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Theoretical and Practical Applications of the Intracerebroventricular Route for CSF Sampling and Drug Administration in CNS Drug Discovery Research: A Mini Review

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Abstract

Clinically, central nervous system (CNS) disorders account for more hospitalisations and prolonged care than almost all other diseases combined. In the preclinical setting, the intracerebroventricular (ICV) route for cerebrospinal fluid (CSF) sampling or dose administration in rodent models of human CNS disorders has potential to provide key insight on the pathobiology of these conditions. Low level neuroinflammation is present in > 40% of patients with severe depression or schizophrenia and so comparative assessment of CSF composition between patients and rodent models of CNS disorders is potentially invaluable for hypothesis generation and for assessing rodent model validity. As molecules in the CSF have relatively low protein binding and are freely exchanged into the extracellular fluid of the brain parenchyma, supraspinal drug administration into the CSF can produce therapeutic drug concentrations in the brain. Direct administration of investigational agents into the CSF of the lateral ventricle of the brain enables intrinsic efficacy and adverse effect profiles to be evaluated without the confounding effects of drug metabolism, due to the low capacity of the CNS to metabolise exogenous compounds. It is our view that the ICV route for CSF sampling and for administration of novel drugs in development is under-utilised in preclinical research on CNS disorders. This is due to the high degree of technical skill and low margin for error associated with correct ICV guide cannula implantation in the rat. However, these technical challenges can be overcome by using standardized procedures and attention to detail during surgery and in the post-operative period.

Keywords: Brain, Central Nervous System, Intracerebroventricular, rat, Cerebrospinal Fluid (CSF), supraspinal, intrathecal

Introduction

Despite considerable research advances in the past two decades, central nervous system (CNS) disorders that encompass developmental, psychiatric and neurodegenerative diseases, remain the world's leading causes of disability and account for more hospitalisations and prolonged care than almost all other diseases combined (de Lange, 2013; Misra et al., 2003). In the clinical setting, brain disorders and other chronic conditions such as persistent inflammatory and neuropathic pain where the pathobiology is underpinned by neuroplastic changes in the CNS (Woolf, 2011), cause enormous human suffering and a huge socioeconomic cost to patients, their care-givers and the general community.

It is estimated that approximately 13% of global disease is due to disorders of the brain, surpassing both cardiovascular diseases and cancer (Collins et al., 2011). In the European Union, disorders of the brain are the largest contributor to the all cause morbidity burden as measured by disability adjusted life years (Wittchen et al., 2011). Brain disorders represent approximately one third of the total disease burden in Europe (Olesen and Leonardi, 2003; Stoeckli, 2012). In 2010, it was estimated that the total annual direct and indirect costs of disorder of the brain was €798 billion (Gustavsson et al., 2011). In 2001 to 2003 in the USA, serious mental illness reportedly accounted for ~$193 billion per annum in lost earnings alone (Kessler et al., 2008) with 2006 estimates of a further $57.5 billion per annum for direct mental healthcare costs in the USA (Soni, 2009). Estimates by the World Health Organization indicate that approximately 22% of the world's primary care patients have debilitating chronic pain and that these individuals are approximately four times more likely to have co-morbid anxiety or depressive disorder compared with pain-free patients in the primary care setting (Alonso et al., 2011; Lepine and Briley, 2004). More recently, population surveys in many countries consistently show that at any given time, ~20% of

people have severe/chronic pain (Blyth et al., 2001; Boulanger et al., 2007; Harifi et al., 2013; Langley, 2011; Ohayon and Stingl, 2012) with the prevalence of neuropathic pain accounting for ~10% in the United Kingdom (Torrance et al., 2006) and Brazil (de Moraes Vieira et al., 2012). Neuropathic pain is underpinned by well-documented neuroplastic changes in the CNS (Mika et al., 2013) and it is notoriously difficult to treat with no more than 40 to 60% of patients achieving partial pain relief with currently available analgesic/adjuvant drug treatments (Dworkin et al., 2007).

Based on the high prevalence and associated costs of CNS disorders and chronic pain in the general population, investment in drug development for new treatments for these large unmet medical needs should be flourishing. However, the reality is that many pharmaceutical companies have withdrawn investment from these therapeutic areas due to the perceived lack of validated drug targets and the failure of many clinical trials in the last decade (Stoeckli, 2012). By contrast, there has been a recent surge in multi-government interest in the societal burden of brain disorders (Poo, 2014). Herein, we highlight the unique value of the intracerebroventricular (ICV) route for CSF sampling and for efficacy assessment of novel drug treatments in preclinical research on CNS disorders.

1.1 Translational Research Bridging Preclinical Studies and Human Clinical Studies

Molecules in the extracellular fluid of the brain parenchyma freely exchange into the CSF and *vice versa*. Hence, CSF sampling in relevant rodent models of human CNS disorders has the potential to provide mechanistic insight on disease pathobiology. On the other hand, ICV drug administration in rat models of CNS disorders to produce therapeutic concentrations in the CNS enables the intrinsic efficacy and adverse event profiles of novel drug treatments

from discovery programs to be assessed due to the low capacity of brain parenchyma to metabolise exogenous compounds (Hanna et al., 1990; Smith et al., 1999).

As already noted, CNS disorders account for more hospitalisations and prolonged care than almost all other diseases combined, despite enormous global research effort to date (Misra et al., 2003). Intriguingly, recent work implicates low level neuroinflammation in more than 40% of patients with severe depression or schizophrenia (Bechter et al., 2010). Hence, comparative assessment of CSF composition between rodent models of CNS disorders and patients with these clinical conditions with a focus on proinflammatory/anti-inflammatory profiles of cytokines, chemokines and other biomolecules of interest is warranted, not only for assessing the validity of rodent models of CNS disorders, but also for hypothesis generation and testing.

It is evident that key factors contributing to the poor track record for translation of findings from basic science research on CNS disorders to new therapeutics approved for patient use, are the poor validity of rodent models of these disorders and the narrow set of experimental conditions used for *in vivo* efficacy testing using these models (Jucker, 2010; Lowenstein and Castro, 2009). Hence, the failure of early phase clinical trials for lack of efficacy of novel drug treatments for CNS disorders compared with placebo is not too surprising, as the challenges associated with disease complexity were never evaluated during preclinical testing (Ledford, 2011; Lowenstein and Castro, 2009). Thus, major imperatives are to develop improved rodent models of CNS disorders that better mimic disease complexity in humans, and to improve the robustness of *in vivo* efficacy study designs in rodent models, by inclusion of a broader spectrum of disease readouts. Apart from inclusion of multiple behavioural endpoints and imaging to assess treatment efficacy in a panel of rodent models of CNS

disorders that each recapitulate a different aspect of the human disease pathology, study designs incorporating CSF collection may have considerable benefit in addressing the > 50% attrition of novel treatments due to lack of efficacy in phase 2 human clinical trials (Hurko and Ryan, 2005; Paul et al., 2010).

1.2 Intracerebroventricular Dose Administration

For *in vivo* research in rodents, the ICV and intrathecal (IT) routes are the two most commonly used central routes of drug administration. The former enables direct injection of test compound into the CSF in the lateral ventricle of the brain whereas the latter facilitates direct injection into the CSF in the spinal subarachnoid space. Following drug administration by the ICV and IT dosing routes, virtually 100% of the administered dose is delivered in close proximity to the target organ (Luger et al., 2005; Misra et al., 2003). ICV dosing enables drug effects mediated predominantly at supraspinal sites to be investigated whereas IT dosing facilitates assessment of drug effects transduced primarily in the spinal cord. Drugs administered directly into the CSF are minimally protein bound and undergo a low extent of metabolism relative to systemic dosing, leading to a much longer half-life in the CSF compared with the systemic circulation (Adler, 1963; Misra et al., 2003). By contrast, the extent to which a drug administered by systemic routes reaches the CNS is highly dependent upon the extent of metabolism in the liver and/or gastrointestinal wall, its pharmacokinetic properties and whether or not it is a substrate for efflux transporters in the blood-brain-barrier (Alavijeh et al., 2005).

Following ICV drug administration, there are very high local concentrations in brain regions adjacent to the lateral ventricle such as the periaqueductal grey matter (Okura et al., 2003). For example, ICV opioid agonist administration induces potent analgesia due to the high

density of opioid receptors in the periaqueductal grey matter and avoidance of systemic metabolism (Adler, 1963; Leow and Smith, 1994; Millan, 1986; Nielsen et al., 2007; Wahlstrom et al., 1988). As the extracellular fluid space of the brain is extremely tortuous, drug diffusion into the brain parenchyma is slow and it is dependent upon the drug's physiochemical properties (Misra et al., 2003) including molecular weight, lipophilicity, and degree of ionisation at the physiological pH of the CSF (Cook et al., 2009). Hence, after ICV drug administration, there are potentially large concentration gradients in the CNS which may result in relatively low drug concentrations at targets in brain parenchyma that are some distance from the lateral ventricle (Cook et al., 2009; Misra et al., 2003). For ICV administered drugs, the two main factors influencing caudal re-distribution to spinal sites to enable potential supraspinal-spinal synergy (Payne et al., 1996), are physicochemical properties of the drug and CSF bulk flow.

1.3 ICV Guide Cannula Implantation: Technical Issues

To achieve a consistently high rate of technical success for implantation of chronic ICV guide cannulae to a position 1mm above the lateral ventricle of the brain in anaesthetised rats, requires a high-quality stereotaxic frame, an experienced surgeon with excellent fine motor skills, and bone cement with strong bonding properties. These stringent requirements are essential as the margin for error in ICV cannula implantation is low, in the millimetre range. Additionally, between-rat variability in presentation of Bregma, the anatomical landmark on the skull (Figure 1), makes it difficult for an inexperienced ICV surgeon to consistently identify this landmark to ensure accurate ICV guide cannula implantation. Hence, standardised surgical protocols for ICV guide cannula implantation in rats may need to be tailored to cater for slight inter-individual differences in surgical techniques are required

together with meticulous attention to detail during each surgery and in the post-operative period.

During the highly intrusive surgical procedure for chronic ICV guide cannula implantation in the anaesthetised rat to a position 1mm above the lateral ventricle, the cannula is passed through the hindlimb area of the cerebral cortex from the cingulum to the corpus callosum (Figure 2), causing damage to these brain regions (Paxinos and Watson, 1986). Investigation of the extent of brain trauma induced by needle injury (2.4 mm external diameter) into the brain at a position 3 mm to the right of mid-line and 2 mm behind the coronal suture (Cavanagh, 1970), showed that the greatest mitotic activity occurred in the brain parenchyma at day 4 post-damage, with rapid recovery thereafter (Cavanagh, 1970). These animals exhibited no obvious functional disturbances or other complications (Cavanagh, 1970), consistent with our own findings in approximately one thousands rats with chronically implanted ICV guide cannulae (A Kuo and MT Smith, unpublished observations). In other work involving induction of a noxious cold-induced lesion to the cerebral cortex of the rat brain, electron microscopy methods showed that by 3-days post-lesion, all visible material was removed by phagocytosis from the extracellular space (Blakemore, 1969). This finding is aligned to within a day of the timeline for cessation of cell division following induction of a traumatic brain injury in the rat (Cavanagh, 1970). These data support commencement of ICV drug dosing and behavioural testing from day 5 onwards after chronic ICV guide cannula implantation surgery, in the rat.

In our laboratory, at completion of experimentation in rats with a chronically implanted ICV guide cannula, rats are anaesthetised and administered an ICV injection of a dilute solution of malachite green dye, followed by euthanasia and decapitation. After removal, the brain is cut

coronally at the point of guide cannula entry to facilitate visual inspection of dye distribution. Correct cannula placement is defined as dye present in the lumen of the lateral ventricle often with spread into the third ventricle. Data from rats where dye distribution does not meet these criteria are excluded from all analyses. In our experience, there is a trend for the number of misplaced guide cannulae to increase when recovery periods longer than 10 days post-ICV cannula implantation, are utilised (A Kuo, unpublished data). Contributing factors include (a) repeated head grooming by rats with a chronically implanted ICV guide cannula that can be minimised by Elizabethan collars in the period immediately following anaesthetic recovery, and (b) a subtle change in ICV guide cannula position due to rat growth. In our laboratory, with the exception of the first 1-2 days immediately post-ICV surgery, mean body weight of male Sprague-Dawley (SD) rats increases at ~10 g/day (A Kuo unpublished data). Our data are aligned with that of others whereby mean body weight of 7-week old male SD rats at 200 g increased to ~270 g at 8-weeks of age (Sengupta, 2012).

1.4 ICV Route of Administration: Impact of CSF Distribution

CSF bulk flow is pulsatile with each cardiac cycle characterised by forth and back movement between the ventricles and the subarachnoid spaces with each systole-diastole, with the net result being rapid CSF redistribution between the ventricles and throughout the subarachnoid spaces surrounding the brain and spinal cord (Bechter, 2011). Apart from the rapid pulsatile CSF movements, there are also slow net flows of CSF within subarachnoid spaces downwards along the neuraxis around the spinal cord, within the subarachnoid spaces from the ventricles to areas around the skull resulting in a "net" uni-directional flow of CSF as minute volumes flow between each systole-diastole cycle (Bechter, 2011). Although the CSF microcirculation provides a mechanism for solute movement from the CSF to deep spaces within the brain parenchyma, it may not necessarily be a quantitatively important pathway for

drug distribution into the brain from the CSF compartment (Pardridge, 2011). Some bidirectional exchange between the blood–CSF and CNS parenchyma–CSF interfaces may also take place (Johanson et al., 2008).

Following ICV dose administration via a chronically implanted ICV guide cannula in the rat, the injectate undergoes "net" uni-directional bulk flow in the CSF from the lateral ventricle via the foramen of Monro to the third ventricle (Bui et al., 1999; Cook et al., 2009; Di Terlizzi and Platt, 2006; Levinger, 1971). From there, the injectate travels via the aqueduct of Sylvius to the fourth ventricle and on into the subarachnoid space via the foramina of Magendie and Luschka (Bechter, 2011; Bui et al., 1999; Cook et al., 2009; Di Terlizzi and Platt, 2006; Levinger, 1971). Next, the drug travels via bulk CSF flow into the venous sinuses and from there to the internal jugular vein (Bechter, 2011; Bui et al., 1999; Cook et al., 2009; Di Terlizzi and Platt, 2006; Levinger, 1971). Although it has been suggested that the ICV dosing route is like a slow intravenous infusion (Christy and Fishman, 1961; Pardridge, 2011), this statement is overly simplistic for several reasons. For ICV administered drugs, physicochemical properties such as relatively low molecular weight, high lipophilicity and incomplete ionization at physiological pH (Carrupt et al., 1991; Cook et al., 2009; Ishizaki et al., 1997; Witt and Davis, 2006; Wood, 1997), are required to facilitate distribution into and out of the brain parenchyma to produce "fast on, fast off" physiological effects (Silva-Moreno et al., 2012). The speed and extent of distribution after ICV drug dosing is also influenced by injectate volume (Cook et al., 2009; Misra et al., 2003).

Another major factor influencing the physiological effects evoked by ICV drug administration is location of the drug target in the CNS. This is emphasized by the fact that the ICV dosing route is used to deliver drug treatments in the clinical setting where the drug

target is in close apposition to the brain ventricles (Misra et al., 2003; Pham et al., 2003; Porreca et al., 1984). Examples include treatment of patients with meningitis with glycopeptide and aminoglycoside antibiotics administered by the ICV route (Cook et al., 2009), treatment of meningeal metastasis with chemotherapy agents given by the ICV route (Cook et al., 2009), and ICV administration of opioids for relief of severe, unremitting, chronic pain such as that associated with head and neck cancer (Raffa and Pergolizzi, 2012; Smith et al., 1999).

In the rat, the volume of injectate that can be delivered into the lateral ventricle is limited. Using polyester resin castings, the mean volumes of the lateral, third and fourth ventricles of male SD rats weighing 370 to 420 g were estimated at 43 mm^3 (43 μL), 38 mm^3 (38 μL), and 10 mm^3 (10 μL) respectively giving a total CSF volume of ~200 μL (Levinger, 1971). However, there is considerable inter-rat variability in these ventricular volumes (Levinger, 1971). The rate of CSF production in the anesthetised SD rat is estimated at 2.2 μL/min (Cserr, 1971; Suzuki et al., 1985; Whittico and Giacomini, 1988).

Digital subtraction radiography with densitometry in the rat has provided useful information on the CSF distribution of injectate following administration of contrast agent (5 μL followed by a 5 μL flush) via a chronically implanted ICV guide cannula. At 90 min post-ICV dosing, radiographic density of contrast agent was 100% in the ventricles and cerebral aqueduct relative to the cannula tip (arbitrarily defined as 100%) (Luger et al., 2005). There was also caudal spread of injectate to the upper cervical region (31.6%) with faint spreading to the lower cervical spine (7.9%), and these findings were verified by serial cryosectioning (Luger et al., 2005).

ICV injection of either water for injection or 0.9% saline at 5 – 20 μL in rats, reportedly did not induce significant changes in behaviour compared with pre-injection behaviour (Cao et al., 1999; Kawasaki et al., 1991; Leighton et al., 1988; Smith and Smith, 1998). Based on our own experience, injectate volumes of 15 μL can be administered into the lateral ventricle of lightly anaesthetized male SD rats in the weight range 270 to 300 g over an approximate 2 min period without leakage by capillary action from the guide cannula or induction of behavioural changes. In the clinical setting, there are no definitive recommendations that provide guidance on the rate of ICV dose administration, although instilling small volumes (< 3 ml) over 1–2 min appears to be safe (Cook et al., 2009). Although injectate administration into the intracranial cavity can theoretically result in detrimental changes in intracranial pressure, clinical evidence confirming this is sparse (Cook et al., 2009; Thomas and Rosenwasser, 1999).

1.5 Brain Functions are Age Dependent

The choroid plexus, located in the lateral, third and fourth ventricles, is the site of elimination of xenobiotics and endogenous waste from the CSF together with convective flow associated with CSF turnover (Gradinaru et al., 2009). Anatomical and/or functional modification of the choroid plexus with aging in rats (Preston, 2001; Serot et al., 2003), sheep (Chen et al., 2012) and humans (Serot et al., 2000) has been reported. Hence, in preclinical studies in rodents, animal age may be a major contributor to inter-laboratory variability in efficacy study outcomes and also contribute to difficulties in research translation from the preclinical to the human clinical trial setting.

1.6 Advantages and Disadvantages of ICV Guide Cannula Implantation

1.6.1 Rodent Studies

A major advantage of chronic ICV guide cannula implantation in rodent models of CNS disorders is that they facilitate supraspinal CSF sampling for pathobiologic investigation. Additionally, ICV administration of novel compounds from drug discovery programs enables their intrinsic efficacy and adverse effect profiles that are mediated predominantly by supraspinal mechanisms to be defined, as brain parenchyma has a low capacity for metabolism of exogenous compounds (Table 1). Disadvantages include the highly invasive surgical procedure for ICV guide cannula implantation in the rat and the high degree of technical skill required to consistently achieve successful guide cannula implantation due to the very low margin for error (Table 1). One should also be aware that the extent to which compounds administered by the ICV route are confined to the supraspinal level depends upon their physicochemical properties.

1.6.2 Large Animal Studies

Apart from use of the ICV route for CSF sampling and/or drug administration in rats as described herein, it also has applicability in larger animal species such as the dog (Fujiki et al., 2003), pig (Salak-Johnson et al., 1997), sheep (Payne et al., 1996) and monkey (Vuillemenot et al., 2014). In the non-anesthetized sheep, administration of opioids by each of the ICV and lumbar IT routes with CSF sampling by the alternate route, showed there were marked differences in the CSF distribution kinetics of lipophilic and hydrophilic opioids (Payne et al., 1996). Indeed, lipophilic opioids (e.g. methadone and naloxone) were largely confined to tissues near the site of injection (Payne et al., 1996). By contrast, the CSF and plasma exposure profiles in cynomolgus monkeys administered a single ICV or lumbar IT infusion of recombinant human tripeptidyl peptidase-1 (hTTP-1), were similar such that the peak plasma concentrations at completion of the infusion were 0.3-0.5% of the corresponding CSF concentrations (Vuillemenot et al., 2014). Additionally, comparison of the brain tissue

distribution profiles of hTTP-1 after dosing by each of the ICV and IT routes showed that ICV administration resulted in distribution into deep brain structures (Vuillemenot et al., 2014).

1.7 **Future Perspectives**

As noted above, concurrent CSF sampling by each of the ICV and IT routes, particularly in larger animal species, enables insight on the *in vivo* distribution dynamics of exogenously administered compounds (Payne et al., 1996; Salak-Johnson et al., 1997; (Fujiki et al., 2003; Vuillemenot et al., 2014)). In addition, cannulation-based sequential CSF sampling in animal models of CNS disorders, in a temporal manner over the disease course coupled with mass spectrometry-based identification and quantification of proteins, facilitates novel insight on the pathobiology of CNS disorders to inform hypothesis generation and assess treatment efficacy (Onaivi et al., 2014).

2. Conclusion

Low level neuroinflammation is implicated in more than 40% of patients with severe depression or schizophrenia. These are CNS disorders that have a high prevalence and represent large unmet medical needs for novel, highly efficacious and well-tolerated therapeutic treatments. It is our view that CSF sampling via the ICV route for generating new insight on the pathobiology of human CNS disorders and for administration of new CNS drug treatments in development is under-utilised in preclinical research due to the technical difficulties associated with this dosing route. These technical issues can be overcome by using standardized procedures and attention to detail during surgery for ICV guide cannula implantation in rats and during the post-operative period.

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Figure Legends

Figure 1. Anatomical landmark "Bregma" (black open circle) on the skull of the rat brain.

Figure 2. Anatomical structure of the rat brain adapted from "The Rat Brain in Stereotaxic Coordinates" (Paxinos and Watson).

Schematic diagram showing a 21-gauge (21G) stainless steel guide cannula (red colour) extending to 1 mm above the right lateral ventricle of the brain implanted 0.8 mm posteria; 1.5 mm lateral; 4.15 mm ventral relative to Bregma in the rat brain. A 25G Hamilton syringe was modified so that it extended only 1mm below the guide cannula (green colour) during drug injections.

Note: Figure not to scale.

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Table 1. Advantages and Disadvantages of the ICV Dosing Route Compared with Systemic Dosing Routes

	Intracerebroventricular Administration
Advantages	• Drug dosing in close proximity to brain parenchyma especially the periaqueductal grey matter
	• Relatively low protein binding in CSF
	• Low extent of drug metabolism by brain parenchyma
	• Dissociation of CNS effects from potentially confounding effects of systemically produced metabolites
	• Ideal investigational route for *in vivo* brain effects, both desired and undesired
Disadvantages	▪ Complex surgery requiring a high degree of technical skill
	▪ Rapid elimination of some compounds from the CSF
	▪ Test compound is not necessarily confined to supraspinal sites

Highlights

- Review of CNS disorders.
- Describes knowledge/applications underlying ICV route of CSF sampling and dosing.
- Bridging preclinical studies and human clinical studies

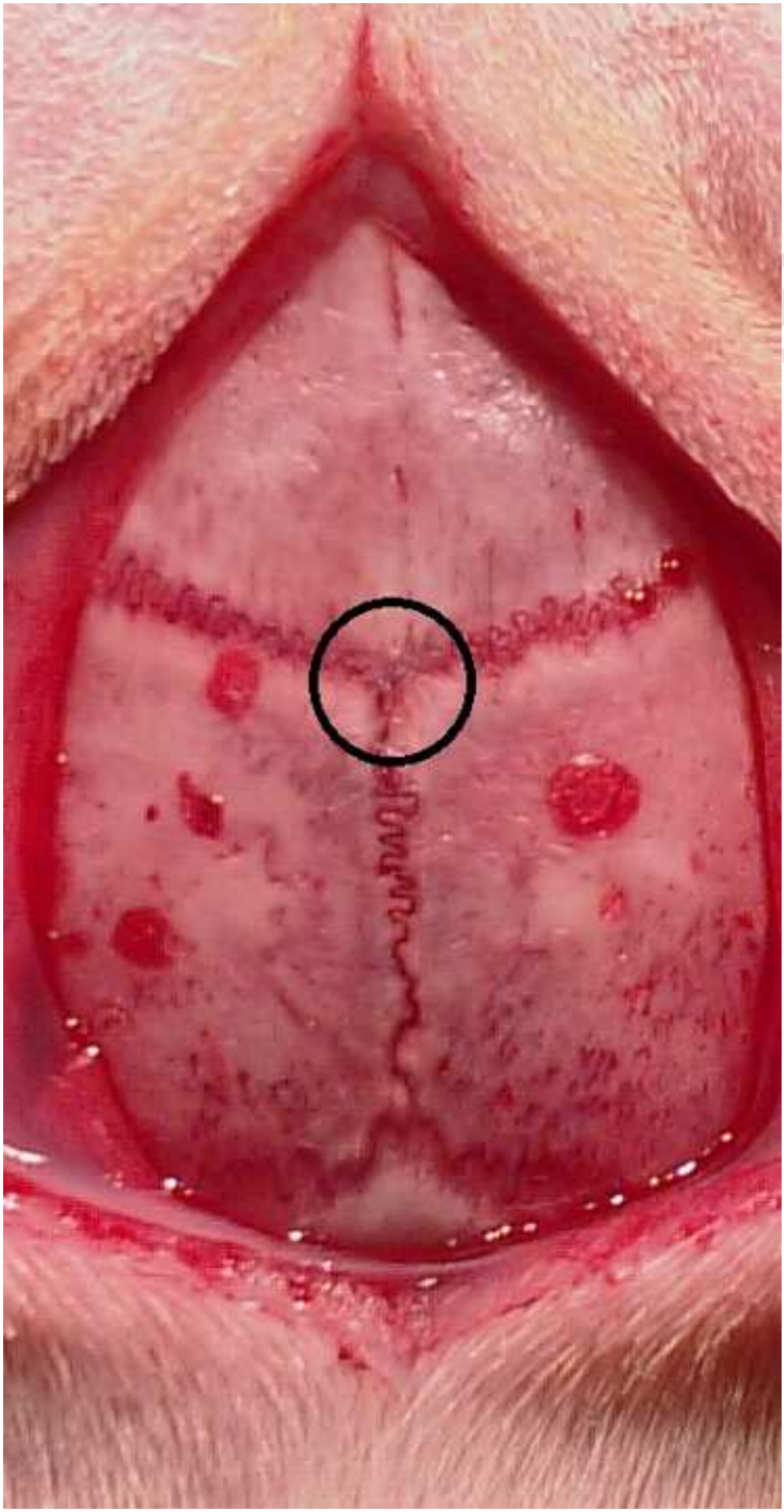

Figure 2