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No man is an island. A personal tribute to Bob Blanchard and ethoexperimental approaches to the study of behaviour

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Abstract

I first met Bob Blanchard at an international conference in Paris some 40 years ago. We collaborated intensively during the late 1980's/early 1990s on the ethopharmacology of antipredator defence in wild and laboratory rats, and remained good friends until his untimely passing in November 2013. Bob will undoubtedly be remembered as one of the most influential behavioural neuroscientists of the 20th Century and, with Caroline, the most eloquent advocate of ethoexperimental approaches to the study of behaviour. In this brief trip down memory lane, I describe when and where Bob and I first met and how, over a lengthy period, he directly and indirectly helped shape my own research career. His profound influence in this regard is illustrated by reference not only to our collaborative research on antipredator behaviour but also my other work on the ethopharmacology of agonistic behaviour, social conflict analgesia, anxiety, and appetite. The element common to all of this work has been ethoexperimental analysis and, for teaching me the true value of this approach, I shall always remain indebted to the big man. Literally and figuratively, Bob was most certainly larger than life.

Key words: Bob Blanchard - Ethoexperimental analysis - Defence - Ethopharmacology - Antipredator behaviour - Social conflict analgesia - Risk assessment - Anxiety - Plus-maze - Appetite

Introduction

No man is an island entire of itself; every man is a piece of the continent, a part of the main

I have always considered these lines of the English poet John Donne [1] to nicely encapsulate the nature of scientific research. As scientists, our work is heavily influenced by others in so many ways, and not only in the form of published literature or critiques of our manuscripts/funding applications. During my career, I have been fortunate enough to have worked with and been directly influenced by many gifted behavioural scientists, including the late Steve Cooper, Paul Brain, Sandra File, Stefano Parmigiani, Guy Griebel, David Sanger, Jozsef Haller and, most recently, John Blundell. However, the person who more than any other influenced my thinking and scientific direction was the big man, the 'bear', Bob Blanchard. Echoing views very recently expressed by Elena Choleris and Steve Kent [2], it was Bob who through his emphasis on ethoexperimental analysis did so much to put behaviour - not least, defensive behaviour - into behavioural neuroscience. This particular emphasis is reflected in my 1996 DSc thesis, somewhat grandiosely entitled 'The Behavioural Pharmacology of Defence', a compendium of work published between 1973 and 1995. That weighty tome, which nowadays doubles as an aide memoire and door-stop, was organised into 5 sections, the titles of which are quite revealing: (i) Neurochemical and neuroendocrine regulation of defensive fighting, (ii) Opiates, opiate antagonists and defensive reactivity, (iii) Ethopharmacology of defensive analgesia, (iv) Ethopharmacology of antipredator defence, and (v) Ethopharmacology of situational defence. This unwavering focus on defence (or as the big man would have consistently had it, 'defense'!) acknowledges Bob's ground-breaking, substantive and sustained contributions to our understanding of the diversity, structure and functionality of mammalian defensive behaviour.

The editors of this special issue of Physiology & Behavior (Jaap, Sietse & Randall) have kindly given me licence to reminisce about when and where I first met Bob and how, over a lengthy period, he helped shape my views on the vital importance of ethoexperimental analysis within the broad church that is behavioural neuroscience.

Early days

In my latter school years, I studied physics, chemistry and biology. However, while biology was my favourite subject, I had no clear idea of which career direction to pursue – that is until I attended an 'open day' at my local university (Queens' University Belfast, QUB). Here, I am assuming that university open days are much the same the world over, i.e. an opportunity for learned institutions and their constituent departments to 'tout' for business by opening up their doors to would-be applicants and providing taster lectures and demonstrations of one form or another. As luck would have it, I

chanced (literally) upon a laboratory demonstration in the Department of Psychology. If memory serves, it wasn't anything more elaborate than watching a white rat explore a classical (circular!) open field environment – but this experience was more than enough to set me thinking about the biology of behaviour and, after a touch more reading around the subject area [3, 4], I applied to QUB specifically to study psychology. To cut a long story short, my first degree culminated in a final-year experimental project in which I adopted a basic understanding of rodent social behaviour (gleaned from the pioneering work of Ewen Grant and John Mackintosh [6, 7] to investigate the influence of scopolamine on resident-intruder interactions in male Wistar rats. Despite a disappointingly low baseline level of fighting behaviour (the reasons for which became apparent just a few years later), this hands-on lab experience well and truly saw me infected with the 'research bug'. When I subsequently commenced PhD research in the same department (role of the amygdaloid complex in the cholinergic mediation of aggression), my advisors convinced me of the need for a less 'subjective'/more 'robust' measure of aggression than that afforded by my undergraduate resident-intruder paradigm. Not really knowing any better at the time, my PhD (1972-75) was therefore based on the so-called 'objective' reflexive fighting model as originally developed by O'Kelly & Steckle [8] and later elaborated by Ulrich and Azrin [9]. In the Summer following a successful thesis defence (there's that word again), I travelled to Strasbourg to visit Pierre Karli's lab where I had the pleasure of also meeting Marguerite Vergnes, the late Pierre Schmitt, Antoine Depaulis and Francoise Eclancher. It was during those discussions that I discovered that Karli's research group had responsibility for organising the 2nd Biennial Meeting of the International Society for Research on Aggression (ISRA) to be held in Paris in July of the following year (1976). As I already knew that the 21st International Congress of Psychology was to be held in Paris at roughly the same time, I rather ambitiously sent off abstracts to both meetings. Much to my surprise, being at the time totally naïve to the financial realities of conference organisation, both submissions were accepted.

Back in the day, and certainly for meetings of relatively small societies such as ISRA, all contributions were given as oral presentations in a single- (rather than parallel-) session format. This was a most fortunate happening in that both Bob (who I had never previously met) and I were on the same programme and could not easily have missed one another's talks – at least not without creating a major breach of conference etiquette! I am afraid that I have long since lost/mislaid/discarded the programme for that ISRA meeting and cannot honestly free recall who spoke first. However, I do remember that my inaugural conference address was well-received but that, during his riveting presentation, Bob argued for the defensive as opposed to offensive nature of reflexive fighting. More specifically, work on rat colony aggression in the Blanchard's lab [9] was beginning to differentiate offensive (colony dominant males) and defensive (colony intruder males) attack patterns. I recall being a little 'precious' in defence (no pun intended) of the conventional interpretation of reflexive

fighting (i.e. as a model of something called 'aggression') - afterall I had just spent three years of my life using this paradigm to explore amygdaloid involvement in the cholinergic mediation of 'aggression'. However, Bob (or, possibly, Caroline) must have detected my unease for, at the close of the conference day, they kindly invited me for a beer with themselves and Holger Ursin. Our discussion about the significance of the offence/defence distinction, and the then emerging evidence favouring amygdaloid involvement in various aspects of defensive responding, encouraged me to re-think my stance on the nature of reflexive fighting. This was just as well since, over the following few years, the Bekesy research group published very convincing evidence on the defensive nature of reflexive fighting, including the now classical distinction between defensive and offensive bite target patterns [10-13]. The impact of these developments on my subsequent reflexive fighting publications is plain to see, not least from the wording of the titles of those papers, i.e. from 'shock-induced aggression' [14], through 'shock–induced attack' [15] and 'shock-induced fighting' [16], to 'defensive fighting' [17] and 'defensive behaviour' [18]. Indeed, I referred to the changing methodology in aggression research when, in 1979, I was invited to comment upon a review article by David Adams on the brain mechanisms of offense, defense and submission [19]. In sum, it was Bob Blanchard who, virtually from the beginning of my research career, set me on the right path by encouraging much greater attention to behaviour and its interpretation.

Antipredator defence & defensive analgesia

In researching drug effects on reflexive fighting, it had obviously been important to control for possible indirect treatment effects - not only on basic motor function but also nociception, i.e. an analgesic effect could easily explain a drug-induced reduction in reflexive fighting. The need for analgesia assays to control for such 'non-specific' drug effects soon brought me into contact with one of the major discoveries of the early-mid 1970s – namely the existence in brain and body of opiate receptors and endogenous opiate-like (opioid) peptides. As the amygdala contains relatively high densities of opiate receptors, I spent some time during my early postdoctoral years looking at the behavioural effects of intra-amygdaloid morphine infusions [20,21]. However, it was the discovery of stimulation-produced analgesia and stress-induced analgesia, together with empirical evidence for the involvement of opioid substrates in both sets of phenomena [22-24], that brought me firmly back to the study of defensive behaviour. At the time, I was beginning to recognise the crucial importance of 'why' as well as 'how' research questions and, in context, became intrigued by speculations concerning the functional significance of intrinsic pain inhibitory systems. In short, John Liebeskind and colleagues [25], and somewhat later Bob Bolles and Mike Fanselow [26], had convincingly argued for a defensive function of the intrinsic analgesia system in that, under dire circumstances, fear would inhibit pain to allow for effective defence i.e. unhindered by pain reflexes. In 1980, Colin Hendrie joined my lab and commenced doctoral studies designed to test this line of thinking in male

resident-intruder interactions – initially in rats but, because they are a much more territorial species, mostly in mice. The basic hypothesis was that attacked, and therefore defensive, intruders would display a post-encounter analgesia that should be blocked by an opiate receptor antagonist such as naloxone. The hypothesis was fully confirmed, not only in our lab [27, 28] but also elsewhere [29-31]. Needless to say, matters turned out to be a touch more complicated leading to a further decade of research and the delineation of both opioid and non-opioid forms of social conflict analgesia (for review, [32]).

Back to the main story. Following our initial encounter in 1976, I am fairly sure that Bob and I did not actually meet again until a NATO Advanced Study Institute held in 1980 at Chateau de Bonas near Toulouse in the south of France. That was a most enjoyable meeting not only because virtually everyone working on The Biology of Aggression [33] was present, but also because it provided Bob, Caroline and myself with ample time to get to know one another just a little better. Over the following decade, we made a point of hooking up annually at International and European meetings of ISRA (Strasbourg 1981, Mexico City 1982, Zeist 1983, Turku 1984, Parma 1985, Chicago 1986, Seville 1987, Swansea 1988, Szombathely 1989) during which I learned much about their work on aggression and defence in rats while they learned of our work on social conflict analgesia in mice. Although I also recall another seminal NATO Advanced Study Institute (Ethoexperimental Approaches to the Study of Behavior, [34]), held in 1988 at the Il Ciocco resort in Tuscany and co-organised by Bob, Caroline, Paul Brain and Stefano Parmigiani, I am fairly confident that it was during the 1985 Parma meeting of ISRA that 'the Blanchards' and I started to seriously contemplate active research collaboration - talk that quite quickly became reality. This cut at least 2 ways. From around 1987, I became increasingly involved in the design, analysis and reporting of a whole series of Honolulu studies on the behavioural pharmacology of antipredator defence in wild and lab rats (e.g. [35-37]). Fortunately, these collaborative research endeavours entailed personal (1987) and family (1989) trips to Hawaii during which the Blanchards were superb hosts. As a result of my growing appreciation for late afternoon margaritas and often highly animated discussions with Bob and Caroline about analgesia as part of the defensive repertoire, I (together with my research assistant, Jill Randall) was better able to understand the need for two forms of social conflict analgesia; a non-opioid response supporting active defence and an opioid response supporting passive defence [32, 38]. This thinking in turn meshed very well with the then developing literature on the functional neuroanatomy of the periaqueductal gray matter of the midbrain (PAG), with its distinct lateral and ventrolateral cell columns mediating the physiological as well as behavioural aspects of active and passive defence patterns, respectively [39, 40]. I am not at all convinced that we would have reached this conceptualisation of 'stress analgesia' had it not been for all those informed Blanchardian interactions at scientific meetings, in the Bekesy Lab, at 'casa Blanchard', and in my Yorkshire home.

Ethoexperimental analysis, risk assessment and the elevated plus-maze

In the period between first meeting Bob (1976) and our subsequent research collaboration (1985-93), I had the great pleasure of working with Sandra File at the School of Pharmacy in London. We had initially met at a Winter School in Zuoz (Switzerland) in January 1978 and had readily agreed to work together on the behavioural effects of intra-amygdaloid morphine injections in rats [41, 42]. One of the tests we used was the social interaction test of anxiety, a popular procedure that Sandra had developed several years earlier with her student Jeremy Hyde [43]. Of greater relevance to the present story is the fact that, during the early-mid 1980s, Sandra's group went on to validate what has become probably the most widely-used of all animal tests for anxiety, the rat elevated plus-maze or EPM [44-47]. However, it was when one of Sandra's PhD graduates, the late Richard Lister, went on to develop the procedure for mice [48] that I really began to take notice of the EPM. Derived from our work on social conflict analgesia, we initially used the mouse EPM to test the hypothesis that exposure to anxiety-provoking stimuli and/or situations would elicit a non-opioid form of analgesia [49-51]. However, based on the deeper understanding of defensive behaviour that I had acquired though my collaboration with Bob and Caroline, I and my then graduate students (chronologically through the 1990s: Claire Lee,the late Jon Shepherd, Jon Cole, Neil Johnson, Ash Dalvi and Andy Holmes) began to look at the murine EPM with an 'ethological eye'.

Most readers will know that the classical EPM is based upon the spatiotemporal distribution of behaviour (entries & time spent) in the open, centre and enclosed parts of the apparatus. Relative to placebo control, anxiolytics (not only but mainly $GABA_A$-related anxiolytics!) disinhibit open arm exploration while anxiogenics (not only but mainly $GABA_A$-related anxiogenics!) enhance open arm avoidance [45-47]. In sound ethological spirit, we decided to investigate not only where in the maze the animals were during the test but also what they were doing whilst there. After many hours of watching videotapes (no DVDs or other digital recording formats available), we had created an EPM ethogram – a list of defined behavioural acts and postures shown by mice during the test. We have long since refined this list using factor analysis [52, 53] but, in the early days, the ethogram was quite extensive and included rearing, grooming, immobility, closed arm returns, head-dipping, flat back approach and stretched attend postures [54, 55]. While most of these measures have proved useful in one way or another, the frequency of stretched attend postures (SAP) has perhaps been the most useful of the so-called 'ethological' measures. In their work on antipredator defence, the Blanchards had found that rats and mice, when confronted with potential as distinct from actual danger (e.g. an environment in which a predator has recently been but is no longer physically present; or a recently worn cat collar), would engage in behaviours such as SAP - a risk assessment behaviour believed to function in an information-gathering capacity [56]. Furthermore, the effects of anxiolytic drugs on such behaviour were found to vary as a function of the defensive baseline: i.e. if the situation

promoted freezing, the drug would reduce that behaviour and disinhibit the lower order SAP response; on the other hand, if the situation promoted a high SAP response, the same drug would reduce that response thereby permitting a return to non-defensive behaviour (for review, see [37]).

Although the EPM profile of some mouse strains is typified by very low levels of exploration and prolonged bouts of immobility [57, 58], most strains show a high level of SAP in the EPM. This typically starts from the moment they are placed onto the central square of the apparatus, with SAP directed towards 'all 4 points of the compass', i.e. into all 4 arms. As exploration of the maze continues, SAP remain directed to the open arms but less so to the closed arms. Consistent with the Blanchard's work on antipredator risk assessment, anxiolytics of various classes (but $GABA_A$ anxiolytics in particular) not only reduce the total SAP score but, more often than not, also reduce the proportion of such behaviours displayed from the closed arms and central square (something we have called percent protected SAP). Interestingly, head-dipping (which we initially also thought of as a risk assessment element) is actually increased by $GABA_A$ anxiolytics although the precent protected form of the behaviour is (like protected SAP) typically reduced by such drugs [45-47]. It is therefore no surprise that principal components factor analysis revealed that total SAP, total head-dipping and open arm avoidance measures (including the percent protected forms of SAP & head-dipping) all loaded on independent factors [52, 53]. What was more striking was the observation that, in many instances, total SAP (as a measure of risk assessment) was often more sensitive to drug action than the conventional open arm measures such as percent open entries and percent open time [44-46, 59]. In this way, the adoption of a more ethoexperimental approach – while more time-consuming – actually increased test sensitivity. It is most gratifying to know that this ethoexperimental approach to EPM scoring and interpretation has since been adopted by research groups the world over [45, 46].

The Present

It should be abundantly clear from the above reminisences that my research career has been strongly influenced by the life and work of Bob Blanchard. His (and Caroline's) perspective is patently obvious in my work on defensive fighting, defensive analgesia and situational defence, not to mention my contributions to all our joint publications on antipredator defence! However, it can be argued that this influence extends even further in that comprehensive behavioural profiling (a cornerstone of ethoexperimental analysis) has also been the hallmark of my research contributions to the behavioural pharmacology both of agonistic behaviour and appetite regulation. The former dates back to the early-mid 1980s when we used video-technology to investigate in depth the effects of naloxone (e.g.[27,28]) and of benzodiazepine receptor ligands [60] on behaviours displayed during resident-intruder encounters in rats and mice. The latter on the other hand is of very much more recent origin, dating from around the year 2000, and entails the use behavioural satiety sequence (BSS)

methodology to fully characterise drug effects on rat behaviour during feeding tests (for review: [61]). Outcome-based research in this field (i.e. the detection of a drug-induced reduction in food intake) says nothing about the process/es involved in reaching that outcome. However, as animals engage in a variety of behaviours during feeding tests, many of which are not directly related to ingestion, analysis of the full behavioural output provides a means whereby the behavioural selectivity of an anorectic drug effect can be determined in one and the same test situation [62, 63]. This is typically done in two ways, examining treatment effects both on (i) ingestive and non-ingestive components of the behavioural repertoire, and (ii) the normal structure of feeding behaviour i.e. the natural progression from feeding, through grooming, to resting (BSS). Of course, all these avenues of research have in common close attention to behavioural detail and an interest in both proximate and ultimate causation – the hallmark of ethoexperimental analysis.

In closing, I shall leave you with a couple of anecdotes that nicely illustrate Bob's dry (if not, at times, downright wicked) sense of humour. During my first visit to Honolulu (1987), and as thoughtful hosts, Bob and Caroline took me for an afternoon's snorkeling at Hanauma Bay. Caroline relaxed on the beach while Bob and I had a great time feeding 'the locals'. When we had exhausted our food supply (I seem to remember bread crusts?), we rested at the reef's edge. All of a sudden, Bob shouted an alarm, gesticulating towards 'a huge moray just behind *you'*. Needless to say, I fell for the ruse – in the process, demonstrating what can best be described as a 'protean' (or is that 'headless chicken'?) display. Prior to my second trip to paradise (1989), I had applied for promotion to the academic position of Reader in my home department. For those unfamiliar with the quaintness of academic nomenclature in the UK, this (unfortunately now fast disappearing) title is awarded in recognition of an international research reputation and is just one rung down from a full professorship. Anyhow, while I was still in Honolulu, my secretary back home faxed (no email in those days) a quick note to the Bekesy Lab confirming that my Readership application had been successful. Naturally, the good news was given to Bob to pass on to yours truly. In congratulating me, Bob simply said *'Well done* John. Perhaps, by next year, you'll have learned how to *write'*!

Thanks for some great memories, Bob.

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