Barry J. Everitt

Born:

Dagenham, Essex, United Kingdom
February 19, 1946

Education:

University of Hull, BSc Zoology (1967)
University of Birmingham Medical School PhD (1970)

Appointments:

Postdoctoral Research Fellow, University of Birmingham Medical School (1970–1973)
Medical Research Council Traveling Research Fellow, Karolinska Institute, Stockholm (1973–1974)
University Demonstrator, University of Cambridge (1974–1979)
Fellow, Downing College, Cambridge (1974–2003)
University Lecturer, University of Cambridge (1979–1991)
Director of Studies in Medicine, Downing College, Cambridge (1979–1998)
Ciba-Geigy Senior Research Fellow, Karolinska Institutet, Stockholm (1982–1983)
Reader in Neuroscience, University of Cambridge (1991–1997)
Professor of Behavioral Neuroscience, University of Cambridge (1997–2013)
Editor-in-Chief, European Journal of Neuroscience (1997–2008)
Master, Downing College Cambridge (2003–2013)
Emeritus Professor of Behavioral Neuroscience, University of Cambridge (2013–present)
Director of Research, University of Cambridge (2013–present)
Provost, Gates Cambridge Trust, University of Cambridge (2013–present)

Honors and Awards (Selected):

President, British Association for Psychopharmacology (1992–1994)
President, European Brain and Behaviour Society (1998–2000)
President, European Behavioural Pharmacology Society (2003–2005)
Sc.D. University of Cambridge (2004)
Fellow of the Royal Society (elected 2007)
Fellow of the Academy of Medical Sciences (elected 2008)
D.Sc. *honoris causa,* University of Hull (2009)
D.Sc. *honoris causa,* University of Birmingham (2010)
European Behavioural Pharmacology Society Distinguished Achievement Award (2011)
American Psychological Association Distinguished Scientific Contribution Award (2011)
Federation of European Neuroscience Societies-European Journal of Neuroscience Award (2012)
British Association for Psychopharmacology Lifetime Achievement Award (2012)
Fondation Ipsen Neuronal Plasticity Prize (2014)
Member of European Molecular Biology Organization (EMBO) (elected 2014)
MD *honoris causa,* Karolinska Institutet, Stockholm, Sweden (2015)
President, Federation of European Neuroscience Societies (2016–2018)
President, Society for Neuroscience (2019–2020)

Barry Everitt is a behavioral neuroscientist who has an enduring interest in understanding the neural mechanisms of motivation, learning, and memory. His early research focused on the neuroendocrine basis of sexual motivation and behavior in primate and rodent species. He then combined neuroanatomical, neurochemical and behavioral approaches to define the functions of monoaminergic and cholinergic neurotransmitter systems in motivation, learning, and attention. He made fundamental discoveries on the functions of limbic corticostriatal systems underlying aversive and appetitive behavior through their mediation of Pavlovian and instrumental learning. He formulated, with T. W. Robbins, a theory of drug addiction that proposes a shift from goal-directed to habitual behavior in the development of compulsive drug seeking, reflecting the shifting engagement of the ventral and dorsal striatum and emergent loss of control over behavior by the prefrontal cortex. He has demonstrated the molecular and systems basis of the reconsolidation of addictive drug and fear memories, highlighting the potential of targeting maladaptive memories in the treatment of neuropsychiatric disorders, including addiction.

Barry J. Everitt

Early Years: From School in Dagenham, Essex to University in Hull, Yorkshire: An Education

I was born in Dagenham in the east end of London (southeast Essex) in 1946, soon after my father was demobilized from the Royal Air Force (RAF) at the end of World War II. Three years later my sister Melanie was born and we lived in a new house on a council estate (social housing in United States terminology) that was built to house the many people relocated from London because of the devastation caused during the Blitz. My mother, Winifred, was born in Old Dagenham village and began her working life at age 14 as a maid in service before being recruited to the Sterling machine gun factory in Dagenham as part of the war effort. My father, Fred, was born in Southampton but grew up in Cadoxton, Glamorgan, in South Wales. His father having died when he was very young, he left school at age 13 to work to support his mother after his three older brothers had left home. He came to London to find work during the depression and settled in east London, and then in Dagenham, where he met and married my mother in 1943. He worked until his retirement in the Ford Motor Company, which had built its massive factory on the banks of the river Thames in Dagenham. I had a very loving and supportive, if strict, home life in a social environment in which there was no expectation that anyone would remain in school after the age of 15, which was then the legal leaving age, let alone go to university. Although never having received anything but a basic education, my parents were both very bright and knew that a pathway out of the low expectation working-class environment of Dagenham was through education. I passed the daunting 11-plus examination and went to the South East Essex County Technical High School rather than a traditional grammar school, which taught a range of academic subjects but also technical subjects such as woodwork, metalwork, and architectural drawing—all of which I enjoyed but in none of which did I ever achieve more than basic competence. I regret that I was never particularly studious at school, but I played a lot of sport and was especially good at basketball (which I continued to play into my late 20s) and cricket, which remains a passion. I was constantly in trouble and frequently caned by the headmaster, but was also regarded by my science teachers as bright but underachieving. Not a promising start.

I sat and passed my O (ordinary) level exams at 16, doing well in the sciences, and remained at school in the sixth form (age 16–18) to study zoology, botany, and chemistry at 'A' (advanced) level. It was then that

I encountered my first significant and influential mentors, and I have come to realize how important such encounters have been at key times during my life and career. As a consequence, I have tried always to take my role as mentor extremely seriously in both research and teaching when interacting with undergraduate and graduate students, as well as postdoctoral researchers spending time in my lab. Vera Clarke ('Miss Clarke'), the senior sixth-form biology teacher, was a dedicated, kind, and shy spinster who traveled miles across London each day to teach us. She was the first to sit me down and give me a stern talking to about the value of study, and most important of all, she transmitted the excitement and wonder of biological sciences. She was joined by Valerie Singleton who had just graduated from the University of London with a degree in zoology and was doing a temporary teaching job before emigrating to Australia. She was the first person I had met who had just been to university, was only four years older than me, and was a brilliant teacher. She more than anyone made me see the importance of applying to university and, because I was good at it, suggested I study zoology. Alas nobody suggested I might think more widely about what to study at university other than a subject I was good at; had someone suggested to me it would be possible to study medicine, I feel sure I would have tried to do so. As it turned out, I was one of only two or three in a sixth form of more than 100 who applied to and was admitted to university. I went to the relatively new University of Hull in Yorkshire in 1964 to read zoology, also studying organic chemistry and psychology in my first and second years, respectively. I was the first in my family to go to university and, given my family's financial circumstances, I would never done so had I not received a grant from my local Education Authority. Higher Education grants were then available to everyone but sadly now have been abandoned. As well as my tuition fees, it provided my maintenance of about £300 per year, which was plenty to live on if supplemented with income from the variety of jobs I held during vacation months.

A very traditional zoology course was offered at Hull based on the systematic study of the anatomy and physiology of the animal classes. The department was led by the eccentric, even exotic, Professor Paul 'Espinasse, an authority on the embryology of feathers, but he was kind and encouraging if occasionally stern (he threw me out of a practical class for incorrectly grasping the lever on a Cambridge rocker microtome). I did not find the first two years of zoology compelling (and played a lot of sport), but in my final year, I took a course on endocrinology, a fairly new subject in the 1960s, taught by Norman Nowell who both stimulated my interest in hypothalamic integration mechanisms and encouraged me to explore a doctoral studentship in neuroendocrinology. I had taken the unusual option of studying psychology in my second year (almost all zoology students read botany) and so had discovered my enduring fascination with motivational theory and hypothalamic mechanisms of motivated behavior. With this combination

of interests, I wrote seeking a graduate studentship to the University of Birmingham Medical School where a number of neuroendocrine researchers were working. My application found its way to Joe Herbert who had just been appointed as a lecturer in the Department of Anatomy having completed his doctoral research on primate sexual behavior at the Institute of Psychiatry in London. He invited me for an interview, showed me the very large and impressive primate colony that had been established there through a Ford Foundation grant to Sir Solly Zuckerman, then chair of anatomy. I was accepted as one of his first two doctoral students (Diane Scruton, now Bunsell, was the other) to begin my research project on the neuroendocrine control of sexual behavior in female rhesus monkeys. Diane began with Herbert the research program on the sociosexual behavior of a just-established social group of talapoin monkeys. Herbert was the perfect mentor for me; extremely intelligent, creative, infectiously enthusiastic, and generous, he taught me to think, write, talk about, and enjoy research. I suspect without his influence, my research career would have been very different and may even have petered out at the postdoctoral stage. We remain very close friends. As it turned out, I remained in Birmingham for six years, first as a graduate student and then, after Herbert had left for the University of Cambridge, as a postdoctoral researcher continuing to work on primate sexual behavior funded by the Medical Research Council (MRC).

Graduate and Postdoctoral Behavioral Neuroendocrine Research in Birmingham

Having been drawn into neuroendocrinology by my interest in the regulation of gonadal and adrenal function and the possibility of investigating the integrative functions of the hypothalamus, about which little was known, it came as rather a shock to find myself learning about primate sexual behavior and ethological methods of study. Previous work by Herbert had indicated that androgens might play an important role in regulating sexual receptivity in female primates, not just in males, and that this might explain why they remained sexually receptive throughout the menstrual cycle rather than just in the periovulatory period characterized by estrus in nonprimate mammals. There were also data on women indicating that there was often little or no change in sexual activity following the menopause or after ovariectomy. The main extra-ovarian source of androgens in females is the adrenal glands, and in my doctoral research, I showed that while ovariectomized female rhesus monkeys maintained only on estradiol were sexually receptive, when adrenal function was suppressed by dexamethasone (resulting in a major reduction in plasma testosterone), or following adrenalectomy with corticosteroid replacement, sexual solicitation of male sexual activity was markedly decreased, and females tended to reject attempts by males to

mount. This change in sexual motivation was reversed dose-dependently by treatment with testosterone (or androstenedione, which was less potent). My first scientific paper describing these data was published in *Nature*, a remarkably fortunate beginning (Everitt and Herbert, 1969). My doctoral thesis was examined by Professor Sir Robert Hinde, FRS, the eminent ethologist and director of the Sub-Department of Animal Behaviour in Cambridge. Hinde was a towering intellect, rather intimidating, and my viva was very long; he ended it with the words "I'm afraid we cannot award you your PhD" (at this point, I could taste my heart in my throat) and, after a dramatic pause, continued "but we can recommend most strongly to the degree committee that they do." I would see this wonderful man regularly over the next 40 years in Cambridge (he died only recently) and he was always supportive, kind, and intellectually uncompromising.

A question arising during my doctoral work concerned the behavioral specificity and site of action of androgens in the female primate brain, at a time when it was beginning to be shown that estradiol and progesterone in female, and testosterone in male, nonprimates exerted their effects on sexual behavior in the ventromedial hypothalamus and medial preoptic area, respectively. The MRC funded a project grant that enabled me to stay as a postdoctoral researcher in Birmingham to investigate the hypothalamic site of action of androgens in female rhesus monkeys. It was a difficult project, and I had to devise the methodology to removably implant testosterone in the brain, but it was successful, and I was able to show that medial preoptic/anterior hypothalamic area implantation of testosterone reinstated receptivity in ovariectomized, estradiol-treated monkeys lacking adrenal androgens (Everitt and Herbert, 1975).

After I left Birmingham, my primate research was interrupted, but it re-started briefly when I moved to Cambridge and resumed working with Herbert and Barry Keverne, who had just arrived from London. We were joined for a year by Michael Baum, who was visiting from MIT, and decided to write a review bringing together the work we had conducted separately and together on the neuroendocrine control of female primate sexual behavior (Baum et al., 1977). This we did within a conceptual framework proposed by the brilliant and pioneering University of Berkeley psychologist, Frank Beach, who made a distinction between proceptivity, receptivity, and attractivity when considering hormonal determinants of sexual interaction in female mammals (Beach, 1976). Our overarching conclusion at that time was that estrogen of ovarian origin facilitated sexual interaction primarily by stimulating the emission of nonbehavioral, mainly olfactory, cues that increased a female's sexual attractivity, whereas progesterone decreased sexual interaction primarily by reducing sexual attractivity though its vaginal actions. We further concluded that while estrogen may enhance proceptivity by acting in the female's central nervous system, there was no clear demonstration that it affected the neural mechanisms controlling

receptivity. Finally, we concluded that proceptivity and, to a lesser extent, receptivity were modulated by adrenal androgens that may act synergistically with estrogen to facilitate sexual behavior.

When investigating the hypothalamic site of action of testosterone on sexual behavior when still in Birmingham, great interest had begun to emerge in the possibility that monoamine neurotransmitters might in some way mediate the effects of sex steroids on motivational systems in the brain. Bengt Meyerson in Uppsala had shown that some drugs that altered monoamines in the brain profoundly affected sexual behavior in male and female rodents. A visiting postdoctoral researcher from Rhodesia (now Zimbabwe), Peter Gradwell, arrived in Birmingham and along with Herbert and me showed that depleting the brain of 5-hydroxytryptamine (serotonin, 5-HT) with para-chlorophenylalanine increased the proceptivity of estradiol-treated female macaques depleted of androgens, whereas treatment with the 5-HT precursor 5-hydroxytryptophan reduced sexual activity in monkeys not depleted of androgens. Intriguingly, estradiol and testosterone also reduced the turnover of 5-HT in the brain (Gradwell et al., 1975). Of course, it could not be established in these initial experiments whether the effects of sex steroids on behavior were actually mediated by altered serotonin in the brain or whether the steroid and monoamine affected behavioral responses through different mechanisms. It also seemed unlikely that decreasing brain 5-HT had a specific effect on sexual behavior, and of course that proved to be the case. Tragically, Peter Gradwell was murdered in the violence and turmoil that engulfed Rhodesia during the Unilateral Declaration of Independence (UDI) and the establishment of the Republic of Zimbabwe.

A Transforming Postdoctoral Fellowship at the Karolinska Institute in Stockholm: Immersion in Monoamine Research

In the midst of this period of behavioral neuroendocrinology experimentation, the European Science Foundation announced that the first of its winter schools—the European Training Programme in Brain and Behaviour Research—would be held in Zuoz in the Engadine Valley in Switzerland in January 1972. I was fortunate to attend two more, one in 1975 also as a delegate, and the 25th and final one in 1997 as a lecturer. It was themed "Transmission and Behaviour" and could not have been more perfectly timed for me, turning out to be a transformational experience in my life as a researcher. I also began to learn to ski and continued to do so for the next 30 years (but never attained the skill that enabled me to give no thought to the color of the slope that had to be descended having arrived at the top of a ski lift). The lecturers at this first meeting were a who's who of eminent researchers in these early years of monoamine and chemical transmission research: Jacques Glowinski, Leslie Iversen, Hans Thoenen,

Nils-Erik Andén, and Tomas Hökfelt among them. Hökfelt was one of the small group of exceptional researchers from the Amine Group at the Karolinska Institute in Stockholm, including Kjell Fuxe, Urban Ungerstedt, Lars Olson, Gösta Jonsson, and Charlotte Sachs. All had been graduate students of Nils-Åke Hillarp who, with Bengt Falck, had developed the histofluorescence technique to visualize monoamine neurons in the brain (Falck et al., 1962). It was through my discussions with Hökfelt that I realized that what I really wished to immerse myself in during the next phase of my career was neuroscience and especially research on the anatomy of monoamine neural systems, the impact of monoamine transmission on behavior and, at least initially, neuroendocrine integration. I wrote to Hökfelt and Fuxe to ask if they would accept me into the lab as a postdoc, and they said yes. I applied for and was awarded an MRC Traveling Fellowship (a scheme that alas was phased out) to go to Stockholm in October 1973. Having spent my doctorate and first postdoc in Birmingham funded by the MRC, this critical international bridge in my career has meant that I have been funded continuously by the MRC to the present day. I realize that I have been immensely fortunate.

At the Karolinska, my intention was to learn all I could about monoaminergic systems in the brain and how to study them. I was trained to perform the Falck-Hillarp technique with all the joys of freeze-drying brains, reacting them with formaldehyde, and sectioning and then viewing broken sections under ultraviolet (UV) light for hours in the midnight space that was the fluorescence microscopy room. Hökfelt and Fuxe are brilliant neuroanatomists, and it was a joy to learn from them. I was also mentored in neurochemistry by Gösta Jonsson, who taught me how to measure regional monoamine levels and turnover using cation exchange chromatography with fluorescence detection and radioenzymatic assays, and monoamine reuptake in synaptosomes and brain slices. However, this turned out to be a period not only of acquiring monoamine neurobiological skills. Hökfelt and Fuxe were very interested in my earlier findings on 5-HT and sexual behavior in primates and had indicated before my arrival in Stockholm that they wished to collaborate with me to investigate the impact of monoamine transmitters on sexual behavior in rats.

I had never touched a rat, let alone observed and measured the sexual behavior of one, and so I set about acquiring these skills before I left Birmingham. Guided by Fuxe's neuropharmacological expertise, I began investigating the effects of the rapidly emerging new drugs targeting monoaminergic receptors, as well as monoamine synthesis and reuptake, initially by systemic injection and then by direct manipulation in the brain. The latter involved some of the first behavioral experiments using the recently discovered 5-HT neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT), which when infused into the rostral midbrain (while cotreating with catecholamine reuptake inhibitors) selectively depleted the forebrain of 5-HT.

These lesion-inducing neurotoxin infusions also caused a buildup of monoamines proximal to the site of neurotoxin injection, enabling the highly transient 5-HT florescence signal to persist under UV light long enough to visualize more accurately the axons of monoamine 5-HT neurons projecting from the raphe nuclei as they merged within the medial forebrain bundle to reach the diencephalon and hypothalamus. This phenomenon had been exploited previously by Urban Ungerstedt in his use of the catecholamine neurotoxin 6-hydroxydopamine (6-OHDA) to map catecholamine neurons in the brain. In doing so, he also revealed that nonhomeostatic features of the syndrome of aphagia and adipsia that followed electrolytic lesions of the lateral hypothalamus were largely a consequence of dopamine depletion in the striatum (Ungerstedt, 1971). This remarkable observation opened the way simultaneously to an understanding of the role of dopamine systems in motivation, ingestive behavior, and the motor functions of the striatum, as well as establishing a still-used model of Parkinson's disease.

It is not unreasonable to suggest that a new era of behavioral neuroscience was beginning, as neuropsychopharmacology now began to emerge as a powerful way to investigate the behavioral functions of monoamine transmitter systems. New drugs with ever-greater specificity were appearing at an incredible rate as big pharma began major discovery programs for new treatments for neurological and neuropsychiatric disorders. It was already becoming apparent that monoamines were key players in these disorders and also potential treatment targets. It rapidly became clear from my own research and that of others that a range of drugs that altered serotonergic, dopaminergic, and noradrenergic transmission had major and dissociable effects on sexual behavior in female rats (Everitt et al., 1975a). I paid careful attention to the distinction between proceptive and receptive behavior in my behavioral analysis of female rats: the distinction between lordosis—the immobile receptive reflex posture—and the extraordinary species-specific proceptive responses of hopping, darting, and ear-wiggling. These appetitive behavioral responses elicited sexual interest and pursuit by males, but they were not often quantified in behavioral experiments. Yet it was clear that proceptive behavior and lordosis, although only displayed at estrus or when ovariectomized females were treated sequentially with estradiol and progesterone, were differentially affected by monoaminergic drugs. For example, dopamine receptor antagonists decreased proceptive behavior but facilitated the immobile lordosis response (Everitt and Fuxe, 1977), and so it became obvious that a distinction had to be made between the general effects of drugs on motor activity and sensory responsiveness to understand any more specific effects on motivational processes. Similarly, while these early experiments showed that sex steroids markedly affected monoamine turnover and reuptake in the hypothalamus, cortex, and brainstem, it was becoming apparent that these hormones were not influencing sexual behavior simply by altering monoamine transmission.

It was increasingly understood that hypothalamic monoamines were involved in neuroendocrine integration (e.g., the role of the tuberoinfundibular dopamine neurons in the control of the release of anterior pituitary hormones). However, it was now clear to me that attempts to understand the behavioral functions of these chemically defined brainstem systems would depend on investigating the way monoamines modulated processing in the many areas of the brain they innervated, not just the hypothalamus. The striatum with its rich dopaminergic innervation was already of growing interest, not least through Ungerstedt's research. Therefore, the manipulation of monoamine transmission in discrete brain regions would have to be combined with precise behavioral analysis to make further progress. In reality, I was gradually realizing that I had to reconnect with the psychology I had studied as an undergraduate and combine this with my now-complete immersion in, and fascination with, neuroscience. My wonderful postdoctoral time at the Karolinska was life-changing; it enabled me to decide what I was really interested in and enabled me to begin establishing my future career as a behavioral neuroscientist. I shall always be grateful to Tomas Hökfelt and Kjell Fuxe for the inspiring time I spent in Stockholm.

However, I had a major obstacle to overcome which was that my postdoctoral fellowship was quickly coming to an end and political turmoil in the United Kingdom as a result of the oil crisis threatened my future employment. A three-day week was introduced in the United Kingdom in the first quarter of 1974 as part of the government's response, and the government of Edward Heath lost its majority in the subsequent election. Harold Wilson then led a minority government and, among the inevitable cuts that followed, the lecturer position I had been expecting to take up back in Birmingham was suppressed. I was about to be unemployed and what my wife, our young son, and I were going to do was very uncertain. It just so happened that an Australian academic was briefly visiting Stockholm and was about to set up a new department of anatomy in New South Wales. After some discussion, he offered me a tenure-track position and I began the process of emigration to Australia, visiting the embassy and even having my medical examination. The plan was to return to the United Kingdom briefly, say goodbye to our families and friends and set off for Australia. We were both excited and apprehensive as emigrating had never been in our thoughts before my imminent employment crisis, now only a month or so away.

Appointment to an Academic Post in Cambridge: Behavioral Neuroscience, Sex, and Monoamines

Then, out of the blue, I received news from Joe Herbert, my doctoral supervisor and mentor, that a vacancy for a demonstratorship position had arisen in the Anatomy Department at Cambridge University, where he had been appointed as a lecturer four years earlier. I returned unemployed to the

United Kingdom to join my family and live with parents, applied for the position, and was shortlisted, interviewed, and then appointed in November 1974. Sadly, my then wife and I were separated and divorced soon afterward. I began teaching topographical anatomy and other alien subjects in January 1975 and set about reestablishing my research. A demonstratorship in the University of Cambridge was an unusual position (it no longer exists) intended to bridge the gap between doctoral studentship or first postdoctoral position and a lectureship. It was a five-year fixed-term appointment with no guarantee of anything beyond, as upgrading to a lectureship depended on there being a vacancy and was highly competitive. The salary was fixed nonnegotiably at £2,700 per year and there were no annual increments. Yet this small salary allowed me to borrow three times that amount and obtain a mortgage on a two-bedroom house and was just about enough to live on. In Cambridge today, it is necessary to borrow more than 10 times a lecturer's salary of around £40,000 per year to buy a house and few are able to do so.

So it was that I rejoined Joe Herbert, and the neuroendocrine research group he was establishing in Cambridge. Barry Keverne had joined the lab during my year in Stockholm and was leading research on the sociosexual behavior of the talapoin monkey group that had been brought from Birmingham. We were later joined by Michael Hastings, who worked on circadian and circannual reproductive rhythms. Although I resumed my research on sexual behavior in macaques, it was becoming obvious that this was unsustainable in the department's poor facilities. I was, in any case, increasingly focused on my research on monoamines and behavior and setting up neurochemistry and neuroanatomy labs with the skills I had acquired in Stockholm.

The Anatomy Department at Cambridge University was a strange and initially hostile place. The great majority of the faculty were research inactive, almost all had Cambridge medical degrees, and they were very suspicious of the three new appointees who had no medical qualification: me, Barry Keverne, and Martin Johnson, a brilliant developmental biologist and also a lifelong friend. However, we had reasonably good experimental space because nobody else was using it, and our neuroendocrine group soon attracted students and visiting researchers. On the back of my experiments in Stockholm that had revealed truly remarkable changes in sexual and aggressive behavior in rats following 5,7-DHT-induced lesions of ascending raphé projections in the midbrain (Everitt et al., 1975b), I began to think about comparing the effects of manipulating each of the major monoamine projections. This work was based on the hypotheses that had emerged from the psychopharmacological data I had collected in Stockholm, as a first attempt to understand their differential contributions to behavioral responses through modulation of diencephalic and forebrain mechanisms. I was increasingly convinced that monoaminergic mechanisms had general,

rather than specific, functions (as I and many others had initially thought) and that comparing the effects of manipulating them on the same behaviors or tasks might be revealing.

Early experiments involved infusing 6-OHDA into the dorsal and ventral noradrenergic fiber bundles in the brainstem before they merged in the medial forebrain bundle to course through the lateral hypothalamus. This resulted in noradrenaline depletions from the hypothalamus and other subcortical sites following lesions of the ventral bundle (composed of axons of the medullary noradrenaline cell groups) or from cortical and limbic structures following lesions of the dorsal bundle originating mainly from the locus ceruleus. A visiting research fellow from Gothenburg, Stefan Hansen, together with a final-year undergraduate in Cambridge, Erica Stanfield, working with me on this project showed that ventral bundle lesions left proceptive behavior intact—females still solicited male sexual interest—but impaired the display of lordosis and also prevented pseudopregnancy induced by cervical stimulation (Hansen et al., 1980, 1981). This behavioral and neuroendocrine outcome was interpreted as an impairment in the response to the somatosensory stimulation during coitus that elicited both lordosis and pseudopregnancy. The effects on receptive behavior of noradrenaline depletion were additionally shown to depend on altered striatal dopamine, one of a growing number of demonstrations of noradrenaline-dopamine interactions controlling behavior.

It was around this time that I attended the European Brain and Behaviour Society (EBBS) meeting in Copenhagen in 1976 and first met Trevor Robbins, who was a demonstrator in the Department of Psychology. We had both been working for a couple of years just 25 meters or so apart in adjacent buildings but had never met each other in Cambridge, although I had met and begun collaborating with his former supervisor, Susan Iversen. We soon realized while socializing that we were both interested in and working on monoamines and behavior but from completely different perspectives. We resolved to meet regularly when we returned to Cambridge and this we did, over lunch and at lab talks over the next couple of years. The outcome was the first of many successful joint grant applications funded by the MRC—this first one on comparative functions of cortical and subcortical noradrenergic systems in visual attention and discrimination learning. This enduring connection with Robbins allowed me to re-immerse myself in psychological theory and a completely different approach to studying behavior, and allowed Robbins rapidly to engage with my neuroanatomical and neurochemical expertise. It was the beginning of what is now a 40-year collaboration that was to our great pleasure recognized by the Distinguished Scientific Contribution Award from the American Psychological Association in 2011 that explicitly referred to our collaborative research and mentorship. We began working closely together, but also retained and developed our own research interests and this we both agree has enabled our highly

fruitful collaboration to thrive while retaining our individuality. We became and remain the closest of friends.

Over the next several years, I led a sort of parallel research life, beginning and often ending the day running experiments and in discussions with Robbins in the Psychology Department as we pursued our jointly funded work on comparative functions of monoaminergic and later cholinergic neural systems, while in the Department of Anatomy, I was investigating the neural basis of sexual motivation and its modulation by monoamines. The conceptual basis of our joint research evolved from Robbins's theorizing (inspired by Broadbent's seminal work on decisions and stress) that the effects on attention, learning, and memory of different central arousal states were dissociably mediated by the noradrenergic, serotoninergic, and dopaminergic systems originating in the brainstem, and later the similarly organized cholinergic basal forebrain projections (Robbins and Everitt, 1982; Everitt and Robbins, 1997). These ideas increasingly influenced my thinking and theorizing about monoaminergic involvement in behavior, mainly sexual behavior at the time, that I had been studying using ethological approaches. I then gradually began to adopt the operant methodologies I was using in collaboration with Robbins in my own investigation of the neural mechanisms underlying sexual motivation, being very much influenced by the great motivational theorists Dalbir Bindra and Jerzy Konorski.

During this period of evolution and transition of my research interests, my personal life was affected by the sudden diagnosis of a malignant melanoma, and I found myself undergoing deep excision surgery and a skin graft. Fortunately, the outcome was good and I was able to escape further screening after several years. A much more positive and exciting event a year or so later was my marriage in 1979 to Jane Sterling, who was in the early stages of her medical career and later embarked on a doctoral degree in virology. We have just celebrated our 40th wedding anniversary. Amidst this period of adaptation and change, Martin Johnson and I completed the first edition of our textbook *Essential Reproduction* (Johnson and Everitt, 1980), an immersive joint venture borne out of the course we taught together and for which no effective textbook existed; it went through five updates and revisions over the next 20 years. I also returned to Stockholm to work again with Tomas Hökfelt, and Jane came with me to spend time in the dermatology department at the Karolinska Hospital. During this sabbatical year, I acquired the then relatively new technique of immunocytochemistry that enabled even more refined neuroanatomical analysis of monoaminergic neurons, including the coexistence of neuropeptides with the classical transmitters that Hökfelt was studying intensively. My collaboration with Hökfelt on some of this work led to a detailed analysis of the coexistence of neuropeptide-Y in brainstem noradrenergic cell groups (Everitt et al., 1984). During this second period in Stockholm, I met

Robert Schwartz who was there collaborating with Fuxe and whose major interest was Huntington's disease. He introduced me to the remarkable axon-sparing lesions induced by ibotenic acid and other excitotoxins when infused in the brain and their potential use in exploring causal relationships between specific neural structures and behavior. This was to have a significant impact on my future research on the functions of both the hypothalamus and limbic-cortico-striatal systems.

Back in the world of sex research, the medial preoptic area had by now been identified as a key structure underlying sexual behavior in male mammals. However, I was intrigued by the observation that although medial preoptic area lesions prevented copulatory behavior by males, other measures of sexual interest and motivation such as olfactory investigation, pursuit, and even mounting of females were spared. I decided that an objective, quantifiable measure of appetitive, precopulatory behavior, or "sexual motivation" that was independent of sexual performance measures (such as the latency to mount, or intromit or ejaculate) would enable a more fine-grained analysis of the neural mechanisms involved in male sexual behavior. This problem had been faced previously when measuring the motivation for other reinforcers, such as addictive drugs, because attainment of the reinforcer—whether a female or a drug—profoundly affected the performance of the very behavioral responses being taken as measures of motivation. The utilization of second-order schedules of reinforcement by a small group of researchers at the National Institute of Drug Abuse (NIDA) to study drugs as reinforcers (Goldberg, 1973) seemed to offer a possible solution in studies of sexual behavior, although they had never previously been used in this way. The principle is that animals will work for an environmental stimulus, such as a light, that previously had been associated with a reinforcer (or "reward") through Pavlovian conditioning. This conditioned stimulus (CS) will support appetitive instrumental behavior for long periods of time before the primary reinforcer is obtained, if delivered response-contingently, therefore acting as a conditioned reinforcer.

My idea was that male rats in an operant chamber might respond on a lever for a CS associated with sexual interaction with a receptive female, and this would provide a measure of sexual motivation independent of species-specific copulatory reflexes. The problem was how to do this without having a female present in the operant chamber where lever presses were made, because if she was there, males would simply copulate. Paul Fray, who had been a doctoral student with Susan Iversen, then joined the lab and he was both a very talented psychologist and a highly accomplished technologist. He had already developed a new online computer control language for automatically controlling and recording animal behavior, and now set about constructing an apparatus in which male rats could make lever press responses to gain access to a receptive female. He designed and constructed a compartment above the ceiling of an operant chamber in which a receptive

female rat could be placed, the door of which could be automatically opened when a male had completed the instrumental response requirements of a second-order schedule to gain access to her. After overcoming some difficult procedural challenges, we eventually succeeded in training male rats to work for periods of 15 minutes to gain access to a receptive female and then subsequently to interact sexually with her so that both appetitive and performance measures of sexual motivation could be obtained. We also established that contingent presentations of the sex-associated CS were critical if males were to respond; instrumental responses collapsed without the conditioned reinforcing properties of the CS that mediated delays to primary sexual reinforcement (Everitt et al., 1987).

Equipped with this new behavioral procedure, I reinvestigated the role of the medial preoptic area and the effects of testosterone on appetitive and consummatory measures of sexual motivation. A key innovation was to use excitotoxic, axon-sparing lesions of the medial preoptic area induced by infusion of ibotenic acid, which, unlike electrolytic lesions, did not destroy axons *en passant* or close to the structure of interest. Although a great technical advance then, this, of course, has been surpassed by the exquisite selectivity of optogenetic and chemogenetic techniques that provide a previously unimagined level of specificity in cellular and circuit analysis of brain mechanisms of behavior. The results showed that male rats with medial preoptic area lesions no longer displayed copulatory behavior, confirming earlier data, but they continued to make high levels of instrumental responses to gain access to a receptive female that they then pursued, investigated, and attempted to mount, but could not copulate with (Everitt and Stacey, 1987). This therefore revealed a dissociation in the hypothalamic basis of appetitive and consummatory sexual responses, but left open the question of which neural systems mediated the associative influence on appetitive behavior (Everitt, 1990).

In parallel with these experiments, I had been deploying excitotoxic lesions to begin what has turned out to be still ongoing investigations of amygdala function where electrolytic and other physical lesions massively disrupted pathways between the diencephalon and other temporal lobe structures. It was quickly apparent that excitotoxic amygdala lesions did not result in the classic Klüver-Bucy syndrome that involved "hypersexuality" in some species (that was later shown to depend on a quite different mechanism involving the inferotemporal cortex). A visiting postdoctoral researcher from Bordeaux, Martine Cador, collaborated with me to show that rats with excitotoxic basolateral amygdala lesions showed completely normal, or unaltered, copulatory behavior when interacting with sexually receptive females, but their appetitive instrumental behavior under the second-order schedule was greatly impaired (Everitt et al., 1989). Therefore, we had shown a double dissociation between appetitive and consummatory sexual responses following amygdala and medial preoptic area lesions (Everitt, 1990). In addition,

we also revealed an interaction between the basolateral amygdala and nucleus accumbens dopamine transmission in appetitive behavior that was explored more mechanistically in Cador's parallel study of the neural basis of conditioned reinforcement (Cador et al., 1989).

The Functions of Limbic-Corticostriatal Systems

My research on the neural basis of sexual motivation and behavior was now drawing to a close, save for studies using c-fos cellular imaging to reveal the structures and circuitry activated in males by sexual interaction with females (Baum and Everitt, 1992), and a detailed analysis of the effects of hypothalamic proopiomelanocortin peptides on sexual motivation and performance (Hughes et al., 1990). My interest had shifted to what has been an enduring focus on limbic cortico-striatal mechanisms underlying the influence of learning and memory on motivated behavior. This interest had in part been stimulated by Anne Kelley, who had worked previously with Susan Iversen and was making a brief visit to Cambridge. She had been working with the eminent neuroanatomist Walle Nauta and demonstrated the major projections from the basolateral amygdala to the ventral striatum, especially the nucleus accumbens core and also from the hippocampal formation (ventral subiculum) to the nucleus accumbens shell (Kelley et al., 1982). These neuroanatomical data provided some of the key evidence for the nucleus accumbens as a "limbic-motor interface" in the landmark theoretical paper by Gordon Mogenson and Larry Swanson (Mogenson et al., 1980). I continue to feel great sadness when I think of Anne, a dear friend and an outstanding behavioral neuroscientist, who died so prematurely in 2007.

It was within this neuroanatomical framework that Jane Taylor, an exceptional graduate student working with Robbins, had shown that the effect of stimulant drugs to potentiate the control over behavior by conditioned reinforcers depended on the mesolimbic dopamine system innervating the nucleus accumbens. They used an acquisition of a new response procedure, which isolates the property of a Pavlovian CS to act as a conditioned reinforcer (as the new instrumental response is reinforced only by contingent presentations of the CS and never a primary reinforcer) to show that depletion of dopamine from the nucleus accumbens, but not the caudate-putamen, completely prevented the potentiating effect of intra-accumbens infusions of D-amphetamine on responding with conditioned reinforcement (Taylor and Robbins, 1986). However, nucleus accumbens dopamine depletion did not impair the conditioned reinforcement effect itself. Cador then tested our hypothesis that the association between CS and a water (later food, sex, or drug) reinforcer was formed in the basolateral amygdala, but the potentiation of the control over instrumental behavior by the CS depended on the dopaminergic modulation of its projections to the

nucleus accumbens. The results showed that basolateral amygdala lesions prevented or greatly diminished the acquisition of a new response with conditioned reinforcement and thereby prevented or reduced the potentiative effect of intra-accumbens amphetamine (Cador et al., 1989). This finding also informed the interpretation of the effects of basolateral amygdala lesions on the second-order schedule of sexual reinforcement (Everitt, 1990). It was of great interest to us that rats with basolateral amygdala lesions continued to make conditioned approach responses to the source of reinforcement, suggesting that not all Pavlovian CS influences on behavior were disrupted—something we later explored in more detail.

My research interest had by now firmly shifted to dopamine-dependent functions of the ventral and dorsal striatum and the limbic corticostriatal systems involved in motivated behavior, learning, and memory. The realization that a major effect of addictive stimulant drugs is to enhance the control over appetitive behavior by CSs strongly suggested that the future experimental investigation of drug addictive behavior should incorporate an understanding of Pavlovian and instrumental learning and memory mechanisms and their interactions. This has turned out to dominate the research of my laboratory ever since.

Alongside this emerging shift in my area of major interest, Robbins and I continued to investigate the comparative effects of manipulating monoaminergic transmission on attention, impulsivity, and learning and expanded this program to include the basal forebrain cholinergic system. The advent of immunocytochemical visualization of choline-acetyltransferase antibodies enabled this system to be mapped in the brain at a time, more or less, when the "cholinergic hypothesis" of Alzheimer's disease was formulated, suggesting a link between basal forebrain cholinergic degeneration and memory deficits in the disorder. At the time, there was no neurotoxin specific to cholinergic neurons, so some researchers had made putative lesions of the nucleus basalis of Meynert (nbM) cholinergic neurons by infusing ibotenic acid into the substantia innominata of rats and reported deficits in tests of memory. However, when I looked carefully at the brain following ibotenate infused into this basal forebrain region, I was struck by the relative sparing of magnocellular nbM cholinergic neurons and instead the major destruction of neurons of the dorsal and ventral globus pallidus. The memory impairments following ibotenate-induced basal forebrain lesions therefore seemed very unlikely to be attributable to cholinergic denervation of the cerebral cortex. It so happened that I had begun to explore the effects of other excitotoxic amino acids infused into the basal forebrain, as well as into the amygdala and striatum, and discovered following careful cellular analysis that quisqualic acid and αamino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) had quite different effects. When infused into the basal forebrain, AMPA much more effectively destroyed cholinergic nbM neurons and relatively spared pallidal neurons. Together with a

graduate student, Keith Page, we showed that this differential sensitivity to AMPA excitotoxicity was correlated with GluR4 AMPA receptor subunit (as it was known then) immunoreactivity in magnocellular cholinergic neurons and hence was a serendipitous finding of a more selective, but certainly not specific, cholinergic neurotoxin (Page and Everitt, 1995; Page et al., 1995)

The AMPA-induced lesions of the nbM in rats resulted in a marked reduction in acetylcholine markers in the neocortex, but they did not affect performance in tests of learning and memory in rats, such as the Morris water-maze and passive avoidance (Page et al., 1991). However, they did result in specific deficits in accuracy in a test of visual attentional performance (the five-choice serial reaction-time task, 5CSRTT) and these deficits were dose-dependently reversed by treatment with systemic physostigmine or nicotine (Muir et al., 1992). Intriguingly, analogous improvements in attention were shown in a clinical trial of the anticholinesterase tacrine in patients with Alzheimer's disease by Barbara Sahakian (who is also Trevor Robbin's wife and a close friend), thereby providing quite early evidence of successful preclinical-clinical translation and reverse translation. Gráinne McAlonan, who had been a very successful undergraduate medical student undertaking her research project with me and who continued to do her doctoral research in my lab, jointly supervised by Gerry Dawson at the Merck, Sharpe, and Dohme Neuroscience Research Centre (now closed), then began investigating the medial septal and diagonal band of Broca cholinergic neurons that innervate the hippocampal formation and cingulate cortex using AMPA to make relatively selective lesions. She showed that medial septal cholinergic neuron lesions resulted in major reductions in hippocampal acetylcholine, and this did produce delay-dependent deficits in spatial working memory in a delayed non-matching-to-position task in rats, which were partially reversed by low doses of physostigmine (Robbins et al., 1997). This approach was extended to investigate nbM cholinergic function in marmosets in a program led by Angela Roberts that was enabled by the first detailed description of the magnocellular cholinergic neurons and some of their projections in the brain of this species (Everitt et al., 1988).

Although the effects of selective excitotoxic lesions were correlated with cholinergic depletion in innervated structures and task performance was later correlated with acetylcholine release (Dalley et al., 2001), it was clear that further progress in analyzing cholinergic function in the forebrain required a leap forward in chemical specificity of the tools being used (as in the study of catecholamine and serotonin function). This was soon the case with the development of 192-IgG saporin, and the final studies conducted in the lab on forebrain cholinergic function using this chemically specific lesion technique generally confirmed our findings and hypotheses, for example, about cortical acetylcholine and attentional function (McGaughy et al., 2002). Later, sophisticated research by, among others, Martin Sarter

(with whom Jill McGaughy had worked before her postdoc in the lab) went on very successfully to deploy selective new tools and to provide a much more detailed and precise understanding of cholinergic basal forebrain function.

My interest in the functions of the amygdala was especially stimulated by our research on the effects of lesions of the basolateral amygdala on appetitive conditioning, because at the time, the great majority of amygdala research was focused on fear conditioning (now much more frequently called threat conditioning, as promoted by Joe LeDoux who suggested researchers should be more cautious about inferring subjective states in animals, a view I share). Indeed, some leading amygdala researchers stated firmly that the amygdala is specialized for processing fear, despite the early fundamental findings published in a landmark paper by a scientific hero of mine, Larry Weiskrantz (Weiskrantz, 1956), showing this could not be the case. However, axon-sparing excitotoxic lesions now offered a significant new and relatively selective tool with which to probe possibly dissociable functions of different amygdaloid nuclei and their associated circuitries. Together with a graduate student, Lawrence Dunn, we already had shown that passive avoidance, conditioned taste aversion, and taste neophobia could be dissociably affected by discrete amygdala and gustatory insular cortex excitotoxic lesions, demonstrating that some effects previously attributed to the amygdala following more gross manipulations were most likely the result of interruption of projections to and from the insular cortex that passed through the medial temporal lobe (Dunn and Everitt, 1988).

Simon Killcross, who had completed a doctorate with Trevor Robbins and joined our Human Frontier Science Programme (HFSP) grant as a postdoc, made the significant discovery of dissociable effects of selective excitotoxic central and basolateral amygdala lesions on two different responses to conditioned threat stimuli within the same task—that is, conditioned suppression (automatic, reflexive) and punishment avoidance (volitional, choice behavior). Rats with lesions of the central nucleus showed a marked attenuation of conditioned suppression of appetitive responses when an aversive CS was presented, but they were still able to direct their actions to avoid further presentations of the aversive CS. In contrast, animals with lesions of the basolateral amygdala were unable to avoid the conditioned aversive CS by their choice behavior, but they showed normal conditioned suppression (Killcross et al., 1997). Thus, animals were both impaired and unimpaired in their conditioned fear behavior depending on the nature of the response to the conditioned threat CS being measured. This was heresy of course, as the dominant serial processing model of amygdala function in conditioned fear required that the CS-unconditioned stimulus (US) association occurred in the basolateral amygdala that relayed this associated information to the central nucleus to effect automatic behavioral (e.g., freezing, suppression), endocrine, and physiological fear responses through its direct projection to hypothalamic and brainstem sites. Such was the dominance

of this model that experimental data that did not conform were classed as rogue (one commentary suggested that although our data were interesting, the task we used was complex, so the data should be viewed with suspicion whilst the model itself should not be questioned!).

One conclusion from our study, however, was not that the notion of serial processing within basolateral amygdala-central nucleus circuitry should be rejected as clearly it explained many data, but that some forms of aversive learning did not require basolateral amygdala-central amygdala serial processing. Our results showed that the central amygdala nucleus could mediate some forms of threat conditioning and fear responses in the absence of the basolateral amygdala and vice versa. The results also emphasized that basolateral amygdala outputs to other structures, especially the ventral striatum and prefrontal cortex, should be taken into account when considering associative influences not only on appetitive (as our conditioned reinforcement experiments had shown) but also on aversive behavior. Optogenetic, chemogenetic, and cellular imaging methodologies have now moved the field well beyond the macroscopic analysis of amygdala function that was possible then and have revealed much more precisely the complexities of discrete and seemingly highly specialized amygdala circuits involved in appetitive and aversive behavior, for example, through the work of Patricia Janak, Kay Tye, and Andreas Luthi.

From Anatomy to Experimental Psychology in Cambridge: Pavlovian-Instrumental Interactions and Addiction Research

By the late 1980s to early 1990s, I had made the decision to orient my lab increasingly toward the field of experimental drug addiction research, but I was in impossibly inadequate research space and increasingly isolated in the Department of Anatomy in Cambridge, although still collaborating very successfully with Trevor Robbins in the Department of Experimental Psychology. Following rather intense discussions with the university when I had come very close to accepting a position in another university, it suddenly became possible to move my entire lab and readership to the Department of Experimental Psychology. This would never have been possible without the incredible support of Nick Mackintosh, the preeminent learning theorist and then chair of the department. This enabled Robbins and me, to establish a joint behavioral neuroscience laboratory, having been planning and scheming to do so for more than a decade. The combination of Mackintosh's negotiating skills and the university's considerable financial investment eventually delivered both new wet labs and a new animal facility with, finally, specially designed animal behavioral facilities.

As I moved to the Department of Experimental Psychology, which felt very much like coming home, one of those magical moments that can happen

during the life of a lab took place in mine with the simultaneous arrival of an exceptional group of graduate students: John Parkinson, Rutsuko Ito, Jeremy Hall, Anastasia Christakou, and Rudolf Cardinal, followed soon afterward by Jonathan Lee and Amy Milton. They helped initiate and progress the research programs on appetitive conditioning and the differential involvement of the amygdala, hippocampus, and prefrontal cortex; their circuit interactions with the ventral and dorsal striatum; and the modulation of these circuits by the dopamine system. It rapidly became clear that manipulations of the basolateral amygdala and central amygdala resulted in dissociable outcomes in appetitive Pavlovian conditioning tasks, as we had seen in aversive tasks (Everitt et al., 2003). For example, John Parkinson and Jeremy Hall showed that central nucleus lesions prevented the acquisition of conditioned Pavlovian approach behavior (more often now called "sign tracking") (Parkinson et al., 2000b). Patricia Robledo, a postdoc in the lab, had previously shown that similar central amygdala lesions, although not affecting conditioned reinforcement, prevented the potentiating effect of D-amphetamine infused directly into the nucleus accumbens (Robledo et al., 1996). This was quite different than the effects of basolateral amygdala lesions and suggested a functional relationship between the central amygdala and activity of the midbrain dopaminergic (DA) system.

Further analysis revealed that central nucleus, but not basolateral amygdala, lesions prevented Pavlovian-to-instrumental transfer (PIT), the motivational impact of a noncontingently presented reward-associated CS to boost separately trained instrumental responses for that reward (Hall et al., 2001b). The involvement of amygdala subsystems in PIT was later analyzed in greater detail by Bernard Balleine and colleagues who showed that the basolateral and central amygdala mediated specific and general PIT, respectively, with the latter reflecting more general arousal elicited by a motivationally salient CS (Corbit and Balleine, 2005). Michela Gallagher and Peter Holland, then at Duke University, with whom we shared an HFSP grant with Betsy Murray at the National Institutes of Health (NIH), also showed that some Pavlovian-conditioned responses, such as orienting to a food-predictive CS, depended on the central amygdala but not on the basolateral amygdala and that this too involved circuitry linking the central nucleus of the amygdala with DA neurons in the midbrain and thence the striatum (Han et al., 1997).

These investigations of amygdala function emphasized to me something that I believe even more now, which is that a strong learning theory framework and a clear understanding of the psychological processes engaged in behavioral tasks is essential for our understanding of neural circuit function. I feel fortunate to have had Tony Dickinson as a colleague in the department and as a collaborator and friend. He helped to inspire the graduate students and postdocs working in the lab to bring learning theory and behavioral neuroscience closer together, something Robbins and I had been committed

to for some time. Rudolf Cardinal was especially concerned to provide a learning theory explanation for the increasing number of dissociable functions of the central and basolateral amygdala (Cardinal et al., 2002). This was a particular challenge for the central nucleus of the amygdala, which generally had been viewed simply as a downstream effector nucleus of the basolateral amygdaloid complex. Even though receiving afferent connections appropriate to support them, no direct evidence suggested that the central amygdala nucleus is itself a site of Pavlovian association as it might, indeed does, receive an already-associated input. Yet it was the case that animals lacking a basolateral amygdala were able to form some kinds of association, the conditioned expression of which was sensitive to central, but not basolateral amygdala, lesions or inactivations, and therefore, the locus of Pavlovian conditioning was unlikely solely to be the lateral and basal amygdala (Cardinal et al., 2002).

Considering the kinds of conditioned responses that seemed to depend on the central amygdala nucleus, our initial analysis led us to suggest that it does form simple CS–unconditioned response (UR; "sensorimotor") associations that do not depend on a specific US and that are independent of the identity and current motivational value of the US, and therefore, the central amygdala is also unable to support second-order conditioning. We further suggested (Everitt et al., 2000) that the responses subserved by central amygdala-dependent associations especially included the modulation of reflexes organized within the brainstem, including some that might conventionally be regarded as "affective," such as conditioned suppression, conditioned orienting, and PIT that are disrupted by central but not basolateral amygdala manipulations. We proposed that responses such as conditioned suppression may influence instrumental behavior nonspecifically (i.e., by influencing the ongoing level of all instrumental responses) but are insufficient to modulate instrumental behavior differentially (i.e., affect choice). We further suggested that the basolateral amygdala stores the associations that allow the CS to retrieve the affective or motivational value of its particular US, a form of Pavlovian stimulus–outcome association (Cardinal et al., 2002). This associative information can then, through its major projection to the central amygdala and then to hypothalamic, midbrain, and brainstem targets, control "affective" responses, such as freezing or fear-potentiated startle, and can modulate arousal and attention. The other perhaps major realization from our research was that Pavlovian representations in the basolateral amygdala also can influence appetitive instrumental actions, for example, through projections to the ventral striatum.

Very much related to this analysis of amygdala function was my increasing interest in the striatum, particularly the ventral striatum, because of its connectivity with the amygdala and also with the hippocampus and areas of the prefrontal cortex. The finding that amygdala-dependent conditioned reinforcement was potentiated by DA transmission in the nucleus accumbens

confirmed that there are behaviorally important interactions between these structures. This led us to explore in more detail behavioral processes dependent on the nucleus accumbens, informed by the then-recent description of discrete core and shell compartments each with its own relatively specific connections (Jongen-Relo et al., 1994; Groenewegen et al., 1999). Parkinson, Hall, and Cardinal working independently and collaboratively with each other and with me undertook a series of studies to show that the nucleus accumbens is a key site mediating the ability of Pavlovian CSs to invigorate and direct behavior, as measured in sign-tracking, PIT, and conditioned reinforcement tasks, with core and shell subregions being differentially involved in these processes, but that the nucleus accumbens itself is not required for goal-directed instrumental behavior (Cardinal et al., 2002). For example, sign-tracking was shown to depend on the nucleus accumbens core but not the shell (Parkinson et al., 1999). Moreover, in our autoshaping procedure that measured sign-tracking, designed by Tim Bussey, in which visual CSs were presented on a touchscreen that was distant from the location of reward, anterior cingulate cortex connectivity with the nucleus accumbens core was shown to be critical for the development of a discriminated conditioned approach (sign-tracking) (Parkinson et al., 2000a). Thus, we were beginning to define separable and quite specific functions of discrete limbic cortical-nucleus accumbens circuits, having previously shown that disconnecting the basolateral amygdala and nucleus accumbens (the disconnection approach will be discussed more later) impaired the acquisition of conditioned "place" preference (Everitt et al., 1991). (In fact, the commonly used conditioned place preference procedures do not truly measure "place" preference unless specially configured to do so, but instead measure learned preference for often-unspecified specific cues in the conditioning context, as will be discussed further.)

The nucleus accumbens core, but not shell, was then shown to be essential for PIT, the potentiation of instrumental responses for food by a food CS (Hall et al., 2001b). I still regret that having made these initial observations about the involvement of the amygdala and nucleus accumbens in PIT, we did not go further and investigate the neural basis of specific and general PIT (but for good reason, as the lab was increasingly focused on behavior related to drug addiction). However, Balleine, who had earlier worked in Cambridge with Tony Dickinson, did do so and, in an impressive series of experiments, showed that specific PIT depended on basolateral amygdala-nucleus accumbens shell circuitry, while general PIT (which we had been measuring in our experiments) depended on the central amygdala and nucleus accumbens core, likely though their interconnection via midbrain DA neurons (Corbit and Balleine, 2011). Indeed, both PIT and sign-tracking were shown to be markedly affected by manipulations of the mesolimbic DA system innervating the nucleus accumbens (Parkinson et al., 2004), which has since been specified more precisely in the work on sign-tracking by Shelley Flagel and colleagues (Flagel et al., 2011).

In pursuing the different functions subserved by the nucleus accumbens core and shell, John Parkinson and Cella Olmstead, a visiting postdoc from Canada, showed that the potentiative effect of psychostimulants on conditioned reinforcement depended specifically on the nucleus accumbens shell, not the core, whereas conditioned reinforcement required the nucleus accumbens core interacting with the basolateral amygdala (Parkinson et al., 1999). The way in which the Pavlovian-instrumental transfer effects, whether PIT or conditioned reinforcement, mediated by amygdala connectivity with the nucleus accumbens core can be modulated by dopaminergic mechanisms specifically in the nucleus accumbens shell still remains fully to be explained.

One of the features of ventral striatal organization that intrigued me at that time was the then recently observed relative compartmentalization of amygdala and hippocampal (ventral subiculum) afferents to the nucleus accumbens core and shell, respectively. Nathan Selden had earlier shown that the amygdala and hippocampus were differentially required for CS and contextual conditioning, respectively, in an aversive trace conditioning paradigm (Selden et al., 1991). Together with Cyriel Pennartz, with whom Robbins and I had secured a second HFSP collaborative grant, we hypothesized that these, in some sense opposing, functions of amygdala and hippocampus (reflecting the earlier ideas of Alexander Cools) were mediated by differentiated projections to the nucleus accumbens core and shell. Rutsuko Ito, now a postdoc having completed her doctoral research (see next section) undertook these challenging experiments in a task that was painstakingly designed together with Carol Barnes and Bruce McNaughton (who were collaborators within the same HFSP grant). Rats were trained sequentially to acquire discrete CS–sucrose conditioning, followed by spatial context–sucrose conditioning in a uniquely designed place preference apparatus characterized by three topographically identical chambers that were discriminable only on the basis of "place" (i.e., path integration). Selective excitotoxic lesions of the nucleus accumbens shell selectively impaired the acquisition of conditioned place preference (truly "place" preference) and the use of spatial information to retrieve information about a discrete CS, whereas nucleus accumbens core lesions attenuated the acquisition of discrete CS responses (Ito et al., 2008). However, the remarkable finding was that disconnection of the hippocampus from the nucleus accumbens shell using unilateral asymmetric lesions of each structure resulted in a pattern of impairment in place conditioning and context-dependent CS retrieval that was very similar to that produced by bilateral nucleus accumbens shell lesions. These results allowed us to conclude that the nucleus accumbens core and shell subserved distinct associative processes but also that the shell and hippocampal formation were functional components of a limbic corticostriatal network involved in spatial context conditioning (Ito et al., 2008).

Our explorations of ventral striatal function revealing that the nucleus accumbens is a key site mediating the ability of Pavlovian CSs to invigorate and direct behavior took another intriguing and exciting turn when Cardinal showed that when rats were offered the choice of an immediate, small reward or a larger, delayed reward, selective lesions of the nucleus accumbens core severely impaired their ability to choose the delayed reward; in other words, they made impulsive choices (Cardinal et al., 2001). This demonstration that the nucleus accumbens is required for impulse control was to have a major impact on our later research on vulnerability to stimulant addiction (Belin et al., 2008).

Neural and Psychological Mechanisms Underlying Addictive Behavior

Robbins, Dickinson, and I began developing our idea that understanding the neuropsychological basis of addiction to stimulants and other drugs may be enhanced by applying theories of learning developed from the more general study of motivational processes and that this might have implications for future therapeutic strategies in the clinic. Much progress had been made, especially by researchers in the United States where the drug addiction field was and continues to be extremely well supported, in defining the specific targets of addictive drugs in the brain and showing that the dopaminergic innervation of the nucleus accumbens is of major importance in mediating the reinforcing effects of addictive drugs. Moreover, particularly through the work and theorizing of Terry Robinson and Kent Berridge (Robinson and Berridge, 1993), attention was already being paid to Pavlovian conditioning mechanisms and adaptations in the mesolimbic DA system to repeated drug exposure to provide an explanation for drug craving. We instead had the notion that the reinforcing effects of addictive drugs, stimulants in particular, depended not only on their initial molecular and cellular sites of action resulting in increased dopamine transmission in the nucleus accumbens, but also on interactions with the associative information conveyed to the nucleus accumbens by its limbic cortical afferents. We felt that progress in the field required more than even a detailed molecular understanding of how drugs act as reinforcers (or "rewards"), or how CSs associated with drug rewards elicit craving as emphasized in Pavlovian theories of addiction, but not readily amenable to operationalization in non-human species. It seemed to us that the fact that individuals must seek and procure drugs before they can take them was extremely important, yet this was relatively neglected in experimental approaches to addiction and therefore that the neural mechanisms of instrumental learning must also be incorporated into explanations of addictive behavior.

Our new approach relied considerably on Dickinson's original analyses of conditioning with conventional reinforcers showing that the general concept of instrumental positive reinforcement conflates at least two

different processes. The first process is the classic stimulus-response (S-R) mechanism by which reinforcers strengthen an association between the response and the contextual and discriminative stimuli present at the time of reinforcement. Behavior controlled by this process is habitual, elicited by these stimuli, divorced from the value of the outcome and autonomous. By contrast, the second process is cognitive and based on knowledge of the causal relation between the instrumental behavior and reinforcement. When controlled by this process, instrumental behavior takes the form of intentional, goal-directed actions that are performed because an individual knows that these actions give access to the reinforcer or outcome and therefore are dependent on the value of the goal (Everitt et al., 2001). We therefore were heading into new and difficult territory as these concepts had not been applied to the study of drug reinforcers, not least because it involves the practical complication of long-term intravenous drug self-administration, a technique that we had somehow to acquire from scratch under strict United Kingdom Home Office regulatory oversight that is a challenge to this day.

We acquired the intravenous self-administration technique during two visits to the lab by Athina Markou, who had recently completed her doctorate with George Koob at the Scripps Institute and who had exquisite technical skills as well as exceptional theoretical insight into the neuroscience of addiction. Athina and her husband Mark Geyer became close family friends, and together with my wife Jane and daughter Jessica, we later spent a wonderful vacation together in her Greek homeland. Tragically, Athina died in 2016 after four years of coping with extremely challenging treatments for cancer; her loss is still acutely felt.

Robbins, Dickinson, and I were then able to initiate our new experimental approach to understanding drug addiction which took the clinical insight embodied within the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV; now DSM-V) criteria for "substance dependence" and sought to decompose and augment this in terms of specified learning and cognitive processes deriving from the rich body of animal learning theory that was in that moment being mapped onto specific neural systems. Our investigation of neurobehavioral constructs related to a psychiatric disorder, avoiding descriptions of behavior in terms of subjective states (such as reward, liking, wanting, or craving) in some sense prefaced the embracing of this approach in the Research Domain Criteria (RDoc) of the NIH. Our overarching hypothesis was that drug addiction can be understood as the aberrant engagement of Pavlovian and instrumental learning processes that underpin drug seeking and drug taking (Everitt et al., 2001). We felt then and continue to think now that the distinction between drug seeking—foraging for drugs over long delays with uncertain outcomes—and drug taking—proximal engagement with and ingestion of a drug—is critically important. In a sense, this is a reexpression of the earlier, widely

accepted distinction between appetitive and consummatory behavior. The field of ingestive behavior had already embraced the notion of distinctive and dissociable neural mechanisms engaged in foraging for and ingestion of food rewards, but the addiction field was not yet ready to do so.

Our first project investigated the role of so-called drug cues in drug seeking and relapse focusing on the amygdala and its interactions with the nucleus accumbens core that we had already established as being important for conditioned reinforcement. To do this, my graduate student Mercedes Arroyo, working with Athina Markou, adapted the second-order schedule of reinforcement that I had employed to study sexual motivation, but now with intravenous cocaine as the primary reinforcer. Second-order schedules had of course been used previously to study responses for drugs by researchers at NIDA, but a key difference in our approach was to focus on each daily, extended period of cocaine seeking before any drug infusion, as this offered a way to measure the motivation for a drug uncontaminated by its behavioral effects once self-administered (stimulants like cocaine enhance responding whereas opiates suppress it, confounding motivational measures) (Everitt and Robbins, 2000). We were able to establish that rats would work for 15 minutes or longer making up to 300 responses for a single infusion of cocaine if, and only if, a cocaine-associated CS was presented contingent on responding during this drug-free seeking period. If the CS was not presented, cocaine seeking collapsed (Arroyo et al., 1998). Furthermore, if the dose of cocaine was increased on one day, rats worked harder the next day, whereas if the dose was decreased, they worked less (Markou et al., 1999). This is quite the opposite outcome of increasing or decreasing drug dose in simple drug-taking sessions (under continuous reinforcement schedules of reinforcement) in which regulatory processes dominate as rats titrate their optimal blood levels of cocaine, as importantly shown by Roy Wise (Gerber and Wise, 1989). We then showed that excitotoxic lesions of the basolateral amygdala prevented the acquisition of cue-controlled cocaine seeking, but had no effect on cocaine self-administration (i.e., cocaine reinforcement) (Whitelaw et al., 1996), further emphasizing the distinction between the seeking and taking of drugs. The circuit linking the basolateral amygdala with the nucleus accumbens core is therefore required for conditioned reinforcement and hence also for the acquisition of drug seeking; this we have recently confirmed by specifically inactivating this circuit using Cre-mediated DREADD expression in basolateral amygdala neurons projecting to the core.

As a graduate student, Rutsuko Ito made a series of significant findings when investigating striatal mechanisms in both the acquisition and well-trained performance of cocaine seeking. First, she showed that selective lesions of the nucleus accumbens core greatly impaired the acquisition of cocaine seeking, but had no effect on cocaine self-administration. Selective nucleus accumbens shell lesions had no effect on the acquisition

of cocaine self-administration, but instead eliminated the potentiative effect of cocaine on responding for the CS (Ito et al., 2004), which was a further demonstration that the shell is a key site mediating the response-amplifying effects of stimulant drugs. Ito, in collaboration with Jeff Dalley, also used *in vivo* microdialysis to measure extracellular DA during prolonged sessions of cocaine-seeking behavior in rats that had been well trained over about a two-month period. These experiments led to the initially surprising finding that during one hour of responding maintained by the cocaine-associated CS, there was no increase in dopamine in the nucleus accumbens core or shell; only after cocaine infusion did dopamine increase in both regions, somewhat more in the shell than in the core. Noncontingent, unexpected presentations of the cocaine-associated CS, however, did increase dopamine, but selectively in the nucleus accumbens core (Ito et al., 2000). The most remarkable finding was the marked increase in extracellular DA in the anterior dorsolateral striatum during the hour when rats were responding with conditioned reinforcement and to a level that was as high as that later caused by the cocaine infusion (Ito et al., 2002).

These and other observations led us to propose the overarching hypothesis, presented in a now highly cited review (Everitt and Robbins, 2005), that drug addiction can be seen as the endpoint of a series of transitions from initial drug use, when a drug is voluntarily taken because it has rewarding and other effects, through loss of control over drug seeking that eventually emerges as a compulsive, maladaptive habit elicited and controlled by salient environmental stimuli. We proposed that these transitions depend on interactions between Pavlovian and instrumental learning processes that may be aberrantly engaged through the actions of self-administered, addictive drugs. The progression from voluntary to habitual drug seeking, we hypothesized, was underpinned by a transition from ventral to dorsal striatal mechanisms mediating the Pavlovian-instrumental interactions controlling behavior. However, we further proposed that the compulsive quality of drug seeking in addicted individuals required an additional mechanism of diminished prefrontal cortical control over behavior that is a consequence of chronic drug use. There was already considerable functional imaging evidence for prefrontal cortical hypofunction from studies of humans addicted to drugs, most notably by Nora Volkow and colleagues (Volkow et al., 2004).

I have been extremely fortunate that many exceptionally talented postdoctoral as well as graduate student researchers have come to work in my lab in Cambridge to undertake experimental addiction research and to test these hypotheses across the drugs cocaine, heroin, and alcohol. They are very difficult experiments that require a dedication to understanding addictive behavior and great persistence to make progress. It has been interesting to see how challenging the notion of "compulsive habits" (Everitt et al., 2008), or even habits, has been to some in the field. It has

been suggested that this is because it trivializes the problem of addiction that faces society, perhaps because it is too easy to dismiss it as a "bad habit," which I've always found surprising. But when it was suggested to me by an experimental addiction researcher at the end of a talk in Mannheim that "people don't have habits, so how can they be important in addiction?" it became clear that it would be very difficult for many to accept the notion that the instrumental behavior of drug seeking might, at some point, be something other than the expression of goal-directed motivational responses. It still is.

A challenging aspect of probing the goal-directed or habitual associative structure underlying drug seeking is that: (1) it pertains to preparatory and not consummatory responses, and (2) the key tests of reinforcer devaluation and contingency degradation are not easily applied to addictive drugs, especially when administered intravenously. However, in rats drinking alcohol and also cocaine (which is not the usual mode of cocaine self-administration), reinforcer devaluation induced by postingestion gastric malaise revealed that habitual responding developed more rapidly for both drugs than for a food reinforcer (Dickinson et al., 2002; Miles et al., 2003), suggesting the potentially aberrant engagement of the habit system by addictive drugs. We have taken the shift to anterior dorsolateral striatal control over drug seeking to be a proxy for engagement of the habit system, because it has been well-established from studies of responding for food (where devaluation is much easier to achieve) that this striatal domain is required for habit learning, whereas the posterior dorsomedial striatum is required for goal-directed behavior (Balleine et al., 2009). Louk Vanderschuren, a visiting postdoc, demonstrated that the dorsolateral striatum was causally involved in well-established cocaine seeking by showing that infusions of the DA receptor antagonist, alpha-flupenthixol, into this site dose-dependently decreased the performance, or maintenance, of cocaine seeking measured in our now-routine second-order schedule procedure, whereas similar infusions into the nucleus accumbens at the same stage had no effect (Vanderschuren et al., 2005).

Perhaps one of the most significant breakthroughs came when David Belin joined the lab as a visiting postdoc from Bordeaux; he is now a tenured member of the faculty in Cambridge, a collaborator and close friend. He tackled the conundrum of how ventral and dorsal striatal processes were intertwined and how, if indeed it was the case, dorsal striatal mechanisms could come to exert dominant control over drug-seeking behavior. I had speculated, following Suzanne Haber's remarkable demonstration of spiraling, serial connectivity between the ventral and dorsal striatum mediated by recurrent connections with midbrain DA neurons (Haber et al., 2000), that this might provide a neural circuit substrate for such functional interactions. Belin, who is a brilliant experimentalist, developed a unique ventral-dorsal striatal disconnection procedure by combining a unilateral lesion of

the nucleus accumbens core with a contralateral DA receptor antagonist infusion into the anterior dorsolateral striatum, thus functionally disconnecting the putative circuit bilaterally (Belin and Everitt, 2008). Again adopting the second-order schedule cocaine-seeking task in which we knew the dorsolateral striatum exerted dominant control over this behavior when well established, Belin showed that the disconnection greatly decreased cocaine seeking, yet had no effect at all on responding in a newly acquired, goal-directed task. This remarkable finding gave credence to the functional importance of nucleus accumbens-midbrain DA neuron-dorsal striatal functional connectivity. It gained further support from a collaborative *in vivo* voltammetry study showing that in a cocaine self-administration setting, the development of dorsal striatal DA transients depended on the integrity of the ipsilateral nucleus accumbens core (Willuhn et al., 2012).

We were now beginning to be able to draw a clearer picture of amygdalo-striatal circuitry mechanisms in cocaine seeking, with the basolateral amygdala-nucleus accumbens core circuit mediating conditioned reinforcement and the acquisition of cocaine seeking and the anterior dorsolateral striatum being required for habitual, well-established cocaine seeking, together with a circuit basis for ventral-dorsal striatal interaction. Jennifer Murray, another talented postdoc experimentalist, made the important observation that dopamine receptor blockade in a different site, the posterior dorsomedial striatum, prevented goal-directed cocaine seeking early in acquisition, but was without effect when well established. By contrast, anterior dorsolateral striatal DA receptor blockade had the opposite pattern of effect, decreasing habitual responding having no effect at an earlier stage—a double dissociation (Murray et al., 2012).

The data presented the conundrum of how the amygdala processing of conditioned reinforcers that support drug seeking could interact with the anterior dorsolateral striatum, a structure to which it does not project. Aude Belin-Rauscent now joined this project with David Belin in Poitiers, France, together with Jenn Murray and me in Cambridge under the auspices of a European Associated Laboratory funded by INSERM and the MRC. Murray combined functional disconnections of basolateral or central amygdala with the dorsolateral striatum in a behavioral study; Belin-Rauscent recorded from medium spiny neurons in the dorsolateral striatum while electrically stimulating the basolateral amygdala to investigate the underlying circuitry (Murray et al., 2015). The data revealed that recruitment of dorsolateral striatum, dopamine-dependent control over cocaine seeking is triggered by the basolateral amygdala via the nucleus accumbens core, while the long-term maintenance of the drug-seeking habit depends instead on the central amygdala interacting with the dorsolateral striatum. Moreover, stimulating basolateral amygdala neurons up- and down-regulated anterior dorsolateral striatum medium spiny neurons by a mechanism that depended on the functional integrity of the nucleus accumbens core. We still do not know

for certain, but strongly hypothesize, that this involves the serial connectivity between the nucleus accumbens, midbrain dopamine neurons, and the dorsal striatum. This putative and complex circuitry continues to be investigated in the lab using optogenetic and chemogenetic techniques.

The second-order schedule of reinforcement, capturing as it does the important role of drug cues acting as conditioned reinforcers to enable an individual to mediate delays to drug taking, has served us well as a behavioral approach to studying drug seeking. However, it was apparent early on that a different behavioral procedure was required to enable us to study instrumental processes sensitive to the contingencies between actions (or responses) and outcomes and also to distinguish between drug seeking and drug taking that depend on different psychological as well as neural mechanisms.

A visiting postdoc from Canada, Cella Olmstead, initially took on this challenge with Tony Dickinson and me and established a heterogenous chained schedule of cocaine reinforcement in which seeking responses were spatially and temporally distinct from taking responses. Responses on a seeking lever under a random interval schedule were never reinforced, but gave access to a second, taking lever, responding on which delivered intravenous cocaine. In initial studies, this methodology revealed the complex interplay between regulatory, activating, and reinforcing effects of self-administered cocaine (Olmstead et al., 2000). In an equally fundamental study of heroin seeking and taking, we established that opiate-dependent rats learned about the enhanced incentive value of heroin when in withdrawal only if they had previously had the opportunity to self-administer the drug while in the withdrawal state. This observation showed that heroin engages appetitive motivational mechanisms in the withdrawn state, in contradistinction to theories emphasizing only negative reinforcement, and was an affirmation of Dickinson's theory of incentive learning applied to the motivation for drugs (Hutcheson et al., 2001).

An especially valuable outcome of investing time and effort in developing an instrumental chained schedule of intravenous drug reinforcement was that it enabled a direct test of our hypothesis that drug seeking becomes habitual when performed over an extended period, becoming resistant to reinforcer devaluation. Because it is difficult, if not impossible, to deploy sensory-specific satiety or gastric malaise to devalue intravenous cocaine as opposed to an orally ingested food or drug reward, our approach was instead to devalue the outcome of seeking responses by extinguishing the taking response. This enabled us to probe the underlying associative structure of seeking: were rats working on the seeking lever to get the opportunity to take drug (i.e., a goal-directed action)? Or was responding a manifestation of stimulus-response learning, independent of the value of the outcome and habitual? Olmstead quickly showed that after limited training, cocaine seeking was indeed goal directed, as extinction of the taking response

led to a prompt and significant decrease in seeking responses (Olmstead et al., 2001). The same was true in a sucrose seeking-taking chain. We were beaten to the post, however, in the key test of our hypothesis that after a protracted history of seeking and taking cocaine, seeking would be resistant to the devaluation manipulation. Agustin Zapata in Toni Shippenberg's lab at NIDA adopted our methodology and replicated our initial demonstration that cocaine seeking was goal-directed after a short drug history (replications in complex behavioral experiments are always reassuring). Importantly, they further showed that cocaine seeking became habitual and resistant to devaluation after protracted training and also that the anterior dorsolateral striatum was critically involved in mediating this transition (Zapata et al., 2010). I am sad to mention here that Toni Shippenberg, a warm and generous friend, died in 2012.

The cocaine seeking-taking chained procedure also gave us the opportunity to develop a model of compulsive drug seeking by unpredictably punishing seeking responses such that animals had to run this risk of danger and punishment to gain access to and respond on the taking lever to receive a cocaine infusion. This seeking-taking punishment task, which in one form or another is now used by a number of labs around the world, was the result of the exceptional work of Yann Pelloux when a postdoc in my lab (Pelloux et al., 2007; Pelloux et al., 2015). I cannot pay sufficient tribute to his dedication and meticulous, patient work that transformed our research and also provided one of its biggest challenges: having to incorporate individual differences in behavior when investigating the neural mechanisms of, and the vulnerability to, compulsive drug seeking in addiction. The major finding by Pelloux was that after a brief cocaine-taking history, all rats ceased seeking cocaine when they contingently and unpredictably were punished in a proportion of trials in a session; they voluntarily abstained. However, after a prolonged history of cocaine exposure, about 80% of rats abstained from seeking cocaine, but 20% persisted despite punishment; in other words, they were compulsive (Pelloux et al., 2007). This observation has been replicated many times both in Cambridge and elsewhere. Because only 20% of each experimental cohort of rats becomes compulsive, exploring its neural basis is very challenging. Nevertheless, Pelloux was able to show that an evolving deficit in forebrain serotonin transmission is causally involved in compulsive cocaine seeking (Pelloux et al., 2012). Sietse Jonkman, another exceptional graduate student and later a postdoc in the lab, showed the importance of an extensive history of cocaine intake in the development of compulsion (Jonkman et al., 2012a) and also that a discrete domain of the anterior dorsolateral striatum was specifically involved in mediating seeking under punishment (Jonkman et al., 2012b).

The seeking-taking-punishment task also has been modified to measure compulsive alcohol seeking by Chiara Giuliano, an exceptionally talented behavioral neuroscientist, to show that in rats with a preference for alcohol,

a subpopulation persists in seeking alcohol in the face of punishment and are therefore vulnerable to develop the compulsive alcohol seeking characteristic of alcohol addiction (Giuliano et al., 2018). Even more significantly, she successfully directly tested our key hypothesis that alcohol-seeking habits predict and underpin the emergence of compulsive alcohol seeking in a vulnerable population and that this propensity is associated with engagement of, and an inability to disengage, the anterior dorsolateral striatal control over alcohol seeking (Giuliano et al., 2019). This perhaps is the clearest demonstration of the maladaptive, inflexible nature of compulsive alcohol-seeking habits (Belin et al., 2013) and is a continuing major research topic in the lab.

The observations on differing susceptibility to develop compulsive drug seeking naturally raised the question as to what the neurobehavioral vulnerability trait or traits in addiction might be. My collaborator and friend Jeff Dalley had made the serendipitous discovery that a subpopulation of rats performing the 5CSRTT were impulsive, responding prematurely instead of waiting for stimulus presentations before making a response choice. These high-impulsive rats, while not differing in their propensity to self-administer cocaine, escalated their intake much more than low-impulsive animals. High-impulsive animals also had lower levels of D2/3 DA receptors in the nucleus accumbens that were highly correlated with their impulsivity before any exposure to cocaine (Dalley et al., 2007). When David Belin arrived in the lab from Bordeaux to work with me, having there established the three-criteria model of addiction that also encapsulated the core notion of individual vulnerability to compulsivity (Deroche-Gamonet et al., 2004), he tested the hypothesis that high impulsivity is a predisposing factor in the development of cocaine "addiction," but most especially compulsivity—the self-administration of cocaine in the face of punishment. The results showed that trait high impulsivity, but not high reactivity to novelty, predicted the emergence of compulsive cocaine self-administration and thereby that impulsivity and its neural basis represent an endophenotype for cocaine addiction (Belin et al., 2008). Karen Ersche, working with Trevor Robbins, subsequently tested this hypothesis in humans, adopting an endophenotype strategy to show that impulsivity is a vulnerability trait in stimulant addiction (Ersche et al., 2010). Daina Economidou, an extremely talented postdoc from Crete further showed that high impulsivity also predicted relapse to cocaine seeking after punishment-induced abstinence in the seeking-taking-punishment task and that the propensity to relapse was decreased by the selective noradrenergic reuptake inhibitor, atomoxetine, suggesting a novel therapeutic approach (Economidou et al., 2009). Tragically, while in a wonderfully productive phase of her research and on the threshold of establishing an independent career, Daina was diagnosed with adenocarcinoma of the lung and died just 18 months later while at home with her family in Nicosia. The study of vulnerability traits predicting compulsivity in cocaine,

heroin, and alcohol addiction continues to be a major research direction in the Cambridge lab, using a combination of *in vivo* imaging, molecular, and circuit tools.

The Search for Treatments to Prevent Relapse to Drug Addiction

I have always believed that the greater the understanding of the neural and psychological mechanisms involved in the compulsive pursuit and use of drugs, the more likely it will be that treatments for addiction will be developed that have a rational basis (Everitt, 2014). There is an enormous unmet clinical need given the devastating impact that addiction has on the lives of individuals, their families, healthcare systems, and society. Sadly, this has always been a Cinderella area in pharmaceutical research, and there is little sign of specific new treatments being developed at present. The approach that I began in the lab was, first, to explore the effects of both new and repurposed drugs as treatments that might decrease drug seeking and relapse in our models.

Our first major success in using this approach was the result of a collaboration with Pierre Sokoloff and Jean-Charles Schwartz in Paris, funded under an EU program, investigating a novel partial agonist at the recently discovered D3 dopamine receptor that they had shown to be enriched in the nucleus accumbens and amygdala. An Italian graduate student, Maria Pilla, joined the lab under this collaboration and showed that this new drug, BP897, selectively reduced cocaine seeking under a second-order schedule of cocaine reinforcement, but had no effect on cocaine self-administration (Pilla et al., 1999). A little later, a full D3 dopamine receptor antagonist was developed by GlaxoSmithKline (GSK) and this we showed had an even more profound and seemingly behaviorally selective effect to reduce cocaine seeking (Di Ciano et al., 2003). Further studies by other groups confirmed this effect of antagonism at the D3 dopamine receptor and also that there were apparently few or no unwanted behavioral effects, for example, on motor activity. A division of GSK in Verona began actively developing this lead and a potentially novel antirelapse treatment for cocaine addiction seemed possible, but alas safety concerns about D3 dopamine receptor antagonists caused GSK to end its program, and to date, there is no sign that this exciting preclinical lead will result in a clinically approved treatment. A salutary lesson.

Another perhaps even more promising lead came from a subsequent collaboration with Ed Bullmore under a GSK academic incubator program in the United Kingdom involving a highly selective μ-opioid receptor antagonist. This compound was revealed to have a remarkable profile of behavioral effects by Chiara Giuliano in a major series of meticulously conducted experiments that showed it dose-dependently and significantly reduced cocaine, heroin, and alcohol seeking as assessed under second-order schedules

of reinforcement (Giuliano et al., 2012; Giuliano et al., 2013; Giuliano et al., 2015). It had no apparent unwanted or general behavioral consequences, while in parallel experimental medicine studies, it was shown to be well tolerated and to have promising effects on neural responses to food and alcohol cues (Ziauddeen et al., 2013). Even more promising was that pretreatment with this compound reduced both compulsive alcohol seeking and alcohol drinking (Giuliano et al., 2018). Despite these compelling preclinical data, which are consistent with data on naloxone (a less specific μ-opioid receptor antagonist) in clinical studies of cocaine and alcohol addiction, this putative treatment has not been developed further. Chronic dosing studies, in any case, would be needed in animal models to further assess efficacy, but there seems little reason to embark on such challenging experiments without the promise of further treatment development. The area of addiction therapeutics clearly has a long way to go before we see clinical translation, but there remains potential in repurposing already clinically approved drugs to this end. For example, serotonin-selective reuptake inhibition reduced compulsive cocaine seeking (Pelloux et al., 2012), perhaps through a mechanism not unrelated to its efficacy in obsessive-compulsive disorder and there is evidence that treatment trials are being undertaken.

Memories are Made of This: Fear and Drug Memory Reconsolidation

I was convinced early on that in addition to identifying the distinct neural systems of learning implicated in drug addiction, we also should investigate the underlying memory processes upon which such learning depends. I reasoned that the memory for drug-associated CSs and contexts is made more permanent by repeated consolidation through multiple episodes of drug taking, perhaps exacerbated by the effects of addictive drugs on dopaminergic and other plasticity mechanisms in limbic-corticostriatal circuitry. Relapse, even following protracted withdrawal and abstinence, is a logical consequence of such processing. I speculated that retrieval of drug memories elicits both drug-seeking habits and, in humans, a subjective state of drug craving that can lead to further drug seeking and taking (Everitt, 2014). I had fortunately managed to persuade Kerrie Thomas to join the lab, bringing with her great experience in investigating molecular mechanisms of long-term potentiation, with the specific intention of exploring molecular correlates of Pavlovian associations between CSs and cocaine, and the retrieval of memories elicited by such CSs that subsequently impact cocaine seeking and relapse. Jeremy Hall arrived at this time for the research element of his MD-PhD program, and we decided to begin by using cellular imaging to explore Pavlovian CS and contextual fear conditioning. Our previous work had invoked competitive hippocampal and amygdala mechanisms and, perhaps more important, the experimental conditions for such studies would be much easier to control than conditioning to addictive

drug effects. The outcome was not what I had expected, as the experiments on conditioned fear drew us into the completely new area of the reconsolidation and extinction of both conditioned fear and drug memories.

In a specially configured, contextual fear paradigm, the rapid and selective induction of brain-derived neurotrophic factor (BDNF) expression in the hippocampal CA1 region, but not zif268 in CA1 or in the amygdala, was shown to be correlated with contextual fear acquisition (Hall et al., 2000). However, contextual or cued fear memory retrieval was then shown to be associated with increased expression of zif268 in the hippocampus and amygdala, respectively, but with no change in BDNF (Hall et al., 2001a). Retrieval of a cocaine memory on presentation of a CS associated with the drug was also then shown to be correlated with increased zif268 expression in the basolateral amygdala (Thomas and Everitt, 2001). These cellular imaging data suggesting different molecular correlates of learning and retrieval emerged just as Karim Nader at McGill had demonstrated amygdala-dependent fear memory reconsolidation. He showed that protein synthesis inhibition in the amygdala within four hours of retrieval of a fully consolidated CS-fear memory resulted in amnesia—subsequent presentation of the CS no longer elicited a conditioned freezing response. Critically, this effect was memory-reactivation dependent, as amygdala protein synthesis inhibition in the absence of memory reactivation was without effect (Nader et al., 2000). The notion that fully consolidated memories might reenter a labile, or active, state previously had been shown in humans (Misanin et al., 1968), but had not been followed up in any great detail, probably in part because the findings went against the dominant consolidation theory, which asserted that once consolidated, a memory is immutable. Nader's findings presented the field with compelling evidence that retrieved memories could reenter a labile state and must undergo restabilization, or reconsolidation, in the brain if they are to persist. In the reactivation-dependent labile state, they become vulnerable to disruption by amnestic agents.

Although the very word "reconsolidation" implied that the molecular signaling events occurring during consolidation were in some sense recapitulated at retrieval, our own data on BDNF and zif268 expression led us to hypothesize that the protein products of these genes were dissociably involved in consolidation and reconsolidation, respectively. Kerrie Thomas set about designing antisense oligonucleotide probes to target and knock down the BDNF and zif268 genes at specified times during learning and retrieval when she was in the very latest stage of pregnancy, and indeed completed doing so just before leaving the lab to give birth to her daughter. Jonathan (Joff) Lee, a new graduate student in the lab supervised jointly by Kerrie Thomas and me, undertook our first gene knockdown experiments to test this hypothesis in the contextual fear paradigm painstakingly designed by Jeremy Hall. The results of the experiments were remarkable in showing that the molecular basis of consolidation and reconsolidation are doubly

dissociable component processes of memory. Contextual fear memory consolidation required the expression of BDNF, but not the transcription factor Zif268, in the hippocampus, whereas reconsolidation required hippocampal Zif268, but not BDNF, expression (Lee et al., 2004).

Later experiments explored the neurotransmitter mechanisms involved in fear memory reconsolidation. For example, Lee demonstrated the bidirectional modulation of reconsolidation and extinction of a CS-fear memory by an antagonist and agonist at the N-methyl-D-aspartate (NMDA) receptor. He showed that an NMDA receptor antagonist prevented both fear memory reconsolidation and extinction leading to the opposite outcomes of memory loss and memory persistence, respectively. By contrast, the NMDA receptor agonist D-cycloserine enhanced reconsolidation and extinction, leading to the opposite outcomes of fear memory persistence and loss, respectively (Lee et al., 2006). Amy Milton, who was a graduate student and then postdoctoral researcher in the lab and is now a tenured member of faculty, later showed the doubly dissociable involvement of GluN2B- and GluN2A-Containing NMDA receptors in the destabilization and restabilization of a reconsolidating fear memory (Milton et al., 2013).

The seemingly dynamic relationship between reconsolidation, induced by brief memory reactivation induced by presenting a single fear-associated CS, and extinction, induced by repeated or prolonged exposure to the CS, was pursued in more detail by a Royal Society visiting research fellow from Argentina, Emiliano Merlo. Merlo had established in his work on memory in crabs that the calmodulin-dependent protein phosphatase, calcineurin, is specially involved in memory extinction. In an ingenious experiment, rats conditioned to a threat CS were exposed 24 hours later to different "doses" of the CS to induce either reconsolidation (1CS) or extinction (10CS), or to the intermediate numbers of 4 and 7 CS presentations. Increasing the number of CSs induced a proportionate decrease in freezing responses to the CS (extinction) and a gradual increase in basolateral amygdala levels of calcineurin. This newly synthesized calcineurin was then shown to be required for the extinction, but not the reconsolidation, of conditioned fear using a gene knockdown approach (Merlo et al., 2014). Intriguingly, during the transition from reconsolidation to extinction (the 4 and 7 CS presentation conditions), Merlo discovered an insensitive state of the fear memory, one in which NMDA receptor agonist and antagonist drugs were completely ineffective either in modulating calcineurin levels in the basolateral amygdala or in altering the reconsolidation or extinction processes. The demonstration that reconsolidation and extinction are mutually exclusive processes also generated the hypothesis of a previously unknown transitional, or "limbo," state of the original memory between these two alternative outcomes of fear memory retrieval, when neither process is engaged (Merlo et al., 2014).

With this now firm evidence of fear memory reconsolidation and a demonstration of at least some of the neurochemical and molecular mechanisms involved, the big question that confronted us was whether appetitive, especially addictive drug, memories also undergo reconsolidation following reactivation, and whether they could be disrupted by knocking down zif268 in the basolateral amygdala or by interfering with key neurotransmitter mechanisms. It was immediately obvious that this might not be a trivial question to answer, not least given the major procedural differences between CS-fear and CS-drug conditioning. In typical fear conditioning experiments, only one or two pairings of a CS with shock are needed to establish an enduring fear memory. This is not the case for CS-drug conditioning except in the particular case of conditioned place preference procedures, for which a relatively small number of experimenter-administered drug injections could suffice.

I was convinced, however, that if preventing drug memory reconsolidation was to have any translational potential, it had to be demonstrated in a drug-seeking and -taking setting. Even in our most straightforward drug self-administration procedures, there were some 300 pairings of a CS with a cocaine infusion over the 10 days needed to obtain stable drug self-administration. We knew that the resultant CS arising from these multiple CS-drug pairings could sustain drug seeking under a second-order schedule or could induce relapse. I decided that the first experiment should involve the acquisition of a new response procedure that depended both on conditioned reinforcement and the basolateral amygdala, and where we had shown that cocaine-associated CS presentations increased zif268 expression. Lee, who is an exceptional behavioral neuroscientist, took on this challenge and trained rats to make nose-poke responses for intravenous cocaine infusions over 10 days, and then later exposed them to cocaine CSs alone to reactivate the cocaine memory (Lee et al., 2005). The problem was that we had no idea how many CS presentations would be required to reactivate the memory, but not initiate extinction processes. We began by adopting an informal 10% rule; if there had been 10 days of 30 cocaine infusions and pairings with a CS each time, meaning 300 discrete CS-cocaine conditioning sessions, then reactivation would involve 30 CS unreinforced presentations. This fortunately turned out to be effective. Cocaine memory reactivation was performed in conjunction with zif268 knockdown in the basolateral amygdala, with the same antisense oligonucleotide infusion in the absence of memory reactivation serving as a control. The rats then entered the all-important test phase during which we measured the efficacy of the cocaine CS to support the acquisition of a new instrumental response, the canonical test of its conditioned reinforcing property. Rather dramatically, we showed that the cocaine-associated CS could no longer act as a conditioned reinforcer and the knockdown rats did not acquire the new instrumental response

(Lee et al., 2005). We then applied the zif268 knockdown in the amygdala at drug memory reactivation to show that it completely prevented the ability of a cocaine-associated CS to support cocaine seeking under a second-order schedule of reinforcement (Lee et al., 2005).

Therefore, we had shown in these experiments both that appetitive, drug-associated memories underwent reconsolidation and that preventing reconsolidation greatly diminished or prevented the effects of the drug CS to influence drug-seeking behavior subsequently, a finding that surely suggested translational potential. Amy Milton went on to demonstrate the involvement of both NMDA and ß-adrenoceptors in cocaine and alcohol memory reconsolidation, also linking the NMDA receptor to zif268 expression in the basolateral amygdala at cocaine memory reactivation (Milton et al., 2008b; Milton et al., 2008a; Schramm et al., 2016). Finally, Kim Hellemans, a visiting postdoc from Canada, showed that opiate conditioned withdrawal memories also underwent memory reactivation-dependent reconsolidation, suggesting that withdrawal memories also might be targeted in a treatment setting (Hellemans et al., 2006).

I am pleased that there has been some realization of the translational potential of this basic behavioral neuroscience research on memory reconsolidation to the clinic. Although the early promise of positive results in the treatment of post-traumatic stress disorder by reactivating traumatic memories under ß-propanolol treatment have not been followed by fully controlled trials, Merel Kindt has shown the extremely successful treatment of severe spider phobia following a single treatment with ß-propranolol at the time of phobic memory reactivation (Soeter and Kindt, 2015). As was always to be expected, successfully applying this approach to drug memories has been much more difficult, but even here, there have been signs of success in the treatment of nicotine and alcohol addiction (Xue et al., 2017; Das et al., 2019). The big challenge has always been and remains defining the precise conditions for memory reactivation that are sufficient to return the memory to a labile state, when amnestic agents can be used effectively, while not engaging extinction mechanisms (when an amnestic treatment actually will have the opposite outcome of memory persistence), or placing the memory in a "limbo" state in which it is impervious to any amnestic treatment. Discovering a biomarker of memory destabilization obviously would be of great utility, but to date, it remains elusive.

Some Final and More Personal Thoughts

Before being invited to contribute this chapter, I had not planned to reflect on my life and my more than 50 years of research in this way. I have enjoyed the opportunity to do so, not least because it has enabled me to reconnect in memory with many people who have had an impact on my life in research. I have been intrigued that many of the questions that interested me when

I began my career continue to do so now. Although the detail has changed, my engrossing interest in the behavioral neuroscience of motivation, learning, and memory has been consistent throughout. I have referred to the irreplaceable importance of mentors at key moments in my life and also my interactions with graduate students, postdoctoral researchers, and collaborators, many of whom quite naturally became friends, really good friends who I know I could always turn to. Surely, a privilege of our academic research careers is that we encounter other researchers, both in our laboratories and also at meetings, to discover that we have more in common than just an academic interest, and we establish friendships. That is really rather special.

I was fortunate to have been appointed to an academic post at a great university at just the right time. I had always thought from my time as a graduate student that a career combining teaching and research would be optimal for me, and so it has turned out to be. I have loved the combination of undergraduate teaching, graduate mentorship, and research in a laboratory that has grown, and I have been very fortunate to have had many wonderful young researchers choosing to spend time in it. I was especially happy to have been promoted to professor at the same time as my close friend and collaborator in research, Trevor Robbins; we have shared much as well as supported each other as individuals throughout the past 40 years.

I have also done things during my career that were not planned and that are still genuinely surprising to me. These include playing an active role in the scientific societies I joined and that in some sense had sustained me in my early career. I found this to be very rewarding as well as, I hope, being of value to my colleagues around the world. I was extremely surprised to be nominated and then elected as president of the Federation of European Neuroscience Societies (FENS), an organization that I had helped to establish in 1998 and then had been privileged to lead during its 20th-anniversary year, culminating in an exceptional FENS Forum in Berlin in 2018. If that was not enough, in fact too much, for someone late in their career, being nominated and then elected as president of the Society for Neuroscience (SfN) in its 50th-anniversary year is something I still find to be frankly astonishing. I previously had served SfN as its first program chair from outside the United States, and in other volunteer roles, and am now proud to be its first president from outside North America. As I write this memoir, I am in the first third of my term, and while I find it extremely engrossing and enjoyable, it is also challenging not least because the world is in the accelerating phase of a coronavirus pandemic. The neuroscience discipline, the way that neuroscience research is funded, and the way scientific findings are published and disseminated are in a moment of great change.

In a quite different domain of my life in Cambridge, I was elected Master of Downing College in 2003, having been a fellow of the college since 1976

and the Director of Studies in Medicine for 20 years. Serving in this educational leadership role was never something I dreamed was possible for someone who grew up on a council estate in Dagenham, was not distinguished at school, and was not educated at either Cambridge or Oxford, where at least I would have grown to understand what collegiate education was all about. Living as a family with my wife Jane and daughter Jessica in the beautiful Georgian Master's Lodge in the heart of this elegant college in the middle of Cambridge was a wonderful experience (and great for sleepovers of my daughter's friends after late nights on the town). This role provided a special opportunity to engage with undergraduate and graduate students in a unique and supportive way at an important time in their lives. While living there, Jessica successfully navigated both school and university, graduating with distinction in psychology from Oxford University and then with a master's degree from London University; she is now forging her career in Sydney, Australia. Jane has enjoyed a very successful career as a clinical dermatologist and molecular virologist, and she too is now serving her discipline as president of the Dermatology Section of the Royal Society of Medicine. My son Alex is a dedicated consultant neurologist and epileptologist at a major London teaching hospital and his three children, our grandchildren, are making their ways through school and university. Family, career, outside fulfilment, and enjoyment are always difficult to keep in balance, and this is as true now as it was in the 1960s when I was a graduate student. I have been quite fortunate to have received recognition of my contributions to research and scholarship. I have been equally fortunate to have worked with so many exceptional colleagues. I have been even more fortunate to have a wonderful family.

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