Epinephrine Increases Mortality after Brief Asphyxial Cardiac Arrest in an In Vivo Rat Model

Conán L. McCaul, MD*†‡**, Patrick J. McNamara, MD*§, Doreen Engelberts*, Gregory J. Wilson, MD¶, Alex Romaschin, PhD¶, Andrew N. Redington, MD#, and Brian P. Kavanagh, MD*†‡**

*The Lung Biology Program, The Research Institute, and the Departments of †Critical Care Medicine, ‡Anesthesia, Pediatrics (§Neonatology and #Cardiology) and ¶Pathology, The Hospital for Sick Children; and the **Interdepartmental Division of Critical Care Medicine, University of Toronto, Toronto, Ontario

Epinephrine may be detrimental in cardiac arrest. In this laboratory study we sought to characterize the effect of epinephrine and concomitant calcium channel blockade on postresuscitation myocardial performance after brief asphyxial cardiac arrest. Anesthesized rats were disconnected from mechanical ventilation, resulting in cardiac arrest. Resuscitation was attempted after 1 min with mechanical ventilation, oxygen, chest compressions, and IV medication. In experimental series 1 and 2, animals were allocated to 10 or 30 μg/kg epinephrine or 0.9% saline. In series 3, animals received 30 μg/kg of epinephrine and were randomized to 0.1 mg/kg of verapamil or to 0.9% saline. In series 1 and 3, left ventricular function was assessed using transthoracic echocardiography. In series 2, left atrial pressure was measured. Epinephrine was associated with increased mortality (0/8 [0%] in controls, 4/12 [33.3%] in 10 μg/kg animals, and 16/22 [72.8%] in 30 μg/kg animals; \( P < 0.05 \)), hypertension (\( P < 0.001 \)), tachycardia (\( P < 0.001 \)), early transient left atrial hypertension, and dose-related reduction in left ventricular end diastolic diameter (\( P < 0.05 \)). Verapamil prevented mortality associated with large-dose epinephrine (0% versus 100%) and attenuated early diastolic dysfunction and postresuscitation hypertension (\( P < 0.001 \)) without systolic dysfunction. Epinephrine appears to be harmful in the setting of brief cardiac arrest after asphyxia.

The optimal drug therapy for treatment in asphyxial cardiac arrest is not known. Epinephrine is universally administered in cardiac arrest (1), but it has never been tested in a placebo-controlled clinical trial. Evidence supporting its efficacy at any dose is limited, and it is considered an indeterminate drug class by the American Heart Association (2). At larger doses, concerns have been raised about a severe toxic hyperadrenergic state and postresuscitation myocardial dysfunction. A meta-analysis of lower versus higher doses of epinephrine (2) published subsequent to the 2000 American Heart Association Advanced Cardiac Life Support (ACLS) guidelines (1) suggests that a small but positive short-term effect (i.e., obtaining an initial detectable pulse pressure) of epinephrine given soon after commencing cardiopulmonary resuscitation (CPR) is offset by a survival disadvantage at time of hospital discharge (2). These studies are supported by laboratory data indicating that large doses of epinephrine, given during resuscitation from cardiac arrest after ventricular fibrillation, result in more frequent recovery of spontaneous circulation but at the later cost of impaired cardiac function (3).

Asphyxia, the clinical correlate of airway loss, is a rare but important cause of anesthetic morbidity and mortality. Because epinephrine, a standard of care in management of cardiac arrest, may have detrimental effects in nonasphyxial cardiac arrest (3), we hypothesized that large-dose epinephrine may be detrimental after asphyxial cardiac arrest. Many studies report on...
myocardial function after resuscitation from ventricular fibrillation, but data after brief asphyxial cardiac arrest have not been reported. This distinction may be critically important because myocardial dysfunction after resuscitation from ventricular fibrillation is different from dysfunction after asphyxial arrest (4).

We hypothesized that epinephrine would have a detrimental effect on survival and would impair myocardial function after resuscitation from asphyxial cardiac arrest. Furthermore, because catecholamines modify myocardial function via increases in intracellular calcium, we hypothesized that epinephrine-induced impairment would be preventable with calcium channel blockade. We therefore compared the effects of large-dose and small-dose epinephrine (versus saline control) on systolic and diastolic function after asphyxial cardiac arrest and explored the possible role of altered intracellular calcium by use of nonselective calcium channel blockade.

**Methods**

Adult male Sprague-Dawley (Charles River, Quebec) rats (340 to 400 g, 16–24 wk) were used in all experiments. Animals had been caged and were nonfasting before experimentation. Institutional Ethics approval was obtained from the Animal Care Committee at the Research Institute at the Hospital for Sick Children. All work conformed to the guidelines of the Canadian Council for Animal Care. Experimental details are provided as per Utstein guidelines (5).

Anesthesia was induced with intraperitoneal ketamine 60 mg/kg and xylazine 5 mg/kg. The depth of anesthesia was confirmed by the absence of response to paw clamp. IV access was secured using the penile vein for administration of drugs and fluids. A tracheostomy was performed with a 14º cannula and secured in place. Teflon catheters (Becton Dickinson, Sandy, UT) were inserted via the femoral artery to the aorta (24º). Limb lead II electrocardiograms were recorded with subcutaneous limb electrodes (right and left upper, right lower).

Pancuronium bromide (0.5 mg) was administered IV. The lungs were ventilated with tidal volume (VT), 8 mL/kg and frequency 38–40 min⁻¹; positive end-expiratory pressure (PEEP), 1 cm H₂O; and FIO₂ 0.21 using a time-cycled, volume-cycled ventilator (Model 683; Harvard Apparatus, South Natick, MA) for small animals. A fluid bolus (lactated Ringer’s solution 5 mL/kg) was given over 15 min. Baseline arterial blood gases were measured 15 min after the fluid bolus. Core temperature was regulated with a homeothermic blanket control unit (Model 50-7079-F; Harvard Apparatus). Mean arterial blood pressure and esophageal temperature were recorded at baseline. Inclusion and exclusion were performed as previously described (6).

### Experimental Protocol

Cardiac arrest was induced by disconnecting the tracheostomy tube from mechanical ventilation. The hemodynamic pattern observed consisted of an initial increase in mean arterial blood pressure and heart rate (data not presented), followed by progressive bradycardia and hypotension. This resulted in eventual asystole/pulseless electrical activity after approximately 3 min (Tables 1, 2). Cardiac arrest was defined by the onset of mean arterial pressure decline to 10 mm Hg. The asphyxial time interval was defined as the period between ventilator disconnection and the commencement of resuscitation efforts. Resuscitation was commenced 1 minute after onset of cardiac arrest and was done by turning on the ventilator (FIo₂ 1.0, V₇ 8 mL/kg, rate 40 min⁻¹), manual anteroposterior compression of the thorax (approximately 200 min⁻¹), and IV administration of medication. Resuscitation medications were determined by random blinded allocation. Successful resuscitation (return of spontaneous circulation, ROSC) was defined as a mean arterial blood pressure of 50 mm Hg. Resuscitative efforts were discontinued if ROSC did not occur within 5 min of commencement of chest compressions.

The lungs were ventilated (VT 8 mL/kg, frequency 38–40 min⁻¹; PEEP 1 cm H₂O; FIo₂ 0.21) using a time-cycled, volume-cycled ventilator (Model 683; Harvard Apparatus) for small animals. No ventilation changes were made before or after resuscitation. Animals received a maintenance infusion of Ringer’s lactate solution (5 mL/h) for postresuscitation circulatory support.

### Table 1. Baseline and Outcome Data: Series 1 (Epinephrine Dose Response) and 2 (Left Atrial Pressure)

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>Epinephrine 10 µg/kg</th>
<th>Epinephrine 30 µg/kg</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Series 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac Arrest Onset (s)</td>
<td>210 (11.8)</td>
<td>196 (5.1)</td>
<td>210 (7.8)</td>
<td>0.65</td>
</tr>
<tr>
<td>ROSC (s)</td>
<td>91.5 (55–151)</td>
<td>34.5 (31–44)*</td>
<td>38.0* (29–41)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Duration of Survival (min)</td>
<td>120 (120–120)</td>
<td>120.0 (46–120)*</td>
<td>13.0 (8–120)*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td><strong>Series 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOAF (s)</td>
<td>181 (150–195)</td>
<td>187 (183–202)</td>
<td>180 (177–189)</td>
<td>0.59</td>
</tr>
<tr>
<td>ROSC (s)</td>
<td>110 (83–171)</td>
<td>55.5 (48–58)*</td>
<td>51 (40–58)*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Survival Duration (min)</td>
<td>15 (15–15)</td>
<td>15 (15–15)</td>
<td>15 (12–15)</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Continuous data are presented as mean (SD) and median (interquartile range). Categorical data are presented as percentage. LOAF = loss of aortic fluctuation; ROSC = return of spontaneous circulation. * P < 0.05 versus saline; † P < 0.05 versus epinephrine 30 g/kg.
Experimental Design

Lung wet/dry weight ratio. After resuscitation. Lung edema was assessed using arterial blood samples before cardiac arrest and 2 cardiac troponin-I by enzyme immunoassay in serum from successive cardiac cycles.

Calculations were obtained using the mean value from 5 determinations (7). For each echocardiographic recording, calculations were made of left ventricular cavity dimensions at end-diastole and end systole. Fractional shortening was calculated as:

\[
\frac{LVEDD - LVESD}{LVEDD} \times 100
\]

where LVEDD and LVESD indicate left ventricular end-diastolic and end-systolic diameters, respectively, and was used as the index of left ventricular contractility (7). For each echocardiographic recording, calculations were obtained using the mean value from 5 successive cardiac cycles.

Myocardial injury was assessed by measuring cardiac troponin-I by enzyme immunoassay in serum from arterial blood samples before cardiac arrest and 2 h after resuscitation. Lung edema was assessed using lung wet/dry weight ratio.

Experimental Design

Series 1: Epinephrine Dose-Response. Experimental animals were allocated to receive saline (control), epinephrine 10 µg/kg, or epinephrine 30 µg/kg given as a single bolus at the commencement of resuscitation. Based on preliminary echocardiographic data we estimated that 8 subjects with complete data per group would be required.

Series 2: Left Atrial Pressure Measurement. To determine the effect of epinephrine on LA diastolic function, an additional series of experiments was performed in which LA pressure was directly measured. Animal preparation and experimental design were identical to the previous series. In addition, a 3 mm Teflon catheter (18°) was inserted into the LA appendage (Becton Dickinson, Sandy, UT) via a sternotomy. Group allocation was by randomization, and resuscitation was done as previously described.

Series 3: Calcium Channel Blockade. To determine whether the effects of epinephrine were mediated by changes in intracellular calcium homeostasis, animals were randomized to receive either verapamil 0.1 mg/kg or saline (control) given as a bolus immediately before the administration of the epinephrine 30 µg/kg. Animal preparation and resuscitation were done as in series 1.

All data were analyzed using Sigma Stat (Version 2.0; Jandel Corporation, San Rafael, CA). Survival data were compared using Fischer’s exact or χ² tests. Parametric data were analyzed by analysis of variance and Student-Newman-Keuls tests. Nonparametric data were analyzed by repeated-measures analysis of variance for ranks. Significance was set at P < 0.05. Nonparametric data are expressed as median and interquartile range, and parametric data as mean ± SEM.

Results

Series 1: Epinephrine Dose Response

There were no differences among the experimental groups in terms of baseline characteristics (Tables 1, 2). Fifty animals were anesthetized for this series, and 8 were terminated before group allocation because of technical issues with dissection. Of the remaining 42, 8 were allocated to the saline group, all of which survived the protocol; 12 were allocated to the epinephrine-10 group, 8 of which survived the protocol; and 22 were allocated to the epinephrine-30 group; 22 of which survived the protocol.
group, 6 of which survived the protocol. The time required for ROSC was shorter in the epinephrine-30 and epinephrine-10 groups, compared with saline controls (Tables 1, 2). Kaplan-Meier analysis demonstrated that the survival was less in the epinephrine-30 group, compared with the epinephrine-10 and saline groups (Fig. 1).

Epinephrine administration was associated with a dose-related effect of epinephrine on systemic arterial blood pressure (epinephrine-30 > epinephrine-10 > saline controls; \( P < 0.05 \); Fig. 2A) and heart rate (epinephrine-30 > epinephrine-10 > saline controls; \( P < 0.05 \); Fig. 2B) after ROSC.

Left ventricular fractional shortening (Table 3) and end-diastolic dimension were reduced in all groups after resuscitation (Fig. 2C). Epinephrine 30 \( \mu g/kg \) administration was associated with the largest reduction in LVEDD (Fig. 2C) and with delayed recovery of fractional shortening (Table 3). Arterial base excess was reduced in all groups after resuscitation (Fig. 2D). Plasma troponin was significantly increased in all groups after cardiac arrest (Fig. 3A). The final level of troponin was larger in the epinephrine-30 group versus saline controls (Fig. 3A).

The lung wet/dry ratio was largest in the epinephrine-30 group (Fig. 3B). \( \text{PaO}_2 \) was increased in all groups after CPR, where \( \text{FiO}_2 \) was 1.0 (Table 3). Arterial pH and bicarbonate decreased in all groups after CPR (Table 3).

**Series 2: Left Atrial Pressure Measurement**

Baseline characteristics were similar in all groups (Tables 1, 2; Fig. 4A, B). Thirty-five animals were anesthetized for this series, and 17 were terminated before group randomization because of technical/dissection issues associated with the open-chest preparation. Of the remaining 18, 6 were randomized to the saline group, all of which survived the protocol; 6 were randomized to the epinephrine-10 group, all of which survived the protocol; and 6 were randomized to the epinephrine-30 group, 4 of which survived the protocol. LA and mean arterial blood pressure were unchanged after resuscitation in controls (Fig. 4A, B). Conversely, there was an early, marked increase in both epinephrine groups (mean peak LA pressure 37 versus 27 mm Hg in the 30 and 10 \( \mu g/kg \) groups, respectively; Fig. 4A, B).

**Series 3: Calcium Channel Blockade**

Baseline characteristics were similar among groups (Tables 1, 2; Fig. 5). Fifteen animals were anesthetized for this series, and 3 were terminated before group randomization because of technical/dissection issues. Of the remaining 12, 6 were randomized to the epinephrine with verapamil group, all of which survived the protocol; and 6 were randomized to the epinephrine without verapamil group, none of which survived the protocol. Administration of verapamil during the resuscitation (i.e., after cardiac arrest but before epinephrine) was associated with improved survival (Fig. 5A). Postresuscitation hypertension (Fig. 5B), tachycardia (Fig. 5C), and reduction in LVEDD (Fig. 5D) associated with epinephrine (as in series 1) were attenuated by coadministration of verapamil. Left ventricular fractional shortening was increased in both groups after resuscitation. Time to resuscitation was longer in animals that received verapamil (Tables 1, 2),
but both the number of animals surviving and the duration of survival were greater in this group (Tables 1, 2; Fig. 5A). The wet/dry ratio of the lung was larger in animals that did not receive verapamil (Tables 1, 2).

Discussion

The current study explores the effects and mechanisms of epinephrine administration after asphyxial cardiac arrest. Epinephrine impaired hemodynamics, caused myocardial damage, and worsened survival. Increased doses were associated with hypertension, tachycardia, myocyte injury, and pulmonary edema. The profound reductions in LVEDD were associated with increases in LA pressure reflecting impaired diastolic relaxation. The adverse effects of epinephrine on mortality as well as on cardiovascular performance were attenuated by calcium channel blockade.

Epinephrine is universally accepted as a critically important therapy in ALCS (8,9). There is concern about the optimal use of epinephrine in human CPR. First, although resuscitation algorithms differ for the initial approach to ventricular fibrillation versus asystolic cardiac arrest, the recommended doses of epinephrine are identical, as are the intervals for repeat dosing. This “lumping together” of all etiologies ignores documented differences in myocardial energetics, postresuscitation

| Table 3. Series 2: Epinephrine Dose-Response: Hemodynamic and Blood Gas Data |
|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|                  | Group            | Baseline         | 2 min            | 10 min           | 30 min           | 60 min           | 90 min           | 120 min          |
| LVFS (%)         | Saline          | 47.8 (4.0)       | 62.0 (7.8)*      | 32.5 (5.0)       | 43.0 (4.0)       | 44.6 (2.7)       | 57.9 (7.6)       | 46.2 (4.4)       |
|                  | Epi. 10 µg/Kg   | 52.2 (3.1)       | 72.9 (6.4)*      | 27.1 (3.6)*      | 39.8 (3.8)       | 46.6 (3.8)       | 45.7 (3.1)       | 54.7 (3.5)       |
|                  | Epi. 30 µg/Kg   | 52.4 (1.9)       | 65.0 (5.9)       | 32.9 (3.2)*      | 31.6 (3.7)*      | 52.1 (6.3)       | 43.3 (4.7)       | 69.6 (13.7)      |
| pH               | Saline          | 7.34 (0.00)      | 7.23 (0.02)*     | 7.33 (0.01)      | 7.34 (0.01)      | –               | 7.29 (0.02)      | –               |
|                  | Epi. 10 µg/Kg   | 7.34 (0.01)      | 7.19 (0.02)*     | 7.28 (0.02)      | 7.34 (0.01)      | –               | 7.34 (0.01)      | –               |
|                  | Epi. 30 µg/Kg   | 7.35 (0.01)      | 7.13 (0.04)*     | 7.26 (0.02)      | 7.26 (0.03)      | –               | 7.35 (0.02)      | –               |
| HCO₃⁻ (mm Hg)    | Saline          | 21.3 (0.4)       | 16.6 (0.7)*      | 20.2 (0.2)       | 20.2 (0.3)       | –               | 18.6 (0.3)       | –               |
|                  | Epi. 10 µg/Kg   | 20.9 (0.4)       | 15.2 (0.7)*      | 18.3 (0.9)       | 19.8 (0.3)       | –               | 21.1 (0.3)       | –               |
|                  | Epi. 30 µg/Kg   | 20.3 (0.6)       | 14.3 (0.5)*      | 17.0 (0.9)       | 16.3 (1.7)       | –               | 21.1 (0.3)       | –               |

Please note that the y-axis does not start at zero in panel B. Data presented as mean ± SEM. *P < 0.05 versus baseline.
function, neurological lesions, and mortality between ventricular fibrillation and other etiologies of arrest (10–12). Data supporting the use of epinephrine in any resuscitative situation are limited (13). In a recent study, no patient survived to hospital discharge in a clinical trial in which 179 asystolic patients had 3 or more doses of 1 mg epinephrine (14). The optimum dose, or dosing interval (if any), is not known. Large doses of epinephrine have limited benefit and have been shown to improve outcome in small series with historic controls (15,16) but do not beneficially alter rates of hospital admission or discharge in prospective randomized trials (2). In the present study we chose to examine the effect of epinephrine in a dose range (10–30 μg/kg) that approximates that which may be encountered clinically. The standard current ACLS dose of 1 mg bolus results in dose variability corresponding to 10 μg/kg in a 100-kg person but 25 μg/kg in a 40-kg person.

An important cause of perioperative cardiac arrest is hypoxia secondary to failed airway management or inadequate ventilation. In this situation, reversal of hypoxia is an immediate priority but may be difficult (e.g., complete airway obstruction), and consequently cardiovascular resuscitation may be continuing during attempts to restore oxygenation. A previous laboratory study provides additional grounds for concern about standardized epinephrine in this context. In a study of asphyxial piglets, an extreme dose of epinephrine (200 μg/kg) was compared with a “standard” dose (20 μg/kg), both administered after a more prolonged duration of asphyxia, which may have better represented a scenario of unwitnessed cardiac arrest (17). In this setting, the larger dose was associated with improved initial resuscitation success but not improved survival at 24 hours (17), but the study did not use techniques to assess cardiac function. More extreme doses of epinephrine (e.g., 1000 μg/kg) have also been studied in the laboratory and demonstrate a characteristic improvement in early resuscitation success at the expense of later survival (18).

In the current study, which may better represent a witnessed asphyxial arrest, the initial resuscitation rate was 100% in all groups, including saline control. Although resuscitation was more rapid in both epinephrine groups (10 and 30 μg/kg), there was also an appreciable mortality associated with both epinephrine doses. Taken together, these data suggest that the current ACLS guidelines allowing epinephrine doses of up to 200 μg/kg may need additional experimentation to estimate the optimal context-specific use (1).

Previously, the functional impact of cardiac arrest and resuscitation has loosely been termed “global contractile dysfunction” (19). This syndrome is seen during CPR and after resuscitation from global ischemia and ventricular fibrillation and increases with duration of arrest (3,19,20). However, the alteration in function noted in the present study is different than previously described. We observed that epinephrine administration caused predominantly diastolic dysfunction, manifested as severe constriction of the left ventricular cavity diameter despite higher left atrial filling pressures, suggesting impaired left ventricular compliance. Circulating troponin was also increased in the epinephrine groups, indicating that cellular injury was an important component of the pathophysiological process. In contrast to the observed diastolic abnormalities, demonstrable systolic dysfunction did not occur in the current experiments.

In the present study the period of no-flow was standardized, and dysfunction was relatable solely to differences in epinephrine administration. Increasing doses of epinephrine progressively reduced postresuscitation survival. Although the explanation for the cardiac impairment is complex, our data are comparable to the previously described cardiotoxicity secondary to catecholamines (21), which was associated with β-adrenergic-mediated increases of intracellular calcium. This may be amplified during cardiac arrest because of hypoxic induction of Na⁺/K⁺ adenosine triphosphatase-dependent calcium removal from the myocyte. The physiological effects of epinephrine involve impaired diastolic function and pulmonary edema and can be inhibited by blockade of L-type calcium channels.

Verapamil has potent negative inotropic and chronotropic effects and reduces vascular resistance. Thus, verapamil could have produced benefit through attenuation of the heart rate, reduction of left ventricular afterload, and/or direct inhibition of intracellular calcium mobilization (22). In our study, verapamil administration did not reduce the epinephrine-induced early increase in fractional shortening but did markedly attenuate the reduced ventricular end-diastolic volume observed with epinephrine administration. The combination of reduced end-diastolic dimension and increased LA pressure (consistent with impaired left ventricular compliance) indicates severe impairment of left ventricular diastolic function and suggests a hydrostatic basis (i.e., increased pulmonary venous pressure) for the severe pulmonary edema.

The current study has several important limitations. First, the model used was a previously healthy, anesthetized animal, and this may not be representative of complex disease states with preexisting cardiopulmonary disease. Second, resuscitation in the current experiment was applied within 3–5 minutes of the onset of asphyxia; this is therefore highly unlikely to represent a large proportion of “unwitnessed” cardiac arrests. However, the protocol may well reflect a witnessed arrest, with the resuscitation procedures reflecting an ideal response in an ACLS-equipped environment. The relatively brief period of asphyxia is shorter than that used by others (17,18), and is short enough that resuscitation should be successful in almost all animals, with any deaths therefore reflecting...
adverse effects of the resuscitative regimen. Third, while the effects and mechanisms of epinephrine were examined, no benefit of epinephrine was observed, and thus no “optimal” dose was identifiable. Fourth, because verapamil is a relatively nonspecific pharmacological probe, there are several mechanisms whereby calcium channel blockade could prevent adverse effects of epinephrine, including a reduction in heart rate. Nonetheless, our findings support the possibility that epinephrine toxicity in the current study was mediated in part by increased intracellular calcium flux, and the finding that verapamil abolished the mortality associated with epinephrine has potential clinical implications. Furthermore, because of projected large numbers of animals required to demonstrate a statistically significant effect, verapamil was not explored in the epinephrine 10 µg/kg group. Finally, this study focused exclusively on cardiovascular events and does not address neurological outcome. However, given the dependence of neurological outcome on cerebral perfusion and given the survival differences noted here, there is a strong likelihood of neurological improvement in at least some survivors.

Resuscitation protocols are strongly influenced by information obtained in laboratory experiments. Specifically, there are no human trials of CPR that address outcome differences between placebo and epinephrine or that examine the role of epinephrine in cardiac arrests of different durations. Our findings should be explored in larger mammalian models, with different epinephrine dosing and timing strategies, with alternative pharmacologic choices, and, if confirmed, with functional neurological assessment in survival experiments.

References