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A Novel Approach to Discovering Treatments for Alzheimer's Disease

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Abstract

At present, learning and memory tests are widely used in the preclinical search for treatments for Alzheimer's disease (AD). However, in most cases these tests do not probe "episodic memory" which is the type most affected in AD. This article describes a different approach to preclinical testing - the use of species-typical tasks. These mirror "activities of daily living," impairments of which are noticeable in the earliest stages of the illness. These impairments are frequently more of a problem to the patient than the loss of cognitive abilities, and lead to a need for carers or institutionalisation, both of which can be costly. Species-typical tasks do not require food deprivation or aversive stimulation to motivate the animals; they are performed spontaneously. Moreover they are simple and cheap to execute. Thus they model an aspect of the disease more closely, are cost-effective, simpler and ethically sounder than current practices.

Keywords: Alzheimer's disease; Mice; Rats; Memory; Cognition; Hippocampus; Species-typical behaviours; ADL (Activities of daily living)

Introduction

As the population ages, so does the incidence of dementia, mostly Alzheimer's disease (AD), and it is widely recognised that a global epidemic of dementia is here and steadily growing. Although AD is widely thought of as a disorder of memory, learning and other forms of cognition, in practice the deterioration in activities of daily living (ADL) is at least as prevalent and troublesome. Toileting, feeding, dressing, bathing, meal preparation, telephone use and putting on shoes (especially if laced) are all affected, as well as many other activities necessary to lead a normal life. This leads to a need for carers or institutionalisation, both of which can be costly and further disrupt the patient.

Most preclinical screening for potential dementia, mostly AD, therapies involves testing rats and mice on learning and memory tasks, which they learn by trial and error in a rote fashion. However, this type of memory is rather different to the episodic memory [1] for past events that is so impaired in AD. Amnesic patients can learn new information, but do not remember the circumstances surrounding the learning in an episodic-like manner. A classic example of this is the "Claparede" effect. Claparede, a Swiss physician, had known an amnesic patient for many years, but she was so densely amnesic that she never remembered him and always had to be introduced afresh each time they met. One day Claparede concealed a sharp pin in his hand and pricked her painfully with it. The next day she refused to shake his hand. When questioned as to why she was avoiding him, she said she really wasn't sure; she just had a sense of unease and danger when he appeared. The analogy with the passive avoidance test, where an animal is taught to avoid an area in which it had previously received an electric foot shock, is clear. Passive avoidance is, however, still routinely used in the search for memory enhancing treatments. So most preclinical tests for therapies may be tapping into the wrong type of memory, the type that is minimally impaired in dementia.

The hippocampal region is one of the first brain regions to deteriorate in AD, and the rate of shrinkage of the medial temporal lobe regions (containing the hippocampi) reliably correlates with the development of clinical deterioration [2,3]. It has long been recognised that the hippocampus is vital for normal memory function [4] and in 1978 it was proposed that it is crucial to spatial learning and memory [5]. Rodents with lesions of the hippocampus typically perform very poorly on spatial memory tasks. In 2005 it was proposed that the hippocampus is also vital for the performance of species-typical tasks, or "activities of daily living" of mice [6]. Several tests of ADL in the mouse have now been developed [6-10]. These tests do not require supplemental motivation in the form of swimming in deep water, appetitive deprivation or electric shock. In fact, since the mice spontaneously perform them, and appear to find the activity rewarding, in effect, they constitute a form of environmental enrichment. This Refinement of experimental procedures conforms to Refinement as discussed by Russell and Birch in their classic work on the principles of human experimentation [11]. Moreover, the effect sizes in these tests are often very large, and some tests have been shown to be among the most sensitive indices of mouse behaviour available.

The present paper reviews and describes the range of tests of species-typical behaviours in the mouse. Many of the tests could probably be adapted for use in rats (as we have in the case of burrowing) [12]. However, as mice are now the most extensively used rodent species for behavioural work, partly due to the better knowledge of their genotype and how to manipulate it, the present short review will concentrate on mouse behaviour. Finally, I propose that these tests of mouse ADL mirror human ADL, and offer a different approach to preclinical screening for new AD treatments. Moreover, they comply with the present *zeitgeist* of experimental refinement and the improvement of animal welfare. They are also generally simple, require less experimenter time than learning and memory tests, and the apparatus is cheap and easy to make. The importance of assessing animal ADL or "global functional deficits" as well as more specifically cognitive tasks, has been strongly made by Lindner et al. [13].

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This mini-review does not attempt to discuss the multitude of models of dementia, primarily genetically modified mice. Its focus is behaviour rather than asking questions about aetiology and histopathology. It proposes that, regardless of aetiology, a mouse that is demented will be detected by impairments in species-typical behavioural tasks. These will detect both genetically modified, familial AD models, as well as the spontaneously occurring "sporadic" forms, the aetiology of which is currently poorly understood. In the next section a number of these tasks will be described, but it will be noted in the results section that only a selection have been used. This is because we concentrated on tests that could easily be adapted to laboratory conditions, and were quick, sensitive and simple to run; in effect, we have brought behaviour from the field into the laboratory and made it user-friendly for the experimenter. Species-typical behavioural tests may be useful in both academia and the pharmaceutical industry, where research costs are critical.

Two very simple models of neurodegeneration, AD or dementia were used in the development of this battery of species-typical behaviour tests: total removal of the hippocampus by multiple injections of the cytotoxin NMDA, or a single injection of scrapie (prion disease) homogenate into the hippocampus.

Tests of Species Typical Behaviours

In the burrowing test, mice or rats spontaneously excavate the contents of a tube ("burrow") of sand, gravel or food pellets [6]. Mice will burrow virtually any substrate, whereas rats prefer earthy substrates which they would encounter while making burrows for themselves in the wild, such as soil, sand or gravel. The animal is placed into an individual test cage containing a burrow, about 3 h before the dark phase of the light-dark cycle. Two hours later, a "snapshot" measurement is taken by weighing the contents of the burrow and subtracting this figure from the weight of the substrate at the start of the test. The non-burrowed substrate is then replaced in the burrow and the test continued overnight, after which a final measurement is taken of the weight burrowed.

The digging behaviour measured in burrowing can also be assessed by the direct-observation digging test and the marble burying test [8]. Both the latter give similar results to the burrowing test. For direct observation of digging, the mouse is placed in a small observation box filled with 5 cm of bedding material and the latency, duration and number of digging episodes is recorded over a 3 min period. The marble burying test uses a slightly larger box or cage with deep bedding. This is firmed and 12-20 marbles placed in a regular pattern on the surface. A mouse is placed inside, and 30 min later the number of marbles buried to 2/3 of their depth is counted. Observation reveals that the mice are not burying the marbles; they rarely interact with them, as most of the time is spent in vigorous digging in the deep bedding. This allows the marbles, being heavier than the bedding, to become buried.

Burrowing is our test of choice in assessing treatments that might affect dementias. It is quick (in terms of experimenter hours) the burrows are cheap and simple to make, and the measurement of the test parameter (weight of food pellets burrowed) can be performed by personnel with no knowledge of science.

Burrowing is an extremely ancient and widespread behaviour. A cynodont, a proto-mammal, living more than 200,000 years ago, was found inside a burrow in southern Africa, probably hiding from the inclement climatic conditions of the End-Permian time of mass extinctions. Mammals (including *Homo sapiens*) may therefore owe their existence to the burrowing abilities of their forebears. Burrows may also provide a place to retreat from predators, to breed and store food, and in certain species, a hibernation site.

In the nest construction test, mice are housed individually overnight with a single "Nestlet" (a pad of pressed cotton) [9] in each cage. The following morning the quality of the nest is assessed using a 5-point scale. The weight of any untorn Nestlet is also measured.

Climbing behaviour can be tested using a large vertical cylinder of wire mesh. The number of mice on the wire is counted at set intervals for a predetermined time [6].

Researchers familiar with mouse behaviour know that as soon as the cage lid is opened, the mice begin to walk around the edge of the cage. This exploratory behaviour is highly susceptible to lesions of the hippocampus. It can be easily quantified by a time sampling technique, counting the number of mice walking around the cage edge at set time intervals, in a similar way to the climbing test [14].

Hoarding behaviour requires a home cage or similar holding area, connected (a wire mesh tube is convenient) to an external source of food pellets. Mice are habituated to the home-like environment during the day without access to the food source (mice, being nocturnal, eat very little during the day). Access to the food is available during the night, when they can retrieve (hoard) food from this source and return with it to the home environment, where it is collected and measured by the experimenter on the following morning [9].

Social animals often huddle together. The primary purpose of this is probably heat conservation, especially for a small animal like the mouse. In an initial study, [13] very little huddling underneath the provided cardboard shelter was observed in a cage of hippocampal lesioned mice. A similar lack of huddling behaviour was observed in hippocampal lesioned degus (*Octodon degus*) [15]. To use this as a test of species-typical behaviour would not appear to be a practical proposition, however, as groups, rather than individuals would need to be tested, and satisfactory end points might be difficult to define.

Further Development of Species-Typical Behavioural Tests

There are a variety of mouse species-typical behaviours which have not yet been fully investigated, chiefly because they would appear to have limited application as preclinical screens. Also, the present work has concentrated on the mouse, as this species is generally used in dementia models. Gnawing is one of the most characteristic rodent behaviours, but apart from direct observation it is difficult to quantify, and laboratory mice do not appear to gnaw very frequently (possibly because their tooth growth is adequately checked by their normal diet, very hard food pellets). Mating behaviour and maternal care have been little studied, although O'Keefe and Nadel [5] noted poor nursing ability in a female rat with a hippocampal lesion. They interpreted this in terms of their spatial theory, as the mother adopted a nursing posture but not over the pups. This might represent an abnormality in maternal care (i.e. a species-typical behaviour), rather than spatial behaviour. Although mating and maternal care may be neuropsychologically interesting, they, like gnawing, would not appear suitable as quick and easy preclinical tests.

Two relatively quick and easy further tests were, however, devised following perusal of a "missing persons" document for working police officers [15]. Demented missing people tend to follow boundaries, such as a wall or fence, without crossing through doors or gates. They also

frequently get stuck if the ground is boggy or swampy, as they do not avoid such dangerous wet ground like other normal people would. In unpublished work, mice with complete lesions of the hippocampus, or selective dorsal or ventral regions, were assessed in these two paradigms. The apparatus for the boundary crossing test was a circular corridor formed by placing a small plant pot saucer inside a larger one. The complete or ventral lesioned mice failed to climb over the plant pot saucer boundary walls after being placed in this corridor, whereas most of the control and dorsal lesioned mice did. To simulate wet ground, wet kitchen sponge cloths were placed at one end only of a rectangular box. After initially being placed on the dry side of the box, the complete lesioned group spent significantly longer on this wet side than the controls [16].

For all these tests, a useful control paradigm is grooming. This is motivated by internal stimuli, unlike the responses to external stimuli (burrows, nesting materials etc.) measured in the other species-typical behaviour tests. Animals with spinal cord lesions groom normally [17]. Grooming can readily be measured by counting and timing the number of grooming episodes in a small observation box [6].

Foraging has not been included in this mini-review, as the digging tests probably rely on similar neural mechanisms, although mapping of surface foraging in a large open field type arena might be expected to reveal deficits in hippocampal lesioned animals.

Thigmotaxis (keeping close to a wall, especially in an open field environment) is a very characteristic rodent behaviour, minimising the chances of predation. Although it is a classic species-typical behaviour, for decades it has been regarded, rightly, as an index of anxiety. Moreover, compared to the other species-typical tests presented here, it is only marginally sensitive to hippocampal lesions.

Results Summary

Complete cytotoxic hippocampal lesions dramatically impair performance on all these species typical tests [6,14].

A useful attribute of species-typical tests is that they can be repeatedly administered, in order, for example, to perform longitudinal monitoring of a disease. An example is our work with prion (scrapie) infection in mice: burrowing and nesting progressively deteriorated as the disease developed, around 10-12 weeks after the intrahippocampal injection of scrapie agent [18,19] about 2 weeks before it can be detected by an operant task [20], and around 10 weeks before the development of clinical signs such as poor coat condition.

An interesting finding in a genetically modified AD mouse (the Tg2576 construct) was that burrowing was impaired at three months of age, before significant other behavioural or histopathological changes occurred [21]. Recent unpublished work has also shown impairments in burrowing in the Chilean degu (*Octodon degus*) which was thought not to show behavioural or systemic abnormalities till around three years. (They are long lived rodents, around seven years, and the first rodent species to show unequivocal human AD-like plaques and tangles). These two findings raise the interesting possibility that burrowing can detect extremely early pathology. This enlarges the time window during which therapeutically interesting compounds could be evaluated, and could lead to prevention of dementia, rather than a treatment or partial cure after symptoms become irreversible.

There are also strain differences in performance of species-typical tasks. Inbred C57BL/10 mice are subtly different to the C57BL/6 strain genetically, and this is reflected in impairments in burrowing, digging and the weight of Nestlets left untorn. However, marble burying and finished nest quality were no different to the BL/6 strain [22]. Burrowing ("food displacement") was also impaired in the 129S2/Sv strain relative to BL/6 mice [23]. Anecdotally, albino rats (Sprague-Dawley, Wistar) have been reported to the author as performing less well than the more vigorous hooded Lister strain.

Conclusion

Present research into possible therapies for AD may be hampered by employing animal models which do not tap into the core memory deficit in AD, namely episodic memory. However, ADL impairment in AD is at least as debilitating and clinically relevant [24]. Tests of species-typical behaviour (mouse ADL) are virtually homologous with human ADL, and using them to screen for treatments might represent a useful addition to a pharmaceutical company's test battery. Hippocampal lesioned or scrapie-infected mice, animal AD models, perform very poorly on such tests. Species-typical behavior tests might even be used as primary screens, due to their cheapness, low demand on experimenter time and simplicity. Species-typical behaviours are innate, therefore the experimenter does not have to spend time training the animals. Positive compounds could then be subjected to secondary and tertiary tests with a more cognitive component, especially alternation tasks. Spontaneous alternation in a T-maze (having visited one arm of the T, the animal is replaced at the start and generally alternates, choosing the opposite arm) may tap into a memory system somewhat similar to human episodic memory, as the animal has to remember where it has just been, tapping into an episodic-like memory trace; rote learning cannot solve the task [25,26]. Although AD is the most prevalent type of dementia, other dementias and amnesias could also probably be treated with drugs discovered by such an approach.

Species-typical behaviour tests probably have many applications outside the dementia field. Already, several groups are using burrowing as a non-reflexive measure of pain or discomfort. Burrowing provides a broad, non-specific but very sensitive assessment of the well-being of an animal, in a similar way to taking its temperature. Comparing burrowing with body temperature measurement, lipopolysaccharide (LPS) depresses burrowing at a dose one thousand times lower than that causing a change in body temperature.

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Verification

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References

1. Tulving E (2002) Episodic memory: from mind to brain. Annu Rev Psychol 53: 1-25.
2. Jack CR Jr, Petersen RC, Xu Y, O'Brien PC, Smith GE, et al. (1998) Rate of medial temporal lobe atrophy in typical aging and Alzheimer's disease. Neurology 51: 993-999.
3. Jobst KA, Smith AD, Szatmari M, Molyneux A, Esiri ME, et al. (1992) Detection in life of confirmed Alzheimer's disease using a simple measurement of medial temporal lobe atrophy by computed tomography. Lancet 340: 1179-1183.
4. Scoville WB, Milner B (1957) Loss of recent memory after bilateral hippocampal lesions. J Neurol Neurosurg Psychiatry 20: 11-21.
5. O'Keefe J, Nadel L (1978) The hippocampus as a cognitive map. Clarendon Press: Oxford.

6. Deacon RM, Rawlins JN (2005) Hippocampal lesions, species-typical behaviours and anxiety in mice. Behav Brain Res 156: 241-249.

7. Deacon RM (2006) Burrowing in rodents: a sensitive method for detecting behavioral dysfunction. Nat Protoc 1: 118-121.

8. Deacon RM (2006) Digging and marble burying in mice: simple methods for in vivo identification of biological impacts. Nat Protoc 1: 122-124.

9. Deacon RM (2006) Assessing nest building in mice. Nat Protoc 1: 1117-1119.

10. Deacon RM (2006) Assessing hoarding in mice. Nat Protoc 1: 2828-2830.

11. Russell WMS, Burch RL (1959) The principles of humane experimental technique. Methuen: London.

12. Campbell SJ, Deacon RM, Jiang Y, Ferrari C, Pitossi FJ, et al. (2007) Overexpression of IL-1beta by adenoviral-mediated gene transfer in the rat brain causes a prolonged hepatic chemokine response, axonal injury and the suppression of spontaneous behaviour. Neurobiol Dis 27: 151-163.

13. Lindner MD, McArthur RA, Deadwyler SA, Hampson RE, Tariot PN (2008). In: Animal and Translational Models for CNS Drug Discovery; McArthur R, Borsini F Eds; Academic Press; 2: pp. 93-157.

14. Deacon RM, Croucher A, Rawlins JN (2002) Hippocampal cytotoxic lesion effects on species-typical behaviours in mice. Behav Brain Res 132: 203-213.

15. Uekita T, Okanoya K (2011) Hippocampus lesions induced deficits in social and spatial recognition in Octodon degus. Behav Brain Res 219: 302-309.

16. Gibb GJ, Woolnough P (2007). Missing persons: Understanding, Planning, Responding. Grampian Police.

17. Osborn JW, Taylor RF, Schramm LP (1990) Chronic cervical spinal cord injury and autonomic hyperreflexia in rats. Am J Physiol 258: R169-174.

18. Deacon RM, Raley JM, Perry VH, Rawlins JN (2001) Burrowing into prion disease. Neuroreport 12: 2053-2057.

19. Guenther K, Deacon RM, Perry VH, Rawlins JN (2001) Early behavioural changes in scrapie-affected mice and the influence of dapsone. Eur J Neurosci 14: 401-409.

20. Deacon RM, Reisel D, Perry VH, Nicholas J, Rawlins P (2005) Hippocampal scrapie infection impairs operant DRL performance in mice. Behav Brain Res 157: 99-105.

21. RMJ Deacon, LL Cholerton, K Talbot, RG Nair-Roberts, DJ Sanderson, et al. (2008) Age-dependent and -independent behavioral deficits in Tg2576 mice. Behav Brain Res 189: 126-138.

22. Deacon RM, Thomas CL, Rawlins JN, Morley BJ (2007) A comparison of the behavior of C57BL/6 and C57BL/10 mice. Behav Brain Res 179: 239-247.

23. Contet C, Rawlins JN, Deacon RM (2001) A comparison of 129S2/SvHsd and C57BL/6JOlaHsd mice on a test battery assessing sensorimotor, affective and cognitive behaviours: implications for the study of genetically modified mice. Behav Brain Res 124: 33-46.

24. Andersen CK, Wittrup-Jensen KU, Lolk A, Andersen K, Kragh-Sørensen P (2004) Ability to perform activities of daily living is the main factor affecting quality of life in patients with dementia. Health Qual Life Outcomes 2: 52.

25. Deacon RM, Rawlins JN (2006) T-maze alternation in the rodent. Nat Protoc 1: 7-12.

26. Dember WN, Richman CL (1989) Spontaneous Alternation Behavior. Springer: New York.