STARTING UP

Molecular biologist Daniel Bose in one of the university lecture theatres he uses for teaching.

Every year, thousands of researchers struggle to launch their own labs. Two of them fought against the odds together through funding anxiety, a pandemic and a personal crisis. Here is their story.

By Kerri Smith, Richard Van Noorden and Heidi Ledford. Photography by Chris Maddaloni.

On the morning of 7 June 2018, neuroscientist Alison Twelvetrees arrives at a slick five-storey building on the traffic-choked Euston Road in London. She has made sure she is wearing her lucky charm — a locket that belonged to her grandmother. Her bag is stuffed with crumpled neuroscience papers and notes that she's been carrying around like a comfort blanket for the past two weeks. She is here to make a pitch to win a £1.14-million ($1.6-million) fellowship — money that she needs to sustain the fledgling research group she started the previous year in Sheffield, UK.

IN SCIENCE

Neuroscientist Alison Twelvetrees in her laboratory at the University of Sheffield, UK.

Outside the interview room, she watches the previous applicant emerge and tries to get a handle on her adrenaline. She has rehearsed for this moment intensively, fielding awkward questions in three practice interviews. The worst moment: when one panel member asked why she'd been so unproductive as a postdoc — a query so aggressive that it took Ali a while to gather herself sufficiently to reel off her scientific accomplishments and point out that she'd also had a baby.

Ali's now-three-year-old daughter, Ada, is back home in Sheffield with her husband, Daniel Bose. Dan knows what's at stake for his wife. Both of them are new principal investigators (PIs) — independent scientists building up their own laboratories at the University of Sheffield. A year earlier, Dan had tried and failed to win a £1-million fellowship from the same scheme, funded by the UK charity Wellcome and the Royal Society. It is one of the most prestigious and lucrative grants available to PIs in the United Kingdom.

When Ali walks into the room at Wellcome headquarters, she finds a panel of 20 judges. The chairperson is so far away that she seems to exist in a different dimension. Ali has five minutes to take the panel through just two PowerPoint slides about her research into how cellular cargo is shuttled up and down neurons, and how these mechanisms break down in disease. She then has 25 minutes to answer the panel's questions. It will take the panel a few weeks to decide whether to award her the money she needs to get her lab off the ground.

The interview is simultaneously painfully slow and over too quickly. At a nearby pub afterwards, Ali sips a half-pint of beer, downcast. "I'm not crying, so there's that," she says. "The next three weeks is going to be stuff popping into my head: 'Why did I answer that? Why did he pull that face when I said that?'"

Every year, a few hundred scientists in the United Kingdom try to establish new labs from scratch; globally, thousands of researchers

Dan in his lab.

become heads of their own labs. From the outset, it's a chase for money and a time of intense pressure as scientists try to build research programmes while juggling teaching, fundraising, publishing and family life. Ali began her lab with just £15,000 in grants to cover equipment and experiments; Dan had £20,000. Both need to recruit PhD students, and Dan must also devise and deliver a programme of lectures.

They arrived at Sheffield in 2017 with three years in which to prove themselves, by attracting bigger, lab-sustaining grants and, they hope, publishing papers. If they can do that, they will pass their probation and their positions will become more stable, although the United Kingdom doesn't have a tenure system like that of the United States. If they fail, the university can terminate their contracts and they'll be out of a job. Dan and Ali agreed to allow a team of reporters from *Nature* to chronicle their lives as they struggled to establish themselves in science.

'Okay, where do I start?'

When Dan first opened the door to his new lab in spring 2017, he was surprised to find that it was stuffed with someone else's junk. Half-full boxes of pipette tips and nitrile lab gloves littered the dusty shelves. In one corner sat a trolley with peeling, dirty white paint. On it stood an ancient computer monitor, a spaghetti tangle of defunct cables and several tired-looking machines.

Dan had one bench and one microcentrifuge. The rest of the space was assigned to people from another lab. He sprayed down his benchtop space, wiped off the dust and walked out again.

For the next two weeks, Dan's mind would race when he walked into the lab. "Okay, where do I start? What do I do? What do I need to get going? How on earth do I afford what I need to get going?" he thought. Then he would retreat to the safety of his office down the hall to think about something else.

Gradually, things came together. Reagents he'd developed as a postdoc arrived from his former lab at the University of Pennsylvania in Philadelphia. And he learnt to scavenge. In corridors, meeting rooms and the backs of cupboards as well as on his new lab's shelves, he found an embarrassment of riches: barely used cartons of 96-well plates used to carry out reactions in small volumes, micropipette tips, forgotten tubes of enzymes.

Dan ordered bright orange tape to mark his territory in the shared space, a fridge freezer, a few test-tube racks and a partial set of micropipettes — his first purchases as a PI. But fiscal realities quickly set in. Dan wanted to study enhancer RNAs, which can control when and where genes are turned on. After years as a postdoc, dreaming of the experiments and projects he'd launch in his own lab, Dan realized he would have to rein in his ambitions. He didn't have the time or the money to race to the bench and get started. He needed to write grants, plan budgets, negotiate rates with vendors and recruit students.

When he did make it to the bench, Dan would sometimes begin an experiment, only to realize that a key reagent was missing. Once a postdoc in an established and well-funded lab, Dan now found himself trying to stretch his scant resources. He borrowed basic molecular-biology staples such as agarose, a compound used to separate molecules on the basis of size, from people in neighbouring labs until he felt their patience wearing thin. When he did place an order, the price tag on some reagents made him shudder.

But at least he had space to play with. For a few weeks after her job started, Ali didn't have a bench — nowhere to actually do any lab work. "I turned up. And I sat in my office for a few weeks, and then at a meeting, I sort of raised … 'Can I have a bench, maybe?'"

Her new home, the Sheffield Institute for Translational Neuroscience (SITraN), had been established seven years earlier by drawing together existing scientists from Sheffield. Ali was the first member of staff to join from outside the university, and most lab space was already allocated. Once she prodded, she got a small spot in a shared space.

Feeling that she needed more support from her peers, Ali set up a group on the messaging app Slack for new PIs in the United Kingdom, together with immunologist Sophie Acton, who had also just started her first lab at University College London. Slack groups are a popular way for PIs to share experiences: one set up in 2016 by cell biologist Prachee Avasthi now has more than 3,000 members.

Despite the slow start, Ali began to make

plans for her science and to collect some preliminary data. She started collaborating with a scientist from another department who had built a microscope she could use in her research.

For a while, Dan and Ali had a game going to see how much they could stretch free samples from vendors. It was a far cry from his postdoc lab, Dan recalls, where all he had to do was wander over to tell the lab manager that he needed a reagent. "Suddenly, you're hit with having a budget and learning purchasing," he says. "I was spectacularly unprepared for that."

Dan was also unprepared for teaching. Unlike Ali, Dan's position came with teaching responsibilities and, in the autumn of his first year at Sheffield, Dan walked into the first of four lectures he was slated to give for a class on RNA biology. He'd had almost no experience or training, and he was a little nervous, as he often is before public speaking events. The lecture started well as Dan ran through the basics of the CRISPR–Cas9 gene-editing technique. But when he delved into the enzyme mechanism underlying how this editing system works, 75 faces went blank. "You hear comedians talk about how you can tell when you lose an audience," he says. "I didn't expect to see this happening myself."

Dan felt a whiff of panic but ploughed on with his material, rather than going back over the topic — a choice he later regretted. Looking back on that day, he describes it as "a little bit soul destroying", but he knows he's not the only scientist who has had a rough first lecture: "It's a rite of passage."

'I finally decided I had nothing to lose'

Ali and Dan met as undergraduates at Imperial College London. They both studied biochemistry, but Dan sat at the back of the lecture theatres; Ali at the front. "I didn't speak to Ali for three years," says Dan. "I was kind of shy. She was kind of dating somebody."

But at a summer ball towards the end of their third year in 2004, Ali persuaded a friend to ask Dan out for her. "I finally decided I had nothing to lose: 'Well, I won't see him again, so it doesn't really matter,'" she says. "But I still wasn't brave enough to ask him out in person."

"You were braver than me," says Dan.

A little over a year into the relationship, the two began what would become a long struggle with the 'two-body problem' — a difficulty encountered by many academic couples as they manoeuvre themselves through a tight job market in search of two jobs in the same location. They both got into graduate school in London, with Dan lingering there as a postdoc while Ali finished her PhD. Then Ali got a fellowship that allowed her to split her time between London and the University of Pennsylvania, where Dan found a second postdoc.

They uprooted and moved to Pennsylvania in December 2011, just three weeks after getting married. A little more than three years later, their daughter Ada was born.

At the end of Ali's one-year maternity leave, the two-body problem loomed large. Ali had to return to London to finish her fellowship, but Dan was preparing to submit a paper — a crucial step in any young scientist's career — and he couldn't leave Pennsylvania just then.

In December 2015, the family packed up its belongings and the cat, and rushed to the airport to fly back to the United Kingdom for the holidays. At the start of the new year, Dan returned to the United States alone.

"It was a really tough time for everybody," he says.

Ultimately, Dan stayed in Philadelphia for another 11 months, while Ali juggled work and Ada alone in London. Dan did his best to parent by FaceTime. "Ada got very used to talking to an iPad," he says. "She would do things like carry the iPad around and tuck it up with her toys at bedtime."

Years later, Dan and Ali still carry the weight of that time apart. The separation seemed a necessary sacrifice to ensure that they both had a solid chance of long-term employment, says Ali. But it also felt like a risk to their family.

"It was a big gamble, or at least it felt that way," she says. "It's difficult. The ideal, perfect image of a mother … what kind of mother would separate their child from a loving father who wanted to be there for her?"

"Similarly, what kind of a father would leave his wife and daughter in another country and go and take a flight back to do science?" Dan says.

Dan has given talks to new postdocs about his academic career path, and likens the two-body problem to running a three-legged race. "You're both trying to go in the same direction," he says, "running with two legs tied together."

After Dan's offer from Sheffield had arrived, Ali faced a protracted wait to see whether her application was also successful. When it finally came through, Ali and Dan counted themselves lucky that they were able to find jobs at the same university. When they first moved to Sheffield in 2017, it was the first time that they'd all lived together as two working parents raising a child. "There was a lot of intense discussion about how it was all going to work because we hadn't had to do it before," says Ali. "It took a long time."

But through trial and error, the family settled into a routine that worked. Ali and Dan alternated Ada's day-care pickup — one week on, one week off — to allow them to work late and tend to experiments.

"The fact that we managed to get here at all is somewhat surprising," says Dan. "There's definitely an element — when you're in the middle of paper reviews and neither of you have

> "YOU'RE BOTH TRYING TO GO IN THE SAME DIRECTION, RUNNING WITH TWO LEGS TIED TOGETHER."

Ali with the couple's daughter, Ada, in their garden in Sheffield, UK.

jobs sorted out — when you think maybe it's not going to happen for us."

Now they're in the same place, the challenge is to sustain their good fortune and use their stability to break new ground in science.

Nanometre steps

When Ali talks about the brain, her eyes widen and sparkle with child-like glee. "All cells are special, but I love neurons the most," she says. "And they have to function your whole life. It's crazy. It kind of gives you vertigo, thinking about all this stuff going on in your own cells."

In every one of these vertigo-inducing neurons, a fleet of motor proteins ensures the safe passage of cargo from the cell's main body down its axon, or nerve fibre, and back again. That cellular shuttle system is crucial because it supplies materials to the ends of the axon, where electrical signals jump from one neuron to another.

Inside neurons, the motor proteins travel along tracks called microtubules. For some long neurons in the spinal cord, the journey from end to end could take two weeks. When motor proteins fail to do their work, as they do in motor neuron disease, the effects on the network can be devastating.

Ali wants to know how these proteins load and lug their precious cargo. They can carry freight many times their size — large organelles such as mitochondria, or proteins contained in bubbles called vesicles. One class of motor protein called kinesins look like upside-down Ys, and their two 'legs' plod along the microtubules in eight-nanometre steps, shuttling newly made components to the ends of the axon.

Ali knows that different cargos can travel to the ends of axons at very different rates. But the kinesin that does the hauling moves forwards at an almost constant speed. Ali wants to understand how kinesin activates — or loads its cargo — and how that sets the pace of the journey. If she can do that, she can take a stab at working out how the process breaks down in disease.

She can just about see these proteins at work in single neurons in a dish, but it's hard to observe them properly. Ali's dream microscope, which would allow her to track motor proteins as they carry cargo in live neurons, is a big part of her grant application and costs a quarter of a million pounds. "£250k is a lot of money, but it's a lot of microscope," she says. "I always think of the money as science tokens — 'I can buy this much science'."

For now, she is having to settle for putting the proteins under a shared microscope and watching them float freely in fluid, spying on them as they open and close like the legs of synchronized swimmers.

Ali describes all of this to the judges in her pitch for the Sir Henry Dale Fellowship at Wellcome in June 2018, outlining how she plans to study kinesin and what she needs to make it happen. Afterwards, Ali recalls, there wasn't much time to take stock of how it had gone because Dan had a deadline a few days later. "I had to finish my interview, and then sort of drop everything to make sure that I was covering everything at home while Dan was finishing his grant application," she says.

Firth Court at the University of Sheffield.

A few weeks after her interview, Ali checks her phone after a lab meeting: "And literally the first thing I saw was this e-mail in my inbox. And I just knew, even though I hadn't seen the content of it, that it was negative."

> "WHEN YOU'RE STARTING OUT, YOU'RE A TEAM OF ONE. THAT'S QUITE A LONELY PLACE TO BE."

She forces herself to read the e-mail, which is brief and confirms her fear: she hasn't got the fellowship. Then she calls Dan. "And then I ran away and had some lunch. And a big piece of chocolate brownie and a small cry and got back to work again."

Ali is immediately pragmatic about what to do next. She knows she can apply to other funding bodies, and probably to the same fellowship programme, which she is most familiar with. She knows her weak spots. "Even though I can do all this stuff, I don't necessarily project impermeable confidence. When you're trying to essentially give a pitch and get people on board with your vision … you kind of have to be bullet-proof in the confidence department."

But it's hard to project that confidence when you know your job is at stake. Ali joined SITraN on a three-year, fixed-term contract and must pass her probation to convert it to an open-ended job. The main way to pass is to win a grant that can support the lab, and she fears she'll be let go if she doesn't get a cash infusion.

At lunch, Dan had suggested cocktails, but they'd decided to hold off until the evening. "He's probably more upset than I am," Ali says. "His phrase is that we win together and we lose together, and I think we get so tied up with wanting each other to succeed."

The couple had lost together before, in 2017, when Dan applied for a Sir Henry Dale Fellowship in a previous round and didn't get it. Dan is reworking his presentation for a second application this year, hoping that he can convince the judges to fund his work on a molecular mystery.

The enhancers that he studies reside in poorly understood regions of the genome that do not serve as templates for proteins, but instead encode snippets of RNA called enhancer RNAs. These are molecular switchboard operators, controlling whether genes are turned on or off at just the right time and place.

When the switchboard goes awry, disease can follow. Errant enhancers have been linked to conditions such as cancer and neurodegenerative diseases. This means that these RNAs could provide an attractive drug target — but to design such drugs, researchers need to know how enhancer RNAs do their job.

During his postdoc, Dan showed that some enhancer RNAs bind to a protein called CBP

that turns genes on by loosening tightly packaged regions of DNA. Now, he wants to use that as a jumping-off point to pick apart how those enhancer RNAs work. A key experiment will be to use Dan's favourite technique — cryo-electron microscopy (cryo-EM) — to see how one particular enhancer RNA changes the structure of CBP when it binds to it. He has been hooked on cryo-EM since graduate school. "I was incredibly taken with the fact that you could look down the microscope and see individual molecules of RNA polymerase in front of you," he says. "It's stuff like that that really stirs your excitement in science."

But to get to that point, there is a slew of molecular biology to be done. Dan and his team must first purify the CBP protein away from all other proteins and cellular gunk, then find the right conditions to coax it to bind to its enhancer RNAs outside the cell. All of that has to happen before Dan even touches the electron microscope and starts collecting data.

These are the crucial steps that molecular biologists sweat over — each represents an opportunity for a project to get hung up for months. The painstaking work to optimize lab conditions and troubleshoot failed experiments rarely makes it into a scientific publication, but it underlies every discovery.

It is also not captivating conversation for a three-year-old.

"Ada shouts at us now," says Ali. "If she's bored by the conversation, like if it's too much work-based, she's like, 'What are you talking about? Stop talking.'"

"Which is a very important perspective," says Dan.

"So we have an extra person to ensure that the work–life balance is enforced at the other end," adds Ali.

It's not just for Ada's benefit that Ali and Dan avoid talking shop. "Scientifically, we're pretty distant," says Dan. "We've tried quite hard to maintain that distance so we don't end up talking about science all the time at home."

Both rely on high-resolution microscopy to peer into the molecular world. But that is where the similarity of their work ends. They both claim to lack the deep background to fully grasp what the other is doing.

"The funny thing is, although I'm very familiar with Dan's work, I have no context for it," says Ali. "I keep having to ask what enhancers are."

"Yeah, which probably means I'm not giving you a very good answer," Dan says.

"What are enhancers again?" Ali laughs, half-serious.

"Outside the realm of the basic techniques, there's a lot of stuff I really don't understand about what she does," says Dan.

They might not sell their science well to each other, but both must think hard about how best to market their work to the grant panel. Around 200 new PIs around the country submit a full application to the Sir Henry Dale scheme each year, and only about 40 will be offered a fellowship. For Dan and Ali, their best shot at keeping their fledgling labs open is to win two of those places.

Team science

On an overcast morning in October 2018, Ali crosses a busy road clutching an ice box of test tubes. She's been carrying this precious cargo once a week to the lab of her chief collaborator, biophysicist Tim Craggs, in the University of Sheffield's physics department.

Their collaboration came about because of a chance encounter before Dan and Ali had even started at Sheffield. On a visit to campus, Dan met Tim and learnt that he had built a special kind of microscope for imaging single molecules. Ali immediately saw the potential for her own work.

"I'm like, 'Yes. That's what I need,'" she remembers.

So now she is making the ten-minute walk through the city at a careful pace to take her samples to Tim's microscope. She is trying to develop a way to study, one by one, the proteins that could form the foundation of her career as a PI. In the depths of the physics building, Ali enters a dimly lit room where a stainless-steel table is kitted out with lasers and lenses. "My son calls it an air-hockey table," says Tim, gesturing at the experimental set-up. It's essentially a way to spy on the shapes of kinesin proteins.

Kinesins are shaped like long sticks that have a hinge in the middle and two feet at one end. They are too small to see directly. But Ali has spent weeks creating kinesin proteins adorned with fluorescent tags at each end. The scientists shoot laser light through a diluted drop of cellular fluid from Ali's test tubes. When an individual kinesin molecule drifts under the laser, its fluorescent tags absorb energy and light up. The frequency of the fluorescence shows whether the motor protein is folded tightly at the hinge or stretched open into its active form. "The actual experiment — don't tell anyone — is incredibly easy," says Tim. The harder job, he says, is Ali's: preparing high-quality protein samples with the right kinds of tags inside cells.

The technique is called fluorescence resonance energy transfer (FRET) microscopy and, much to Ali's delight, it seems to be working. She hopes to use it to see how single molecules of kinesin change shape when they grab cellular cargo, and then to ask what conditions inside a cell affect that process. This could be the key advance that answers Ali's burning questions about how cargo travels down neurons at different speeds and what causes this process to go wrong. But it will take months, if not years, to progress from a "quick and dirty" experiment to a repeatable, high-quality protocol, Ali says.

For Ali, the collaboration with Tim and his team, which has been running for about a year, is crucial. It means she has access to a microscope despite having little cash of her own, and other people to bounce ideas off. "When you're starting out, you're a team of one. That's quite a lonely place to be," she says.

Now, 18 months on, Ali's lab is a team of three. She's taken on a PhD student, Evie Smith, who will work full time on the FRET experiment. A final-year undergraduate student, Ashleigh Davey, has also joined the group to work on a 'neuron-on-a-chip' project, which will enable the team to watch motor proteins move in cells. Ali has managed to negotiate a move to a larger lab space. But money is still incredibly

Ali carries samples to the university's physics department.

tight. Evie is funded by a research grant for PhD students, but if Ashleigh is to stay for a PhD, Ali will need to get a grant to support her.

Dan's team has also grown. Several short-term students have come and gone, but PhD student Petra Celadova is a central figure. Petra joined in November 2017 with years of lab experience already under her belt, and quickly took ownership of her own project even as she helped Dan to set up the lab.

One of her first steps will be to use the gene-editing technology CRISPR–Cas9 to mutate an enhancer in leukaemia cells, looking for those mutations that impair the enhancer RNA's function. Less than a year into her project, Dan says, Petra is already independent. "She will tell you what to do, which I think is a great sign in a student," he says. "I take a lot of pleasure when she is like 'no, this is how we should be doing this'."

Petra is also wise to the harsh realities of lab work: she hopes to have her cell lines ready by the end of the year, but she knows that research will not necessarily bend to her timetable. "In science, you always think you will have done more than you actually can do in a year," she says.

Second shot

It's the eve of Dan's interview for the prestigious Sir Henry Dale Fellowship, which he first applied for in 2017 but didn't get. It's worth five years of lab funding that he desperately needs. He arrives at a hotel near King's Cross Station in London just in time to hold a goodnight video call with Ada.

The next morning, 19 October, Dan wakes up and has an extra-large breakfast to keep his stomach full and his mood positive. As Ada sends him a string of incoherent emoji texts, Dan runs through his practice talk. The interview will take place at the Royal Society's headquarters near Trafalgar Square, and will consist of a five-minute presentation followed by questions. "It's quite a lot to get in five minutes," Dan says. "So I've drunk lots of coffee."

With a bag full of snacks and his nerves jangling, Dan arrives too early at the Royal Society. He strolls through nearby St James's Park, pausing to watch a group of schoolchildren chase the geese. Then it's back to the Royal Society, where he sits in the lobby, has some water and tries to gather his thoughts.

There is cause for optimism: the reviews of his proposal were generally positive. He feels confident in the experiments he has proposed. And, in a way, he welcomes the interview experience, however agonizing it might be. The ability to stand up and defend your work is a great skill to have, he thinks. "Do I like it? No. I'm scared," he says that morning before he leaves for the interview. "But I can see the value of it."

The panel members call him in after their lunch break, and he walks into a room with about 20 people seated in a horseshoe shape. Only two or three seem to be leading the discussion; Dan focuses his eye contact on them. Some of the questions are surprisingly technical, but nothing catches him flat-footed. The 30-minute interview seems like only 10. "I felt like I gave a good impression of myself, and I think that's all I can do here," he says. "Other than that, I feel kind of tired."

Once out of the Royal Society building, his mind returns immediately to his omnipresent to-do list: exam questions to write and all the other things he has pushed to the side while preparing. But first he is going to meet university friends for drinks in a few hours.

He calls Ali, who has spent the day distracting herself at the microscope, and Dan remembers his own anxiety during her interview for the same fellowship four months earlier. He wonders whether the family might go for a walk in the Peak District, a scenic national park near Sheffield, over the weekend to celebrate his new-found liberation from interview prep. "Ada has been bouncing off the walls," he says. "I wasn't a very diligent parent for a couple of weeks. I think we might unleash her on some sheep."

Personal best

On 1 November 2018, Dan is on a train, sweating over the final details of a presentation he will give that afternoon at University College London. He used to think he would never be the sort of person who would still be preparing their talk on the way to give it. But now he's alternating between presentation prep and procrastination as he tries to avoid eye contact with his inbox, where a notification from Wellcome could arrive at any moment. "Waiting for e-mails to come in is very anxiety-inducing," he says.

Temptation eventually gets the better of him and, when he checks, the e-mail is there. "Dear Dr. Bose, I am pleased to inform you …" he reads. Dan's eyes jump forward to the word "successful" and then he looks away. He glances back and revisits the same words to be sure he hasn't misread them. "And then I sent it to Ali to double-check and probably read it again," he recalled later. "But I never made it beyond the first two lines."

Dan forwards the e-mail to collaborators, his department and people who'd helped with the application. He calls Ali. "We do all of this together. You ride the ups and the downs and the pain and the rejections and all of the stuff you do at the same time," he says. "So it's just lovely to be able to share that kind of happiness and release with her." After a few minutes, the train goes through a tunnel and cuts the call short.

The fellowship will give his lab £1.34 million over 5 years, half of which will be soaked up by salaries for the people who work there. But there will be some money for new equipment. "I've been promising Petra here for the last year, all the things we're going to buy when we're rich," says Dan. He thinks about completing her set of micropipettes, buying extra time on the electron microscope and purchasing a machine for fast protein liquid chromatography (FPLC) that purifies proteins. Instead of spending time trying to find ways to stretch his funds to get particular experiments done, the lab could just do them, he thinks. "It just enables everything," he says. "It makes such a big difference to everything we want to do."

The grant also buys Dan time. It funds him for research, guaranteeing some set-aside periods free from the demands of university

An icebox in Ali's lab.

Dan waiting for his fellowship interview in central London.

teaching and committees. Many grants run for only three years; the five-year length of his would allow Dan and Ali to feel more settled. After years of juggling careers, coping with uncertainty and separation, they could look for a school for Ada with the knowledge that she would probably spend much of her primary-school education there. "It's the first time we've been able to say, 'This is where we're going to be in five years,'" says Dan. "And that is just making a big difference to everything."

Would the fellowship affect Dan's salary? He isn't sure. "I haven't really looked at it," he says. "It's really very much secondary."

But for the lab, he has lofty ambitions ready to hand. "Yeah, it's just going to let us take off as a group," he says. "That's what we hope for."

The next day, Dan kneels down in the middle of the corridor leading to his lab, cradling a bottle of champagne.

"Are we ready?" He shuffles slightly on his knee.

Behind him a gaggle of his colleagues monitor his position on the floor. Their chatter turns to whoops as Dan ejects the bottle's cork and watches it ping down the corridor and hit the wall just above a poster about a cancer biomarker.

It's the lab's tradition to fire off a champagne cork for each new grant; Dan's last effort, a small award from the Royal Society that began at Easter, was worth £20,000.

> **"I WASN'T A VERY DILIGENT PARENT FOR A COUPLE OF WEEKS."**

Where the cork hits the wall, his colleague affixes a piece of orange tape with Dan's achievement and the date.

"That's a PB!" someone shouts.

Dan having the security of a fellowship "takes the pressure off a little bit", says Ali. Emotions have been running high as they both digested the news. "Knowing that I didn't have to worry about Dan any more just kind of came over me and I just felt very overwhelmed."

The feeling of relief is fleeting. As 2018 draws to a close, Ali is more than halfway through her three-year probation, and time for her to win a grant is fast running out.

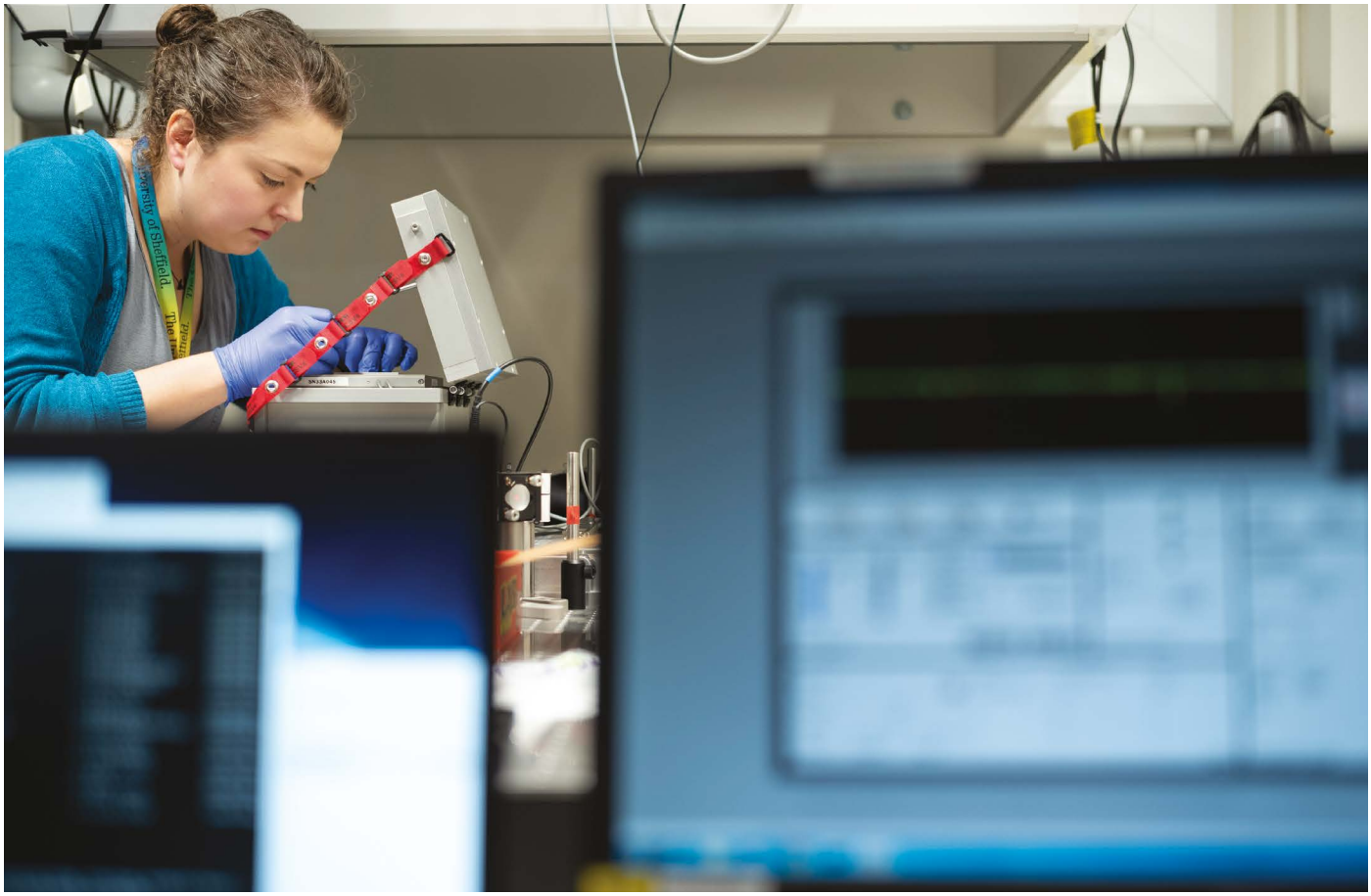
In late December, they put those concerns aside to celebrate Christmas with their labs. Last year, Ali and Dan organized a dinner with another new PI, molecular biologist Emma Thomson, who shares lab space with Dan. There were just five diners, including the three lab heads. This year, Ali says, she and Dan had to move a table, chairs and plates to Emma's house so a dozen people would fit, and "suddenly it was, 'Ah', I have built something.'"

'I can see myself within that data'

In the new year, the family rents a larger, more comfortable house and instantly feels happier, after two years in what Ali calls an "adequate" rental home. Their kitchen now has a dishwasher, the garden has a swing and room for plants — Ali and Dan start growing Tuscan kale — and the walk to work now takes them through Sheffield's floral botanic gardens. Ada's school, which she'll start this year, is only ten minutes away.

After so many years of moving around, the family feels more settled and able to bring out more of the books and other possessions they'd kept in storage. They make resolutions to throw away what they don't need. "We've accumulated three continents' worth of possessions," Dan says.

At work, Ali has spent much of the past few months setting up protocols and tweaking them over and over again until they work. "It seems so glacially slow sometimes," says Ali. "But I feel like that's inevitable, because you're pulled in so many directions at once.



Ali prepares a sample of motor proteins for fluorescence resonance energy transfer (FRET) microscopy.

It takes real effort to find the time to do the stuff that you need to do in lab."

One notable success is that Ali and Emma Thomson have joined together to offer a PhD position split between their labs. Ali's undergraduate student Ashleigh applied for and won that position, and will start in October 2019 using the neuron-on-a-chip that she had developed to grow single neurons in channels and study ribosomes in the axon. "It's super, super exciting," says Ali — but it also puts pressure on her to bring in more research funding.

The Slack messaging group she helped to set up with immunologist Sophie Acton at University College London in 2018 has generated its own workload. Ali and Sophie kicked off a survey of new PIs and their experiences, and by March 2019 they had crunched the data and posted their analysis to the preprint server bioRxiv — since published in the journal *eLife* (S. E. Acton *et al*. *eLife* **8**, e46827; 2019).

Ali has been diverted by some stark results from the survey, which attracted responses from more than 350 PIs who had launched their first UK labs between 2012 and 2018. Women starting their first research labs tended to have a lower salary and receive less university start-up funding than did their male peers, the results show. Female PIs were also less successful at securing further funding in the first five years.

"I can see myself within that data," Ali says. Of the two, Dan got a higher salary and more start-up funding — although, Ali adds, they were hired into different positions in different departments, so aren't directly comparable. "It's difficult to not compare myself to Dan a lot of the time, because he's right there. And that's not particularly healthy," she says. For Ali, the survey made it clear that the gender pay gap applies to new female lab heads as soon as they start work. "When you're appointed, you believe that you're equal to everybody who's appointed at that stage," she says. "Actually, it's not true. We're being appointed at a deficit immediately."

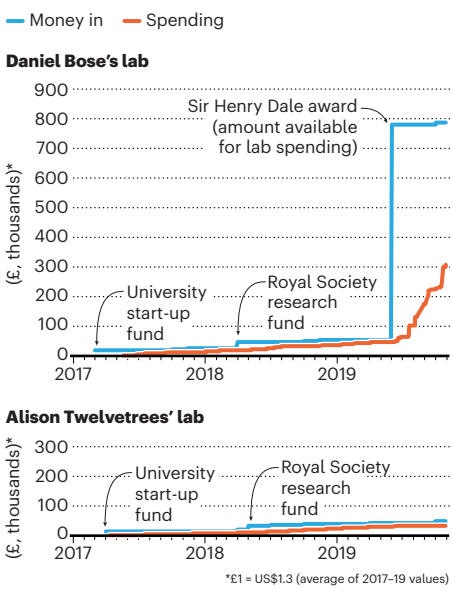
Ali takes immediate action on one of the survey findings: that women without mentors reported the lowest level of optimism about their careers. "I could do with more mentorship," she says. She signs up for a mentorship programme at Sheffield.

Dan is finding ways to progress some key experiments while dealing with a burst of teaching, admin and recruitment. He taught a group of students in the lab to clone and purify some of the proteins he'll need for his cryo-EM experiments. Those students have moved on, but the samples are nearly ready.

He's also interviewing for new PhD students. With the United Kingdom's formal exit from the European Union approaching, he's noticing a drop in applications from Europe. "It's quite sad. You try to build a culturally diverse laboratory and suddenly no one wants to come because of the uncertainty," he says.

CONTRASTING FORTUNES

Whereas Dan's large grant allows him to buy new equipment and reagents, Ali has to watch her spending carefully. (Chart excludes staff salaries.)



*£1 = US$1.3 (average of 2017–19 values)

In late March, Ali decides not to apply for the Sir Henry Dale Fellowship by the rapidly approaching deadline. She is juggling too many priorities to make a strong showing, and is gambling that if she waits until a new round for applications in August, there will be stronger preliminary data from her lab's work to bolster her bid. But it is a big internal struggle to postpone. If Ali doesn't win a grant in the next year, she could be out of a job after her university reviews her three-year record. "That will be a disaster for everybody."

The stress is starting to shake her. "I'm quite up and down. I think I am struggling generally at the moment," she says when we catch up in April. Starting at Sheffield has not been easy, and before that she felt she was still playing catch-up after maternity leave. "It's just constantly felt like an uphill battle to try and have any sort of confidence in what I'm doing, and it's really difficult to kind of be upbeat. Sometimes I just do still feel like I want to quit."

Still, there are jubilant moments. Evie and Ali make their FRET microscope results public for the first time at a conference in Chile in April 2019. "We're really, really excited about what this method can show us in the future about kinesin activation, although we haven't discovered any new biology just quite yet," Ali tells scientists at a small session titled 'The next generation of cytoskeleton researchers'. Dan flies out with Ada to Chile, so that the family can take advantage of the conference to hike in Patagonia — one of Dan's bucket-list destinations.

Rainbow tape

Dan's fellowship begins on 1 June 2019, and he quickly sets about laying purchasing plans. He agonizes over what he should buy first, consulting Ali at length. "I had this quandary: you get this grant, what's the first thing you buy with it?" he says. "It has to be semi-useful but also frivolous. So I got a seven-roll tape dispenser and rainbow tape to put on it." Then he needs to move on to more-practical purchases, such as centrifuges and shaking incubators. The biggest purchase is the FPLC machine for purifying proteins, with a price tag hovering around £65,000 — more than triple Dan's budget when he first opened his lab.

Is he at risk of forgetting his humble beginnings? "Maybe ask me in six months' time, when the gold-plated centrifuge turns up."

But the clock keeps ticking and, a little more than two weeks after his fellowship began, Dan still hasn't been able to bring himself to buy the FPLC machine. "Every time I think about it, it brings me out in some kind of horrible sweat," he says, with a tone that conveys a blend of guilty shopaholic and anxious spendthrift. "It's kind of terrifying, especially when I've been getting by on scavenging the backs of cupboards for a couple of years."

Petra's project seems to be going well: she has engineered the different leukaemia cell lines that she will need, and is on the verge of collecting data. Dan's goal is to get some samples on the cryo-EM over the summer to see how RNAs bind to CBP, which switches genes on and off. Thus far, he has dealt with these molecular complexes only in a test tube. "To actually physically see an interacting RNA and CBP for the first time is going to be super exciting for me," he says.

Ali, meanwhile, is starting to seriously worry about her finances (see 'Contrasting fortunes'). She's managed to convert a master's student at Sheffield, Emma Turner, into a PhD student in her lab, starting in the autumn. For her project, Emma will be engineering mutations into kinesin — the same suite of mutations seen in people with motor neuron disease — to see what effects they have on kinesin walking and unfolding. Her presence will mean that Ali will have doubled the size of her lab team this year, but not increased her income for instruments, reagents and other consumables. "This is my biggest anxiety and stress," she says.

The students get money to cover their tuition fees and living expenses, but they don't always get cash for research materials. Evie does have a support grant of about £5,000 a year, which is largely spent on essential equipment such as pipettes and biochemical reagents. Her project can go ahead only because they are using Tim Craggs's microscope. "I have to be upfront about what we can and can't afford," Ali says. She is eyeing a couple of small

Dan pipettes reagents in his lab.

grants to kickstart and maintain projects for her students.

To top it all, Evie's microscope experiments, which seemed to be going well, have mysteriously stopped working. "It's super frustrating," says Evie. Ali calls it a "summer of anguish".

By the end of October 2019, as Dan is slowly and anxiously spending his grant money, Ali is midway through her second application for the Sir Henry Dale Fellowship. "Feeling unexpectedly excited/proud of it!" she writes in an e-mail after submitting the full pitch. But this is only step two of four; if they like her application, the judges will interview her in March next year. Then it will be another anxious wait to see if she, too, has secured the money she needs for her lab to survive.

Protein universe

In a small, dark room inside the university's biology building, Dan carefully transfers a sample onto a metal grid that will go into a large transmission electron microscope. The microscope takes up half the space in the basement room.

"I always find this the most exciting step," he says. "When you've spent a long time with a protein, you feel how it behaves in a test tube and how it interacts. To stick it under a microscope and see it for the first time is always quite a nice experience."

It's September 2019, and Dan is finally starting the experiment he's long dreamt of doing. For most of the past decade, he has been studying a protein known as CBP, which controls how easily a gene can be switched on. Researchers have glimpsed some of CBP's structure, but they haven't worked out all of it — and they don't know how CBP interacts with an 'enhancer RNA' that Dan's lab is studying.

> "YOU CAN SPEND HOURS FLYING AROUND IN THIS DIFFERENT PROTEIN UNIVERSE."

Now, with a break in his teaching schedule, Dan will try to decipher the RNA–CBP structure.

The microscope hums faintly as, inside it, electrons rain down on the sample. For Dan, it's a nostalgic sound. "I did this for years, before my first postdoc. I used to be regularly falling asleep in a dark room with a hum in the background."

The electron beam shoots through the protein sample, and hits a fluorescent screen below, forming a shadow-like image. Dan patiently moves the beam around the grid, hoping to spot a glimpse of a RNA–CBP complex; the molecules are just 30 nanometres across. He's turned up the zoom to 50,000× magnification. "You can spend hours flying around in this different protein universe," he says.

Eventually, he spots some promising-looking blobs. They seem to be bigger than CBP on its own, suggesting that he has got CBP and RNA binding together. "This is the first time I've seen it. That's pretty cool," he says.

It's just the start of what will be a long series of imaging experiments to construct the 3D details of the complex that Dan is using his £65,000 FPLC machine to purify; Dan aims to recruit a postdoctoral student to do most of the work next year. That will involve preparing more samples for use on this machine and the larger, more sophisticated cryo-EM next door. Not one to show his feelings readily, Dan allows himself a smile. It's hard to hear his soft voice over the hum of the machine. "It feels like quite a big deal. This is a sample we can look at, and to build up the structure side of the lab is pretty exciting. It's a nice victory."

Pack mentality

January 2020 ushers in a wave of productivity and optimism. Both labs are teeming with workers; at the work Christmas dinner a couple of weeks earlier, when they collected their own

Dan studies samples using an electron microscope.

Ali in Sheffield's physics department.

groups together with that of fellow biologist, Emma Thomson, they found themselves catering for more than 20 people, where 2 years ago there were 5.

Dan's students go together to the pub or to the cinema, bring in snacks for their colleagues and share memes on Slack. Dan has been concentrating on kickstarting their projects, and they are becoming increasingly autonomous. "Things move without me in the lab," he says. The result is an atmosphere in which the students feel relaxed, and free to bounce ideas off one another, says master's student Luc Costello-Heaven. "I enjoy coming in and seeing everyone," he says.

The camaraderie helps them endure the pain of inevitable failed experiments. "If you're having a bad day, if your experiment is not working, everyone's always like, 'It's fine. What's the worst that can happen? We just do it again,'" says Katie Gelder, a PhD student who started in the lab in October 2019. PhD student Petra Celadova is still working towards identifying key regions of the enhancer RNAs in leukaemia cells, but her cell lines are misbehaving and she's spent months trying to sort out what is wrong. She's still hoping to salvage the lines after investing so much time in them.

Petra has enjoyed the work, and her role as mentor to the crew of students now crowding the lab. But as she heads into the last year or so of her PhD, she has already decided against setting up a lab of her own some day. Dan's position seems like several jobs rolled into one. "You're supposed to be a lecturer, you're supposed to be leading a lab, you're supposed to be doing research to collect data for your grants," she says. "And you should be doing all of this full time, but obviously you can't."

In the last months of 2019, Ali managed to tick a few big goals off her list. She resubmitted her application, and she finally published a paper she refers to as the 'albatross'. It presented the last tranche of data from her postdoctoral stint, which describe two proteins that team up to help neuronal motors bind to their cargo (A. E. Twelvetrees *et al. J. Cell Sci.* **132**, jcs215822; 2019). And in her lab, work on the motor proteins and their different shapes is proceeding apace after a difficult few months in which the samples weren't behaving.

Just as in Dan's lab, Ali's group is close-knit.

"My lot are crazy busy today," Ali says, when we visit her and Dan in Sheffield at the end of January. "There's a definite pack mentality."

Dan chips in. "It's very pronounced, you've obviously built a cohesive team."

"In a really nice way," says Ali.

"Just mildly aggressive," Dan replies. His own group is not so pack-like. They're collegial, he says, but "they don't have the same air of intimidation. They might go for lunch together."

"I'm kind of encouraging it, to a certain extent," Ali says. "Their confidence is going to feed off each other."

Ali's own confidence got a boost in mid-January when she heard that she'd secured a second interview for the fellowship. But her self-assurance was already quietly growing, and she says that was reflected in her latest application. "Your science comes from your personal creativity. And if you're feeling not very good about yourself, it's difficult to stop that leaching into how you perceive what you're capable of," she says.

The first time around, "I kept that grant within what I was capable of, and they wanted more from me". She says she was afraid to ask such fundamental, unaddressed questions about neuronal cell biology as she has in her latest plan, and to prove that she was the scientist who could answer them. "And now I'm like, yeah, fine. You either think 'I can do it now' or you don't, basically, but I'm pretty confident I can do it."

'Such a stereotypical scientist's nightmare'

The interview is in London on a torrential day in early March 2020. That morning, Ali had woken at 5.30 a.m. from a nightmare in which she had left the lab's −80 °C freezer open. She is disappointed that her sleeping brain wasn't more inventive. "Such a stereotypical scientist's nightmare to have. Such a cliché."

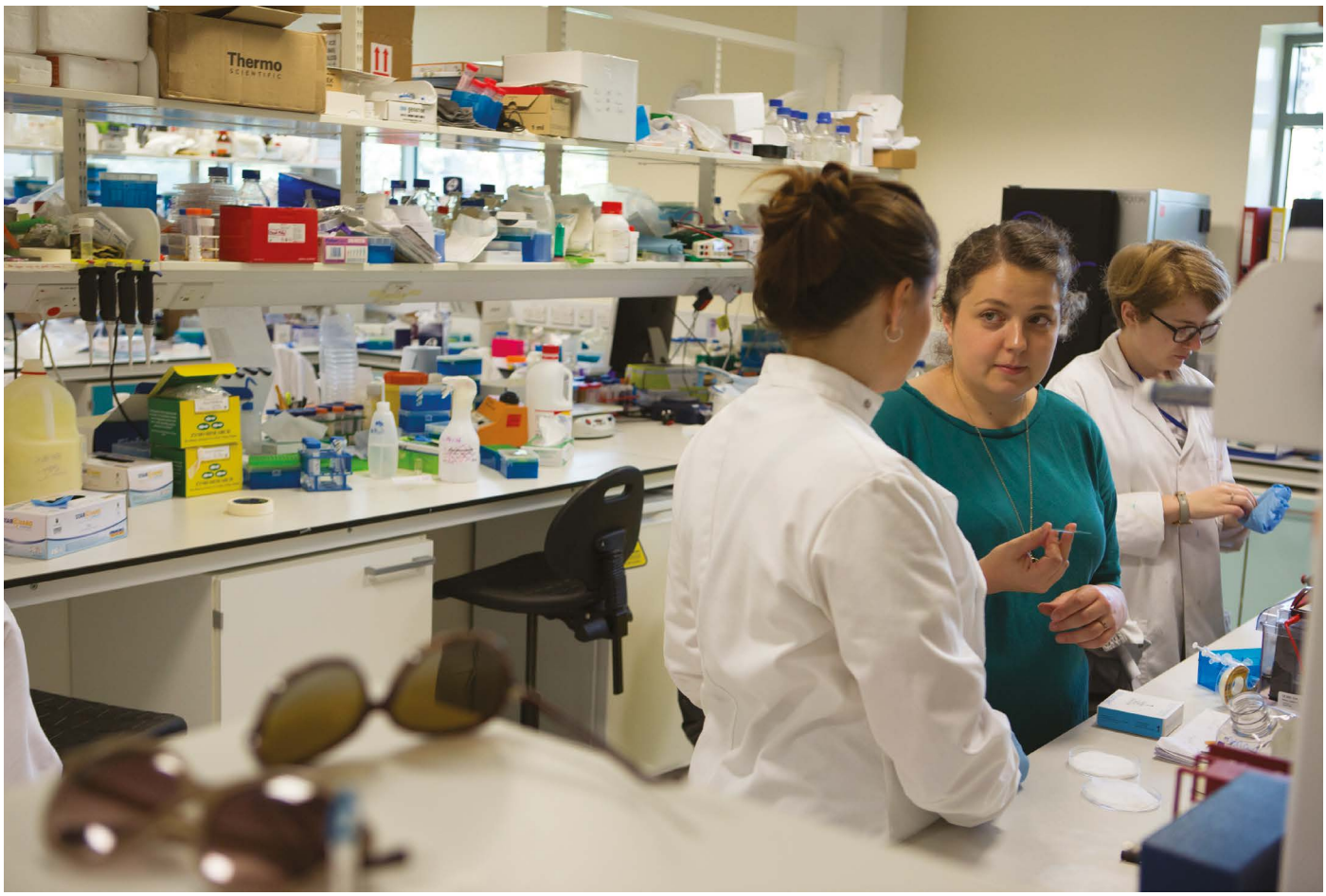
She has memorized her five-minute talk, practised it over and over again and had three mock interviews with colleagues. Her lab members gave her a good-luck card and she is wearing a locket in which there are some strands of Ada's hair and some of Dan's.

She has left her lab in a state of flux. The coronavirus SARS-CoV-2 has established itself in the United Kingdom; COVID-19 cases are ticking up steadily. The university hasn't yet decided on a plan of action, Ali says, but she and Dan are thinking ahead. "Potentially, they're going to shut the university down, which is what they've done in Italy."

For now, she focuses on the task at hand. What's the first line of her presentation? "My lab studies slow axonal transport," she says without hesitation. "Hopefully, once I go in, it'll all just come out."

And mostly it does. After the interview, she is giddy with relief and pretty satisfied with how it went. "I didn't say anything that I regret," she says, her sentence dissolving into high-pitched laughter.

Ali barely has time to reflect on how her interview went before global events intrude. COVID-19 cases continue to rise, and Ali starts talking to her students about the possibility of shutting the lab. "You could see what was happening in Europe," she says. Finally, on 17 March, the day after UK Prime Minister Boris Johnson made a televised address to advise



Ali with her PhD student Evie Smith (right) and Olivia Hill (left), a PhD student in a collaborator's team.

people to stop 'non-essential contact', she shut the lab down. That day, her department e-mailed the same advice. (It would be another week before lockdown began officially.)

"I don't think anybody has begun to grasp how massive an effect this is going to have," she says on a video call the next day.

It's frustrating for Ali to see scientists from large lab groups saying on Twitter that they'll make the best of the enforced break by writing up papers. "If you are sitting on papers like that, you have too much research money," she says, viewing it from the perspective of her own group, in which two PhD students are only half a year into their projects. "It's going to have such a damaging effect."

Ali starts video calls with her team. "I want to make sure they're coping OK." And she's angry that, it seems, universities still expect researchers to be as productive as ever. "This concept that we can continue to work from home while caring for sick people or looking after children. What fantasy land do people live in who can imagine we can do that?"

That day, Dan goes into work to give his lecture to an empty theatre — filmed for the students. "My jokes usually go flat anyway, so no difference there," he says. The lockdown is necessary, but to both Dan and Ali, it means unknown territory for their science, their lab group and their home lives. Ada has had only six months at school — and she knows it's going to close soon. "She's got her red boots on today to keep cheerful," Dan says.

In the afternoon of 25 March, Ali is making a sandwich for Ada when she checks her e-mail on her phone — and sees the grant decision has arrived. The subject line is the same as in her rejection e-mail. But when she opens up the message, the content is different: "Dear Dr. Twelvetrees. I am pleased to confirm that your application was successful." She's won £1.26 million — a grant that saves the lab.

"This is everything I've worked for," she tells us after a raucous call with her lab group. "We can do all of the things that we're excited about for five years. That's the most certainty you ever get in a scientific career. It's like winning the science lottery."

"It's not just the science. It's the security that it brings as well — it's so precious. To have me and Dan both have that now. We've never had that before."

How to run a lab during a pandemic

Two months into England's first lockdown and with schools closed, Ali and Dan have forged a routine: one spends the morning with Ada, the other the afternoon. With Ada they work on phonics, draw birds and grow crystals. At work, they are coping with an endless stream of online meetings, while trying to ensure their students continue to progress — and stay in good mental health. Dan is also teaching, grading papers and holding virtual office hours for undergraduates.

It's impossible to do it all well, says Ali. She struggles to focus with Ada underfoot, even when Dan is on teaching duty. "Her voice, it just shuts my brain down completely," she says.

Both have tried to shift their lab members on to work that can be done at home. Ali ticks through her students' projects on a call: Evie Smith has been developing an automated data-analysis pipeline; Ashleigh Davey and Emma Turner are doing database searches … Ali trails off. "Ada, could you just be quiet for a minute?" she says calmly. Ashleigh and Emma are searching databases to gather data to back up their hypotheses, so that they can return to the lab focused and ready to push their projects forward. Nicola Carruthers, one of Dan's students, has taken a leave of absence to volunteer in a coronavirus testing lab.

"They're so resilient," Ali says of her team. But each student has a strict window of time to complete their degree, and Ali knows that the uncertainty about how the pandemic will affect their education is adding to their lockdown stress. Students in both their labs are

> I DON'T THINK ANYBODY HAS BEGUN TO GRASP HOW MASSIVE AN EFFECT THIS IS GOING TO HAVE."

worried about being unproductive. For Dan and Ali, seeing other scientists brag on social media about attending online conferences and writing grants is frustrating when their major goal is to look after Ada and keep their students afloat remotely.

But there has been good news. Dan has passed his probation and switched from a fixed-term to a more permanent contract with the university. Although the criteria for promotion technically included publications, which Dan has not yet had, he was able to demonstrate that he had made progress towards publishing, and had met the other two criteria of teaching and bringing in funding.

Ali still bristles at mention of the publishing requirement. "Three years is not enough," she says. "I think if you're, you know, a theoretical physicist, right, you don't have to set up a lab or work at the pace at which things grow."

In Ali's case, the criteria are not as clear and the process is not as formal, but she has learnt that her department has shifted her to an open-ended contract and included her in its financial projections, suggesting that her lab will be able to stay.

When the labs reopen in the summer, Dan and Ali's tidy schedule is demolished. Their household routine is continually shifting as their work conditions change. Dan's lab is in a classical university building, with long corridors and closed doors; Ali's is in an open-plan building with shared spaces and a large atrium — their return to the lab is markedly different. Dan is able to bring back his students, and they work almost normally, minding the new social distancing and safety protocols.

For Ali, however, lab work is a logistical nightmare. The students are assigned to shifts, some working in the morning, some in the afternoon. This means that an experiment started in the morning must now be handed over to someone on the afternoon shift to finish. It is a level of collaboration, planning and restricted movement that leaves no margin for error, and progress has slowed as a result.

Some experiments remain off-limits: Ali's lab can no longer access the shared microscopes in other buildings on campus. And perhaps hardest of all is the physical separation, which makes it difficult to recapture the collaborative atmosphere that Ali had so cherished.

"At the start of the year, I felt like we'd finally turned a corner and started to build momentum," she says. "People were motivated, experiments were working. There was an energy."

Ali thinks back to her pre-pandemic lab and struggles to hold back tears. "Mostly I'm crying because I just miss them. For that brief window it was really nice to work with them and support them and see everything coming together."

But she is still moving forwards. At the end of October, Ali sends e-mails to start the release of her grant money, allowing her and the team to begin planning purchases. They buy a couple of small pieces of bench-top equipment to make lab life easier and save them having to move between rooms.

Lockdown has disrupted Dan's plan to recruit a postdoc to run the cryo-EM prong of his work, but in October, he finally puts out a job advert. It's a "big deal" to start this work, to be able to look at the 3D structure of the RNA–CBP complex and see how it is organized and how it behaves. The rest of the lab has weathered the storm relatively well — Dan keeps on top of projects through Slack and a morning video meet-up, and the students support each other when he isn't in the lab. He is delighted that they decided on their own to rearrange and organize the lab fridge, even if some of his old stuff got thrown out.

Ali's PhD student, Ashleigh Davey, prepares tissue cultures.

The lab seems to have changed gear. In March and December, Petra and Dan's names appear on papers for work led by other researchers to which they contributed some data and experiments. A new PhD student has just joined. And Petra — his first-ever PhD student — is starting to think about writing up her project and could soon be moving on.

'It'll be fine. Eventually.'

On 3 December 2020, Dan is wrapping up work when Ali texts him: "Ugh miserable weather. Are you late home again?" Dan replies that he plans to leave around 6 p.m.. But he never shows up.

What happened that evening is still a bit fuzzy, even now, so many months later. Dan was cycling home along his usual route. But he either fell off his bike or dismounted as he passed a children's hospital. A team of doctors walking out of the hospital found him having a seizure and diagnosed him on the spot: a brain haemorrhage.

An ambulance arrived quickly and took Dan to the university hospital, where doctors placed him in a coma. "They thought he wasn't going to make it," says Ali. "His condition going into the hospital was so bad, and that's correlated with how well you recover."

Because of pandemic restrictions on hospital visits, Ali was allowed to see Dan for only a short visit before he was transferred to the neurology department. "My department," Ali says with a sharp laugh. "But I wasn't allowed to cross the threshold into the ward."

> "PERSISTENT CAREER INSECURITY OVER MANY MANY YEARS LEAVES ANXIETY SCARS."

Dan was in a coma for 24 hours. After that, he gradually spent more and more time awake each day. Ada sent him lots of messages and pictures, practising her handwriting. Dan chatted with Ali by phone, but only after she had ensured he couldn't use most of the apps. "She disabled everything except FaceTime," says Dan. "So that I couldn't check work e-mails." While Dan made optimistic plans for his return to work, Ali was haunted by the statistics: only about half of people with Dan's condition survive long enough to leave hospital, and even then they face varying degrees of disability.

But Dan was one of the lucky ones: he went home on 22 December. His haemorrhage had been caused by a weak blood vessel in his brain, called an aneurysm, which had burst. Dan had had a headache for weeks before the rupture, but had dismissed it as being the result of too much coffee, or stress, or screen time.

His medical team scanned his brain to find out if he was at risk of a repeat haemorrhage, and found nothing.

"In the doctor's words: my brain is 'pristine'," he says with a mischievous smile.

"Dan brings that one out on a regular basis," says Ali.

After the holidays, another COVID-19 lockdown closed the schools, so Ada was at home, too, wanting to check in on Dan when he was trying to rest. "She would tiptoe up," says Ali. "But she wasn't very quiet."

Ali unloads new lab equipment.

Dan with PhD student Nicola Carruthers.

While Dan recovered, Ali and Emma worked to keep his job responsibilities at bay. Colleagues in the department took over his teaching, and Emma helped to supervise his team's day-to-day work. Ali kept a close eye on Dan to make sure he gave himself the time he needed to recover.

"He was planning all of these things, and I was like, 'you know you can't walk?'," says Ali. "He would say, 'It'll be fine. Eventually.'"

"Medication is a powerful thing," says Dan.

Gradually, Dan was able to do more and more. He checked in on his students over video calls, and selected a postdoc from applications he had received just before his accident. He interviewed candidates in February, and selected a new recruit to start in May.

As Dan recovered, Ali was able to turn back to her own work, pushing her own students' projects forwards and making some changes to their shift patterns to enable a bit more overlap and make everyone happier.

By the summer, she had begun making plans to spend her grant money: she had finally found a place in her institute for the expensive fluorescence microscope — an act of diplomacy more than design — and begun the "miles of paperwork" its purchase would require (see 'Finally funded'). The total cost would ultimately come to £272,906. "Sort of weird to think it costs the same as a house," Ali says.

Meanwhile, using the FRET microscope, her team had made some intriguing discoveries about their motor proteins: they seem to exist in more than just 'on' and 'off' states, which could have implications for how they do their jobs carrying cargo in neurons. "Still planning that slightly elusive, but hopefully closer, publication," she says.

Funding remained a nightmare — literally. One weekend in June, Ali woke up in the middle of the night after an oppressive dream about losing her job. "Persistent career insecurity over many many years leaves anxiety scars," she tweeted.

A few months earlier, she had asked fellow new PIs on Twitter how many times they'd thought about quitting academic science in the past year. More than one-third said they'd done so at least every week.

In June, about six months after his haemorrhage, Dan still got tired doing tasks that were routine before, and one of his feet would go numb if he forgot to do his physiotherapy. He worried about how he would deal with large crowds once the lecture theatres were open again.

But he was improving quickly, and both he and Ali were thinking about the future and the never-ending quest for research funding. "It feels like there's this sort of balance between doing work that's related to the fellowship and then doing work with half an eye down the road to where the next fellowship or the next bit of funding is going to come from," Dan said.

It has now been more than four years since Ali and Dan arrived in Sheffield. If she could give advice to her younger self, Ali says, she would have suggested nurturing more faith in her own abilities. "I can see how little confidence I had in myself and my ideas when I started, and how paralysing that was in terms of being able to write anything that someone was going to fund," she says.

Dan says that, at the beginning, he beat himself up about all the things he wasn't getting done. But, in retrospect, he can see that work in his lab was progressing — just more slowly than he'd hoped. "First off — know that slow is OK," says Dan. "I had expectations of just carrying on at postdoc speeds, and, actually, that's not healthy. And it wasn't possible."

In September, the UK school year starts. Ali and Dan are searching for their routine: they alternate week by week who picks Ada up after school so that they can each count on some evenings to work late.

Dan is once again the first one in the household to wake up each morning, which Ali finds comforting. "The weirdest thing for me after the aneurysm was how much he slept," she says.

Although he will be returning to teaching in a few weeks, Dan keeps his early mornings sacrosanct. He gets up, feeds the cats, brings a sleeping Ali some tea and sits reading a book until Ali or Ada wakes up. He tries to avoid checking e-mails.

He now tries to make sure that he has time set aside each day to think about his science, and he guards his free time much better. "It's not normal to wake up in hospital after that and your first instinct is 'I've got to get back to work because I've got teaching in a few weeks'," says Dan.

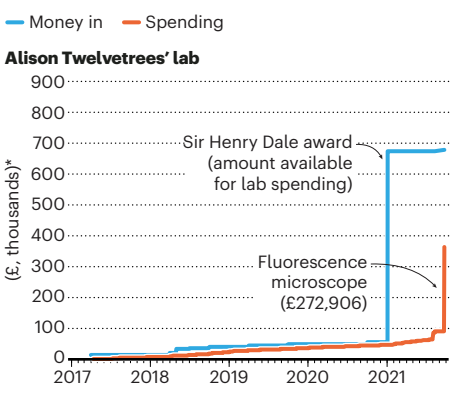
Ever since his stint in the hospital, he can separate from work more easily. "I will often turn off the phone over the weekend and play with Ada for longer," he says. "I feel much lighter now."

Kerri Smith and **Richard Van Noorden** are features editors for *Nature* in London. **Heidi Ledford** is a senior reporter in London.

Additional reporting by Benjamin Thompson.

FINALLY FUNDED

With her grant secured, Ali can at last order the microscope of her dreams. (Chart excludes staff salaries.)



*£1 = US$1.3 (average of 2017–19 values)