MY FIRST ANIMAL EXPERIMENT

My first animal experiment wasn't one. I knew what was going to happen; in a proper experiment you don't know what's going to happen. Experiments have been called *dialogues with nature*; in the dialogue the experimenter is trying to understand how nature works and uses an experiment to ask a question, and then sees what nature's answer is.

I was just starting my PhD studying parasitic nematode worms. These are worms that really matter: they very commonly parasitise people and animals, living inside their guts. About a billion people have these worms, mainly the young, poor of the developing world. They used to be very common in the UK and other now-developed countries, but sanitation largely got rid of them. All farm animals are normally wormy too so they have to constantly be de-wormed. If you've got a pet dog or cat you'll will be familiar with de-worming them too.

I needed to collect some of these worms and then mash them up, so that I could extract proteins from them to use in a lab experiment. To get these worms I was going to have to deliberately infect some rats with worms and then, some days later, either collect the worms that would come out in the rats' faeces, or kill the rats and dissect out the worms from their guts. The rats I was going to infect would be living incubators for the worms. I needed a lot of worms for my experiments, so I needed to infect a group of rats.

In the wild, worms naturally infect their hosts either by the host accidentally eating the worm's infective stages, or by these penetrating the host's skin – different species of worms infect in different ways. Once the worms are inside the host, they grow, and then migrate, through the host's body so that they end-up in their host's gut as adult worms. For my species of worm the infective stages were juvenile worms, about half-millimetre long. Naturally they would infect a rat by penetrating the rat's skin, which they do by spitting out digestive enzymes to make a small tunnel in the rat's skin. I was going to infect my rats by giving them an injection of these infective stages just below the skin – a sub-cutaneous injection – in the scruff of the neck. This is a good site for an injection because here there is plenty of loose skin. The injection was going to be much like that a vet gives to a cat or dog, though the contents of my syringe were very different. With my injection I could accurately control how many worms the rats would get, and I could do the infections quickly and easily, which was good for me and for the rats.

But looking at my first rat, there was a problem: firstly, the rat's neck was rather near to its head and, importantly, its teeth; secondly, the rat really didn't look like it wanted a sub-cutaneous injection at all. I wasn't enjoying the whole thing either. Would I get bitten, would the injection go wrong – there must be lots of important bits just underneath where I was going to inject – would I hurt the rat? If I called the whole thing off both the rat and I would be happier.

I took a thick, leather gauntlet and put it on my left hand ready to hold the rat. Even if the rat did bite the gauntlet it wouldn't get through to me. But the glove was so thick and stiff that it was difficult to tell if I was holding the rat sufficiently firmly so that it couldn't move, or whether I was

holding it too firmly so that I was squashing it. I got the rat steady in my gloved hand. In my right hand I had a syringe loaded and ready with 0.2mL – a twenty-fifth of a teaspoon – of water in which there was 500 infective worms. I went to inject the rat. As soon as the needle went into its skin the rat squealed and made a quick movement, I loosened the grip of my gloved left hand, the rat escaped scarpering off along the desk at high speed and I dropped the syringe on the floor. Not only was my first animal experiment not an experiment, but it had failed.

Now I know that this sort of experience is common. Quite a few years later a colleague and I wanted to mark rats so that we could identify individuals during our experiment. Then the standard way to do this was to punch a small hole in the ear of the rats – not unlike how farm animals are marked (or perhaps more like the modern vogue for large ear-lobe piercings). For rats and mice a combination of a different number of holes in right and left ears can be used to uniquely mark many animals. Now days you'd mark an animal by putting a small microchip under the animals' skin that can be read by a machine to identify the animal. We had never punched holes in rat ears before, so we did the proper thing and asked the university vet to help us. She was very experienced and could do almost anything with any animal. My colleague and I were fairly apprehensive about doing this at all, both because we were squeamish, but also – again – because the ears we needed to punch were close-up by the rats' teeth. We had got the leather gauntlet to hand, as well as an ear puncher. The vet arrived and we offered her the gauntlet – literally. She kindly declined, explaining that it was much easier to restrain an animal if you could feel what you were doing; she was right, just as I had found out when I first tried to inject a rat. She held the rat in one hand – it looked a good firm hold. We were paying close attention because it would be our turn next. She went to punch the ear, the rat made some quick movement and bit her, she dropped the rat and a chase ensued. I've only once been bitten by a rat – it really hurts; rats have got long and sharp top and bottom incisors that easily cut right through the end of a finger. Now in the room there was one rather angry rat on the loose, one bleeding vet, and two rather startled scientists.

Not all university vets I've known have been comfortable working with rodents. There was one whose great favourite was horses. He had spent so many years in the saddle that he had something of a John Wayne roll when he walked, though that was as far as the similarity went. I think he was actually frightened of rats and mice and he was never seen to go up close to them. If there was one that really did need his attention he would always ask an assistant to have a look at it while he stood well back. For a while there were various failed attempts to corner him into having a look at some rats when his assistants were out of sight.

My first go at injecting a rat had failed but I had to get the worms for my experiments, so there was no getting away from having another go at injecting the rat. I re-caught the escapee rat and, with the gauntlet again, held it rather more firmly, but making a mental note not to loosen my grip whatever happened. I got a newly charged syringe, put the needle into the skin, the rat squealed again (perhaps it thought 'if it worked before, why not again?') but the larvae were

injected. In thirty seconds it was all over. The only evidence of the injection was a small bump under the rat's skin; there wasn't any blood. I put the rat back in its cage. It was still, calm, and appeared relaxed and would even let me stroke it. I was a sweaty wreck.

I needed lots of worms for my experiments. My routine for a couple of years became to infect a pair of rats every month or so. I probably used about 50 rats in all. The rats I used were nothing like the rats you might occasionally see in the streets. Mine were of a strain called Wistar, named after the Wistar Institute in Philadelphia in the USA, where this strain was bred, starting at the turn of the last century. Over the years the Wistar rat has become one of the standard breed of rats commonly used in labs. Others are Sprague Dawley, Lewis, PVG. All these lab rats are breeds of rats – *Rattus norvegicus,* the common brown rat. Wistar rats are albinos – their fur is white and their eyes are red, because they have no eye pigment so their eyes are coloured by the blood going through their eyes. Because their eyes are unpigmented they are easily damaged by light they can go blind; this is the same with human albinoism. To try and stop this happening to our rats we keep them in rooms where the light is always dim. This is probably generally desirable for rats anyway, since naturally they will rather rarely go into open, brightly lit spaces. Importantly, Wistar rats, and most other commonly used laboratory strains too, have been bred to be docile so that they're easy to handle – though clearly 'easy to handle' is a relative term.

My rats had come from a commercial animal supplier. These suppliers keep extensive breeding colonies of rats and other species, so that they can supply large numbers of animals of the same breed, sex and age, something that is important for experiments. Each of my month-old rats cost just over £30 (at current prices), older rats cost quite a bit more. Keeping each rat also costs – my university charges £10 per rat per week for what's euphemistically called 'bed and breakfast', which covers the cost of the cage space, the rat food as well as the changing and cleaning of cages.

My rats were bought from one of the main UK suppliers; there are a few of these in the UK and some international companies too. I have to buy rats from a registered and approved supplier; it would be illegal to buy rats from a pet shop and to take them back to the lab. My main rat supplier now sends me a Christmas card and a calendar each year. This year's calendar has a different laboratory animal for each month – the one I'm looking at now shows the head of a white mouse peering-out from behind some straw bedding; there's a hamster for next month, and a brown mouse for the month after that. All the pictures are of rats and mice – the animals most used in labs – though for August and December there are dogs, and there's a rabbit for the spring. Because of the activities of anti-vivisectionist movements there's lots of security surrounding these animal supply companies. I've just done an internet search to try and find our main supplier, but without using their company name. I searched 'laboratory rat', 'laboratory animal', 'rat purchase' and 'life sciences supplies' and this didn't find them. If I go directly to their website it's very clear what their business is, but that I can't easily search for them is because they want to a discreet profile. Once ordered, my rats are delivered in an unmarked company van. The rats themselves

are in a fancy white cardboard box with filtered air holes in the lid. Inside the box there's bedding material and some half oranges for the rats to feed and drink on during their journey.

All the work I do with rats is done in an animal house, sometimes called a ‘mouse house’, even if there’s more than just mice in it. Animal houses are rather dull places to look at. Largely for reasons of security they are either in basements or on the top floor of buildings and there are never any windows. The animal house I work in now is a series of rooms off a main corridor. Each room houses one species of animal: one for rats, one for mice, one for toads, one for birds. In large medical research institutes a whole animal house may keep only mice, but thousands of them. I once visited an overseas top-of-the-range animal house that kept 35,000 mice. This particular institute had several animal houses across the city, keeping about 100,000 mice in total. An average mouse weighs about twenty grams so this institute was keeping about two metric tonnes of mice.

Disease can be a big problem in an animal house. When there are very many animals in one space – and especially if they are all of the same species – if any disease gets in it would very quickly go through the whole place. Unwanted infections are also a real problem because they can interfere with experiments, especially those studying animal immune responses. Because of this, one needs to be very sure that the animals have no other infections, because if they did this would mess-up the results, and make the experiment – your dialogue with nature – confused. Serious precautions are taken against diseases. When I went to the 35,000-mouse animal house, the mice were in cages where each one had its own sterile air supply. The whole animal house was divided into about ten completely separate rooms. The idea here was that if an infection did get in, it could be confined to one room. All the bedding, food and water – indeed everything – that came into the animal house was sterilised. The only really dirty things that came into the animal house were the people who worked inside it and visitors like me. To deal with this, to get into this animal house everyone had to change out of their usual clothes and into a single-use, disposable set of overalls, and then stand under an ‘air shower’ – a stream of forced, sterile air – for a minute before being allowed in. Once you were finally inside the animal house there was a one-way system: if you went into one animal room you couldn’t get to any other rooms, and the only way was out. If you then wanted to go back in you had to change again and then go back to the other room. All of this was a tremendous amount of faff, but unless this was properly done it wasn’t worth doing at all.

All animal houses have a core staff of technicians who look after the animals on a day-to-day basis. There are three or four technicians in the animal house I work in now. Their main job is to change and clean the animals’ cages – my rats are moved to clean cages once a week – to replace the food, and check the water levels. The law requires that each animal is checked each day, so even if it isn't time for a cage change, every rat is looked at each day. This happens seven days a week, every day of the year, including Christmas day. On top of this, the rest of the animal house has to be kept clean and supplies replenished. New experiments start, experiments end so there's a constant coming and going of animals.

I like going into our animal house for two reasons: the people and, during the summer, the air conditioning. The animal house technicians are always good for a chat. There's talk, banter and laughter ringing along the corridors and it's also always a good place to get the latest gossip, or so I hear. There's usually a radio on, which makes it one of the jollier places in the building. I once worked in a large animal house – where there was the unfortunate incident of the vet trying to punch holes in rat ears – that was run with military precision. The chief technician was extremely proud of how well kept and how clean his (and it was 'his') animal house was. He didn't like his staff carrying their radios from room to room. He was a canny man, and he took this problem to the head of department and explained to him that because rodents can hear at higher frequencies than humans, that the cheap transistor radios being used by his staff were broadcasting unnecessary noise to the animals: it became a question of animal welfare. His suggestion to deal with this ‘animal welfare problem’ was to install a centralised radio system and wire-in good quality speakers to each room. The head of department bought the idea and this animal house certainly had the best sound system of any animal house I’ve ever worked in.

In my current animal house each room is much the same – plainly painted with water-proof walls and a sealed floor. There is a small drain set into the floor so that the whole room can be hosed down. All the animal rooms are immaculate, if spartan. The engineering behind them is also remarkable. Each room has a separate ventilation system which changes the air 15-20 times each hour. The temperature is also accurately controlled and constantly monitored. This makes it a great place to be in the summer because it's always a steady twenty degrees no matter what the weather outside. In the animal rooms the lights are automatically controlled too. The ones for my rat room give a 18 hours light : 6 hours dark cycle, and when the lights come on or go off they gradually increase in brightness and then dim, mirroring how the sun behaves. It is a standing joke amongst animal experimenters that the facilities provided for the animals are better than those provided for the experimenters.

Inside my rat room is a metal rack on wheels that holds 25 cages. Each cage is a plastic box about two-by-one feet and holds two rats. It has a metal lid that holds a water bottle and some food, and the floor is covered with sawdust and a handful of tissue paper, which the rats use as bedding material. There is also an eight inch long piece of plastic drain pipe that the rats can use as a nest. Rather grandly this is called ‘environmental enrichment’. Here the idea is to make the rats’ environment more rich and varied so that they don’t develop odd behaviours, as many caged animals can. Each of the other rooms in the animal house has cages specific for the animals being kept. Mice cages are much the same as rat cages, but of different sizes and often holding a dozen mice or more. Toads have aquaria, birds have cages with perches.

There’s a whole part of the animal house where fresh food and bedding is stored. The rats get ‘rat cake’ to eat. This looks like dog biscuits but to my palate doesn’t taste as nice. Toads get chopped ox heart (from an organic butcher, no less); birds get a whole mix of seed and fresh vegetables – it does look rather better not being a rodent in an animal house. The other important

room in the animal house is the Procedures Room. This is the room that is designated for doing things to animals. This is the room where I'll take an animal to give it an injection. Animals are also killed in this room. It is much the same as the other rooms in the animal house except that it has a large work area, scales for weighing animals, 'a sharps' bin' for disposing of used needles, and various other paraphernalia.

My second attempt at injecting rats was much like the first, but over the months I slowly got better, learning new tricks along the way. I had started off by having the infective worms that I was going to inject in water, but when water is injected into a rat (or into you or me) it stings; this is probably why the rats squealed as I injected them. An easy way around this was to put the worms into a saline – a slightly salty solution, much like oral rehydration fluid. When this is injected then there is no sting and there is usually not a sound from the rats. Instead, probably all the rat feels is the sharp scratch of the needle going in, and the delivery of the liquid is probably hardly noticed. I only say this from experiences of having injections myself. It's getting the needle in that is unpleasant; emptying the syringe and taking the needle out is difficult to notice. Of course, there are exceptions – injections of large volumes can hurt; injection of cold liquids is very noticeable. I once had a large volume of a cold solution injected into a vein in my arm, and felt an odd wave of coldness move along my arm. With my rats, the solution being injected is at room temperature and the volume is pretty small. Also, because I was injecting the rats in the scruff of their necks where there's plenty of skin and fat, this makes the injection less likely to hurt or feel uncomfortable for the rat.

You might be thinking that both for the rat and me that it would be better to anaesthetise it before giving it an injection. This is certainly possible and it has some advantages, though it turns out that these advantages are for the experimenter only, not for the rat. If the rat is unconscious, then the injection could be done far more carefully and the rat wouldn't feel it at all. Me as the young experimenter would have been far more calm too. Indeed, for a while I did anaesthetise rats before giving them the injections but this anaesthesia probably caused more distress to the rats than did a simple injection. Administering the anaesthetic either needs an injection – so you're no further forward – or using an anaesthetic gas. Anaesthetising a rat either way is probably fairly distressing for the rat – it will feel woozy or queasy and then later 'wakes up' again feeling slightly odd. I've never had a general anaesthetic, but I believe that they aren't pleasant experiences. Anaesthesia isn't without risks either – a small (probably very small) proportion of rats might die under anaesthesia. (It is almost impossibly unlikely that a rat will die because of a sub-cutaneous injection.) These are the same reasons why doctors don't give anaesthesia when giving sub-cutaneous or intra-muscular (into the muscle) injections to us. An injection is momentarily uncomfortable, but it is all over very quickly.

You can also buy fancy equipment to help restrain an animal to make it easier to give injections, or to take a blood sample. We have one for when we need to take a small sample of blood from a rat's tail. The restraint is a Perspex tube (about the diameter of a drain pipe), with air

holes along its length. The far end of the tube is closed, but the position of its end is adjustable to take different sized rats. You encourage a rat to go into the tube while gently holding its tail. When it's in the tube, you then close the near end of the tube with a Perspex door, which has a gap cut in to it to allow space for the tail. When this is all secured the rat is in the Perspex tube, but its tail is poking out of the end of the tube. Because rats don't really like being in open, lit spaces, once the rats in the tube I cover it with a towel, to darken it and to help the rat relax. At the base of a rat's tail are some blood vessel just below the skin, and it's from here that we take small samples of blood Because the rats are safely in the Perspex tube, one person can easily restrain a rat and quickly take a blood sample, which causes minimum distress to the rat, and to the experimenter.

There are similar devices for mice, they're just smaller. Another mouse-sized restraint that I've used is a little like a see-through plastic icing bag. The mouse goes in head first, and its nose ends-up poking out of a hole (where an icing nozzle would be). The mouse can then be wrapped up in the rest of the bag. This lets the experimenter easily hold a mouse, and you can then give the mouse an injection directly through the plastic bag, all the while the mouse being properly restrained.

Now when I'm injecting a rat with worms I use a much better technique. I actually picked this up from watching a science programme on the television. I remember little of the programme now except that it was something to do with drugs and brains. The narrator was telling us about some recent discovery and the pictures were of an American laboratory where a rat was being given an injection of a drug. What so took my attention was that the person with the needle injected the rat without the slightest fuss or nonsense from the rat. This was so different from my experience. At first I thought that it was some special event staged for the television cameras, or that the rat had been given so many other drugs during its laboratory life that it was pretty much stoned and one could have done pretty much anything to it, without it noticing. But, I watched carefully what was being done, and then tried the technique myself. The trick was to work with rats' natural behaviour.

Now when I inject a rat I take it out of its cage and place it on a small towel. The rat is immediately happier on this surface where it can get a grip, compared with the easily washable, slippery work surfaces where it can't. I then form a small towel cone with my left hand. A rat will immediately go head-first into this and will happily and calmly stay there; if I then take away my hand it won't stir as long as its head is covered and it's in the dark. This works because rats' natural behaviour is to go into darker, more confined spaces. Rats will also behave this calmly on your arm. If you fold your left arm across your body, so that there is a crook at your elbow against your chest, and if you then put a rat on your forearm it will run along your arm to the crooked elbow and bury its head in the crevice and then happily sit there. Mind you, this can go too far: I've had rats nosing their way at the cuff of my lab coat. This obviously looks a nice place too – it's dark, warm and confined. A former colleague of mine (and now a famous Professor) with something of a nervous disposition got himself rather closer to his experimental mice than he wanted. He was very

particular when working in the animal house and took to wearing an all-in-one white jump-suit, which he would zip-up to the neck. One rather adventurous mouse got up the arm of this get-up, but then couldn't get out. It ran up one arm, over his shoulders, headed briefly down the other arm, before thinking better of it, and instead headed for his torso. After this incident he took to putting rubber bands over the ends of his cuffs to make sure that this never happened again.

Once a rat has got its head in the towel cone you next gently hold its head and neck, pushing up a fold of skin on the scruff of its neck between a thumb and a first finger. This leaves your right hand free to do the injection, aiming the needle between, but just below, your finger and thumb. The whole thing takes twenty seconds. The rats are not obviously distressed – they don't squeal or squeak. All this means that I can now do my experiments knowing that what I've done to the rats, I've done in the least distressing way possible, which is good for them, for me, and for my nerves.

Now when I work with rats I have to take much more care because I've become very allergic to them. This is actually pretty common among people who work with rats and mice – about a third of people develop this allergy. This allergy is so common because rat and mouse urine is very allergenic, but also because animal houses keep large numbers of animals in quite small spaces, which compounds the problem. I now use a face mask whenever I'm in an animal house; without it I soon start sneezing and my breathing gets wheezy. My skin's also very sensitive so that if I pick up a rat and its claws touch the skin on my hand, this ends up as a little wheal, like a nettle sting. If I've been holding rats I also have to be very careful about washing my hands very thoroughly afterwards; if I don't then hours later if, by chance, I touch my eye then it will start to water and go red. When I leave an animal house I scrub my hands and arms and wash my face with soap and lots of water. I'm so sensitive that if someone brings a lab coat or an injecting towel to the lab and I touch it then I start sniffing too.

These allergies can be very bad especially for the staff who work in the animal house every day. Some of these staff have to wear face masks that cover their whole face – not unlike astronauts' helmets – and which have a separate breathing supply of filtered air coming from a pack strapped to their waist. Yvonne who used to work with me and who did a lot of rat work had to use one of these systems soon after starting. As she became allergic to the rats, she also became allergic to her eye make-up and had to change make-up brands. I was probably very likely to get the rat allergy since I've always had hay fever. In fact I'm also very allergic to cats. I've wondered whether I've got a rhyming allergy: so far it's cats and rats so I predict I should be allergic to bats, but I haven't had the chance to test this yet. One other good reason for keeping a high rate of air change in animal houses is to keep the level of allergens down. All of us have our lung function checked once a year by an occupational health nurse, which looks for long-term changes in how our lungs are working. Mine are absolutely fine, last time my lung function was more than one hundred per cent.

After I inject the infective worms they migrate through the rat. Four to five days later the juvenile worms have grown into adult worms that are in the rats' small intestine. The adult worms are just over two millimetres long and these worms lay eggs that get released into the rats' guts, so that they eventually pass out of the rats in their faeces. It is from here that the fresh infective larvae develop. We put the rats in special cages to collect their faeces. These cages have a metal mesh floor, so that when the rats defecate their faeces fall through on to trays, which are lined with damp paper. This keeps the faecal pellets wet, which is necessary to keep the worm eggs inside the pellets alive. Because the rats are crepuscular – active at twilight – this is when they defecate most, so we put them into these cages at about five in the evening and then take them out again at nine the next morning. In the lab we keep the rats' faecal pellets damp and slightly warm and in two to three days infective worms have grown, and they then crawl out of the faeces. It's these that we collect and these that I was using in my experiments, and which I then used to infect rats.

The rats aren't passive when they have a worm infection; rats make immune responses against the worms. These immune response damage the worms. You can first see this because the worms in the gut start laying fewer and fewer eggs. We know that if rats have their immune systems suppressed then the worms produce more eggs. This proves that the reduction in the worms' egg laying is because of the rats' immune responses and not just because the worms are getting old. The rats' immune responses eventually kills the worms and the rats are then immune to being re-infected. If they are re-infected with another injection, a few worms do get to the gut but they live for only a very short time before the immune responses kills them. Because of this we have to infect new rats every month. Rats that have become immune to worms can't be reused and have to be killed.

My first attempt at injecting a rat was twenty years ago and I've moved on a lot since then. I can now inject rats easily. I've worked out the best way to give an injection, and I know why it works: I only wish I had been taught this when I started. Many things have changed since I started out. Now, before anyone can work with animals they are trained in many general aspects of animal care and then trained specifically for the species they're going to be working with, such as how to hold and restrain the animal. They'll also be trained in the specific procedures they're going to need to use, such as how to give an injection or collect blood.

I now teach my students how to inject rats. We start off by practising on dead rats, but this doesn't help that much. The tricky thing about injecting rats is holding the live animal so that it can't move, but doing so without hurting it. With live animals I'll let my students watch me give a few injections, explaining what I'm doing and why. Then next we'll do one together – they hold the rat and I inject it, then the other way around. Then they can do one by themselves, with me watching; anyone's first injection is never easy. I'll then do another one – this time they are watching after having done it themselves, so they now know what to watch for – then they can have another go. After someone's injected ten or twenty rats they'll probably be very good at it.

For new people it also takes a little time to get used to an animal house. They are not normal places. The security can be off-putting; it’s like no other part of the building we all work in. There are different animals being used in different experiments. It's both exciting and fascinating, but also rather weird. It's a 'public' place compared with the privacy of our offices; it’s somewhere where I meet many of my colleagues. But, it's also a very private because of the low profile that 'security' demands. It's a place full of banter, of every-day work, but it's also a place that is covered by many rules, regulations and officialdom. This every-day place has led others to campaign both peacefully and violently to stop the 'cruelty' that goes on inside. How has my every-day life become a secret and un-trusted world?

Mark Viney, 2014