory neurotransmitters define the excitotoxic insult, secondary progression of the injury after LFP can affect selectively specific cell types, as demonstrated by neuronal damage across brain regions, including the cortex, hippocampus, thalamus and striatum using FluroJade (Sato et al., 2001; Hallam et al., 2004). Acute damage progresses to apoptotic and necrotic neuronal death after LFP in rodents and remains detectable for months (Dietrich et al., 1994b; Rink et al., 1995; Hicks et al., 1996; Yakovlev et al., 1997; Bramlett et al., 1997b; Conti et al., 1998; Keane et al., 2001; Bramlett et al., 2002; Raghupathi et al., 2002; Knoblach et al., 2002b). Moreover, glial susceptibility to the pathology associated with LFP has been demonstrated at both acute and chronic time points throughout the brain (Cortez et al., 1989; Hill et al., 1996; Conti et al., 1998; Carbonell et al., 1999; D’Ambrosio et al., 1999; Hill-Felberg et al., 1999; Zhao et al., 2003; Grady et al., 2003). Regional degeneration can be identified by astrocyte, macrophage and microglia activation (Lowenstein et al., 1992; Soares et al., 1995a; Okimura et al., 1996; Bramlett et al., 1997a; Hill-Felberg et al., 1999; Zhao et al., 2003; Grady et al., 2003). The initial increase in neutrophils lining the vasculature spreads into surrounding contused tissue (Soares et al., 1995a), while damage and loss of endothelial cells demonstrate significant alterations to the microvascula-
tured inflammation (Fan et al., 1995; Toulmond et al., 1999; Polderman et al., 2000; Hlatky et al., 2002), affording the LFP model further construct validity in terms of the secondary pathophysiology.

Subcellular and molecular response. The focal and diffuse nature of LFP affects most, if not all, components of the CNS. Reports have documented pathology associated with subcellular organelles (Vink et al., 1990a; Hovda et al., 1991; Lifshitz et al., 2003a), gene transcription (O’Dell et al., 2000c; Natale et al., 2003), protein translation and folding (Tanno et al., 1993; Lowenstein et al., 1994; Raghupathi et al., 1995b; Fan et al., 1999), cytoskeletal components (Saatman et al., 1998), and synapses (Emery et al., 2000). In injured tissue, metabolic function is impaired, which may reflect damage directly to mitochondria or trafficking of metabolic substrates. Not only cell death genes, but an array of housekeeping, neurotrophic, cytokine, cell adhesion and immediate early genes are known to change their expression directly to mitochondrial or trafficking of metabolic substrates. The focal and diffuse nature of LFP affects most, if not all, components of the CNS. Reports have documented pathology associated with subcellular organelles (Vink et al., 1990a; Hovda et al., 1991; Lifshitz et al., 2003a), gene transcription (O’Dell et al., 2000c; Natale et al., 2003), protein translation and folding (Tanno et al., 1993; Lowenstein et al., 1994; Raghupathi et al., 1995b; Fan et al., 1999), cytoskeletal components (Saatman et al., 1998), and synapses (Emery et al., 2000). In injured tissue, metabolic function is impaired, which may reflect damage directly to mitochondria or trafficking of metabolic substrates. Not only cell death genes, but an array of housekeeping, neurotrophic, cytokine, cell adhesion and immediate early genes are known to change their expression significantly after LFP brain injury (Hayes et al., 1995; Raghupathi et al., 1995a, 1996; Hicks et al., 1997b; McIntosh et al., 1998; Truettner et al., 1999; Giza et al., 2002; Natale et al., 2003). Moreover, the integrity of DNA lies susceptible to injury-induced cascades (Conti et al., 1998; Zhang et al., 1999; LaPlaca et al., 1999). Additionally, the levels and distribution of proteins involved in cell death, cell signaling, inflammation, cytoskeleton, and synaptic transmission also are altered by this model of TBI (Yakovlev et al., 1997; Knoblach et al., 1999; Dietrich et al., 1999; O’Dell et al., 2000c; Lotocki et al., 2003). Stretching and shearing of axons, both in the direct vicinity of the injury and in areas remote to the impact, contribute to the cytoskeletal damage that disrupts the cytoarchitecture related to network function and cellular transport (Hicks et al., 1997a; Bramlett et al., 1997b; Saatman et al., 1998; Carbonell et al., 1998). Total cellular disruption, from transcription to function, has been explained by increases in the intracellular calcium concentration from plasma membrane disruption and the activation of calcium-permeable ion channels (Fineman et al., 1993; McIntosh, 1994; Osteen et al., 2001). Subsequent kinase activation initiates transcription factors, heat shock proteins and signaling cascades that effect the delayed sequelae of brain injury. These intracellular signaling cascades occur concurrently with cytokine-mediated inflammation (Fan et al., 1995; Toulmond et al., 1995; Raghupathi et al., 1995a; Fan et al., 1996). In contrast to tissue pathology, the injury-induced activation of signaling pathways extends into the contralateral hemisphere (Raghupathi et al., 1995a; Raghupathi et al., 1996; Fan et al., 1996). Whether the cellular and molecular responses to LFP result from the initial injury, secondary pathology or reflect a recovery process remain to be determined.

Physiology

Vital signs and arterial blood gases. Cerebral autoregulation is impaired to some degree in severely brain-injured patients (Overgaard et al., 1974; Bouma et al., 1990; Golding et al., 1999), with increased likelihood for exacerbation of secondary injury by hypotension (Struchen et al., 2001). In LFP brain injury, severity-dependent increases in mean arterial blood pressure (MAP) immediately after injury became depressed in severely-injured animals (McIntosh et al., 1989a). In more moderate injury severities, MAP returned to baseline and remained stable by five minutes post-injury (McIntosh et al., 1989a). Subsequently, the severity-dependent pattern of hypertension has become a physiological hallmark of LFP (Dietrich et al., 1994b; Prins et al., 1996; Fukuda et al., 1996; Marklund et al., 2001a). Interestingly, adult rats subjected to LFP brain injury lose vascular tone after induction of secondary hemorrhagic shock (Law et al., 1996).

Episodes of hypoxemia, defined as apnea/cyanosis or an PaO2 arterial blood gas less than 60 mm Hg in the acute phase of severe brain injury increases morbidity and mortality (Brain Trauma Foundation, 2000b). Similarly, periods of apnea and unconsciousness (McIntosh et al., 1989a; Smith et al., 1991; Dietrich et al., 1994b; Prins et al., 1996; Fukuda et al., 1996), as well as PaO2 decreases and PaCO2 increases, are evident in severe LFP-injured, spontaneously breathing rats (McIntosh et al., 1989a).

Intracranial pressure, cerebral blood flow, and metabolism. Intracranial pressure (ICP) increases over the initial minutes across a wide range of ages following LFP brain injury (Prins et al., 1996; Armstrong, 1999b). The magnitude of the ICP increase depends on injury severity and is accompanied by an initial increase in mean arterial blood pressure (MAP; Cushings’s response). However, the Cushings’s response is attenuated markedly in developing animals (post-natal day 17) (Prins et al., 1996).

Although injury-induced increases in ICP have been thought to contribute to secondary reductions in local cerebral blood flow (ICBF) after LFP, this concept is somewhat simplistic. In fact, a transient hyper-acute increase in ICBF (Muir et al., 1992) is followed by a pro-

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longed decrease starting within 15–30 min after injury (Yamakami et al., 1989; Ozawa et al., 1991; Muir et al., 1992; Ginsberg et al., 1997), and lasting up to 4 h (Yamakami et al., 1989; Muir et al., 1992). Similar dynamic responses in ICP and ICBF have been documented after human TBI (Kelly et al., 1996; Kelly et al., 1997; Martin et al., 1997). Underlying mechanisms responsible for changes in ICBF following brain injury may include a loss of vasoreactive signaling, spasm and chronic constriction due to ionic disturbance. Although CBF is reduced after LFP, it rarely approaches a CBF range traditionally considered to be below the ischemic threshold, unless the injuries are severe (Yamakami et al., 1989; Graham et al., 2002). However, the contribution of cerebral ischemia after TBI may be related more to the metabolic demands of the tissue, rather than the actual rate of flow.

The metabolic demands of LFP-injured brain, as it relates to glucose utilization, has been explored using 14C-2-deoxy-d-glucose autoradiography (Sunami et al., 1989a,b; Yoshino et al., 1991; Kawamata et al., 1992; Hovda et al., 1992). During the acute phase after LFP, both the developing and adult brain exhibit a marked increase (up to 150%) in the utilization of glucose, primarily within the cerebral cortex and dorsal hippocampus ipsilateral to the injury, and evident in the contralateral hemisphere also (Thomas et al., 2000). Glucose metabolism increases due to the increase in extracellular potassium (Katayama et al., 1990) and concomitant activation of glutamate receptors (Yoshino et al., 1991; Kawamata et al., 1992). The short-lived increase in glucose utilization after LFP can last for over an hour, depending on the severity of injury, after which the same regions enter a state of glucose metabolic depression that can last for up to 2 weeks. More importantly, during the days after LFP, the delivery of glucose may be compromised, given a reported loss of coupling between local cerebral glucose metabolism and cerebral blood flow, a condition consistently seen in human TBI (Jaggi et al., 1990; Bergsneider et al., 1997), particularly in areas previously associated with neuronal necrosis (Kawamata et al., 1992; Ginsberg et al., 1997).

Over the last 10 years, similar dynamic changes in cerebral glucose metabolism have been reported in patients following severe TBI using 18F-deoxy-d-glucose incorporating positron emission tomography (Hovda et al., 1995; Bergsneider et al., 1997; Bergsneider et al., 2000, 2001; Wu et al., 2004), further validating the clinical relevance of the LFP brain injury model. The changes in glucose metabolism are more prolonged in patients than those after LFP, lasting for as long as 2 weeks both regionally and globally. In contrast, the subsequent glucose metabolic depression in brain-injured patients is evident for over a year. Interestingly, the degree of increase or subsequent decrease in glucose metabolism after human TBI is not related to the severity of injury, in agreement with the LFP model. In fact, the severity of injury may be defined by the length of time animals or patients exhibit the ensuing metabolic disturbances.

In addition to glucose utilization, oxidative capacity after LFP exhibits prolonged reductions in the cerebral cortex and underlying hippocampus as assessed using cytochrome oxidase histochemistry (Hovda et al., 1991). Deficits in oxidative capacity may stem from mitochondrial dysfunction imposed by increases in intracellular calcium after LFP (Lifshitz et al., 2003a). In human TBI, monitoring oxidative metabolism remains a hallmark of neuro-intensive care management. As the LFP model demonstrates, patients exhibit a pronounced decrease in oxidative metabolism for days to weeks after injury (Obrist et al., 1984, 1987; Glenn et al., 2003). Similarly, the mechanisms behind oxidative metabolic reductions in human patients appear to be similar to those reported for LFP brain injury (Verweij et al., 2000).

Dynamic changes in metabolic demand, dysfunctional mitochondria, and the loss of coupling between cerebral blood flow and metabolism demonstrate that the injured brain may be in a state of energy crisis. The energy crisis may explain how secondary activation of the injured brain could overwhelm the energy demands of the tissue and result in secondary cell death (Ip et al., 2003). Thus, the reductions in ATP after LFP are associated with energy crisis, rather than ischemic levels of cerebral blood flow (Lee et al., 1999; Lifshitz et al., 2003a).

Electrophysiology

The examination of ensuing electrophysiological alterations began with the initial description of the LFP model. At the higher levels of injury, disturbances in brainstem auditory-evoked potentials were observed, suggestive of disruptions in auditory neuronal processing (McIntosh et al., 1989a). The initial response to injury includes an indiscriminant recurrent release of excitatory neurotransmitters, leading to widespread depolarization (Faden et al., 1989; Katayama et al., 1990; Hayes et al., 1992). Only recently have electrophysiological disturbances in cortical neuronal activity been examined in the acute post-injury period. The number of cortical spreading depression cycles increases as injury severity increases, with almost constant activity at more severe injury levels (Rogatsky et al., 2003). And spontaneous seizures recorded by electrocorticography up to 2 months post-injury demonstrate post-traumatic epileptic episodes associated with hyperexcitability of the neocortex (Kharatishvili et al., 2003; D’Ambrosio et al., 2004).
In the hippocampus, however, significant injury-induced electrophysiological changes repeatedly have been confirmed, and are almost entirely confined to the hemisphere ipsilateral to the injury. Pathological alterations in GABA-mediated inhibition are implicated in LFP hippocampal dysfunction (Reeves et al., 1997; Toth et al., 1997; Santhakumar et al., 2001). At seven days post-injury, the disruption of dentate gyrus excitability manifests as an injury severity-dependent lowering of seizure threshold (Lowenstein et al., 1992). The greater disinhibition in the dentate gyrus lowered thresholds for developing self-sustained seizure activity 1–12 weeks post-injury compared to sham (Coulter et al., 1996; Santhakumar et al., 2001). A proposal for the reduction in inhibition includes the selective loss of inhibitory interneurons, since the frequency of miniature inhibitory synaptic currents decreases (Coulter et al., 1996; Toth et al., 1997; Santhakumar et al., 2000), which reverses months after injury potentially due to enhanced excitatory input onto preserved inhibitory interneurons (Santhakumar et al., 2001). Seemingly contradictory results that demonstrate opposite shifts in excitability have been attributed to proximity of the recordings to the injured cortex and the remaining anatomical connections in the slice preparation (Reeves et al., 1997; Muraoka, 2002). Similarly, LFP disrupts neuronal activity, primarily inhibition, in area CA1 of the hippocampus (Reeves et al., 1997; D’Ambrosio et al., 1998; Akasu et al., 2002), establishing regional shifts in excitability within the injured hippocampus. Global functional disruption is paralleled by the inability to induce or express long-term potentiation up to 8 weeks post-injury (Miyazaki et al., 1992; Sick et al., 1998; D’Ambrosio et al., 1998; Sanders et al., 2000). The regional shifts in excitability and impairments in long-term potentiation may contribute to injury-related learning and memory deficits (Smith et al., 1991; Floyd et al., 2002).

Behavioral Outcomes

Significant neuromotor, sensory and cognitive dysfunction are frequent sequelae of human TBI (Capruso et al., 1992; Sullivan et al., 1994; Levin, 1995; Koelfen et al., 1997; NIH Consensus Conference, 1999). Thus, behavioral analyses have been adapted or developed for use with LFP brain injury in an attempt to replicate events associated with human TBI. To date, behavioral evaluations after LFP brain injury have been limited to the rat and mouse models. Behavioral assessments can be used to determine the severity of the LFP injury (McIntosh et al., 1989a) and functionally evaluate treatments with various therapeutic interventions. The use of specific behavioral outcome measures after experimental brain injury has been reviewed recently (Fujimoto et al., 2004). With behavioral testing after LFP, the choice of tests should address appropriately the recovery process or treatment paradigm to maintain clinical and experimental relevance throughout the investigation (Hamm, 2001). Most importantly, behavioral evaluation should consist of several tests at numerous post-injury time points to document various sequelae and recovery occurring after LFP brain injury. As the aim of experimental brain injury research is to allow brain-injured patients to live long and relatively normal lives, long-term behavioral testing needs to be incorporated into the study design, particularly in interventional studies.

Return of reflexes. After LFP, the corneal, pinna1, paw flexion, and righting reflexes are transiently lost and rapidly return (Floyd et al., 2002; Hallam et al., 2004; Lee et al., 2005). Individual reflexes substitute reasonably well for motor components of the Glasgow Coma Scale (GCS) (Teasdale et al., 1974; Dixon et al., 1987), and reflect the loss of reflexes often seen in the acute phase of human head injury, providing the model additional validity. As such, the return of the righting reflex, and other reflexes, has served routinely as an indicator of severity of LFP injury (Morehead et al., 1994; Schmidt et al., 1995; Carbonell et al., 1998; Hallam et al., 2004; Lee et al., 2005), which appears to correlate with neuromotor deficits.

Neuromotor outcome. After TBI in humans, many long-term neuromotor deficits including difficulties with coordination, posture and steadiness of movement have been shown years after injury (Schalen et al., 1994). The degree of neurological motor impairment depends upon the severity of injury, allowing the testing of motor function to further characterize the magnitude of injury, to evaluate the progression of motor impairment, and to evaluate the efficacy of pharmacological treatments targeting the mechanisms responsible for motor dysfunction. After LFP, a composite neuromotor score (composite neuroscore or neurologic sum score) has been used routinely assess neurological motor function and corresponds to motor components of the GCS in the clinical setting (Hamm, 2001). Components of the composite score vary by laboratory, but generally include evaluations of gross limb strength and reflex function, which may include a measure of activity level (Faden et al., 1989; McIntosh et al., 1989a; Sun et al., 1995a; Knoblauch et al., 1998). The composite score of neuromotor function remains a reliable test to assess the level of neurological motor impairment from 48 hours to one year after LFP injury (Sun et al., 1995a; Saatman et al., 1997; Pierce et al., 1998; Knoblauch et al., 2002a; Furukawa et al., 2003).
In addition to reflexive motor tests, vestibulomotor tests determine the degree of fine motor coordination after injury (Table 1). Through tests such as the rotating pole (Mattiasson et al., 2000; Piot-Grosjean et al., 2001), beam balance (Hamm, 2001; Floyd et al., 2002; Alessandri et al., 2002), beam walk (Saatman et al., 1996; Lyeth et al., 2001; Piot-Grosjean et al., 2001; Floyd et al., 2002; Alessandri et al., 2002), rotarod (Hamm, 2001), and rope grip (Long et al., 1996), vestibulomotor deficits have been shown days to weeks after LFP.

Sensorimotor outcome. Recently, sensorimotor function has been tested after LFP brain injury. The sticky paper test (adhesive paper test) has been adapted for use in LFP from other rodent models of neurologic injury (Schallert et al., 1982; Hernandez et al., 1988), and is sensitive to detect sensorimotor deficits and treatment efficacy (Schallert et al., 1982; Riess et al., 2001). Also, limb placement (whisker test) has been adapted for use in LFP from ischemia models (De Ryck et al., 1989; Schallert et al., 2000), and measures injury-induced deficits in somatosensory and motor integration (O’Dell et al., 2000b). The progression of these sensorimotor impairments (and recovery) over time provides insight to sensory information processing in the injured brain.

Cognitive outcome. Cognitive tests in a clinical setting include both verbal and visual recognition tests, whereas laboratory tests typically rely on performance in a variety of hippocampal-dependent spatial mazes. After LFP, both anterograde and retrograde cognitive function is impaired, as tested in several cognitive tasks. Designed to assess the cognitive processes of learning and working memory (Morris et al., 1982; D’Hooge et al., 2001), the Morris water maze (MWM) was the first cognitive test used with the LFP model (Smith et al., 1991) and remains

### Table 1. Outcome Measures Used to Detect Neurological Impairment after Fluid Percussion Brain Injury

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<td>Hamm, J. Neurotrauma, 2001</td>
</tr>
<tr>
<td>Beam Walk</td>
<td></td>
<td>Lyeth et al., J. Neurotrauma, 1993</td>
</tr>
<tr>
<td>Rotarod</td>
<td></td>
<td>Lyeth et al., Exp. Neurol., 2001</td>
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<td></td>
<td></td>
<td>Hamm et al., J. Neurotrauma, 1996</td>
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<tr>
<td>Rotating Pole</td>
<td></td>
<td>Hamm et al., J. Neurotrauma, 2001</td>
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<tr>
<td></td>
<td></td>
<td>Mattiasson et al., J. Neurosci. Methods, 2000</td>
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<tr>
<td></td>
<td></td>
<td>Hoover et al., J. Neurotrauma, 2004</td>
</tr>
<tr>
<td>Rope Hang/Grip Test</td>
<td></td>
<td>Long et al., J. Neurotrauma, 1996</td>
</tr>
<tr>
<td>Tapered Beam</td>
<td></td>
<td>Klint et al., J. Neurotrauma, 2003</td>
</tr>
<tr>
<td>Cognitive deficits</td>
<td>Morris Water Maze (Memory)</td>
<td>Hicks et al., J. Neurotrauma, 1993</td>
</tr>
<tr>
<td></td>
<td>Smith et al., J. Neurotrauma, 1991</td>
<td></td>
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<tr>
<td></td>
<td>Lyeth et al., Exp. Neurol., 2001</td>
<td></td>
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<tr>
<td></td>
<td>Sanders et al., J. Neurotrauma, 1999</td>
<td></td>
</tr>
<tr>
<td>Morris Water Maze (Learning)</td>
<td></td>
<td>Hoover et al., J. Neurotrauma, 2004</td>
</tr>
<tr>
<td></td>
<td>Griesbach et al., Neuroscience, 2004</td>
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<tr>
<td></td>
<td>Pierce et al., Neuroscience, 1998</td>
<td></td>
</tr>
<tr>
<td>Radial Arm Maze</td>
<td></td>
<td>Lyeth et al., Exp. Neurol., 2001</td>
</tr>
</tbody>
</table>
the most frequently used test of cognitive function after LFP brain injury. Cognitive deficits in memory are observed 48 h to 2 weeks after injury LFP (Smith et al., 1991; Sinson et al., 1997; Pierce et al., 1998; Bramlett et al., 1999a; Leoni et al., 2000), while learning deficits are detectable up to 1 year depending on injury severity (Pierce et al., 1998; Sanderson et al., 1999; Schmidt et al., 1999; Sanders et al., 1999; Lyeth et al., 2001). The Lashley maze and the 8-arm radial maze, which evaluate working memory, have demonstrated injury-induced cognitive deficits after LFP (Lyeth et al., 2001; Piot-Grosjean et al., 2001). Furthermore, cognitive function related to classical conditioning and associative memory has been tested using conditioned fear and shows hippocampal-dependent cognitive deficits after LFP (Hogg et al., 1998a,c). Similarly, severe human TBI leads to enduring memory and learning dysfunction (Barth et al., 1983; Bennett-Levy, 1984; Leplow et al., 1997).

**VALIDITY OF THE FLUID PERCUSSION MODEL OF TBI**

**Face Validity**

When evaluating the validity of LFP to model clinical TBI, one must address what is known about human TBI, to date. In human closed head injury, biomechanical, physiological, neurological, and morphological alterations result from the primary injury. Although other experimental TBI models may appear to better replicate the mechanisms involving the primary impact, the pathophysiological sequelae and functional deficits after LFP represent an injury to the brain that closely reproduces those seen following clinical TBI. The LFP model reproduces several aspects of human TBI, including focal contusion, petechial intraparenchymal and subarachnoid hemorrhages, tissue tears and traumatic axonal injury (McIntosh et al., 1989a; Graham et al., 2000b). Other sequelae of human head injury include blood–brain barrier (BBB) disruption, axonal injury, neuronal loss, changes in glucose metabolism, alterations in cerebral blood flow, seizure activity, excitatory amino acid release, and altered levels of consciousness (vide supra, and see Narayan et al., 1996). After LFP, the sequelae include BBB disruption (Cortez et al., 1989; Tanno et al., 1992; Soares et al., 1992), white matter damage (Hicks et al., 1997a; Graham et al., 2000b), neuronal loss (Cortez et al., 1989; Toulmond et al., 1993a; Hicks et al., 1996; Smith et al., 1997a; Saatman et al., 1998; Sullivan et al., 2000; Sato et al., 2001), altered cerebral metabolism (Hovda et al., 1990; Yoshino et al., 1991), altered cerebral blood flow (Yuan et al., 1988; Yamakami et al., 1989; Kelly et al., 2000), altered brain electrical activity (Miller et al., 1990; Lowenstein et al., 1992), and both acute and chronic behavioral abnormalities (McIntosh et al., 1989a; Pierce et al., 1998; Sanders et al., 1999; Faden et al., 2001; Lyeth et al., 2001; Hamm, 2001). Although LFP injury, like most experimental models, cannot fully reproduce the entire heterogeneous and multifaceted spectrum of clinical TBI, the distinct correlations between clinical and preclinical pathophysiological sequelae afford LFP face validity in modeling human TBI.

**Predictive Validity: Evaluation of Potential Therapeutic Strategies Using LFP**

**Stabilization therapies.** Brain-injury patient management focuses on post-injury stabilization, and the Guidelines for the Management of Severe Traumatic Brain Injury (Brain Trauma Foundation, 2000a) offers options, not recommendations, for treatment. Despite the tremendous need for the evaluation of interventions in the initial management phase, experimental evidence is sparse. With the energy demands and metabolic uncoupling following both experimental and human TBI, the infusion of certain fluids can influence the duration and extent of acute metabolic/cerebrovascular alterations and ensuing neuropathology. Intravenous infusion of lactate after LFP increased brain lactate levels in brain-injured animals when compared to a saline infusion, and recovery of dialysate glucose occurred more rapidly (Chen et al., 2000), suggesting that lactate infusion reduced the characteristic fall in brain glucose observed after LFP (Kawamata et al., 1995; Chen et al., 2000; Bentzer et al., 2000). Brain-injured animals treated with lactate have also been shown to have significantly shorter learning latencies in the MWM at 2 weeks post-injury than saline-treated animals (Rice et al., 2002), indicating that lactate may serve as a potential clinical therapy for moderately brain-injured patients (Bondoli et al., 1978). In addition, intravenous infusion of high-dose and high-concentration human serum albumin reduced contusion volume and improved ipsilateral CA3 neuronal survival after LFP (Belavie et al., 1999). Albumin treatment reduced local cerebral metabolic rate of glucose compared to both vehicle-treated injured animals and sham-injured animals, indicating that albumin fluid resuscitation may be a potential acute management strategy for the TBI patient (Ginsberg et al., 2001). However, a multi-center, randomized, double-blind trial comparing the effects of albumin and saline fluid resuscitation detected increased mortality at 28 days in a sub-group of brain-injured ICU patients treated with albumin (SAFE Study Investigators, 2004). But the low patient numbers and acute 28-day time point leave ambiguity as to albumin treatment. Since the initial physiologic responses af-
ter LFP (e.g., changes in BP, O2, CO2) closely parallel those observed in human TBI, a tremendous opportunity exists to explore stabilization therapies using the LFP model. To date, however, studies concentrating on factors in the initial management of TBI have been limited and are worthy of further investigation.

**Pharmacology.** The disturbance of cognition and neurological function, the development of cerebral edema and the progressive tissue loss in selectively vulnerable brain regions ipsilateral to injury have been used as outcome measures in the preclinical assessment of the therapeutic efficacy of various pharmacological compounds after LFP (McIntosh et al., 1998; Laurer et al., 2000; Royo et al., 2003) and see Tables 2–4. Due to the clinically relevant features of the LFP brain injury model, high expectations have emerged for the development of new pharmacological treatments. Owing to the complexity of the secondary post-injury cascade, no single target treatment is likely to attenuate all post-injury behavioral and histological alterations. Although a detailed overview of the pharmacology of TBI is beyond the scope of this re-

| Table 2. Pharmacological Modulation of Excitotoxicity after Lateral Fluid Percussion Brain Injury |
|---|---|---|
| **Drug and reference** | **Time and route of administration** | **Outcome** |
| Glutamate release inhibitors | | |
| BW1003C871 | Iv, 15 min | Edema ↓ |
| Riluzole²-⁵ | 2-5Iv, 15 min | ²²LV ↓, ²⁵NS ↑, ⁵Edema ↓, |
| 619C89⁶ | Iv, pre | ⁴CA3 →, ⁶memory ↓, ⁵LV → |
| Lubeluzole⁷ | Iv, 15 min | NS ↑, CA3 ↓ |
| Competitive and non-competitive NMDA receptor antagonists | | |
| MK-801⁸-¹⁰ | 8-10 Iv, 8 pre, 8-50 min, 10⁶h | |
| Dextrophan¹¹-¹² | ¹¹Iv, 30 min, ¹²Iv, 30 min | |
| CPP¹³ | icv, pre | |
| Remacemide¹⁴ | Iv, 15 min | |
| Ketamine¹⁵ | Iv, 10 min | |
| CP-101,606¹⁶ | Iv, 15 min | |
| CP-98,113¹⁶-¹⁷ | Ip, 15 min | |
| NMDA (polyamine site) receptor antagonists | | |
| CP-101,581¹⁶ | Ip, 15 min | |
| Eliprodil¹⁰,¹⁸ | ¹⁰Ip, 15 min - 18h | |
| AMPA/KA receptor antagonists | | |
| Talampanel¹⁹ | Iv, 30 min or 3h | |
| YM87⁲⁰-²¹ | Iv, 15 min | |
| RPR117824²² | Iv, 15 min | |
| NMDA (glycine and Mg²⁺ sites) receptor antagonists | | |
| I2CA²³ | Iv, 15 min | |
| KYNA²³-²⁴ | Iv, 15 min | |
| Mg¹,¹⁵,²⁵-³¹ | ¹,²⁵,²⁹IV, 15 min, ²⁵IV, pre, ²⁶IV, 30 min, ²⁷IV, 60 min, ²⁸IV, 20 min, ³⁰IV, 10 min, ³¹Icv | |
| Zinc chelation (CaEDTA)³² | Icv, pre | |
| Inhibitors of NMDA subunits | | |
| Antisense oligos³³ | icv, pre | |
| Metabotropic receptor (group II–III) agonists | | |
| DCG-IV and (R,S)-PPG³⁴ | Intracerebr., <5 min | |
| LY354740³⁵ | Iv, 30 min | |

(continued)
Glutamate receptors are classified as the \( N \)-methyl-d-aspartate (NMDA; the subunits NR1, NR2A, NR2B, NR2C and NR2D), a-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), kainate (Glur5-9, KA1 and KA2), and the Group I (mGlur1 and mGlur5), Group II (mGlur2 and mGlur3) and Group III (mGlur4, mGlur6, mGlur7 and mGlur8) metabotropic receptors. Administration of glutamate release inhibitors (Riluzole and 619C89) reduces the indiscriminate rise in extracellular glutamate to minimize the excitotoxic influence on cellular glutamate to minimize the excitotoxic influence on post-injury pathology, although normal physiological glutamate concentrations may be toxic in injured brain (Di et al., 1999).

### Table 2. Pharmacological Modulation of Excitotoxicity after Lateral Fluid Percussion Brain Injury (Cont’d)

<table>
<thead>
<tr>
<th>Drug and reference</th>
<th>Time and route of administration</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Metabotropic receptor (group 1) antagonists</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCPG</td>
<td>36–38 Icv pre, 37 Iv 15 min</td>
<td>36 LV ↓, 36NS →, 36MWM → 36Mort ↓, 37icv: NS ↑, 37CA3 ↓, 37Iv: NS ↑, 38MWM ↑, 38BW ↑</td>
</tr>
<tr>
<td>AIDA</td>
<td>36 Icv, pre, 39 Intracerebr., 5 min</td>
<td>36MWM →, 36NS ↑, 36LV ↓, 39MWM ↑, 38CA3 ↓, 39BW ↓</td>
</tr>
<tr>
<td>MPEP</td>
<td>Icv, pre</td>
<td>LV ↓, NS ↑, MWM ↑</td>
</tr>
<tr>
<td><strong>NMDA receptor agonist</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cycloserine</td>
<td>Ip, 24h</td>
<td>MWM ↑</td>
</tr>
</tbody>
</table>

**Abbreviations used in Tables 2–4:** All time points are given as minutes or hours post-injury, except for pre, which means administration prior to LFP brain injury. Icv, intracerebroventriculatry; Intracerebr, intracerebral and/or intraparenchymatous; Po, per oral; Sc, subcutaneous; Iv, intravenous; IA, intraarterial; →, no effect; ↑, increased; ↓, decreased; mort, mortality; LV, lesion volume; MWM, Morris water maze (learning and memory); NS, composite neuroscore; CA3, CA3 cell death; CA1, CA1 Cell death; TUNEL, TdT-mediated dUTP nick end labeling; IgG, blood-brain barrier disturbance; BW, beam walk; BB, beam balance test; RP, rotating pole test; ctx, cortex; SP, Sticky paper.

**Abbreviations for compounds in Table 2:** BW1003C87, [5-(2,3,5-trichlorophenyl) pyrimidine 2,4-diamine ethane sulphonate]; Riluzole, 2-amino-6-trifluoromethyl benzthiazole; 619C89, [4-amino-2-(4-methyl-1-piperazinyl)-5-(2,3,5-trichlorophenyl)pyrimidine mesylate monohydrate]; Lubeluzole, [(2S)-2-(2-benzothiazolylmethyl-amino)-a-[(3,4,3,4 difluorophenoxo)methyl]-1-piperidino-neethanol]; MK-801, Dizocilpine maleate; CPP, 3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid; Ramacemide, 2-amino-N-(1-methyl-1,2-diphenylethyl) acetamide hydrochloride; NPS 1506, 3,3-Bis-(m-fluorophenyl)-N-methylpropylamine hydrochloride; CP-101,606, (1S,2S)-1-(4-hydroxyphenyl)-2-(4-hydroxy-4-phenylpiperidino)-1-propanol; Talampanel, (R-7-acyl-5-(4-amino-phenyl)-8,9-di-hydro-8-methyl-7H-1,3,5-dioxolo(4,5-h)(2,3) benzodiazipine); YM872, [2,3-dioxo-7-(1H-imidazol-1-yl)-6-nitro-1,2,3,4-tetrahydron-1-quinoxinalyl]-acetic acid monohydrate; RPR117824, 9-carboxymethyl-imidazo-[1-2a]indenone[12e]; 12CA, Indole-2-carboxylic acid; KYNA, kynurenic acid; Mg, Magnesium; CaEDTA, calcium disodium ethylenediaminetetraacetate; Oligos, oligonucleotides; DCG-IV, 2,2′,3′-dicarboxycyclopropylglycine; (RS)-PPG, (R,S)-4-phosphonophenyglycine; LY354740, (1S,2S,5R,6S)-(+)-2-amino-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid; MCPG, (S)-α-4-carboxypheynylglycine; AIDA, (RS)-1-aminoindan-1,5-dicarboxylic acid; MPEP, 2-methyl-6-(phenylethynyl)-pyridine; Cycloserine, (R+)–Amino-3-isoxazolidinone.


view (McIntosh et al., 1998; Royo et al., 2003; Marklund et al., 2004b), several classes of targeted pharmacologic therapies, tested in the LFP model, are reviewed below.

**ATTENUATION OF EXCITOTOXICITY.** After LFP, an early, marked increase in extracellular glutamate (Faden et al., 1989; Katayama et al., 1990; Panter et al., 1992) establishes excitotoxicity as a component of post-injury pathology, although normal physiological glutamate concentrations may be toxic in injured brain (Di et al., 1999).
cellular pathology. The improved outcomes reported with the glutamate release inhibitor Riluzole using the LFP model (McIntosh et al., 1996; Bareyre et al., 1997; Wahl et al., 1997; Zhang et al., 1998) (Table 2) have prompted the current clinical trial with this drug that is ongoing in European centers.

NMDA receptor activation and calcium influx are contingent on the binding of glutamate and the release of the

<table>
<thead>
<tr>
<th>Drug and reference</th>
<th>Time and route of administration</th>
<th>Outcome</th>
</tr>
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<tbody>
<tr>
<td>Anti-inflammatory drugs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-ICAM-1</td>
<td>Icv, 15 min, 4Iv, 15 min, 43 Icv, 15 min</td>
<td>↑</td>
</tr>
<tr>
<td>IL-1ra</td>
<td>Icv, 15 min</td>
<td>↓</td>
</tr>
<tr>
<td>sIL-1R</td>
<td>Icv, 15 min</td>
<td>↓</td>
</tr>
<tr>
<td>IL-10</td>
<td>Icv, 30 min, Icv, 30 min, Icv, 25 min</td>
<td>↓</td>
</tr>
<tr>
<td>TNF/IL-6 MAB</td>
<td>Icv, 1h</td>
<td>↓</td>
</tr>
<tr>
<td>sTNFR-Fc</td>
<td>Icv/Iv, 15 min</td>
<td>↓</td>
</tr>
<tr>
<td>P-selectin</td>
<td>Iv, 3 min</td>
<td>↓</td>
</tr>
<tr>
<td>Prostacycline</td>
<td>Iv, 20 min</td>
<td>↓</td>
</tr>
<tr>
<td>Cyclosporin</td>
<td>Icv, 15 min</td>
<td>↓</td>
</tr>
<tr>
<td>VCP</td>
<td>Intracerebr., 15 min</td>
<td>↓</td>
</tr>
<tr>
<td>Free radical scavengers and NOS inhibitors</td>
<td></td>
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<tr>
<td>Tirilazad mesylate</td>
<td>Iv, 30 min, 5Iv, 3 min</td>
<td>↑</td>
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<tr>
<td>PEG-SOD</td>
<td>Iv, &lt; 5 min</td>
<td>↑</td>
</tr>
<tr>
<td>MDL 74,180</td>
<td>Iv, &lt; 5 min</td>
<td>↓</td>
</tr>
<tr>
<td>PBN, SPBN</td>
<td>Iv, 5 min</td>
<td>↓</td>
</tr>
<tr>
<td>STAZN</td>
<td>Iv, 5 min</td>
<td>↓</td>
</tr>
<tr>
<td>LY341122</td>
<td>Po, pre; Iv, 5–30 min</td>
<td>↓</td>
</tr>
<tr>
<td>7-NF</td>
<td>63–64 Iv, pre, 64 &lt; 5 min</td>
<td>↓</td>
</tr>
<tr>
<td>L-arginine</td>
<td>Icv, &lt; 5 min</td>
<td>↓</td>
</tr>
<tr>
<td>SIN-1</td>
<td>IA, &lt; 5 min</td>
<td>↓</td>
</tr>
<tr>
<td>L-NAM</td>
<td>63–64 Iv, pre, 64 &lt; 5 min</td>
<td>↓</td>
</tr>
<tr>
<td>AG</td>
<td>63–64 Iv, pre, 64 &lt; 5 min</td>
<td>↓</td>
</tr>
<tr>
<td>Neurotrophic factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGF</td>
<td>Icv, 15 min</td>
<td>↓</td>
</tr>
<tr>
<td>NGF</td>
<td>Intracerebr., 24h</td>
<td>↓</td>
</tr>
<tr>
<td>BDNF</td>
<td>Intracerebr., 4h</td>
<td>↓</td>
</tr>
<tr>
<td>BFGF</td>
<td>28,72–73 Icv, 20 min</td>
<td>↓</td>
</tr>
<tr>
<td>Estrogen</td>
<td>Ip, pre</td>
<td>↓</td>
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<tr>
<td>Inhibitors of apoptosis and endocrinological approaches</td>
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<td></td>
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<tr>
<td>z-DEVD-fmk</td>
<td>Icv, pre</td>
<td>↓</td>
</tr>
<tr>
<td>1-ARA-35</td>
<td>Iv, 30 min</td>
<td>↓</td>
</tr>
<tr>
<td>YM-14673</td>
<td>Icv, 30 min</td>
<td>↓</td>
</tr>
<tr>
<td>4(5)-NO2 TRH</td>
<td>Icv, 30 min</td>
<td>↓</td>
</tr>
<tr>
<td>D-phe CRH</td>
<td>Icv, 15 min</td>
<td>↓</td>
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</tbody>
</table>

(continued)
TABLE 3. Classes of Compounds Evaluated after Lateral Fluid Percussion Brain Injury (Cont’d)

<table>
<thead>
<tr>
<th>Drug and reference</th>
<th>Time and route of administration</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-emopamil84</td>
<td>Ip, 15 min</td>
<td>Memory ↑, NS ↑, Edema ↓</td>
</tr>
<tr>
<td>LOE90893</td>
<td>Iv, 15 min</td>
<td>LV →, MWM →, NS ↑</td>
</tr>
<tr>
<td>BMS-20435284</td>
<td>Iv, 10 min</td>
<td>Edema ↓, NS ↑, MWM →, LV →</td>
</tr>
<tr>
<td>AK29585–86</td>
<td>Ia, 15 min</td>
<td>85 MWM ↑, 84 NS ↓, 83 LV →, 86 TUNEL+ cx/CA3 →</td>
</tr>
</tbody>
</table>

Abbreviations for compounds in Table 3: NOS, Nitric oxide synthase; ICAM, Intercellular adhesion molecule; IL1, Interleukin-1 receptor antagonist; sIL1ra, soluble IL1ra; IL10, interleukin-10; TNF, tumor necrosis factor; IL-6, interleukin-6; sTNFR:Fc, soluble TNF alpha receptor fusion protein; VCP, vaccinia virus complement control protein; PEG-SOD, polyethylene glycol-conjugated superoxide dismutase; MDL 74,180, 2,3-dihydro-2,2,4,6,7-pentamethyl-3-(4-methylpiperaizino)-methyl-1-benzofuran-5-ol dihydrochloride; PBN, α-phenyl-N-tent butyl-nitronite; S-PBN, 2-sulfophenyl-N-tent butyl nitronite; STAZN, stilbazulenyl-bis-nitronite; Y341122, 2-(3,5-di-t-butyl-1,4-hydroxyphenyl)-4-(2-(4-methylethylaminomethyl-phenolxy)-ethyl)xoxazole; 7-NI, 7-nitroindazole; SIN-1, 3-morpholino-sydnonimine; L-NAME, N(G)-nitro-L-arginine methyl ester; AG, Aminoguanidine hydrogen carbonate; IGF-1, insulin-like growth factor-1; NGF, nerve growth factor; BDNF, brain-derived growth factor; BFGF, basic fibroblast growth factor; Z-DEVD-FMK, N-benzyloxycarbonyl-Asp-Glu-Val-Asp fluoromethyl ketone; I-ARA-35b(35b), 57a, 4(5)NO2 TRH, 2,4 diiodo TRH, and 2-ARA-53a are analogues and derivatives of thyrotropin-releasing hormone; YM-14673, Z-Leu-aminobutyrlic acid-CONH(CH2)3-morpholine.


Voltage-sensitive magnesium block (Nowak et al., 1984). The post-LFP loss of intracellular magnesium may promote NMDA-receptor activation (Vink et al., 1990b; Golding et al., 1994; Vink et al., 1996). The competitive NMDA NR2B antagonist CP101, 606 has shown efficacy in the LFP model (Okiyama et al., 1997) (Table 2) and is currently being evaluated in a multi-center clinical trial in TBI patients in the United States. Limiting NMDA-receptor activation by the blockade of co-agonist glycine, polyamine and zinc sites (Fields et al., 1991) also have proven differentially successful in LFP brain injury (Table 2) (Lea et al., 2003; Marklund et al., 2004b). NMDA channel blockers including, but not limited to dextromethorphan, dextrophan, ketamine, MK-801, NPS1506 and remacemide dihydrochloride, decrease neuronal death, edema and/or neurological dysfunction after LFP brain injury (Table 2). Inhibiting NMDA-receptor activation (MK-801 and PCP) after LFP may disrupt LTP and cognitive processes necessary for post-injury recovery (Davis et al., 1992; Hamm et al., 1994a). However, these compounds often show strong psychomimetic side-effects, may be neurotoxic, and have not fared well in clinical trials (Bullock et al., 1999; Maas et al., 1999). Synthetic competitive polyamine site blockers (eliprodil) and AMPA-receptor antagonists (e.g., RPR117824, YM872 and Talampanel) can reduce cortical lesion volume and im-

54
prove neurological motor function after LFP (Toulmond et al., 1993b; Belayev et al., 2001; Mignani et al., 2002) (Table 2). The administration of magnesium post-injury attenuates behavioral deficits and histological alterations (Bareyre et al., 1999; Saatman et al., 2001) (Table 2). Magnesium administration is currently being studied in a single-center NIH-sponsored clinical trial to evaluate its effects to reduce post-traumatic epilepsy in TBI patients. Other glycine-site NMDA receptor antagonists shown to be effective in the LFP model (e.g., KYNA, I2CA; Table 2) have not been developed for clinical trials.

Activation of Group I mGluR receptors can potentiate neuronal excitation and exacerbate excitotoxic cell death (Mukhin et al., 1996), whereas Group II and III agonists reduce excitation and may provide neuroprotection after LFP brain injury (Faden et al., 2001). Group I metabotropic antagonists (MCPG, AIDA and MPEP) improve neurological motor deficits, cortical lesion volume and cognitive performance after LFP (Gong et al., 1995; Mukhin et al., 1996; Faden et al., 2001; Lyeth et al., 2001; Movsesyan et al., 2001a). Group II metabotropic agonists (LY 354740 and DCG-IV) can attenuate neurological motor deficits, and reduce the number of degenerating hippocampal neurons (Allen et al., 1999; Zwienenberg et al., 2001) (Table 2).

Of interest, several reports have demonstrated beneficial actions of glutamate and glutamate receptor activation. For example, the NMDA agonist D-cycloserine improved cognitive performance 24 h after LFP (Temple et al., 1996) (Table 1). Although the attenuation of excitotoxicity presents a promising treatment strategy, the inherent neuronal functions served by glutamate receptor activation must be considered carefully.

### Table 4. Miscellaneous Compounds Evaluated after Lateral Fluid Percussion Brain Injury

<table>
<thead>
<tr>
<th>Drug and reference</th>
<th>Time and route of administration</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lidocaine58, 87</td>
<td>58Iv, 30 min; 87Iv, pre</td>
<td>58MWM ↑, 58NS →, 87NS ↑, 87MWM →</td>
</tr>
<tr>
<td>Naloxone77</td>
<td>Iv, 30 min</td>
<td>NS ↑</td>
</tr>
<tr>
<td>Nalmefene12, 88</td>
<td>12Iv, 30 min; 88Iv, 30 min</td>
<td>NS ↑</td>
</tr>
<tr>
<td>Haloperidol89</td>
<td>Ip, 24h</td>
<td>MWM ↓</td>
</tr>
<tr>
<td>Olanzapine89</td>
<td>Ip, 24h</td>
<td>MWM →</td>
</tr>
<tr>
<td>Amphetamine90</td>
<td>Ip, 24h</td>
<td>BW →, MWM →</td>
</tr>
<tr>
<td>Melthoxamine90</td>
<td>Ip, 24h</td>
<td>BW →, MWM →</td>
</tr>
<tr>
<td>Prazosin90</td>
<td>Ip, 24h</td>
<td>BW →, MWM →</td>
</tr>
<tr>
<td>Exonaparin91</td>
<td>Iv, 2h</td>
<td>Edema ↓, MWM ↓, NS ↓</td>
</tr>
<tr>
<td>Lactate92</td>
<td>Iv, 30 min</td>
<td>MWM ↑, CA3/CA1 →</td>
</tr>
<tr>
<td>GPI 615093</td>
<td>Ip, 30 min</td>
<td>LV ↓, TUNEL ↓</td>
</tr>
<tr>
<td>Topiramate94</td>
<td>Ip, 30 min</td>
<td>LV →, CA3 →, Edema →, memory →, MWM ↓, NS ↑, RP ↑</td>
</tr>
<tr>
<td>TPC95</td>
<td>Icv, 15 min</td>
<td>MWM →, NS ↓</td>
</tr>
<tr>
<td>BMY-2150296</td>
<td>Sc, 7 days</td>
<td>MWM ↑ (injured), MWM ↓ (sham)</td>
</tr>
<tr>
<td>High-fat sucrose diet97</td>
<td>P.o., pre</td>
<td>MWM ↓</td>
</tr>
<tr>
<td>Suritozole98</td>
<td>Ip, 24h or 11 days</td>
<td>early: MWM ↑, delayed: MWM →</td>
</tr>
<tr>
<td>Albumin99</td>
<td>Iv, 15 min</td>
<td>NS ↑, LV ↓</td>
</tr>
<tr>
<td>Diazepam100</td>
<td>Ip, pre or 15 min</td>
<td>MWM ↑, pre: Mort ↓, post: Mort→</td>
</tr>
<tr>
<td>Bicuculline100</td>
<td>Ip, 15 min</td>
<td>MWM ↓</td>
</tr>
<tr>
<td>Fluoxetine101</td>
<td>Ip, 24h</td>
<td>MWM ↑</td>
</tr>
<tr>
<td>S100B102</td>
<td>Icv, 10 min</td>
<td>MWM ↑</td>
</tr>
</tbody>
</table>

Abbreviations for compounds in Table 4: GPI 6150, 1,11b-dihydro-[2H]benzopyrano [4,3,2-de]isooquinolin-3-one; Topiramate, 2,3:4,5-di-O-(1-isopropylidene)-B-D-fructopyranose sulfamate;TPCK, N-Tosyl-L-phenylalanyl-chloromethyl ketone;BMY-21502, 1-[[1-2-fluoromethyl]-4-pyrimidinyl]-4-piperidinyl[methyl]-2-pyrroli dinone;Suritozole, MDL 26,479, 5-aryl-1,2,4-triazole.

**Anti-Inflammatory Agents**

After LFP, the secondary injury includes an acute inflammatory response with breakdown of the blood-brain barrier (BBB), edema formation, infiltration of peripheral blood cells, activation of resident immunocompetent cells and intrathecal release of immune mediators such as cytokines (Toumoul et al., 1995; Soares et al., 1995a; Philips et al., 2001; Morganti-Kossmann et al., 2002). Although the detrimental CNS inflammation can cause sustained functional impairment and cell death, inflammatory pathways may be crucial to regenerative responses. In particular, inflammation following injury is believed to be detrimental, but after days or weeks may contribute to restoration and repair (Philips et al., 2001; Lenzlinger et al., 2001a). Therefore, mixed results follow inflammation of the inhibitory cascade, including the blockade of ICAM-1, TNF-α, IL-6, IL-10, and prostaglandin actions (Table 3). Furthermore, global suppression of inflammatory responses with cyclosporin or vaccinia virus complement control protein (VCP) depends on route, duration and dose of administration for efficacy (Table 3). Currently, the nature of inflammation after TBI remains elusive, not to mention the need for cautious clinical anti-inflammatory administration, particularly in light of multiple organ damage and infection (pneumonia/sepsis) (Griffin et al., 1994; Riess et al., 2001).

**Reactive Oxygen Species.** Despite a short half-life in biological tissue, oxygen radicals (reactive oxygen species [ROS]), in particular superoxide anion (O$_2$•$^-\,$), hydroxyl radical (OH•), peroxynitrite (ONOO•), and nitric oxide, cause tissue pathology due to high reactivity (Lewen et al., 2000). Brain tissue is susceptible to oxidative damage because of its high rate of oxidative metabolism, low antioxidant defenses, low repair mechanism activity, and the high membrane surface to cytoplasm ratio (Tyurin et al., 2000; Marklund et al., 2001a). Predominantly, oxidative damage after LFP manifests as lipid peroxidation. Varying degrees of success have been reported with inhibitors of lipid peroxidation evaluated after LFP, including the vitamin E (α-tocopherol) analogue MDL 74,180 and LY341122 (Petty et al., 1996; Wada et al., 1999a) (Table 3). Conjugated polyethylene glycol-superoxide dismutase (PEG-SOD, peg-SOD, Dismutec) attenuated neurological motor and cognitive deficits after LFP brain injury of moderate severity (Hamm et al., 1996) (Table 3). The initial results of a randomized, phase III multi-center clinical trial of Dismutec in severely brain-injured patients revealed a trend towards more favorable outcomes and less disability at three months post-injury (Muizelaar et al., 1993). However, a follow-up randomized, multi-center clinical trial of Dismutec did not demonstrate therapeutic efficacy (Young et al., 1996). Additionally, the 21-aminosteroid Tirilazad mesylate also was shown to reduce brain water content, post-injury mortality and improve motor function after LFP (McIntosh et al., 1992; Sanada et al., 1993) (Table 2). However, Tirilazad administered to severely, not moderately, head-injured patients of all types (not just those where edema was a primary response to injury) showed no benefit on outcomes in over 2300 patients worldwide (Marshall et al., 1998).

The spin-trapping agent α-phenyl-tert-N-butyl nitrotrone (PBN) and its sulfonated derivative, sodium 2-sulfo-phenyl-tert-butyl nitrotrone (S-PBN) provide neuroprotection in the LFP model through the formation of stable adducts with ROS (Oliver et al., 1990; Marklund et al., 2001b) (Table 3). Additionally, nitroxides (stilbazulenyl nitrotrone, STAZN) are stable free radicals serving as cell permeable antioxidants that improve neurological motor function and reduce lesion volume after LFP (Belayev et al., 2002) (Table 3). Inhibition of nitric oxide synthase (NOS) after LFP has demonstrated a role for nitric oxide in the post-injury free radical pathology, but neither 3-bromo-7-nitroindazole (7-NI), a relatively specific inhibitor of neuronal NOS, nor nitro-l-arginine methyl ester (l-NAME) and aminoguanidine, an iNOS inhibitor, substantially improved functional or anatomical outcome (Wada et al., 1998a,b) (Table 3).

**Neurotrophic Factors.** The peptide growth factors (e.g., nerve growth factor [NGF], basic fibroblast growth factor [bFGF], brain-derived neurotrophic factor [BDNF], insulin-like growth factor [IGF-1], neurotrophin-4/5 [NT-4/5]) function to support neuronal survival, induce sprouting of neurites (neuronal plasticity), and facilitate axon guidance (Conte et al., 2003). In addition, neurotrophins delay apoptosis, prevent atrophy of axotomized neurons, and enhance the expression of growth-associated genes (Fournier et al., 1997; Kobayashi et al., 1997; Bregman et al., 1998; Broude et al., 1999).

After LFP, marked changes in neurotrophin mRNA and protein expression have been reported in vulnerable brain regions (O'Dell et al., 2000c; Royo et al., 2002; Shimizu et al., 2002). Further pharmacological augmentation of the inherent post-injury NGF increase improved memory function and reduced apoptotic cell death, but not motor function or hippocampal cell loss (Sison et al., 1995; Sinson et al., 1997) (Table 3). IGF-1 administration improves learning and neuromotor function, while post-injury bFGF administration reduces lesion volume, necrotic cortical neurons, and memory dysfunction in the LFP model (Dietrich et al., 1996; McDermott et al., 1997; Saatman et al., 1997) (Table 3). Yet, the combined sequential administration of bFGF and magnesium chloride
diminished the neuromotor improvement observed with magnesium treatment alone (Guluma et al., 1999). Prolonged administration of NT-4/5 attenuates hippocampal cell loss, without affecting cortical lesion volume (Royo et al., 2002). Alternatively, no improvement in either cognitive function or histological preservation are observed after BDNF infusion (Blaha et al., 2000). Neurotrophin administration promises to be a potential clinical therapy due to extended treatment windows.

INHIBITORS OF APOPTOSIS. Despite the extensive research establishing the apoptotic pathways that contribute to the pathology of LFP brain injury, sparse interventions to divert cell death signaling exist (Yakovlev et al., 1997). Future interventions aimed at pro-apoptotic and anti-apoptotic gene expression, caspase activation, mitochondrial preservation, and the inhibitors of apoptosis may limit tissue pathology.

ENDOCRINOCOLOGICAL TARGETS. Neuroendocrinological abnormalities and their pharmacological restoration have been explored in LFP brain injury (Yuan et al., 1991; Childers et al., 1998). For example, analogues of thyrotropin-releasing hormone (TRH) consistently improve neuromotor function (Faden, 1996; Faden et al., 2003) (Table 3). In addition, pre-injury treatment with estrogen improves neurological motor function after LFP brain injury (Emerson et al., 1993; Bramlett et al., 2001).

CATION CHANNEL BLOCKERS AND CALPAIN INHIBITORS. The marked ionic disturbance after LFP results in downstream activation of calcium-dependent enzymes. Several compounds that target the acute post-traumatic ionic disturbance have shown improved behavioral outcome and include the calcium channel blocker emopamil, the broad-spectrum cation channel blocker LOE 908, and the potassium channel opener BMS-204352 (Cheney et al., 2001) (Table 3). Likewise, the selective calpain inhibitor AK295 attenuates motor and cognitive dysfunction after LFP brain injury, without affecting lesion volume, apoptotic cell number, or spectrin degradation (Saatman et al., 1996, 2000) (Table 3). To date, these compounds were administered shortly post-injury, and more delayed administration paradigms are needed to establish their clinical utility.

MISCELLANEOUS THERAPEUTIC STRATEGIES. Compounds outside the categories discussed above have been evaluated in the LFP model (Table 4). Miscellaneous compounds target specific neurotransmitter systems (dopamine, GABA, serotonin) and/or include various anesthetics and dietary changes.

CAVEATS AND RECOMMENDATIONS FOR PHARMACOLOGICAL STUDY DESIGN USING THE LFP MODEL. To date, numerous treatment targets have been explored in the LFP brain injury model and ongoing strategies (Tables 2–4) are being developed and studied routinely. However, few evaluations have explored the extent of the efficacious time-window to improve applicability to the clinical setting. Furthermore, numerous experimental studies have employed routes of administration that may not be accessible in mild or moderate clinical TBI. Moreover, single high-dose treatment in the laboratory contrasts with the clinical setting, where continuous infusions control drug concentrations and minimize adverse effects. The observation times for treatment efficacy are commonly short, usually a few weeks, whereas clinical outcomes are 6 months post-injury or longer. Again, we challenge the TBI research community to adopt long-term behavioral and histological outcome measures to increase the clinical relevance of pre-clinical studies.

HYPOTHERMIA. The treatment strategy to induce hypothermia following LFP brain injury slows cellular processes to provide substantial neuroprotection, as demonstrated by attenuated neural injury (Dietrich et al., 1994a; Bramlett et al., 1995, 1997a; Chatzipanteli et al., 2000; Matsushita et al., 2001a). Post-injury hypothermia reduced cortical contusion volume and the number of necrotic cortical neurons compared to normothermic controls (Bramlett et al., 1997a). Additional efficacy in neuromotor and cognitive functioning in animals treated with hypothermia have been reported (Bramlett et al., 1995). When the positive findings from the LFP model were translated to human studies, preliminary results were positive (Marion et al., 1997). However, a subsequent NIH-sponsored multi-center trial showed no statistical improvement in outcome (Clifton et al., 2001), with minor time and age-dependent treatment effects (Clifton et al., 2002).

TRANSPLANTATION. Since cell loss is a hallmark of TBI, cellular transplantation may replace lost cells or aid self-repair mechanisms in damaged tissue (Schouten et al., 2004). In the first transplantation studies, the transplantation window for fetal cortical tissue in adult non-immunosuppressed rats was 2 weeks after LFP brain injury, where cognitive and motor function can recover in LFP-injured rats after transplantation alone or in combination with locally infused NGF (Soares et al., 1991, 1995b; Sinson et al., 1996) (Table 5). Later studies with post-mitotic human neurons (hNT) demonstrated substantial integration between the injured cortex and the graft; however, no behavioral improvements were found up to 2 weeks post-transplantation (Philips et al., 1999; Muir et al., 1999) (Table 5).

Transplantation of immortalized progenitor cells improve outcome after LFP and provide a limitless supply
TABLE 5. SUMMARY OF TRANSPLANTATION STUDIES AFTER LATERAL FLUID PERCUSSION BRAIN INJURY

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Transplantation time (post-injury)</th>
<th>Transplantation location</th>
<th>Histological time point</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal cortical E16</td>
<td>2d, 1wk, 2wk, 4wk</td>
<td>Ips. cortex</td>
<td>4wk</td>
<td>Soares and McIntosh, J. Neural Transplant. Plast., 1991</td>
</tr>
<tr>
<td>E16</td>
<td>2d</td>
<td>Ips. cortex</td>
<td>4wk</td>
<td>Soares et al., J. Neurotrauma, 1995</td>
</tr>
<tr>
<td>E16 (with NGF)</td>
<td>24h</td>
<td>Ips. cortex</td>
<td>72h, 1wk, 2wk</td>
<td>Simson et al., J. Neurosurg., 1996</td>
</tr>
<tr>
<td>hNT</td>
<td>24h</td>
<td>Ips. cortex</td>
<td>2wk, 4wk</td>
<td>Muir et al., J. Neurotrauma, 1999</td>
</tr>
<tr>
<td>HiB5 (NGF)</td>
<td>24h</td>
<td>Ips. cortex</td>
<td>1wk</td>
<td>Philips et al., J. Neurosurg., 1999</td>
</tr>
<tr>
<td>MHP 36</td>
<td>48h</td>
<td>Ips. and contra. cortex</td>
<td>4wks</td>
<td>Lenzlinger et al., J. Neurotrauma, 2001</td>
</tr>
<tr>
<td>C17.2</td>
<td>48h</td>
<td>Ips. and contra. corpus callosum</td>
<td>72h, 2wk</td>
<td>Boockvar et al., Neurosurgery, 2005</td>
</tr>
</tbody>
</table>

of transplant material. Significant neuromotor and cognitive improvements, as well as a reduction in hippocampal (CA3) cell death, were afforded by HiB5 cell transplantation with NGF at 24 h post-injury (Philips et al., 2001). Similarly, detailed evaluation of neural stem cell survival, migration and terminal differentiation in the injured brain after transplantation demonstrate the utility of cell replacement as a viable therapy for TBI (Boockvar et al., 2005). To date, none of these transplantation therapies is available for human clinical trial in TBI, but the LFP model has proven useful for examining potential therapies, graft/brain interaction, and cellular repair post-injury (Table 5).

Predictive Validity

The LFP brain injury model is the most commonly used model for preclinical evaluation of pharmacologic therapies (Bullock et al., 1999). Although several compounds, have shown efficacy in the LFP model, the translation to the bedside was unsuccessful (Bullock et al., 1999). We posit that both the inability of any experimental model to reproduce all types of clinical TBI and issues with study design using severely brain-injured patients contributed to the relatively poor success rate of past clinical trials. Preclinical studies employing LFP injury of moderate severity have been translated to clinical trials involving severely head-injured patients. In fact, injury levels classified as moderate severity typically result in mortality rates approaching 20%. The expectations that the LFP model would have full predictive validity for any trial involving severe TBI clearly are unreasonably high.

Optimal clinical trial design should be based on appropriate dosing regimens and patient population for the specific therapy (Bullock et al., 1999; Statler et al., 2001). Administration paradigms in laboratory studies are based typically on animal body weight, whereas clinical trials establish a single dose for all enrolled patients, regardless of body weight (Bullock et al., 1999). Reciprocally, preclinical designs can be designed to include various dosing strategies, multiple severity levels within a single brain injury model, incorporating secondary insults evident in human TBI, or more than one model of TBI (Narayan et al., 2002). Compounds likely to be brought to clinical trials should be evaluated in the laboratory using models like LFP to better mimic the clinical situation.

Construct Validity

Construct validity assesses the degree to which the LFP model can unambiguously be interpreted to mimic the clinical condition (Jenck et al., 1995; Willner, 1997; Hamm, 2001). Additionally, the internal workings of the model should respond in a similar manner to experimental manipulations (i.e., injury severity, physiological fluctuations) as the clinical condition. High construct validity in the LFP model is afforded by the reproduction of human TBI contusion, diffuse white matter injury, and varying severity (Laurer et al., 2000). Additionally, the persistent neuromotor and cognitive deficits up to one year after severe LFP brain injury, with the concomitant temporal pattern of cellular death add construct validity to the model (Smith et al., 1997a; Pierce et al., 1998; Bramlett et al., 2002). The anesthesia necessary for LFP surgery, in addition to the required craniectomy, introduces factors that disrupt the congruent relationship between the model and what is being modeled. However, LFP clearly is not a model of open or penetrating TBI, as some have suggested. Histological examination after LFP shows white matter tissue tears that are very similar if not identical to the shearing injury seen in human closed head injury (Graham et al., 2000b). Moreover, the patterns of cell death after LFP predict those later observed in post-mortem tissue (Rink et al., 1995; Smith et al., 2000). Brain regions, in particular white matter versus grey mat-
ter, are known to respond differently after either human TBI or LFP (Yakovlev et al., 1997; Conti et al., 1998; Williams et al., 2001; Wilson et al., 2004). The active, progressive enlargement of the cortical contusion and ventricles after LFP (Smith et al., 1997a; Bramlett et al., 1997b; Pierce et al., 1998) also parallels that seen after human head injury (Shiozaki et al., 2001).

IS LFP A RELEVANT MODEL OF HUMAN TBI?

As an experimental model of closed head injury, lateral fluid percussion has provided the consistency, reproducibility and reliability required of a laboratory model. In contrast to human TBI, LFP necessitates prior anesthesia and craniotomy, which presents some potential confounds to the ability of this (or any) model to reproduce the clinical condition. But, based on mortality, physiological alterations, and neurological impairments, the model achieves face validity. Due to finite laboratory resources, the construct validity with regard to mild and moderate traumatic brain injury in the young adult human male population has been achieved. However, certain restrictions on the model somewhat limit the predictive validity of LFP and preclinical therapies tested in this experimental model have yet to translate to effective clinical interventions. By expanding the lateral fluid percussion model to include additional aspects of the clinical presentation, both construct and predictive validity of the model likely can be maximized.

Combined Experimental Models May Afford Additional Construct Validity

Head injured patients often present with additional complications, such as hypotensive or hypoxic episodes, which occur acutely for prolonged intervals in severely head-injured patients (Ananda et al., 1999; Robertson et al., 1999). In order to transfer the true clinical condition to animal models, clinically relevant TBI pathophysiology (hemorrhage, hypotension, ischemia, or hypoxia) can be integrated into the existing model (Statler et al., 2001). Several studies have incorporated ischemia (Dietrich et al., 1998; Perez-Pinzon et al., 1999), hemorrhage (Law et al., 1996; Stover et al., 2002), hypoxia (Tanno et al., 1992; Nida et al., 1995; Dave et al., 1997; Bramlett et al., 1999a,b; Bauman et al., 2000; Matsushita et al., 2000), hyperglycemia (Kinoshita et al., 2002) or hypotension (Matsushita et al., 2001b) after LFP to assess the contributions to brain injury pathology of these secondary insults. Furthermore, postrauumatic coma, a common clinical indicator for morbidity and mortality (Gennarelli, 1983), has proven difficult to induce in small animal models. Rodents are lissencephalic, have smaller brain mass, and lack cortical gyri, which may provide protection from injury, limiting the mechanical induction of coma (Statler et al., 2001). Animals can cope with the postrauumatic secondary episodes without further damage to CNS structures or exacerbation of behavioral impairments, as long as the secondary episodes are moderated in terms of severity and duration. When the duration or severity exceeds a threshold, injury-induced pathology substantially increases.

Although a smaller fraction of the clinical TBI patient population, women remain an understudied component of basic science studies. Susceptibility to brain injury is greater in female rats, which may be related to levels of free magnesium (Emerson et al., 1992). However, endogenous circulating hormones in the female rats surviving brain injury after LFP provide histopathological protection after injury compared to males or ovariectomized females (Bramlett et al., 2001; Suzuki et al., 2003). Yet, postrauumatic hypothermia therapy remains an effective treatment in male, but not female, brain-injured animals (Suzuki et al., 2003). Without further investigation, the paradoxes between gender and treatment efficacy cannot be resolved.

Age is one of the most important predictors of outcome after human traumatic brain injury (Hukkelhoven et al., 2003). Substantial age-dependent injury-induced differences in aging-associated and regeneration-related gene expression in the hippocampus may contribute to the increased vulnerability of the aged brain to injury (Shimamura et al., 2004). However, behavioral dysfunction and vulnerability to injury have not been explored in aged animals after LFP. Moreover, the metabolic stress and free radical cascades after injury can establish conditions that promote aging-associated mitochondrial DNA deletion and oxidation, suggesting that the underlying pathology between TBI and aging may be similar (Liffshitz et al., 2003b). In fact, clinical and preclinical links between Alzheimer’s pathology and TBI have been explored using the LFP model (Pierce et al., 1996; Kay et al., 2003) and may be crucial to treating the surviving population of TBI patients.

Traumatic brain injury (TBI) is the leading cause of injury-related death and disability among children under the age of 15 years in the United States (Prins et al., 2003). The biomechanical and physiological data indicate that reproducible traumatic brain injuries can be generated using the LFP model in developing animals, where physiological responses increase with injury severity. Compared to adult animals, young or developing animals exhibit pronounced hypotension associated with excitatory amino acid–induced pial dilation (Armstead et al., 1994; Prins et al., 1996; Armstead, 1999a; Armstead,
An injury-induced premature elevation of NMDA receptor subunits inhibits experience-dependent developmental plasticity, cortical thickening, and dendritic arborization (Fineman et al., 2000; Giza et al., 2002; Ip et al., 2002). The LFP brain injury further disrupts the coordinated expression of age-dependent genes in the hippocampus (Giza et al., 2002; Griesbach et al., 2002) that may have deleterious consequences for the developing animal. Despite the blunted neuronal damage and preserved blood–brain barrier in young injured animals, an acute hyperglycolytic period is compounded by biphasic regional calcium accumulation and an enhanced microglial/macrophage infiltration (Thomas et al., 2000; Osteen et al., 2001). Yet, developing animals that survive LFP brain injury show intact cognitive function compared to older injured animals (Prins et al., 1998; Prins et al., 2001). Within developing animals, the newborn remains more sensitive to post-traumatic vascular, as presumably other, components of the injury compared with juvenile animals (Armstead, 1999a). Age-related differences in the response to TBI reinforce the hypothesis that outcomes and therapies for head-injured patients may be age-dependent (Prins et al., 2003).

Incorporating additional components of the clinical presentation of traumatic brain injury into the LFP model would add additional construct validity. Gender, old age, and developing animals complete the description of traumatic brain injury in the laboratory and permit clinically relevant therapies to be pursued.

Redirecting the LFP Model:
Mild Versus Severe TBI

The clinical classification of TBI encompasses a broad spectrum of dysfunction, ranging from patients who remain in a coma from the moment of injury until death, to those who are mildly impaired after the injury and recover over time. With the increased use of safety equipment and restraints, the previously large population of severely brain-injured patients has been inflated by a new population of mild and moderately brain-injured patients. Although the classical histopathological damage of LFP is more consistent with severe human TBI, the absence of coma, intravenous fluids, ICP management, nursing care, or vegetative state mandates that the focus of animal brain injury models remains on mild-moderate injury severity. Moreover, the LFP model resembles mild human TBI in its acute pathobiology and behavioral recovery. Redirecting the basic science focus of the LFP brain injury model to mild TBI will help ensure the appropriate translation of pharmacological, behavioral and histopathological findings to the clinical setting (Hicks et al., 1993; Sick et al., 1998; Saatman et al., 1998; Raghu-pathi et al., 2002; Griesbach et al., 2002). The predictive validity of the model is enhanced and directed towards a significantly larger population of traumatic brain injured patients. As such, the LFP injury model has sufficient overall validity and can provide a strong basis for translational research as part of a well-designed program of research for mild to moderate traumatic brain injury.

CONCLUSION

It is possible to gain new insight into clinical problems even if full construct or other validity cannot be established from use of an animal model. The true power of a model does not lie exclusively in its validity, but in its “productive generativity” (Shapiro, 1998), defined as the amount of new information discovered or new hypotheses generated about the disease or condition from the animal model. The productive generativity of the LFP injury model over the past 15 years has been extensive, and includes information regarding gene and protein expression, mechanisms of cell death, the inflammatory response, and potential therapeutic strategies, rehabilitation strategies (e.g., exercise) and cellular transplantation into the injured brain. The amassed literature on LFP brain injury, which has helped to refine clinical trials in human head injury and vice versa, authenticates that sometimes “you get what you need.”

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LATERAL FLUID PERCUSSION BRAIN INJURY


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