

THE ACCIDENTAL EPIGENETICIST

BY STUDYING DISADVANTAGED CHILDREN, RICHARD TREMBLAY HAS TRACED THE ROOTS OF CHRONIC AGGRESSIVE BEHAVIOUR BACK AS FAR AS INFANCY. NOW HE HOPES TO GO BACK FURTHER.

BY STEPHEN S. HALL

Hochelaga was the original Iroquoian name for the village that ultimately became Montreal, but it is also the name of a rough-hewn French-Canadian neighbourhood located east of — and a world away from — the cosmopolitan city centre. The district's tidy two- and three-storey brick duplexes, adorned with Montreal's characteristic wrought-iron staircases, predominantly house families that have, because of poverty and lack of education, never quite attained thriving middle-class status.

During the 1980s, public-school officials identified Hochelaga and many other impoverished neighbourhoods in the eastern part of Montreal as places where kindergarten children disproportionately displayed severe behavioural problems, such as physical aggression. The school system asked a young University of Montreal psychologist named Richard Tremblay for help.

"Their parents didn't have a high-school diploma, and many of the mothers had their first child before the age of 20," Tremblay says of the families he began to study, as he walks along Rue Ontario in Hochelaga on a sunny afternoon in September. Those were

the women, he adds, "most at risk of having children who have problems".

Over the past three decades, Hochelaga and similar neighbourhoods have served as living laboratories in the study of the roots of aggression. Since 1984, Tremblay and his collaborators have followed more than 1,000 children from 53 schools in the city from childhood into adulthood. And in 1985, he initiated a groundbreaking experiment in which some families of at-risk children were given support and counselling to help curb bad behaviour. His research overturned ideas about when aggressive behaviour first emerges, and showed that early intervention can deflect children away from adult criminality.

The idea that a nurturing environment provides better outcomes for children hardly qualifies as news, but Tremblay has taken this idea in a provocative direction in the past ten years. He has joined researchers at McGill University in Montreal and the US National Institutes of Health (NIH) in Bethesda, Maryland, to investigate how nurturing or adverse environments might exert their effects at the molecular level, influencing gene expression through a mechanism known as epigenetics. Tremblay's Canadian cohorts are part





ROGER LEMOYNE/REDUX PICTURES/EVINE

of a growing trend for using longitudinal studies, which follow the same individuals over an extended period of time, to look for epigenetic signatures that might affect health and behaviour later in life. Research in this area is still preliminary — and not without its critics — but Tremblay believes that a firm grasp of early epigenetic effects could inform interventions to influence everything from obesity to mental illness.

“There is a body of evidence, from natural experiments and actual experiments, showing that early-life experiences affect long-term outcomes such as crime, health and wages,” says James Heckman, a Nobel-prizewinning economist at the University of Chicago in Illinois who is currently working with Tremblay on an early-intervention study with at-risk pregnant mothers in Dublin. The work of Tremblay and others, he says, “has established a firmer biological basis for how early-life experiences affect these processes”.

SETTING GOALS

Tremblay’s own early life revolved around sport. His father, Wilfrid Tremblay, played Canadian football from 1938 to 1951, and Richard was an accomplished ice-hockey goalie. When Jacques Plante, the Hall of Fame goalie for the Montreal Canadiens, suffered an injury during the Stanley Cup Playoffs in 1961, a team representative called the then-17-year-old Tremblay asking if he could report to the minor league practice rink the next morning. Tremblay, soft-spoken and mild-mannered, allows that he was “invited to join” the most illustrious franchise in Canadian sports, but concluded that he was too small to play at the professional level. He decided to attend college instead.

Tremblay studied physical education at the University of Ottawa. But before his final year, he read a cult novel by J. R. Salamanca called *Lilith* (Simon & Schuster, 1961), about a recreational therapist who falls in love with a young female patient at a psychiatric hospital. To a naive 20-year old, the work sounded fascinating, and when he returned to college that autumn he applied for a job as a recreational therapist at a high-security psychiatric hospital in Joliette, Quebec. He quickly found himself in over his head, working with convicted murderers and violent criminals. But it was during this time that he first started to wonder about the psychology of aggression. “It shows how a novel can change a life,” he says.

The hospital agreed to send him to get a master’s degree in psychology, which he pursued at the University of Montreal. As Tremblay likes to say: “The first thing I did after finishing my master’s degree was to go to jail for three years.” That was the Pinel Institute, a new maximum-security psychiatric hospital in Montreal. Most of the people there, he says, “had killed someone or were dangerous to the point of killing themselves, or others”. Despite

the danger, he found himself going to work on his days off to play sports with the residents. “I loved it,” he recalls.

Then, in 1971, the University of Montreal decided to create a school focused on children with behavioural problems. The university wanted to hire Tremblay, one of the most promising students to come out of its psychology programme, to join the faculty. But he needed a PhD first, so the university paid for his training at the University of London’s Institute of Education.

That turned out to be a defining experience. Tremblay arrived in London with a sheaf of Rorschach blots and a grounding in psychoanalysis, but there he was exposed to the ‘longitudinal’ philosophy of pioneering human-growth biologist James Tanner, child psychiatrist Michael Rutter and others. He came away with a lesson that has informed the rest of his scientific career: the best way to study any aspect of human development is to conduct longitudinal studies. He threw away his Rorschach blots and, in the late 1970s, headed back to Montreal.

AGGRESSIVE START

By then, Tremblay was eager to launch his own longitudinal study. He got his chance in the early 1980s. School officials came to him with the problem of hyperactive, physically aggressive kindergarten boys. He had never worked with children before and never imagined doing so, but he recognized it as an opportunity to explore the origins of aggressive behaviour. “The idea became very clear,” he says. A longitudinal study of kindergarten children would give him a chance to link childhood behaviours with adolescent and adult outcomes.

In 1984, he started tracking boys from dozens of schools. Funding was initially provided for three years, but nearly three decades later Tremblay and his colleagues continue to follow many of the men involved. They have published more than 160 papers on the group.

Just one year in, when the boys were seven years old, Tremblay obtained a grant to add a randomized, controlled experimental intervention. Teams of four psychologists would visit the families of about 50 boys every two weeks. They counselled parents on identifying and correcting aggressive behaviour, and trained teachers to do the same. In addition, they attempted to socialize unruly boys, and they integrated problematic boys with well-behaved children to provide positive peer role models.

The Montreal intervention began at a time known informally among criminologists as the ‘nothing works’ era, when there was widespread pessimism about the potential to rehabilitate juvenile delinquents and adult criminals. Tremblay’s intervention was

labour-intensive and extremely expensive, and he recalls fretting that he was spending millions of dollars on a study but might end up with nothing to show for it. “I guess I lost hope — in working with juvenile delinquents — that we could make a difference,” he says.

The intervention lasted about two years, but the results would take much longer to become apparent. One of the first people to see hints that it was working was Joan McCord, a criminologist at Temple University in Philadelphia, Pennsylvania. McCord had a reputation for ferreting out data that challenged conventional wisdom, most notably when she demonstrated

behaviour in early childhood that continued into their teens. The roots of physical aggression — and, by extrapolation, the origins of violent behaviour later in life — lie before the age of six, says Nagin. That is, before Tremblay’s kindergarten cohort even began.

Even as Nagin and Tremblay were analysing the original Montreal data, Tremblay had begun another longitudinal study designed to look at aggression before kindergarten. It was a birth cohort based in Quebec, and the resulting data suggested that aggressive behaviour was evident at 17 months and peaked at around 42 months⁴. This and later work culminated in Tremblay’s ‘original sin’ hypothesis: that physical aggression is the default setting in human behaviour⁵. It peaks between the ages of two and four, and is usually socialized out of children by the time they enter school (see ‘Aggression regression’). “We took the view that violence, and physical aggression, is a part of us as a species,” says Nagin, “so the issue is not how we learn it, but rather how we learn to control it.”

Many criminologists dismissed the findings. They argued not that the idea was wrong, but that it was irrelevant — that chronic childhood aggression is trivial compared with murder and rape in adulthood, and that the former does not explain the latter. Most still focus primarily on delinquency during adolescence, and for good reason, says sociologist Robert Sampson at Harvard University in Cambridge, Massachusetts. “Early childhood is centrally important, but it’s not determinative, because there are still changes [in behaviour] later on.”

Yet the Montreal and similar longitudinal studies show that heightened physical aggression at a young age correlates with serious antisocial behaviour in adolescence and adulthood, says Tremblay. He is fond of citing the view that Saint Augustine offered some 1,600 years ago: “It is not the infant’s will that is harmless,” he wrote, “but the weakness of infant limbs.”

Yet the Montreal and similar longitudinal studies show that heightened physical aggression at a young age correlates with serious antisocial behaviour in adolescence and adulthood, says Tremblay. He is fond of citing the view that Saint Augustine offered some 1,600 years ago: “It is not the infant’s will that is harmless,” he wrote, “but the weakness of infant limbs.”

MARKING TIME

With Saint Augustine’s headstrong infants in mind, Tremblay increasingly pondered the effects of the environment at earlier and earlier ages. Like many researchers studying behaviour, he had looked into what role genes might have in aggression, but he was dissatisfied. Genetics did not tell the whole story. Tremblay was primed, therefore, to hear about the work of Moshe Szyf, a cancer biologist at McGill, at a small Vancouver meeting in 2004.

Szyf had been tracking the addition and removal of methyl groups to DNA, which can silence or activate genes. Scientists were interested in whether these methylation marks

**THE ISSUE IS NOT HOW
WE LEARN VIOLENCE,
BUT RATHER HOW WE
LEARN TO CONTROL IT.**

in the 1970s that a famous US longitudinal experiment — the Cambridge–Somerville Youth Study, in which juvenile delinquents were mentored and supported — had actually harmed the young men it had aimed to help¹. Conversely, the Montreal intervention seemed to be working as intended. With each follow-up assessment, boys in the intervention arm displayed not only less delinquent behaviour than controls, but also better school performance, lower consumption of drugs and alcohol, and better social skills.

Data gathered 15 years after the intervention ended revealed that it produced persistent positive effects. The boys whose families received support had a 46% graduation rate as opposed to 32% for controls. And, at the age of 24, fewer of them had criminal records: 22%, versus 33% for controls².

But Tremblay wasn’t just seeking ways to mitigate bad behaviour — he was looking to uncover where it began. In the mid-1990s, he began to collaborate with Daniel Nagin, a criminologist at Carnegie Mellon University in Pittsburgh, Pennsylvania. Nagin applied a more sophisticated statistical metric to the burgeoning Montreal data set. The results, published in 1999, made it clear that the trajectory towards antisocial behaviour and criminality in adolescence begins very early in life³. Most children exhibit decreasing aggression between the ages of 6 and 15: they learn to control their aggressive impulses. Only about 4% of the boys displayed highly aggressive

might allow the environment to influence gene expression over an organism's lifetime. Michael Meaney, a developmental neurobiologist also at McGill, collaborated with Szyf to show that newborn rat pups generously licked and groomed by their mothers had different patterns of DNA methylation from those that received less maternal attention⁶. These changes reached the brain, where the methylation pattern altered the activity of a gene that plays a central part in the animal's response to environmental stress. Maternal nurture, Szyf argued, was a form of 'environmental programming' that altered the activity and function of genes in ways that persisted throughout life.

For Tremblay, it was "as if the roof blew off" the room. The McGill experiments suggested a biological explanation for what he had been tracking for 20 years. As he walked to dinner with Szyf that evening, Tremblay pressed for a possible collaboration.

Human studies of this sort were uncharted territory. So Tremblay initiated a parallel line of animal research with Stephen Suomi, who heads the primate laboratory at the NIH's Eunice Kennedy Shriver National Institute of Child Health and Human Development in Bethesda. Both scientists had noted behavioural similarities between the chronically aggressive, hyperactive boys in the Montreal study and a group of aggressive monkeys that Suomi had raised under conditions of early maternal deprivation. Tremblay, Suomi and Szyf set out to run DNA-methylation studies on two sets of monkeys: a group nurtured by their mothers, and another deprived of maternal nurturing from shortly after birth. It took nearly a decade of difficult molecular-biology work headed up by Nadine Provençal at McGill, but in the past year or so, the researchers have begun to publish their findings.

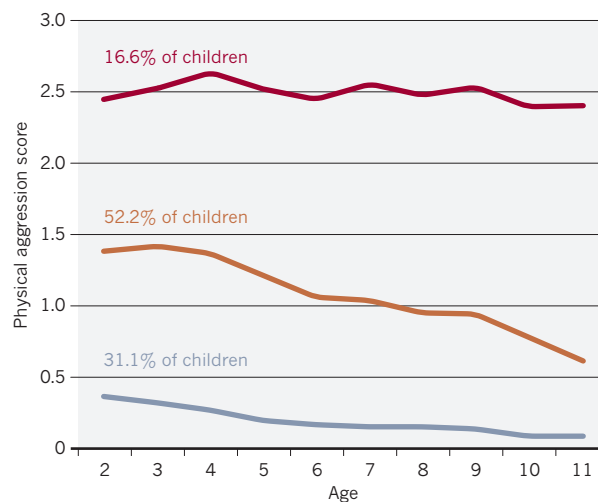
The first primate study found distinct differences in DNA-methylation patterns between nurtured monkeys and those separated from their mothers⁷. The epigenetic residue of post-natal adversity was broad, according to Suomi, affecting more than 4,000 genes — about one-fifth of the genome — and tending to cluster in certain chromosomal regions. Moreover, the epigenetic modifications seemed to alter expression of a gene that Suomi's group had shown to be crucial to the function of the neurotransmitter serotonin⁸, low levels of which have been associated with elevated stress and aggression in humans. "These are not random changes," Suomi says. "They follow particular pathways." The marks remained stable in monkeys up to 8 years old — an age roughly equivalent to 30 in humans.

Although the team was able to test both

brain and white blood cells from the monkeys, they only had access to blood from the men of the Montreal cohort. Even so, studies are starting to offer a complementary human picture. In July, Szyf and Tremblay reported that men with a history of chronic aggression dating back to kindergarten had significantly lower blood levels of immune molecules called cytokines than normal controls from the cohort⁹. These molecules are typically

AGGRESSION REGRESSION

A study of more than 10,000 Canadian children pointed to three basic trajectories for physical aggression. Most become less aggressive between the ages of 2 and 11 years, but a minority maintain a high level of aggression throughout childhood.



activated during the body's response to stress, and animal studies have demonstrated a link between aggression and lower levels of a cytokine called interleukin-6, which was also lower in the chronically aggressive men. In a second study, Szyf and Tremblay showed that members of the Montreal Longitudinal Study with a long-standing history of aggression had a distinctly different pattern of DNA methylation in the genes encoding the cytokines, compared to men with a less aggressive behavioural profile¹⁰.

The early human research has its shortcomings. For starters, the sample size is very small: only seven males with a history of aggression could be tracked down from the cohort for testing, along with 25 controls. And white blood cells are by no means the same as neurons, although Suomi notes that there is considerable overlap between the methylation patterns of the two cell types in the primate studies. Moreover, many researchers remain cautious about recent human epigenetic studies. Attributing behavioural consequences to DNA methylation may be overreaching, says Adrian Bird, a geneticist at the University of Edinburgh, UK. "These are all correlations," he says, "and often the magnitude of the change is very small indeed."

Tremblay is the first to admit that the story

is far from simple: hundreds of genes are involved, and any single expression change is probably subtle. Yet, he says, "it seems relatively clear that there are large differences in DNA methylation between those who have a history of chronic aggression compared to those who have normal development". He is convinced that the benefits of nurture merit early intervention programmes, regardless of the uncertainties in the biological part of the story. And he thinks that earlier intervention may produce even better results. "If we support these parents during pregnancy and if we help these women have a better lifestyle during pregnancy, with less stress, it should affect brain development, and these children should be better able to learn how to control their aggressive behaviour," he says.

He is already testing that hypothesis. In 2007, he accepted a ten-year appointment at University College Dublin, where he is assisting on several early-childhood longitudinal studies. One, called Preparing for Life and headed by economist Orla Doyle, is testing a preventive intervention in 200 pregnant women from a disadvantaged area of north Dublin. During their pregnancies, the women received intensive home visits covering everything from nutrition, smoking, alcohol and drug counselling to support in marital relationships. And the support continues until the children reach the age of four. James Heckman, who is also collaborating on the study, says that the plan includes future epigenetic studies of the cohort.

"To solve the aggression problems, which are mainly a male problem, we need to focus on females," Tremblay says. "If you ameliorate the quality of life of women, it will transfer to the next generation." ■

Stephen S. Hall is a science writer in New York and teaches public communication to graduate students in science at New York University.

- McCord, J. *Am. Psychol.* **33**, 284–289 (1978).
- Boisjoli, R., Vitaro, F., Lacourse, E., Barker, E. D. & Tremblay, R. E. *Brit. J. Psychiatry* **191**, 415–419 (2007).
- Nagin, D. & Tremblay, R. E. *Child Dev.* **70**, 1181–1196 (1999).
- Côté, S. M. et al. *Arch. Gen. Psychiatry* **64**, 1305–1312 (2007).
- Tremblay, R. E. *J. Child Psychol. Psychiatry* **51**, 341–367 (2010).
- Weaver, I. C. G. et al. *Nature Neurosci.* **7**, 847–854 (2004).
- Provençal, N. et al. *J. Neurosci.* **32**, 15626–15642 (2012).
- Bennett, A. J. et al. *Mol. Psychiatry* **7**, 118–122 (2002).
- Provençal, N., Suderman, M. J., Vitaro, F., Szyf, M. & Tremblay, R. E. *PLoS ONE* **8**, e69481 (2013).
- Provençal, N. et al. *PLoS ONE* **8**, e71691 (2013).