Chapter 19

Monitoring Renal Hemodynamics and Oxygenation by Invasive Probes: Experimental Protocol

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Abstract

Renal tissue hypoperfusion and hypoxia are early key elements in the pathophysiology of acute kidney injury of various origins, and may also promote progression from acute injury to chronic kidney disease. Here we describe methods to study control of renal hemodynamics and tissue oxygenation by means of invasive probes in anesthetized rats. Step-by-step protocols are provided for two setups, one for experiments in laboratories for integrative physiology and the other for experiments within small-animal magnetic resonance scanners.

This publication is based upon work from the COST Action PARENCHIMA, a community-driven network funded by the European Cooperation in Science and Technology (COST) program of the European Union, which aims to improve the reproducibility and standardization of renal MRI biomarkers. This experimental protocol chapter is complemented by a separate chapter describing the basic concepts of quantitatively assessing renal perfusion and oxygenation with invasive probes.

Key words Renal hemodynamics and oxygenation, In vivo methods, Rats, Magnetic resonance imaging (MRI)

1 Introduction

Kidney diseases are a global health burden with steadily increasing incidence [1–5]. Animal studies indicate that acute kidney injuries (AKI) of various origins share one common link in the pathophysiological chain of events, ultimately leading to AKI, as well as to progression from AKI to chronic kidney diseases (CKD): imbalance between renal oxygen delivery and oxygen demand [3, 6–13]. Renal tissue hypoperfusion and hypoxia have also been suggested to play a pivotal role in the pathophysiology of other kidney diseases including diabetic nephropathy [14–18].

The majority of the preclinical studies that generated this concept utilized a set of invasive probes to measure renal hemodynamics and oxygenation in anaesthetized rats [12, 14–16, 19–22]. These probes

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typically include (1) a perivascular flow probe for measurement of total renal blood flow, (2) laser-Doppler-probes for assessment of local tissue perfusion, (3) Clark-type electrodes or fluorescence-quenching optodes for measurements of local tissue partial pressure of oxygen (pO_2), and (4) devices for invasive measurement of arterial blood pressure. These methods are considered the gold standard for the study of renal hemodynamics and oxygenation because the methods—with the exception of the laser-Doppler—provide calibrated quantitative data [23–25]. The methodological principles of these techniques are detailed in the chapter by Cantow K et al. "Quantitative Assessment of Renal Perfusion and Oxygenation by Invasive Probes: Basic Concepts." Besides the study of the pathophysiology of AKI and CKD, these methods have also been used to study (1) mechanisms of control of renal hemodynamics and oxygenation in healthy rats, (2) the effects of various substances on this control, and (3) several putative preventive or therapeutic approaches for AKI and CKD [20–22, 26–30].

It is well known that, due to the considerable capacity of the organism's homeostatic control systems to—at least partially—compensate for disturbances of, or injury to, certain control elements, these alterations are often not easily detectable when studied by measuring baseline data only. Therefore, the control systems must be "challenged" in order to unmask such alterations. This is done by dedicated test interventions (*see* the chapter by Cantow K et al. "Reversible (Patho)Physiologically Relevant Test Interventions: Rationale and Examples") [19, 27, 28, 31].

As all established modalities available in today's experimental and translational research, these techniques have shortcomings and methodological restraints, in particular, the invasiveness that preclude the survival of the animals and therefor the implementation in long-term studies, and, of course, their use in humans. Magnetic resonance imaging (MRI) offers noninvasive techniques to obtain insight into renal perfusion and oxygenation under (patho)-physiological conditions. MRI affords full kidney coverage, soft tissue contrast that helps to differentiate the renal layers, seconds to minutes temporal resolution, support of longitudinal studies, and high anatomical detail [24, 25, 32–34].

However, the validity and efficacy of functional MRI techniques for quantitative characterization of renal tissue perfusion and oxygenation and its changes in various (patho)physiological scenarios remains to be established [24, 33, 35–37]. In particular, the weakness of MRI, its qualitative nature, needs to be addressed by calibration with quantitative methods, that is, the gold standard physiological techniques. Realizing the need of tracking invasive physiological parameters and MR parameters simultaneously for the same kidney, an integrated multimodality approach designated as MR-PHYSIOL was developed by our group [24, 33, 38]. It combines the measurements by the invasive probes described above

(hereafter called *PHYSIOL*) with renal functional MRI data acquired by an ultrahigh field small animal scanner. By means of this hybrid setup, the first steps toward calibration of the blood oxygenation-sensitive parameter T_2* (so-called blood oxygenation level-dependent MRI, BOLD-MRI) were done. Dedicated (patho)physiologically relevant test intervention including short periods of suprarenal aortic occlusion, hypoxia, and hyperoxia were applied to modulate renal perfusion and oxygenation, in order to detail the relationship between renal T_2* and tissue oxygenation [24, 33, 39]. Of course, the MR-PHYSIOL setup can be used for calibration of functional MR parameters other than oxygenation-sensitive T_2* as well.

In the following, step-by-step protocols are provided for two setups, one for stand-alone experiments in laboratories for integrative physiology (*PHYSIOL*) and the other for experiments within dedicated small-animal magnetic resonance scanners by use of the hybrid setup (*MR-PHYSIOL*).

This experimental protocol chapter is complemented by a separate chapter describing the basic concepts, which is part of this book.

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2 Materials

2.1 Animals

For *PHYSIOL*, male Wistar rats with body mass of 300–400 g are used. For *MR-PHYSIOL*, the spatial constraints dictated by the MR environment require the use of relatively small rats ($\leq$350 g). The rats are allowed ad libitum food (standard maintenance diet) and water and must be housed at standardized conditions (e.g., group housed in Makrolon type IV cages with elevated lids under conventional SPF conditions; for cage enrichment paper towels as nesting material and pieces of wood for gnawing should be provided).

2.2 Surgical Preparation

1. Anesthesia: urethane solution (Sigma-Aldrich, Steinheim, Germany; 20% in distilled water).
2. Temperature controlled operation table.
3. Operation microscope (e.g., Leica MZ6; Leica Microsystems, Wetzlar, Germany) with magnification range between 6.3× and 40×.
4. Set of microsurgical instruments (including dissecting scissors, microsurgical forceps, needle holders) and threads (including Vicryl polyglactin 910, 4/0; Prolene 6/0; Ethicon, Norderstedt, Germany).
5. For blood pressure measurement: (a) a pressure transducer (e.g., DT-XX, Viggo-Spectramed, Swindon, UK) connected

to an amplifier (TAM-A Plugsys, Hugo Sachs Elektronik—Harvard Apparatus, March, Germany); (b) a femoral artery catheter designed and custom-made in our laboratory using Portex Tubing (polythene). For *MR-PHYSIOL* the catheter must be longer than 1 m to allow placing the pressure transducer well outside the bore of the MR scanner. Safety information: Make sure that the positioning of the amplifier meets the safety requirements of the MR environment.

6. For monitoring of total renal blood flow for *PHYSIOL*: a perivascular flow probe (1RB, Transonic Systems, Ithaca, NY, USA), for *MR-PHYSIOL* a perivascular flow probe (MCV2PSB-MRI; Transonic Systems) as depicted in Fig. 1. Please note that the flow probe employed in *MR-PHYSIOL* uses an acoustic reflector made of Macor ceramics instead of a stainless steel or brass reflector to meet the safety and compatibility requirements of MRI. The respective flow probe is connected to a perivascular flow module (TS420; Transonic Systems). Safety information: Make sure that the positioning of the flow module meets the safety requirements of the MR environment.
7. Combined optical laser-Doppler-Flux and pO_2 probes (pO_2 E-Series Sensor; Oxford Optronix, Oxford, UK) for measurements of local tissue oxygenation and local tissue perfusion as

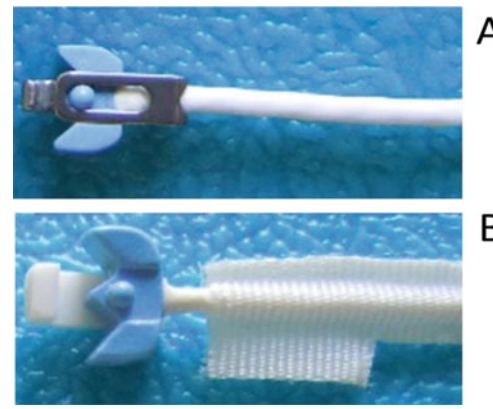

Fig. 1 Probes for monitoring of total renal blood flow. (**a**) Perivascular flow probe (1RB, Transonic Systems, Ithaca, NY, USA) for *PHYSIOL*: the acoustic reflector is made of steel; closing the probe's steel "lock" prevents detachment from the renal artery. (**b**) perivascular flow probe (MV2PSB-MRI; Transonic Systems) for *MR-PHYSIOL*: due to the long extension leads necessary to meet MR safety requirements, this probe has a larger body size. The reflector is made of ceramics (it does not induce MR artifacts), is L-shaped and offers no mechanism to lock the vessel. Therefore, a gauze is attached to the probe's cable, which will be fixed to the retroperitoneal muscles by means of sutures to avoid probe displacement

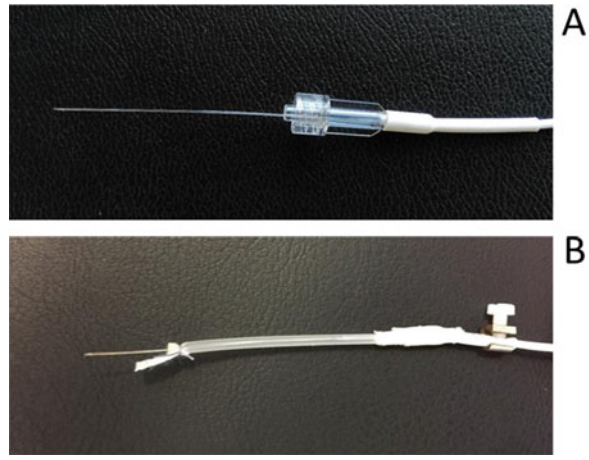

Fig. 2 Probes for monitoring of tissue laser-Doppler-flux and pO_2 (pO_2 E-Series Sensor; Oxford Optronix, Oxford, UK). (**a**) Unmodified probe for *PHYSIOL*. (**b**) Modified probe for *MR-PHYSIOL*: the customary Luer-Lock connector is removed, the fiber glass cores are fixed to the sheathing by means of a clamp. Silicone tubing with its length adjusted to the distance between the caudal and the cranial extremities of the individual kidney enables the exact placement of the cortical probe. A patche of gauze attached to the end of silicone tubing is used to fixed the probe on the kidney surface by Histoacryl glue to prevent displacement

depicted in Fig. 2. The probes are attached to an OxyLite/OxyFlo-apparatus (Oxford Optronics, Oxford, UK). Safety information: For *MR-PHYSIOL* make sure that the positioning of the OxyLite/OxyFlo-apparatus meets the safety requirements of the MR environment.

8. For continuous logging of the signals from the probes for arterial blood pressure, renal blood flow, cortical and medullary pO_2 and flux, their analog outputs must be digitized and recorded. An analog–digital converter (e.g., DT 9800-16SE-BNC, Data Translation GmbH, Bietigheim-Bissingen, Germany) permits connection to the USB port of a PC. A dedicated data acquisition software (HAEMODYN, Hugo Sachs Elektronik—Harvard Apparatus, March, Germany) allows for calibration of the probe signals and their continuous recording.
9. A vascular occluder (*see* Fig. 3): the remotely controlled hydraulic occluder is custom-made; it was designed and manufactured in our laboratory. The occluder consists of an inflatable tube ("head" of the occluder) connected by a catheter to a syringe. The "head" of the occluder is made of a high-grade silicone elastomer tube (Silikonkautschuk, Detakta Isolier-und Messtechnik GmbH & Co KG, Germany). For the connection between the "head" of the occluder and a regulative syringe an inextensible extension catheter (Portex Polythene Tubing; Ref

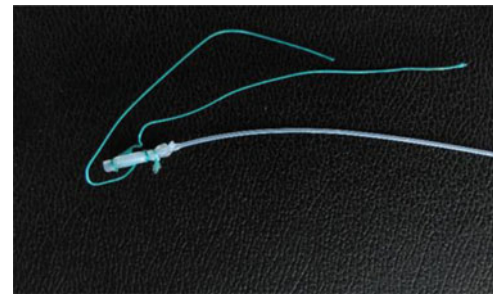

Fig. 3 Custom-made remotely controlled hydraulic vascular occluder as designed and manufactured in our laboratory. An inflatable tube ("head" of the occluder) is connected by an inextensible catheter (about 1 m length for *MR-PHYSIOL*) to a syringe. Inflation of the "head" (which is made of a high-grade silicone elastomer tube) compresses the respective vessel due to its fixation to the vessel by means of the (green) surgical threads

800/110/200; inner diameter 0.58 mm) is used. For *MR-PHYSIOL*, the extension catheter must be longer than 1 m to allow remote control of the occluder from outside the bore of the MR scanner.

10. Vascular catheter(s) for administration of fluids (e.g., isotonic saline) and/or solutions of substances used for selective test interventions (e.g., drugs), and for repeated blood sampling (e.g., for measurements of blood gases and hemoximetry). Catheters for venous and arterial insertion are made in our laboratory using Portex Tubing (polythene).
11. For fixation and stabilization of the probes for *PHYSIOL*: two micromanipulators (e.g., type M-44 and MN-153, Narishige group, Tokyo, Japan) mounted on a rotatable magnetic pedestal (e.g., type M9, World Precision Instruments, Sarasota, FL, USA) as depicted in Fig. 4. The operation table needs a steel surface (e.g., type Micro-g, Technical Manufacturing Corporation, Peabody, MA, USA) to fixate the magnetic pedestals in positions required by the individual placements of the probes within the rat. For *MR-PHYSIOL*: to achieve stabile positions of the probes and to ensure safe transfer of the animal equipped with the probes to the scanner, a custom-made portable animal holder must be used (*see* Fig. 5). It was designed and built in our laboratory using 3D CAD (Autodesk Inventor 2012; Autodesk, San Rafael, CA, USA) and rapid prototyping (BST 1200es; Alphacam GmbH, Schorndorf, Germany). The holder must meet the geometry of the MR setup; it has a half-pipe shape with a section of reduced diameter to allow for the 4-element surface RF coil to be placed beneath. A mark on the holder indicates the center of the RF coil. A bridge-like construction, positioned at the end of the hind paws of the rat, enables fixation of all leads that connect the physiological

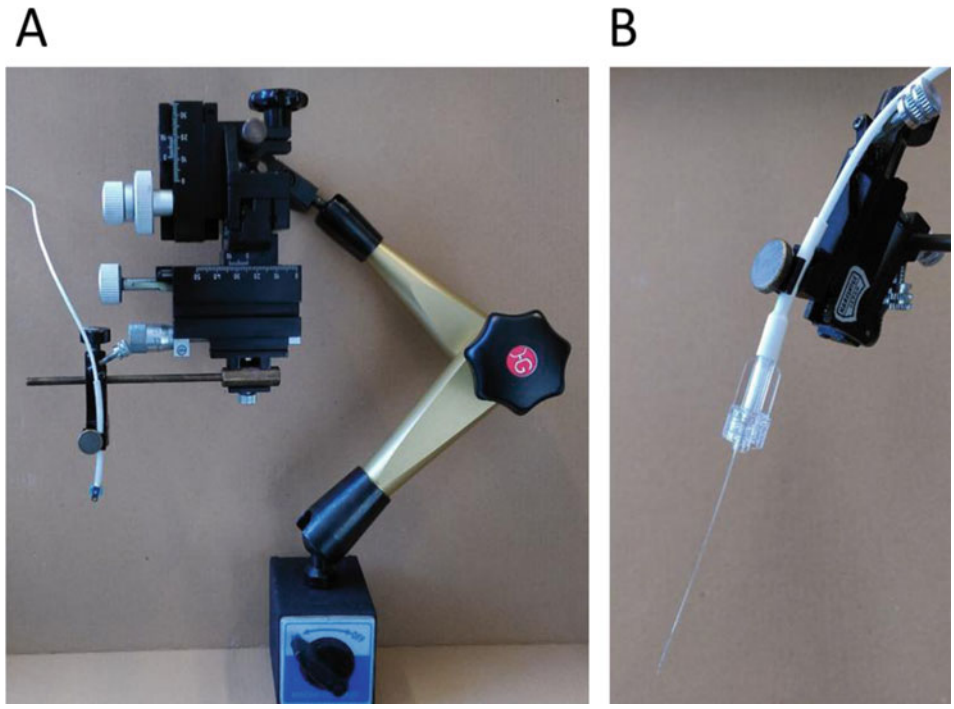

Fig. 4 Micromanipulators used for placement/insertion and fixation of probes for *PHYSIOL*. (**a**) micromanipulator (type M-44 and MN-153, Narishige group, Tokyo, Japan) mounted on a rotatable magnetic pedestal (type M9, World Precision Instruments, Sarasota, FL, USA). A perivascular flow probe (1RB, Transonic Systems, Ithaca, NY, USA) is attached to its "clutch" (type M-44). (**b**) Detail of the "clutch" with a laser-Doppler-flux and pO_2 probe (pO_2 E-Series Sensor; Oxford Optronix, Oxford, UK) attached

Fig. 5 Portable animal holder for *MR-PHYSIOL*. The holder enables fixation and stabilization of the probes and a safe transfer to the scanner of the animal equipped with the probes. To meet the geometry of the MR setup, it is custom-made by our lab in a half-pipe shape with a section of reduced diameter to allow for the surface RF coil. A bridge-like construction, positioned at the caudal end, enables fixation of all leads that connect the physiological probes with the equipment positioned outside the MR scanner room

probes with the equipment positioned outside the MR scanner room. The portable rigid animal holder in conjunction with the adjustable cable support bridge enables a safe transport of the animal to the MR scanner.

12. A respiratory (anesthetic) mask through which the spontaneously breathing rat is provided with air or other gas mixtures at

a supply rate of about 1000 mL/min. Such a mask can be easily built: take a 20 mL plastic syringe, cut off the tip approximately 15 mm from the bottom of the syringe, yielding a funnel shaped mask. Finally deflash and smooth the cutting edges of the mask using a file. The cone of the syringe can be connected to the gas supplying tube (*see* **Note 1**).

13. Patches of Dacron gauze (Woven Mesh Spacer; Merck Millipore, Billerica, USA).
14. Histoacryl glue (Braun Surgical GmbH, Melsungen, Germany).
15. Medical sticky tape.
16. Silicone elastomer tubes (Silikonkautschuk, Detakta Isolier- und Messtechnik GmbH & Co KG, Germany).

2.3 Magnetic Resonance Imaging (for MR-PHYSIOL)

Magnetic resonance imaging (MRI) requires access to an ultrahigh field MRI system including suitable accessories for the MR acquisition (radio frequency antennas), positioning, warming, and monitoring of physiological parameters, as well as trained personnel for operating the MRI system. The general hardware requirements for renal ^{1}H MRI on rats and mice are described in the chapter by Ramos Delgado P et al. "Hardware Considerations for Preclinical Magnetic Resonance of the Kidney." The methods described in this chapter were tailored for in vivo studies in rats by means of the dedicated MR-PHYSIOL techniques.

Due to the small size of rats in comparison with humans a much higher spatial resolution is required to depict the kidney with adequate detail. This, in turn, demands a high signal-to-noise ratio (SNR), which must be achieved by use of tailored MR equipment.

1. MR system: a dedicated small animal MR system with a magnetic field strength of 7 T and higher is recommended. We use a 9.4 T 20 cm bore system (Biospec 94/20, Bruker Biospin, Ettlingen, Germany) equipped with a gradient system with integrated shim set (B-GA12S2, Bruker Biospin, Ettlingen, Germany; gradient amplitude 440 mT/m, max. Slew rate 3440 T/m/s).
2. Radio frequency (RF) coils: use RF coils (antennas for RF transmission and reception) suitable for abdominal imaging, such as a transmit/receive rat body volume coil (72 mm inner diameter, quadrature; Bruker Biospin, Ettlingen, Germany) or preferably a transmit only *rat body volume coil* (72 mm inner diameter, linear; model T10325V3, Bruker Biospin, Ettlingen, Germany) in combination with a receive only *rat heart RF coil array* (curved, 2 × 2 elements; model T12814V3, Bruker Biospin, Ettlingen, Germany). Use of the latter coil setup is assumed here, as it allows for much higher spatial resolution

due to its superior SNR when compared with the transmit/receive *volume coil.*

3. Animal holder: an MRI animal holder designed for the size of the rats and the geometry of the RF coils (here model T11739 with 68 mm diameter, Bruker Biospin, Ettlingen, Germany).
4. Device for sustaining the rat's body temperature: use a circulating warm-water based heating system, consisting of a flexible rubber blanket with integrated tubing (part no. T10964, Bruker Biospin, Ettlingen, Germany) connected to a conventional warm water bath (SC100-A10, ThermoFisher, Dreieich, Germany). For alternative coil setups water pipes may be integrated into the animal holder.
5. Monitoring of physiological parameters: for monitoring of respiration and core body temperature throughout the entire MR experiment use a small animal monitoring system (Model 1025, Small Animal Instruments, Inc., Stony Brook, NY, USA), including a rectal temperature probe and pneumatic pillow.

2.4 Test Interventions

1. Gases: O_2, N_2, and compressed air, as well as a gas-mixing system (e.g., Föhr Medical Instruments GmbH, Seeheim-Ober Beerbach, Germany) to achieve required changes in the oxygen fraction of inspired gas mixture (FiO_2). The following gas mixtures are required during the experiment: (a) for hypoxia: 10% O_2–90% N_2; (b) for hyperoxia: 100% O_2; (c) for normoxia: 21% O_2 (air) (*see* **Note 2**).
2. Device for monitoring FiO_2 and $FiCO_2$ in gas mixtures: for example Capnomac AGM-103 (Datex GE, Chalfont St Gils, UK).
3. Vascular occluder as listed under 2.2.9. already.
4. Vascular catheters as listed under 2.2.10. already.
5. Solutions for fluid supplementation (e.g., isotonic saline) and drug administration for dedicated test intervention according to the scientific purpose of the respective study.
6. Equipment for blood sampling via vascular catheters (e.g., capillaries for hemoximetry).
7. Instruments for analyses of blood samples according to the scientific purpose of the respective study, for example, regarding blood gases and hemoximetry (e.g., ABL80 FLEX CO-OX, Radiometer, Copenhagen, Denmark).

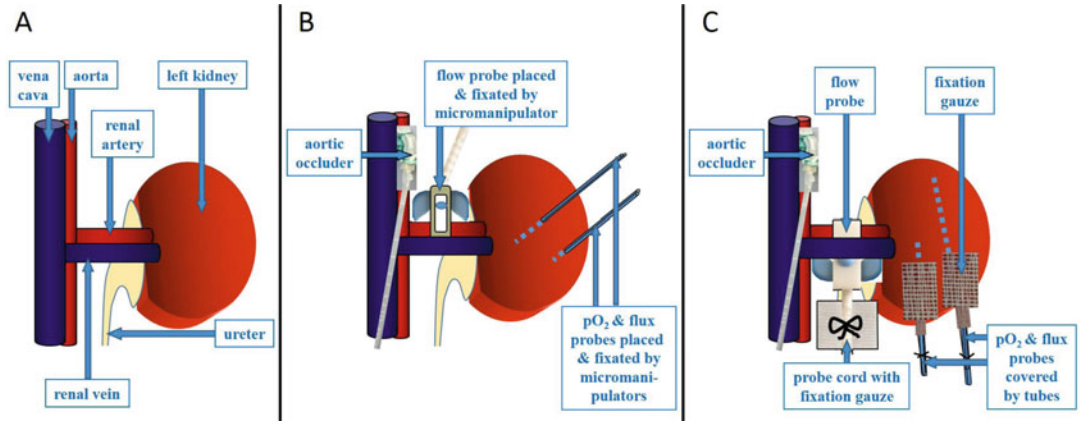

Fig. 6 Schemes depicting (**a**) the rat anatomy relevant for the placing of probes, (**b**) positions and fixations of probes for *PHYSIOL*, and (**c**) positions and fixations of probes for *MR-PHYSIOL*. For details see text

3 Methods

Figure 6 provides schemes of (a) the rat anatomy relevant for the placing of probes, (b) positions and fixations of probes for *PHYSIOL*, and (c) positions and fixations of probes for *MR-PHYSIOL*.

3.1 Surgical Preparation

For *PHYSIOL*, surgery is performed at the same operation table at which the subsequent experiment is executed. For *MR-PHYSIOL*, surgery must be performed outside the MR scanner room (in a neighboring preparation room) for safety reasons.

1. Anesthetize the animal by intraperitoneal injection of urethane (20% solution, 6 mL/kg body mass) (*see* **Note 3**).
2. After reaching the required depth of anesthesia, that is, the state of surgical tolerance (determined by specific physiological signs such as muscle relaxation degree, absence of the paw withdrawal and eye lid reflexes, absence of the swallowing reflex, and whisker movement), carefully shave the coat in the abdominal, inguinal, and ventral neck areas of the rat (hair clipper Elektra II GH2, Aesculap AG, Tuttlingen, Germany).
3. Place the rat in supine position on a warmed-up (39 °C) temperature-controlled operating table and fix the extremities of the animal to the table by means of sticky tapes.
4. Make an incision into the skin of the left inguinal area (approx. 12 mm) along the natural angle of the hind leg by lifting a fold of the skin in order to avoid an injury of the underlying blood vessels.

5. Bluntly dissect the connective tissue until the femoral vein, artery, and nerve are exposed.
6. Gently separate the nerve. Do not cut or damage the nerval tissue.
7. Separate the vein from the artery by using fine tip forceps, while trying to release an approximately 7–8 mm length vein fragment from the surrounding tissue.
8. Place three pieces of 4.0 threads under the femoral artery: situate the *first* thread distal (i.e., toward the leg), the *second* thread proximal (i.e., toward the body) and the *third* one between them.
9. Pull the *first* thread toward the leg and tie a ligature to the distal artery by using a triple knot.
10. Prepare loops with loose surgical knots on the remaining two threads.
11. Pull the *second* thread slightly toward the body so that the blood flow from femoral artery is inhibited.
12. Make a tiny incision in the exposed segment of the femoral artery behind the third thread using fine tip scissors. Fill a catheter with tapered tip with saline.
13. Grasp the catheter with the forceps and gently insert through the incision into the lumen of the femoral artery.
14. Tie the *third* knot slightly, fix catheter and arterial wall with a forceps, relax the tensed *second* thread, and push the catheter with a second forceps slowly deeper (≈10 mm) into the artery in direction of the abdomen.
15. Rinse the catheter carefully with saline, and make sure that it is patent.
16. Tie the prepared loose knot of the *third* and *second* threads.
17. Start the monitoring of arterial blood pressure.
18. For insertion of additional vascular catheters for volume supplementation (e.g., by saline infusion), for test interventions by drugs, and for blood sampling (if warranted by the specific scientific goal of the experiment) surgically prepare the proximal external jugular vein (catheter tip toward vena brachiocephalica) and/or the left common carotid artery (catheter tip toward aorta) similar to **steps 5–16** detailed for the femoral artery catheter implantation.
19. Open the abdominal cavity by a midventral incision (4–5 cm) into the skin. Then open the abdominal cavity by an incision along the linea alba, thereby preventing damage to the abdominal organs. Carefully displace the bowel to the right side of the abdomen to expose the aorta and the left kidney. Carefully detach the aorta directly cranial of the renal arteries from the

surrounding tissues without damaging the very tender lymphatic vessels of that area.

20. Fix the hydraulic occluder around the aorta cranial of the renal arteries in a manner, that the occluder is well attached to the aorta without impairing the blood flow while being deflated (*see* **Note 4**).
21. Carefully separate the left renal artery from the renal vein using fine tip forceps. Try to expose an approximately 6–7 mm long fragment. Do not cut or damage the nerves.
22. For *MR-PHYSIOL*, transfer the animal onto the portable animal holder such that the kidney is aligned with the mark on the holder that indicates the center of the RF coil.
23. Start the HAEMODYN software (*see* Subheading 3.3).
24. Place the Transonic flow probe around the left renal artery and start monitoring RBF. It is important that the head of the flow probe is filled with fluid. For *PHYSIOL*, attach the cable of the probe on the "clutch" of a micromanipulator (*see* Fig. 4a). Position and fixate the probe (Type 1RB) by means of the micromanipulator in the appropriate position of the artery (*see* Fig. 6b). For *MR-PHYSIOL*, the placement of the probe upon the artery must be done without the benefit of a micromanipulator. A gauze is attached to the probe cable (Type MV2PSB-MRI) as shown in Fig. 1b. To avoid displacements of the probe, the gauze is fixed to the retroperitoneal muscles by sutures (*see* Fig. 6c) (*see* **Note 5**).
25. For *PHYSIOL*, attach the cortical and medullary laser-flux-pO_2 probes, respectively, at one of the two "clutches" (*see* Fig. 4b) of a micromanipulator. With the help of the micromanipulator, the probes are placed into the renal cortex (depth about 1–1.5 mm below the capsule) and the medulla (3–4 mm below the capsule), respectively, as shown in Fig. 6b (*see* **Note 6**).
26. For *MR-PHYSIOL*, remove the customary Luer-Lock connectors of the laser-flux-pO2 probes and carefully fix the fiber glass cores to the sheathing using a clamp. Provide the probe with a customary silicone tubing; its length must be adjusted to the distance between the caudal and the cranial extremities of the left kidney. Attach tailored patches of gauze to the end of silicone tubing (*see* Fig. 2b). Measure the distance between the caudal and the cranial extremities of the left kidney by a caliper gauge. Based on this measurement, the cortical laser-flux-pO2-probe must be carefully prepared so that the distance between insertion point and the tip exactly matches the individual kidney's diameter minus 1.5 mm.

27. For *MR-PHYSIOL*, advance the cortical laser-flux-pO_2-probe meticulously from the caudal extremity of the kidney along the caudocranial axis (*see* Fig. 6c). To prevent craniocaudal displacement, the patch of gauze fixed to the silicone tubing of the probe (*see* Fig. 2b) must be stuck to the capsule of kidney's ventral surface by a thin layer of Histoacryl glue.
28. For *MR-PHYSIOL*, prepare the medullary laser-flux-pO_2-probe so that the distance between insertion point and the tip exactly matches the distance 3–4 mm. Carefully advance the medullary laser-flux-pO_2-probe from the caudal extremity of the kidney along the caudocranial axis. Stick the patch of gauze fixed to the silicone tubing of the probe to the capsule of kidney's ventral surface by Histoacryl glue (*see* Fig. 6c).
29. For *MR-PHYSIOL*, carefully fix the two probes' tubing to the retroperitoneal muscles by sutures in order to prevent displacement (*see* Fig. 6c).
30. Fill the abdominal cavity with warm saline (37 °C). For *PHYSIOL*, intermittent exchange of this fluid throughout the experiment is done via the open abdominal wall. As the abdomen will be closed for *MR-PHYSIOL* (see below), for replenishment of abdominal saline a catheter must be placed into the abdominal cavity. Furthermore, for *MR-PHYSIOL*, a fiber-optical temperature probe is placed in close proximity to the left kidney, in order to monitor the temperature of the kidney throughout the experiment.
31. Connect the probes with OxyLite/OxyFlo-apparatus and start the monitoring of tissue pO_2 and laser-Doppler-flux.
32. For *MR-PHYSIOL*, mark the localization of the investigated kidney's upper and lower pole from the outside on the abdominal skin using a pen. Check that the kidney (pen markings on skin) is still aligned with the mark on the portable animal holder that indicates the center of the RF coil—if necessary carefully correct the animal's position. This is essential for optimal positioning of the rat in the MRI scanner (i.e., optimal position of the rat's kidney relative to the MR coil).
33. For *MR-PHYSIOL*, place the special bridge of the portable animal holder right behind the rat's hind paws and fix all the technical extensions (temperature probe, Transonic probe, laser-flux-pO_2-probes, aortic occluder line and abdominal flushing catheter) to the bridge (*see* Fig. 5). Close the abdominal cavity of the rat by continuous suture while passing all extensions through the caudal cutting edge of the median abdominal incision. The extensions of the laser-flux-pO_2-probes must be led through the abdominal wall using a small incision in the left inguinal region.

34. Place a respiratory mask loosely around the muzzle of the spontaneously breathing rat. Open air supply to a rate of 1000 mL/min.
35. Restart the HAEMODYN software (to start a new data file) and check the quality of all physiological signals, that is, arterial pressure (measured caudal the aortic occlude and therefore equivalent to renal artery pressure, RAP), renal blood flow (RBF), cortical and medullary laser-Doppler-flux, and cortical and medullary pO_2.

3.2 Setting Up Animal for MR-PHYSIOL Examination

1. Transfer the animal into the MR scanner room using the portable animal holder (*see* **Note 7**). Position portable animal holder with the rat on the MR scanner's animal bed.
2. Switch on the small animal monitoring system. Place the pneumatic pillow on the abdomen, and cover the animal with the warming blanket. Watch the respiration trace on the monitor of the small animal monitoring system and adjust pillow position until the respiratory motion is captured well (*see* **Note 8**). Set the trigger options of the small animal monitoring system such that the trigger gate opens for the duration of the expiratory phase.
3. Position the animal bed in the MR scanner such that the kidney of interest is located at the isocenter of the magnet.
4. Perform anatomical imaging as described in the chapter by Pohlmann A et al. "Essential Practical Steps for MRI of the Kidney in Experimental Research."
5. Perform localized shimming on the kidney imaging as described in the chapter by Pohlmann A et al. "Essential Practical Steps for MRI of the Kidney in Experimental Research."

3.3 Experimental Procedures

A number of dedicated test procedures and the rationale behind the usage of the respective procedure is detailed in the chapter by Cantow K et al. "Reversible (Patho)Physiologically Relevant Test Interventions: Rationale and Examples." If adequate for the specific purpose of the study, reversible procedures (such as short periods of hypoxia, hyperoxia, and suprarenal aortic occlusion) are preferred, to keep the number of animals as small as possible as required by the 3R principle (*see* the chapter by Hosszu A et al. "Animal Models of Renal Pathophysiology and Disease"). Following each reversible intervention, appropriate time is given for complete recovery. The exact duration of the reversible intervention is dictated by the duration and number of MR measurements. Because MRI can interfere with parameters acquired by invasive probes (as has been observed for laser-Doppler flux), short intervals without MR measurements must be implemented. In the following, an exemplary protocol is given for an *MR-PHYSIOL*

experiment. It must be noted, that for these experiments, the closest coordination among the operator(s) of the MR scanner, the person(s) who perform the test interventions and those who run the electronic data storage (including setting markers for events such as start and end of an intervention) is of the essence.

1. On the PC that acquires the physiological data set a "START" marker in the HAEMODYN software to monitor and store a first set of baseline data.
2. On the MR system run the protocol(s) of your choice (T_1-, T_2-, T_2*-mapping, DWI, etc.) to acquire a set of baseline data. Duplicate these scans for repeated measurements during/between/after the test interventions and ensure that all parameters remain identical, including the shim and receiver gain.
3. *Start of hypoxia*. Change the gas flowing through the respiratory mask to 10% O_2–90% N_2.
4. Set markers and acquire both physiological and MR data during the hypoxic challenge.
5. *End of hypoxia*. Change the gas flowing through the respiratory mask back to air (21% O_2).
6. Set markers and acquire both physiological and MR data during the recovery.
7. *Start of hyperoxia*. Change the gas flowing through the respiratory mask to 100% O_2.
8. Set markers and acquire both physiological and MR data during the hyperoxic challenge.
9. *End of hyperoxia*. Change the gas flowing through the respiratory mask back to air (21% O_2).
10. Set markers and acquire both physiological and MR data during the recovery.
11. *Start of occlusion*. Inflate the remotely controlled suprarenal aortic occluder.
12. Set markers and acquire both physiological and MR data during the occlusion (*see* **Note 9**).
13. *End of occlusion*. Rapidly deflate the occluder.
14. Set markers and acquire both physiological and MR data during the recovery.

3.4 End of Experiment

1. Carefully remove the respiratory mask from the animal's muzzle.
2. Cut all sutures; remove the fluid from the abdominal cavity using a pipette.
3. Control and note the overall condition of the kidney after experiment (e.g., surface coloring and its homogeneity).

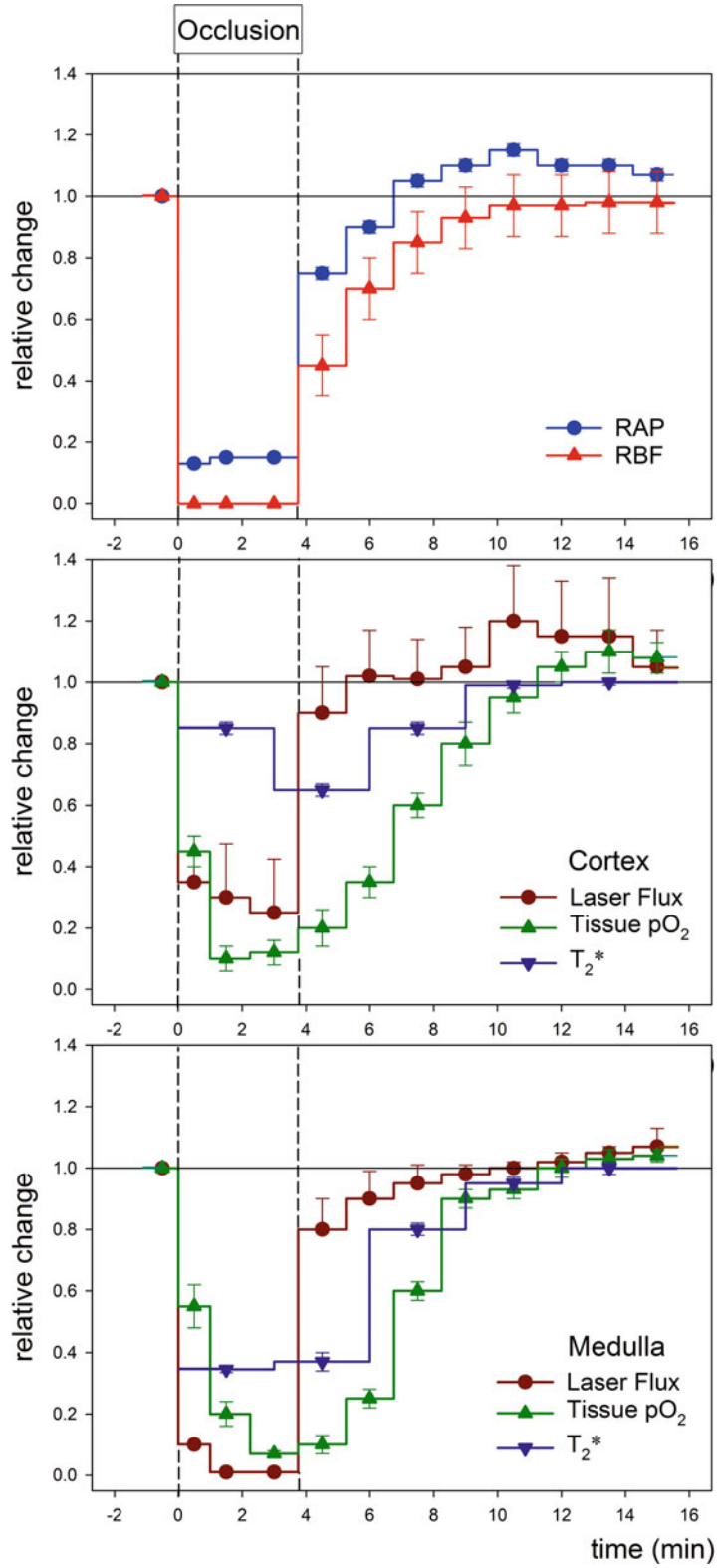

Fig. 7 Example of data obtained with *MR-PHYSIOL* in anesthetized rats (adapted from Ref. 39). Time courses of renal arterial pressure (RAP), total renal blood flow (RBF), cortical and medullary tissue perfusion (Laser Flux), cortical and

4. Carefully untie all sutures and knots; remove all probes and catheters, as well as the occluder.
5. Exsanguinate the animal by cutting the abdominal aorta.

3.5 Data Analysis

The analyses of MRI data acquired for *MR-PHYSIOL* experiments depend on the respective MR protocol and are detailed in dedicated analysis chapters for each MR method, which are part of the book Pohlmann A, Niendorf T (eds) (2020) Preclinical MRI of the Kidney—Methods and Protocols, Springer, New York.

Temporal alignment of MR and PHYSIOL data is achieved by identifying the starting point of the experiment within both data sets. For a direct comparison of the *MR-PHYSIOL* parameters only PHYSIOL data acquired during the relevant MRI acquisitions can be used. While the PHYSIOL parameters are measured with sub-second temporal resolution the acquisition of the MR parameters (derived from a MR image) requires much more time (e.g., about 60 s for a typical T_2* mapping). PHYSIOL and MR parameters can only be compared based on the (low) temporal resolution of the MRI. For this purpose the average value of each physiological parameter over the acquisition time of each MRI scan is calculated. For PHYSIOL data that are influenced by MR acquisition (such as Laser flux signals) the averages over the times without MR acquisition must be taken. Finally, group analyses of the results (e.g., relative changes of MR and PHYSIOL data; *see* **Note 10**) are done, as shown by exemplary data obtained by *MR-PHYSIOL* during a short suprarenal aortic occlusion and recovery in Fig. 7.

4 Notes

1. Instead of a respiratory (anesthetic) mask, a tracheal cannula can be used, as it is custom-made in our lab using designed and made in our laboratory using polythene tubing. The ventral region of the throat and the trachea are opened surgically, the cannula is inserted and fixated by suture.
2. As another dedicated test of control of hemodynamics and oxygenation, hypercapnia can be used with an inspiratory fraction of CO_2 of 5% in air.

Fig. 7 (continued) medullary tissue partial pressure of oxygen (Tissue pO_2) as monitored by invasive methods (PHYSIOL) simultaneously with cortical and medullary T_2* data (MR) acquired by a 9.4 T small animal MR scanner during suprarenal aortic occlusion and recovery. Date are given as relative changes (mean ± SEM) versus baseline (immediately before the occlusion)

3. Urethane supports anesthesia throughout the surgical preparation and the MRI examination (for several hours) and leaves cardiovascular and respiratory reflexes largely undisturbed.
4. (a) In order to prevent additional pressure on the aorta (and therefore development of an unintended kidney ischemia) the positioning and fixation of the aortic occluder must be performed under careful monitoring of the overall condition of the kidney (e.g., surface coloring and its homogeneity); (b) since the occluder consists of silicone and polyethylene and is positioned about 15 mm away from the kidney, it does not cause artefacts in MRI that affect the kidney.
5. (a) The signal of the probe used for *PHYSIOL* is too weak due to the long extension leads. For *MR-PHYSIOL* a probe with a larger body size and a reflector made of ceramics instead of metal must be used (*see* Fig. 1). Its reflector does not induce MR artifacts, is L-shaped and offers no mechanism to lock the vessel, which presents a significant challenge for the implantation if the probe. (b) The bulk of the intestine bears the risk to cause additional pressure that can dislocate the probe and cause probe pressure on the aorta, the renal artery and vein, or the kidney itself. (c) Since Transonic measurements rely on ultrasound an appropriate coupling into tissue is of high relevance. To this end the abdominal cavity must be filled with saline solution (37 °C) and no air bubbles must remain between probe and vessel. An additional catheter was placed in the abdominal cavity to replenish saline leakage in time course of the experiment.
6. Before inserting the tip of the respective probe, a small incision about the diameter of the probe is made into the renal capsule by means of the tip of a hypodermic needle to facilitate insertion of the rather dull tip probe.
7. Special attention during transfer must be paid to the tube/cable extensions (aortic occluder, abdominal flushing, and all probes). Make sure to keep tubes or cable close to the animal bed so they cannot get caught anywhere on the way into the magnet!
8. The peak-to-peak amplitude of the respiratory trace should span about 2/3 of the vertical axis on the display. Any gross movement (for instance during repositioning the pillow) will lead to large peaks and force the monitoring system to adapt the signal amplification, so that temporarily the signal will become much smaller on the display. Keep an eye on the magnification, which is given left next to the display, this will drop to a low value such as 15×—wait until it recovered back to a value around 100× before further adjusting it.

9. If unsuccessful, repeat the occlusion and inflate the occluder with higher hydraulic pressure and make sure that the fluid reservoir for the inflation is sufficiently filled. Always check that the inflation is sufficient to bring total renal blood flow (RBF) rapidly toward zero.
10. It is usually more practical and useful to compare relative changes in the parameters rather than absolute changes. To do this divide all parameter values by that of the last baseline value (e.g., *see* Fig. 7).

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