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A QUESTION OF SURVIVAL

International collaboration and a can-do spirit have allowed some Russian scientists to flourish. **Alison Abbott** watches an extraordinary field test for mutant mice in the Russian wilderness.

As she watched Mikhail Gorbachev's resignation speech on Christmas day 1991, Inga Poletaeva, a Russian behavioural geneticist, was in Switzerland, on her first visit to the West. For her, as for most Russians, the fall of the Soviet Union seemed inevitable. Unfortunately, the same fate also seemed imminent for her research. Poletaeva was a guest of Hans-Peter Lipp, a Zurich University neuroscientist to whom she had sent a forlorn letter a year earlier.

Poletaeva was looking to forge a collaboration, applying Lipp's neurobiological methods to strains of mice bred at Moscow State University. Even with perestroika in full swing, science funding had collapsed and prospects were grim. Poletaeva's department didn't have enough money to feed its mice, let alone experiment on them.

During that short visit, she and Lipp became firm friends. Talking science into the night, they laid plans for a project — correlating the size and distribution of one sort of brain cell, the mossy fibres of the hippocampus, with the behaviour of her mice. As conversation drifted on, Poletaeva mentioned her friend Valentin Pazhetnov, who had set up an outpost at a place called Bubonizi, deep in the Russian Tver forest, for raising and releasing abandoned baby bears. Her words, she recalls, "were like a trigger".

Lipp had a problem with the direction behavioural genetics was taking. The 1980s revolution in genetic engineering had made it easy to produce mutant mice — by targeting a single gene and knocking it out of the genome, for example, or transferring a new one in. New mice were being created, and they would emerge from the standard battery of lab-based behavioural tests with labels such as 'smarter', 'anxious', 'depressed' or 'psychotic'. But did these laboratory measurements have any real-life meaning, he wondered? Bubonizi sounded like just the sort of place to answer this question.

Lipp, still restless today at 60, says he saw the opportunity to test mice in wild conditions in Russia, something that would never fly in his home. "I would never embark on anything that was against Swiss laws or European Union rules — or Russian rules," says Lipp. But he recognized how time-consuming and exhausting it would be to try and set up such a station in Switzerland, where popular movements against genetic engineering regularly delayed research plans.

The outpost

Poletaeva took Lipp to visit Pazhetnov that summer, the only time of year such trips are easy. Bubonizi is about 500 kilometres northwest of Moscow — an 8-hour drive on the narrow, pot-holed road to Riga, where the only signs of civilization are the occasional road-side stalls where peasants sell berries, honey and wood-smoked river fish.

Arriving at the outpost, and with Poletaeva acting as interpreter, Lipp lost no time in arranging for Pazhetnov and his family to organize the building of the first animal house there. He put US$500 of his own cash on the table to cover the costs. It was a risk. "I couldn't know whether my money would evaporate into vodka or anything else," he says.

When he returned the next year, Lipp was pleased to find a splendid building, constructed from local logs. Over the next couple of years, other facilities were added. Pazhetnov's people built a log cabin field laboratory where mouse brains can be analysed histologically to determine neurobiological differences between mice coping well or badly with the great outdoors. Upstairs they installed simple living quarters. Old furniture, discarded lab equipment and computers brought in from the West were supplemented with whatever could be found locally. An old vodka still, for example, was resurrected to distil water.

The facility's centrepiece is eight pens the size of basketball courts that spread out over a large clearing. Looking somewhat akin to overgrown swimming pools, they are fortresses designed to mimic life on the wild side, while still keeping things monitored, controlled and secure. Their metre-tall concrete walls are dug deep into the ground to prevent the mice from tunnelling out and forest animals from tunnelling in.

Electric fences atop the walls stave off larger animals and in the unlikely event that mice escape, they would have a long way to travel to reach the kind of human settlements in which they could thrive or multiply. The pens are equipped with small wooden shelters where the mice nest. The environment closely mimics the real world of wild mice except for one thing — they don't have to forage for food. It is provided for them at covered plates set down at strategic distances from the shelter and fitted with sensors to detect the individually coded transponders inserted under the skin of the mice. Narrow wooden covers run between the shelters and the plates. Without them, owls would easily pick off exposed subjects on their way to dinner.

By changing the timing and placement of food, scientists can examine, for example, how nervous or curious mice are, or how good they are at remembering, or predicting, where the food is going to be. When a transponder no longer shows up at any of the plates, it's assumed that the mouse has either died or become a meal for an owl.

Over the years, Lipp has run more than a dozen experiments with Poletaeva — whose research group, she says, was saved by Lipp — and other collaborators around the globe. Short experiments can be run to follow behaviours in the pen, and longer experiments, sometimes lasting years, can look at the changing genetics in a mixed population of mutant and wild-type mice.

"It may be that the most important thing for life in the wild is not so much smart brains, as respect for danger." — Hans-Peter Lipp

Different in the real world?

One of Lipp's first long-term experiments was on mice that were not mutant, but bred. He began by looking at mossy fibres in the hippocampus, a brain area associated with learning and memory. Projections from these fibres reach up from one part of the hippocampus to form synapses with neurons in another part. Many studies in different labs had shown that the length of these projections correlate positively with learning performance in very specific lab-based tests, such as different types of mazes. "I wondered if natural selection would favour the longer mossy fibres," says Lipp, "or if those very specific lab tests were misleading in terms of what really matters in the 'real world.'"

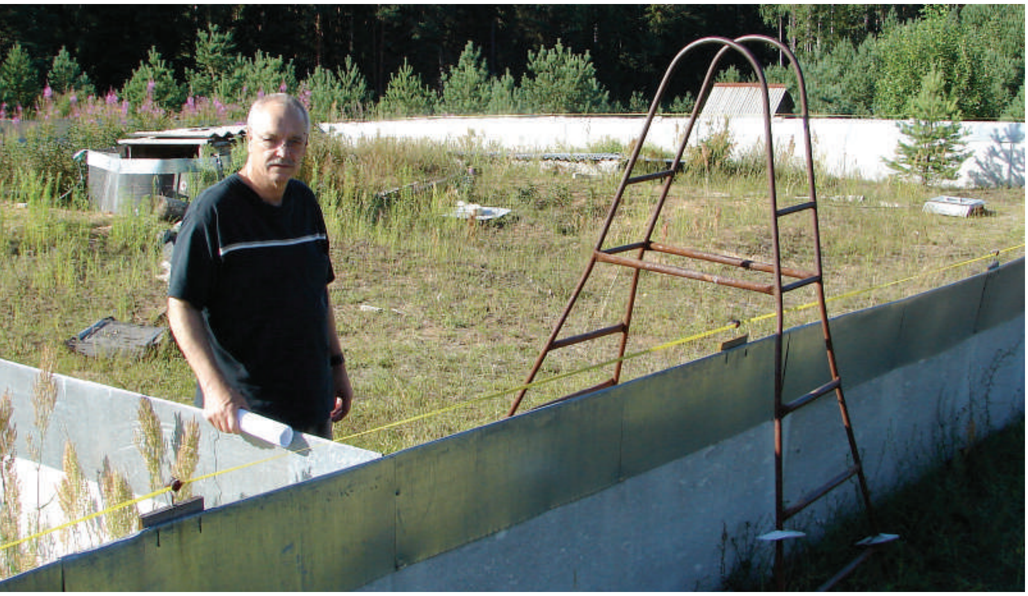
So Lipp and Poletaeva bred four strains by crossing mice that had long hippocampal mossy fibres with those that had short fibres. They divided the offspring into three independent 'founder' populations in which each mouse inherited one gene for long fibres and one for short fibres. One founder population remained in the lab, breeding randomly. The other two made their way down the bumpy road to Bubonizi where Lipp placed them in separate outdoor pens. There they bred for more than four years.

With each generation change, around three per year, Lipp and his postdocs caught the mice, selected a random sample for brain histology and released the others. Members of Pazhetnov's family helped look after the experiments when the scientists dispersed for the harsh winters.

During the fifth winter, the population in both pens mysteriously crashed, a phenomenon that Lipp says has played out again and again in long-term experiments. Nevertheless, with 12 generations' worth of data still being analysed, the emerging trends were disconcerting. Ongoing analysis seemed to suggest that natural selection in the 'wild' was weeding out the long-mossy-fibred animals, not the short-fibred ones. The control founder population breeding in the lab in Moscow had not changed.

The trend was visible within a year. When Lipp put mice from each pen through the normal routine of laboratory tests, they seemed to be more timid and less inclined to explore than the control, lab-bred mice. Nevertheless, they performed about as well in learning and memory tests. When the experiment was repeated in fully covered outdoor pens, safe from predators, mossy-fibre length did not change through the generations. Lipp says he thinks that earlier studies on learning and mossy-fibre length may have underestimated the impact of this trait on exploratory behaviour. "It may be that the most important thing for life in the wild is not so much smart brains, as respect for danger," says Lipp. "Maybe the mice with longer fibres were being picked off by owls because they were less afraid to leave familiar, sheltered areas." If the complete analysis of his data confirms the trends, he says, it implies a shift to defensive behaviours.

Mutant 'smart' mice may make interesting test cases. In 2002 behavioural geneticist Shigeyoshi Itohara's group from RIKEN Brain Science Institute in Saitama, Japan, engineered mice to lack the *S100b* gene. Previous studies had shown that overexpression of the gene limited a neuronal process called long-term potentiation, which has been correlated to learning and memory. Itohara's knockout mice have enhanced long-term potentiation in the hippocampus



A. ABBOTT

Hans-Peter Lipp in a pen that allows him to monitor mice in a close-to-wild environment.

and perform well in spatial memory and fear conditioning tests (H. Nishiyama, T. Knopfel, S. Endo and S. Itohara. *Proc. Natl Acad. Sci. USA* **99,** 4037–4042; 2002).

Similarly a group led by Hee-Sup Shin, a behavioural geneticist from the Korea Institute of Science and Technology in Seoul, has bred mice that lack a neuronal membrane protein called NCX that controls levels of calcium in neurons and thus their electrical activity (D. Jeon *et al. Neuron* **38,** 965–976; 2003). Both types of mice perform better in laboratory maze tests, learning their way around faster than their wildtype counterparts. They are potentially very interesting tools for the learning and memory community.

More brains than sense

Lipp says he thinks that in the wild most smart mice will be more active, and that activity will be their undoing. Indeed, a short-term study over a few weeks this summer, which challenged the intelligence of the Japanese mutant by changing positions of eight food plates, seemed to suggest that they were more active than the wildtype in seeking out the plates, but not very precise at remembering where they all were.

Long-term studies on the Korean group's mice will commence next year, and whereas Lipp sees a foregone conclusion, Shin says the potential is clear. "My NCX mice learn quicker how to find their way to the submerged platform in a water maze and other tests of spatial learning and memory," he says, "so they are smart in the way that London taxi drivers are — but would that smartness help them survive in the real world?" Only experiments at Bubonizi can help, he says.

In addition to his mice currently in the field, Itohara wants to test mice that seem in lab tests to be unaffected by their mutations. "Maybe there are subtle phenotypes which we don't see in our battery of tests looking for very specific deficits — which is after all a very limited way to understand mouse brain function."

But Bubonizi has its own limitations. "The only questions we try to answer here are to do with biological fitness and learning — does the genetic change have any relevance for real life?" says Lipp. Although the set-up is in some ways contrived, even coming close to 'real life' means contending with nature's unpredictable whims. Aside from the mysterious crashing of mouse populations in the pens after four or five years, uninvited guests have beaten the tight

A. ABBOTT

H.-P. LIPP

In the woods: Russian scientists work inside the isolated field station (left).

security. One particularly brutal winter, a snow drift breached a pen wall allowing a mink to climb in and eat all the mice.

Logistics are a problem as well. It's a long and rough journey from Moscow — and it's also a bureaucratic nightmare to bring mice into the country from abroad. Getting all the right forms has delayed some experiments for up to a year. That's not to say things never work: Lipp was amused to receive a consignment of Itohara's mice from a world courier service that drove right up to the Bubonizi log huts. "Really just like the television advertisements where the courier shows up at the most unlikely place at the end of the Earth saying 'we deliver anywhere!'"

Time running out

This is a far cry from the early days of the station when Poletaeva insisted Lipp was always accompanied on the drive from Zurich for fear of the Russian mafia. He would load a two-tonne truck with all the necessary items, lab equipment, chemicals, Italian wine and of course Swiss chocolate. He got used to the long drives but never to border checks, which he says could last from hours to days, often requiring a bit of cash to ease passage.

The trip remains impossibly tiring. Serge Daan, a behavioural biologist from the University of Groningen in the Netherlands, is looking at circadian rhythms using a popular mutant that is missing a fundamental clock gene, *Per2*. He has visited Bubonizi three times, and enthuses that the people are "so helpful and reliable". Regardless, he won't be returning. Together with postdoc Kamiel Spoelstra he is setting up a similar station a 15-minute drive from Princeton University in New Jersey where he has teamed up with Martin Wikelski, an expert in telemetry from Princeton. Using this technology, he will be able to monitor the movement of his mice fitted with telemetry chips 24 hours a day. "Our station is very much inspired by Bubonizi," he says. "But the technology system is better, and the infrastructure for scientific back-up is more secure." And most of all, it is easier to get to. "There is a limit to how many times you can make that forbidding trip."

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— Hans-Peter Lipp

According to Swiss regulations, Lipp will have to retire from his university in five years' time and the fate of the station thereafter is uncertain. Its log housing won't last without extensive repair work for which he is unlikely to be able to raise money. "Maybe someone will take it over — or maybe I'll use it as a holiday dacha," he says, using the Russian word for country home. Otherwise it will be abandoned to the forest and Pazhetnov's bears. But when contemplating retirement and Bubonizi's end of life, Lipp is already thinking about the experiments still to do. One burning wish: testing mutant mice that have shown remarkable longevity. "I wonder if there will be a biological price to pay in the real world," he says. ■

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