



## Modeling traumatic brain injury using controlled cortical impact injury

Jan C. Frankowski, Robert F. Hunt

Department of Anatomy & Neurobiology, University of California, Irvine

Correspondence: robert.hunt@uci.edu

### Abstract

Traumatic brain injury (TBI) occurs when an external mechanical force damages the brain. Controlled cortical impact (CCI) is an experimental neurotrauma model that has been widely used to produce graded, reproducible injuries in animals that mimic important histological, physiological and behavioral aspects of closed-head TBI seen clinically. CCI takes advantage of an electronically controlled pneumatic piston to deliver a precise contusion injury to neocortex. Here, we describe a step-by-step protocol for performing CCI in mice. Expected histological outcomes of injury as well as strengths and limitations of the model are also discussed.

### Background

Traumatic brain injury (TBI) afflicts nearly 6 million Americans (National Institute of Neurological Disorders and Stroke, 2015). It is a serious neurological disorder that occurs after an external mechanical force damages the brain (e.g., from a bump, blow, or jolt to the head). Trauma substantially increases the risk for a variety of physical, cognitive, emotional, social and psychiatric health problems, and it is one of the most common causes of drug-resistant epilepsy in humans (Rao and Lyketsos, 2000; Herman, 2002; Frey, 2003; Faul and Coronado, 2015; Scholten et al.,

2015). Despite the prevalence of TBI, there are no effective therapies for brain trauma. A number of animal models have been developed to investigate basic mechanisms of TBI, injury dynamics and to test new therapies. Here, we describe a protocol for performing controlled cortical impact (CCI) injury in mice, a widely used experimental model of closed-head injury. We routinely use CCI to study injury-related synaptic reorganization in the hippocampus, and we recently adapted the model to investigate neural circuit mechanisms of post-traumatic epilepsy (Hunt et al., 2009, 2010, 2011, 2012).

CCI uses an electronically controlled pneumatic (or electromagnetic) impactor to deliver a precise, focal contusion injury to the brain surface. Initially described in ferrets (Lighthall, 1988), the CCI device has since been adapted for use in rats (Dixon et al., 1991), mice (Smith et al., 1995), sheep (Anderson et al., 2003), pigs (Alessandri et al., 2003), and non-human primates (King et al., 2010). The CCI injury device described here (TBI-0310, Fig. 1) uses a small bore, double-acting stroke-constrained steel pneumatic cylinder. The impactor is mounted vertically on a crossbar perpendicular to the brain surface (though the animal can be angled in the stereotactic device). A removable impactor tip (3-5 mm, with either a flat or rounded edge) is attached to the end of the lower rod, and the

upper rod is attached to a sensor system that detects impactor velocity. The impactor tip is pneumatically-driven by a control unit to compress brain tissue at a user-selected velocity, dwell time (i.e., the amount of time the cortical tissue remains depressed) and depth. Our laboratory typically uses a target velocity of 3.5 m/s, dwell time of 400-500 ms and injury depths of 0.5 mm (moderate injury) to 1 mm (severe injury).

## Materials

### Reagents

Adult, 6-8 wk old CD-1 mice weighing approx. 30g on the day of surgery (Charles River Laboratories, cat. no. 022)

Isoflurane (Western Medical, cat. no. 7263)

Buprenex (Buprenorphine hydrochloride; Western Medical, cat. no. 7292)

Ketaset (Ketamine hydrochloride; Western Medical, cat. no. 565)

Anased (Xylazine hydrochloride; Western Medical, cat. no. 5530)

Oxygen gas (100%, Airgas, cat. no. OX USP200)

Betadine surgical scrub (Fisher, cat. no. 19-027132)

Puralube vet ointment (Fisher, cat. no. NC0138063)

Cotton-tipped applicators (Fisher, cat. no. 23-400-115)

SILK 6/0 C-3 18" sutures (CP Medical Sutures, cat. no. 667S)

Surgicel absorbable hemostat (Ethicon, cat. no. ETH-1951)

Ethanol (use as 70% v/v)

### Equipment

Head Impactor (Precision Systems Inc., cat. no. TBI-0310)

Jun-Air 3-4 air compressor (Precision Systems Inc., supplied with TBI-0310)

Small Animal Stereotaxic U-Frame Assembly (David Kopf Instruments, Model 900R-B)

Universal Clamp (David Kopf Instruments, Model 925-A-C)

Mouse Gas Anesthesia Head Holder (David Kopf Instruments, Model 923-B)

Mouse Non-Rupture 60 Degree Tip Ear Bars (David Kopf Instruments, Model 922)

Dumont SS Forceps - Standard Tips/Straight/13.5cm (Fine Science Tools, cat. no. 11203-23)

Student Fine Scissors - Straight/11.5cm (Fine Science Tools, cat. no. 91460-11)

Graefe Forceps - Serrated/Straight/10cm (Fine Science Tools, cat. no. 11050-10)

Halsey Needle Holder - Straight/Serrated/13cm/with Lock (Fine Science Tools, cat. no. 12501-13)

Ideal micro drill (CellPoint Scientific, cat. no. CP67-1200)

Burrs for Micro Drill - 0.5mm Tip Diameter/Carbon Steel (Fine Science Tools, cat. no. 19007-05)

Compact mini rodent anesthesia machine (DRE Veterinary, cat. no. 9280)

Passive scavenging hose, 19 mm Blue Corr-A-Flex II Circuit Hose (DRE Veterinary, cat no. 12384)

Oster™ Animal Clippers (Fisher Scientific, cat. no. 01-305-10)

## Procedures

### Assembling the device

Assemble the PSI TBI-0310 Head Impactor and connect the Jun-Air compressor and DRE anesthesia machine according to the manufacturer instructions. Two impactor tips are supplied by PSI; one 3 mm beveled (flat) tip and one 5 mm rounded tip. We typically use the beveled impactor tip in our studies, because we have found that it produces more consistent hippocampal damage and epilepsy in mice (Hunt et al., 2009, 2012). The KOPF 900 small animal stereotactic U-frame is attached to the stand post (1 cm diameter) with the KOPF model 925 swivel mount.

### Preparation of the CCI device

1. Turn on the air compressor to pressurize the CCI device (Fig 1a). The compressor tank gauge should display between 90-110 PSI of pressure while the outflow gauge should display approximately 80 PSI of pressure.

2. Turn on the control box.
3. Prior to surgery, it is necessary to calibrate and test the CCI device to confirm that it produces the desired impact velocity and dwell time. This can be accomplished by following the step-by-step procedures on the control box.

## Surgery

4. All procedures must be approved by and comply with Institutional Animal Care and Use Committee (IACUC) regulations and should be performed using aseptic techniques. Record all surgical and procedural items on Form 1 or a similar form (see Form 1).
5. To induce anesthesia, place a mouse into a vented induction chamber supplied with 2-4% isoflurane for 60s.
 

ALTERNATE APPROACH: An injection of ketamine/xylazine (80-100 Ketamine + 5 - 10 Xylazine; mg/kg) delivered IP may be used in place of isoflurane gas anesthesia. Record the pre-operative body weight.
6. Ensure the mouse is deeply anesthetized by a suppression of a toe-pinch response.
7. Shave the scalp using scissors or an electronic hair clipper.
8. Secure the mouse into the stereotactic frame and insert the ear bars. Supply 2-4% isoflurane through KOPF Model 923-B mouse gas anesthesia head holder. Adjust the inspired concentration of isoflurane as necessary, and ensure suppression of a toe-pinch response.
9. Administer a preemptive injection of buprenorphine (0.05mg/kg, IP) prior to initiating surgery.
10. Apply Puralube ointment to eyes, to keep eyes moist during the procedure.

11. Apply Betadine surgical scrub to the scalp using a sterile cotton swab.
12. Make an approx. 1 inch midline incision to the scalp. Reflect the skin and clean skull with sterile cotton swab (Fig 2a).

ALTERNATE APPROACH: The skin on either side of the incision can be retracted using hemostat forceps.

13. Make a 4-5mm craniotomy centered between lambda and bregma, ~1mm lateral to the sagittal suture using the hand-held Ideal micro drill with a 0.6mm round carbide drill bit (Fig 2b). This is achieved by lightly scoring the surface of the skull to produce the circular craniotomy. It is critical not to penetrate the skull with the drill, as this could damage the underlying dura mater and/or brain.
14. Using fine micro-dissection forceps, carefully remove the bone flap from the craniotomy (Fig 2c). If necessary, the craniotomy can be enlarged further to ensure sufficient clearance for the impactor tip by carefully drilling along the edge of the craniotomy. Make sure not to damage the dura mater. This procedure should not result in bleeding at the craniotomy site.

## Delivering contusion injury

15. Adjust the position of the head in the frame to achieve skull-flat; i.e., lambda and bregma should be approximately level.
16. Select "Experiment" on the electronic control unit and choose the desired experimental parameters. Our laboratory typically uses a velocity of 3.5m/s, dwell time of 400-500ms and injury depths of 0.5 mm (moderate injury) to 1 mm (severe injury).
17. Follow the instruction on the control box to zero the impactor tip to the cortical

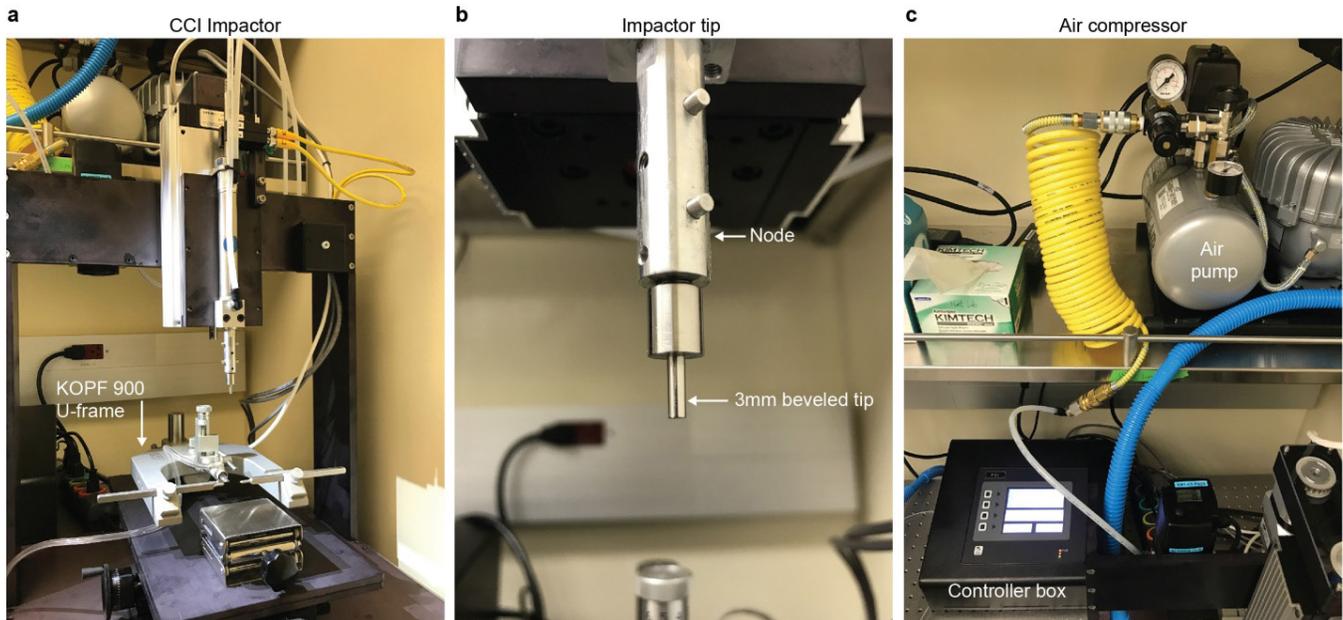


Figure 1. The PSI TBI-0310 CCI injury device equipped with KOPF Model 900R-B frame with Model 923-B Mouse Gas Anesthesia Head Holder and Model 922 Non-Rupture 60 Degree Tip Ear Bars. a. CCI impactor. b. Image showing the 3mm beveled impactor tip attached to the node. c. Top, Jun-Air air compressor. Bottom, electronic controller box used to control the injury device.

surface. Use the X and Y control wheels on the base of the impactor to move the animal into position and align the impactor tip directly above the area to be impacted.

18. Initiate the cortical impact by pressing the “Impact” button.
19. A sterile cotton swab can be used to control any bleeding that may occur immediately following impact.
20. Apply Surgicel to the dorsal surface of the brain, and close the incision using 6-0 silk sutures.

**ALTERNATE APPROACH:** A circular plastic disk (cranioplasty) can be glued to the skull, covering the craniotomy site.

21. Remove the mouse from the stereotactic frame and return to a clean holding cage for recovery. Animals should be monitored until evidence of withdrawal reflex after foot pinch and righting reflex can be observed. Mice may occasionally ex-

perience seizures during the first couple hours after severe trauma (Hunt et al., 2009).

22. Animals should be closely monitored for recovery, signs and symptoms of pain and distress or other adverse effects after surgery. A post-operative injection of buprenorphine (0.05mg/kg, IP) should be administered within 24hr after surgery. Skin sutures should be removed within 14d of surgery. We perform a qualitative postoperative health assessment each day for the first 5d after surgery and periodically thereafter. Record all findings on Form 2 or a similar form. (See Form 2). Animals normally recover from the CCI procedure without complication and remain otherwise healthy.

## Expected outcomes

CCI produces a graded morphological and histological injury response, but craniotomy in the absence of injury does not produce an overt cortical lesion (Fig. 2). In hippocampus, cell death peaks around 48hr after CCI and

is nearly complete by seven days following injury (Baldwin et al., 1997; Hall et al., 2005; Kaya et al., 1999). In mice, impact depths of 0.5 mm typically produce “moderate” injuries that include a cortical cavity generally restricted to the neocortex, and depths of 1.0 mm produce “severe” injuries extending through the thickness of the neocortex and occasionally impinge upon hippocampus (Saatman et al., 2006; Hunt et al., 2009; Hunt et al., 2012). Often, very severe injuries also include distortion of the principal cell layers in hippocampus (Fig. 2j). In addition, mice develop axon sprouting in CA1 of hippocampus (Norris and Scheff, 2009) and dentate gyrus (Hunt et al., 2012; Hunt et al., 2009, 2010, 2011; Semple et al., 2017) within weeks following injury. Sprouting is more robust following 1.0 mm impact depths as compared to more moderate injuries (Hunt et al., 2009). Many laboratories routinely use a battery of neurobehavioral assays to evaluate neurological function after CCI and to test preclinical therapies. Injured mice exhibit gross motor impairments during the first week after injury that typically recover to sham-control levels within two weeks of injury, though some fine motor deficits may persist (Fox et al., 1999). Cognitive deficits are also observed shortly after CCI and are long-lasting (Ham et al., 1992; Scheff et al., 1997). In our hands, approximately 15% of mice will experience behavioral seizures within 2 hrs after CCI injury of 1.0 mm depth, and spontaneous seizures are observed in at least 40% of the animals within 10 weeks following injury (Hunt et al., 2009).

## Advantages and limitations

An advantage of CCI is the high degree of precision that can be achieved over injury dynamics. Injury severity is primarily managed by adjusting the depth of tissue compression and impactor tip shape and size (Mao et al., 2010a; Mao et al., 2010b; Pleasant et al., 2011), but other external injury parameters can also be controlled with precision, such as impact velocity, dwell time, number of impacts and number of craniotomies. The ability to produce accurate, reproducible contusion injuries relies on appropriate calibration of the device and precisely zeroing the impactor tip to the cortical surface using the provided contact sensor. The use of a stereotactic frame allows one to choose whether the tip is perpendicular or angled with respect to the injury site. CCI is a model of focal, closed-head contusion injury. However, histopathology following neurotrauma is not exclusive to the site of injury (Hall et al., 2005), and there is likely a diffuse component to the injury (Hall et al., 2008). Although injury is delivered through a craniotomy, it is considered a “closed-head” and not “penetrating” injury, because the dura remains intact. The presence of a craniotomy likely alters injury-related changes in intracranial pressure following TBI (Zweckberger et al., 2003); this concern can be somewhat alleviated by attaching a cranioplasty over the craniotomy after impact. Alternatives to single unilateral CCI in adult mice include recent adaptations of the model for use in juvenile mice (i.e., at P21; Semple et al., 2017), repeated mild injuries (Bolton et al., 2016) and multifocal injuries (Vonder Haar et al., 2013). In all, these features allow for good control over biomechanical parameters in order to generate relatively consistent and reproducible focal injuries that can be adapted for use in a variety of animal species and developmental ages.

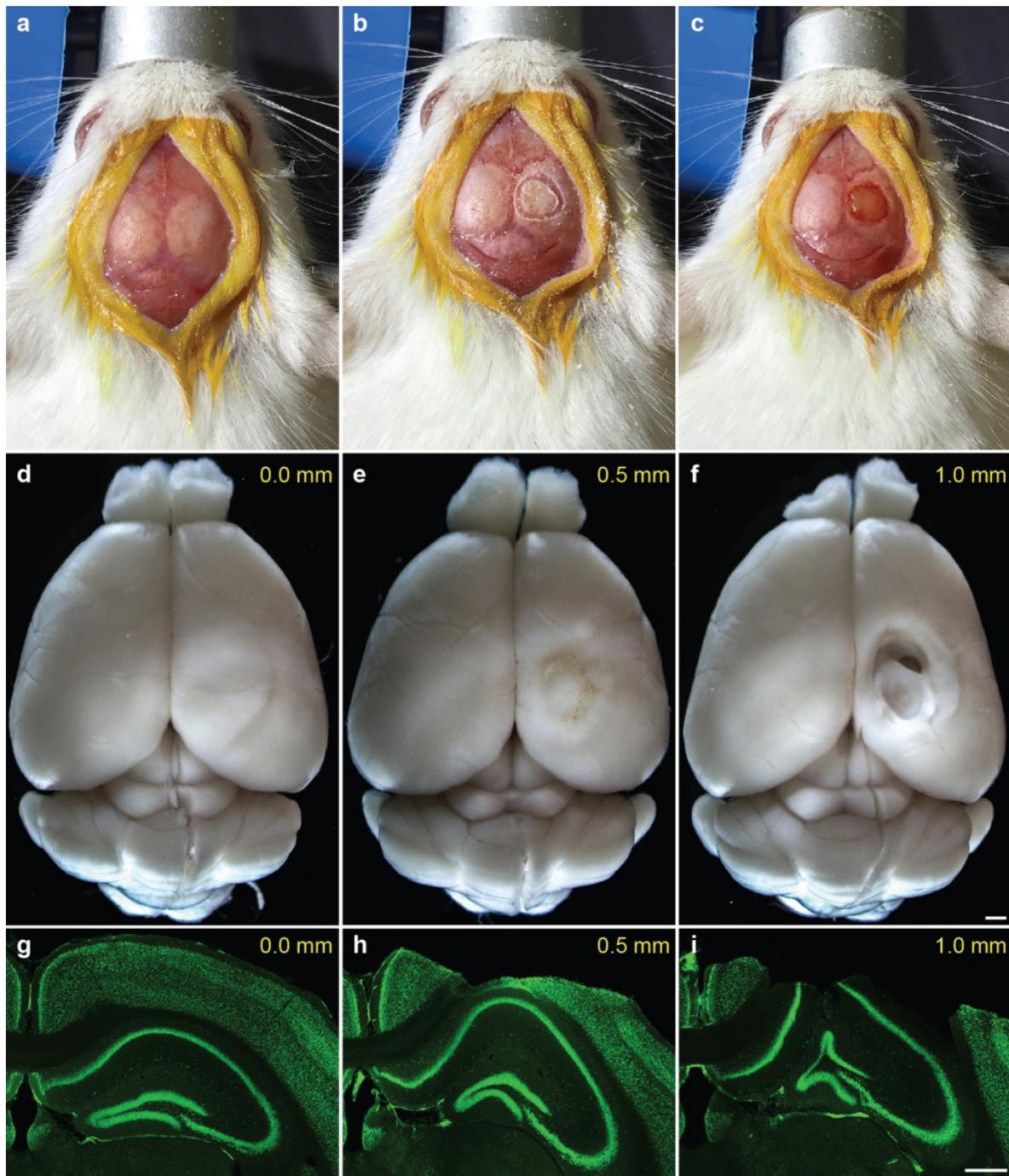


Figure 2. CCI can be used to deliver graded, reproducible injuries. a Mouse in Model 900R-B small animal Stereotaxic U-Frame. The mouse's nose is placed in the Model 923-B mouse gas anesthesia head holder. The head is held in position by Model 922 non-rupture 60 degree tip ear bars, and the skin cleaned with surgical scrub prior to making the incision. b. A 4-5 mm craniotomy is made over the right somatosensory neocortex approximately halfway between bregma (B) and lamda (l). c. Skull cap is carefully removed, leaving the underlying dura intact. d-f. Whole-brain images 30 days following CCI injury in a sham injured control mouse, 0.0 mm (d), moderate 0.5 mm (e) and severe 1.0 mm (f) impact. g-i. NeuN immunostaining (green) 30 days following sham (g), 0.5mm injury (h) and 1.0mm injury (i). Cavitation into the underlying hippocampus and distortion of hippocampal principal layers is common following 1.0 mm injuries. Scale bars: 250  $\mu$ m.

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# Post-operative Evaluation and Care Record

Animal ID: \_\_\_\_\_

| Protocol Information |  | Notes |
|----------------------|--|-------|
| IACUC protocol #     |  |       |
| Lead researcher      |  |       |
| Surgeon              |  |       |
| Species              |  |       |
| Procedure / date     |  |       |
| Date sutures removed |  |       |

| Post-operative inspection          |  |  |  |  |  |
|------------------------------------|--|--|--|--|--|
| Date / time                        |  |  |  |  |  |
| Days post-procedure                |  |  |  |  |  |
| Observer                           |  |  |  |  |  |
| Weight                             |  |  |  |  |  |
| Analgesic<br>(drug / dose / route) |  |  |  |  |  |

|                    |   |   |   |   |   |   |   |   |   |   |
|--------------------|---|---|---|---|---|---|---|---|---|---|
| Active             | y | n | y | n | y | n | y | n | y | n |
| Inquisitive        | y | n | y | n | y | n | y | n | y | n |
| Rough hair coat    | y | n | y | n | y | n | y | n | y | n |
| Crusty eyes        | y | n | y | n | y | n | y | n | y | n |
| Eating / drinking  | y | n | y | n | y | n | y | n | y | n |
| Feces              | y | n | y | n | y | n | y | n | y | n |
| Urine              | y | n | y | n | y | n | y | n | y | n |
| *Dehydration       | y | n | y | n | y | n | y | n | y | n |
| **Breathing        |   |   |   |   |   |   |   |   |   |   |
| Vocalization       | y | n | y | n | y | n | y | n | y | n |
| Wound edges red    | y | n | y | n | y | n | y | n | y | n |
| Incision swelling  | y | n | y | n | y | n | y | n | y | n |
| Incision discharge | y | n | y | n | y | n | y | n | y | n |
| Sutures missing    | y | n | y | n | y | n | y | n | y | n |
| Normal gait        | y | n | y | n | y | n | y | n | y | n |

y=yes, n=no

Post-operative evaluation- Frankowski & Hunt, KOPF Carrier, 2018  
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\*To evaluate animals for dehydration, gently pinch up a fold of skin (e.g., back of neck).  
Skin of dehydrated animals will stay pinched up.

\*\* N=Normal, L=Labored, R=Rapid, S=Shallow