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ALIGNING COGNITIVE STUDIES IN MOUSE MODELS AND HUMAN INFANTS/TODDLERS: THE CASE OF DOWN SYNDROME

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Professor Annette Karmiloff-Smith and LonDownS: Aligning mouse and human phenotyping

Of Annette Karmiloff-Smith's many contributions to developmental psychology, the thrust of her work in developmental disorders can be summarised as follows: in order to truly understand neurodevelopmental disorders, we need to study development across levels of description, across disorders and across species. This approach was evident in how she led the Infant stream within the *London Down Syndrome* (LonDownS) *Consortium*. LonDownS is a large interdisciplinary collaboration between human geneticists, cellular biologists, psychiatrists, psychologists, neuroscientists and mouse geneticists, whose aim is to understand the link between Down syndrome (DS) and Alzheimer's disease (AD), and to identify protective and risk factors that could inform interventions. One of Annette's goals within LonDownS was to nurture cross-talk between mouse and human phenotyping. In particular, Annette focused on one key issue – aligning the designs of memory tasks for human infants/toddlers with DS with behavioural tasks that are used with mice.

In this chapter, we discuss how mouse models can deepen our understanding of neurodevelopmental disorders more generally, before focusing on a specific example of an attempt to align mouse model designs with human infant/toddler studies in DS within LonDownS. We present this cross-species study to illustrate Annette's thinking on the subject. While the design is not fully complete, it serves to demonstrate what is required to make cross-species comparisons scientifically valuable. We begin by considering mouse models of neurodevelopmental disorders.

How can mouse models deepen our understanding of neurodevelopmental disorders?

The mouse is a commonly used model organism in neurodevelopmental disorder research (Sukoff Rizzo, & Crawley, 2017). The advantage of the mouse model is that it permits a high degree of intervention in the system to establish causality, while human studies are understandably usually confined to natural experiments and correlations. In mouse studies, genetic background as well as environment can be tightly controlled and manipulated in order to establish causal pathways between genotype and phenotype. Any tissue can be accessed at any stage of development. This makes it possible to study where and when particular genes in the mouse are expressed. Furthermore, the lifespan of the mouse is short (one mouse year equals about 40 human years) and hence development is rapid (e.g., the time from birth to reproductive age is around 8 weeks) (Sukoff Rizzo, & Crawley, 2017). This allows researchers to repeat longitudinal experiments multiple times within a relatively short period of time and several generations can be observed. Finally, mouse models present an opportunity to test potential therapeutic interventions.

The value of each mouse model of a neurodevelopmental disorder depends on two sorts of alignment: (1) How well the genetics and physiology of the mouse align with the human; and (2) How well the cognitive and behavioural phenotyping of the mouse maps to the human.

Genetic and physiological alignment

Using the mouse as a model organism is particularly advantageous as the genes conserved between mouse and human are well mapped, and the mouse genome can be relatively easily manipulated. The mouse and human genomes share many similarities, including large syntenic genomic regions that directly map between the two species (Breschi, Gingeras, & Guigó, 2017). Evolutionary divergences vary between functional gene clusters, with genes involved in immunology and reproductive organs being particularly divergent, and regulation of gene expression and splicing often varying between the two species (Brawand et al., 2011; Yue et al., 2014). However, many biochemical and physiological processes are conserved in humans and mice, allowing us to draw conclusions about the function of conserved genes and genetic networks across these species.

Cognitive and behavioural phenotyping alignment

When comparing the cognitive processes and behaviours of mice and humans, it is important to use cross-species tasks that measure the same variable. Here, it is crucial to ascertain that task analogues across species not only look similar

from a behavioural perspective, but also that they have the same cognitive demands and neural underpinnings (Edgin, Mason, Spanò, Fernández, & Nadel, 2012; Karmiloff-Smith et al., 2012). Certain variables may be easier to align tasks on than others. Particularly, it may not be feasible to conduct cross-species studies on higher-level cognitive functions, such as language. This is a significant limitation, because such functions are often the core phenotypic difficulties described across many neurodevelopmental disorders, including DS.

In Annette's presentations and writings, she would often highlight the need for better alignment of tasks across species by using an example from spatial cognition (e.g., Karmiloff-Smith, 2007). To study this domain in rodent research, the Morris water maze is commonly used (see Figure 16.1a; Morris, 1981). In this task, a rodent is placed in a water basin with opaque water, which contains a platform just below the surface of the water. Learning of location of this platform is assessed across multiple trials. In contrast, in humans, spatial abilities are often assessed using tests which involve the manipulation of spatial relations between objects while the participant remains seated at a table (e.g., the Block design subtest from the Wechsler Intelligence Scale for Children [WISC, Wechsler, 2014]; see Figure 16.1b). Even though both the rodent and human tasks are supposed to measure spatial abilities, it is difficult to directly compare these two tasks as a number of differences exist between them. For example, in the case of the Morris water maze, the rodent needs to constantly update its changing body position in space, in contrast to the Block design where the body remains static while the human needs to represent the changing relations between objects (Karmiloff-Smith, 2007). These are viewed as different types of spatial cognition even within humans (Uttal et al., 2013). To improve the alignment of tasks across species, a Morris-type virtual water maze task for humans was developed in which human participants navigate through space on a computer (e.g., Kallai, Makany, Karadi, & Jacobs, 2005). An even closer analogue to the rodent task has been used in humans – a spatial test where humans have to find targets in an arena (Kalová, Vlček, Jarolímová, & Bureš, 2005; Laczó et al., 2017; Smith, Gilchrist, Hood, Tassabehji, & Karmiloff-Smith, 2009). Even though this may seem like a close analogue to the rodent task in terms of body position changes, these tasks still differ on a number of dimensions. For example, most of the spatial cognition tasks used in humans rely on understanding verbal instructions, something that is not possible to implement with either mice or young children with/without neurodevelopmental disorders.

Annette argued that we need to think about how to better align tasks across species and age groups. In her own research, she would try to come as close as ethically possible to a Morris water maze with children by using a ball pit with hidden treasure (see Figure 16.1c; Westermann, Thomas, & Karmiloff-Smith, 2011). However, even with this task, Annette would argue that better

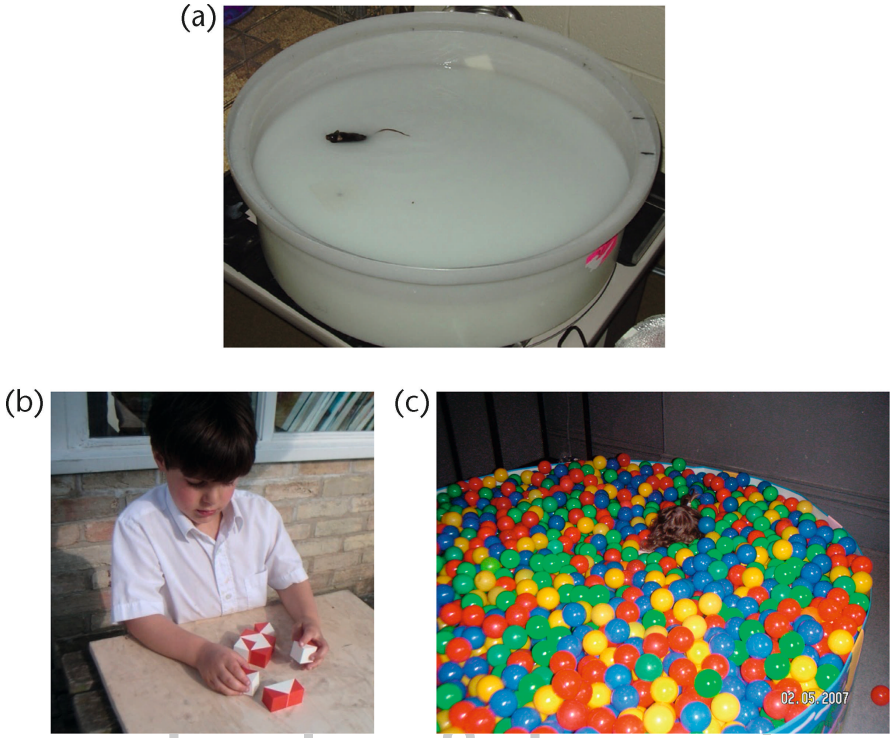

FIGURE 16.1 Comparisons of rodent-human analogues: (a) Morris water maze used with rodents to study spatial cognition (adapted from Hamilton, & Rhodes, 2015); (b) Block design used with humans to study spatial cognition; (c) Ball pit with hidden treasure used with children

alignment is necessary because motivation remains different across the species: fear in the case of the rodent to escape drowning, and reward in the case of the child to find the treasure. Here, ethical considerations prevent closer alignment from human to rodent. Therefore, it would be necessary to adjust the design in the opposite direction, with a version of the animal task that emphasises reward (e.g., the Barnes Maze [Barnes, 1979], Radial Arm Maze [Olton, & Samuelson, 1976]). To optimise alignment, researchers working with the different species therefore need to collaborate at the design stage.

We now turn to consider mouse models in the specific context of Down syndrome, the disorder that Annette focused on in her final work within LonDownS.

Focus on Down syndrome

Down syndrome (DS) is the most prevalent neurodevelopmental disorder of known genetic origin (trisomy 21) associated with intellectual disability, occurring approximately in one in 1,000 live births (Wu, & Morris, 2013). One of the core difficulties described in DS is memory (for review, see Godfrey, & Lee, 2018). This is of particular interest as individuals with DS show higher prevalence of

Alzheimer's disease (AD) than typically developing (TD) individuals (Zigman, & Lott, 2007), and the defining clinical feature of AD is an acquired memory deficit (Carlesimo, & Oscar-Berman, 1992). By the age of 40 years, individuals with DS show universal neuropathology associated with AD, including depositions of plaques and tangles, likely due to trisomy of the amyloid precursor protein (*APP*) gene which lies on chromosome 21. Ninety-seven percent of people with full trisomy of chromosome 21 develop dementia by the age of 80 years (McCarron et al., 2017; Zigman, & Lott, 2007). However, despite the elevated risk, a great deal of variation is observed in the age of onset of dementia in people with DS (McCarron et al., 2017). The cause of these differences in disease course is not clear, and both genetic and environmental factors are likely to play a role. Understanding these factors is not only of interest for understanding AD in DS, but also AD in the general population.

Mouse models of Down syndrome

Mouse models present a useful tool for unpacking mechanisms through which trisomy 21 contributes to AD in DS, as well as to memory difficulties in DS more broadly. Humans have 23 pairs of chromosomes, while mice have 20 pairs. Human chromosome 21 is the smallest human autosome (non-sex chromosome), representing about 1.5% of the total DNA (Hattori et al., 2000). It contains 222 protein-coding genes and 325 non-protein-coding genes (Gupta, Dhanasekaran, & Gardiner, 2016). Out of the protein-coding genes, 158 are conserved in the mouse but are distributed across three different mouse chromosomes. Most of these genes (102) lie on mouse chromosome 16, and a few on mouse chromosome 10 and 17 (37 and 19, respectively) (Gupta et al., 2016). Out of the non-coding genes, 75 are distributed across these three mouse chromosomes (Gupta et al., 2016). The distribution of conserved genetic material across the three different chromosomes, as well as some genes which are not conserved across the species, makes it challenging to create a complete mouse model of trisomy 21 (Antonarakis, Lyle, Dermitzakis, Reymond, & Deutsch, 2004). Nevertheless, a number of mouse models of DS have been developed (see Figure 16.2), which greatly advance our understanding of how trisomy of chromosome 21 relates to the DS phenotype (e.g., Herault et al., 2017; Ruparelia, Pearn, & Mobley, 2013).

Comprehensive reviews of the extent to which findings from mouse models of DS map onto findings in humans with DS have been conducted elsewhere (e.g., Gupta et al., 2016; Herault et al., 2017; Zhao, & Bhattacharyya, 2018). Here, we focus in detail on a specific attempt led by Annette to design memory tasks to be used with infants/toddlers with DS based on memory tasks used with the trisomy 21 mouse model – the Tc1 mouse (O'Doherty et al., 2005; see Figure 16.2). The Tc1 mouse is a transchromosomic model of DS – it carries a freely segregating copy of human chromosome 21 (with approximately 75% of gene content; Gribble et al., 2013). However, unlike individuals with DS, Tc1 mice are not

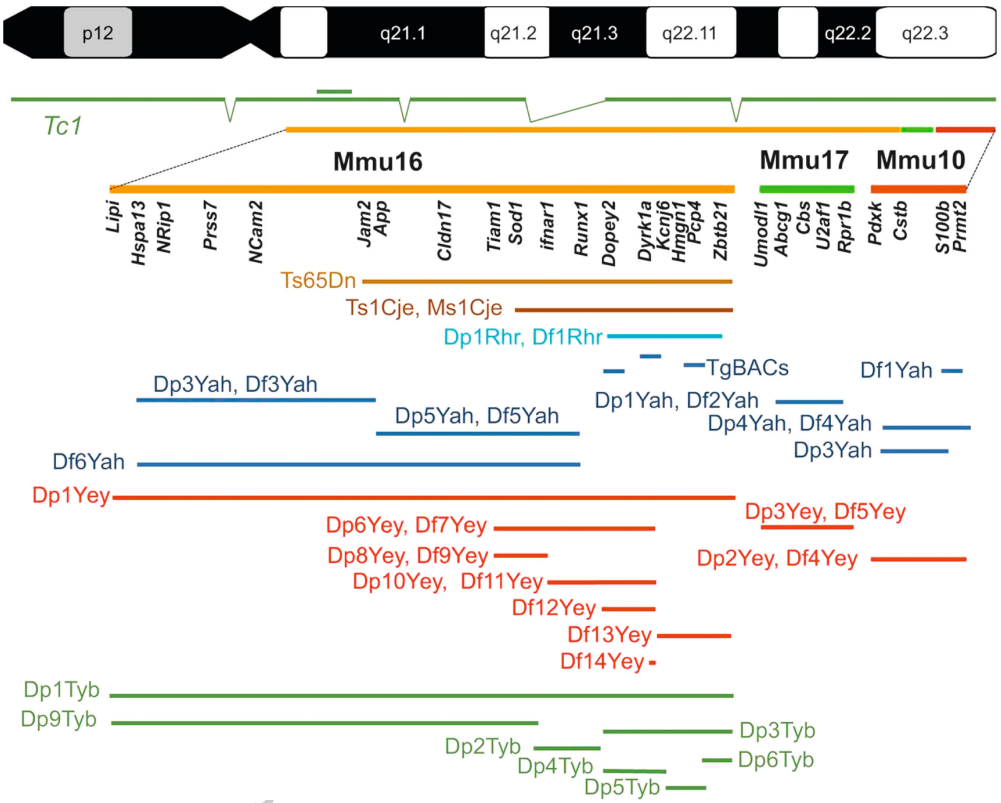

FIGURE 16.2 Mouse models of DS. Human chromosome 21 is depicted at the top of the figure; Mmu10 = mouse chromosome 10; Mmu16 = mouse chromosome 16; Mmu17 = mouse chromosome 17. Tc1 mouse which is of interest in the current chapter is shown in dark green under the human chromosome, with deletions and a duplication (double bar) depicted. Other codes and lines correspond to other mouse models of DS. Adapted from Herault et al. (2017)

trisomic for the *APP* gene, the gene thought to cause early onset AD (Gribble et al., 2013). Therefore, the Tc1 mouse allows us to investigate the contribution of chromosome 21 genes to memory, isolating it from contributions caused by the overexpressed *APP* gene.

Memory phenotyping of Tc1 mouse

Hall et al. (2016) investigated memory function in the Tc1 mouse across four different tasks: (1) novel object recognition task, (2) object-in-place memory task, (3) object location memory task, and (4) novel odour recognition task (Figure 16.3). All tasks were administered using the same apparatus – a square arena (60 × 60 × 40 cm) with a pale grey floor and white walls. An overhead camera was used to record the mouse's object exploration. This was defined as the time spent actively attending to (sniffing or interacting with) the object at a distance no greater than 1 cm.

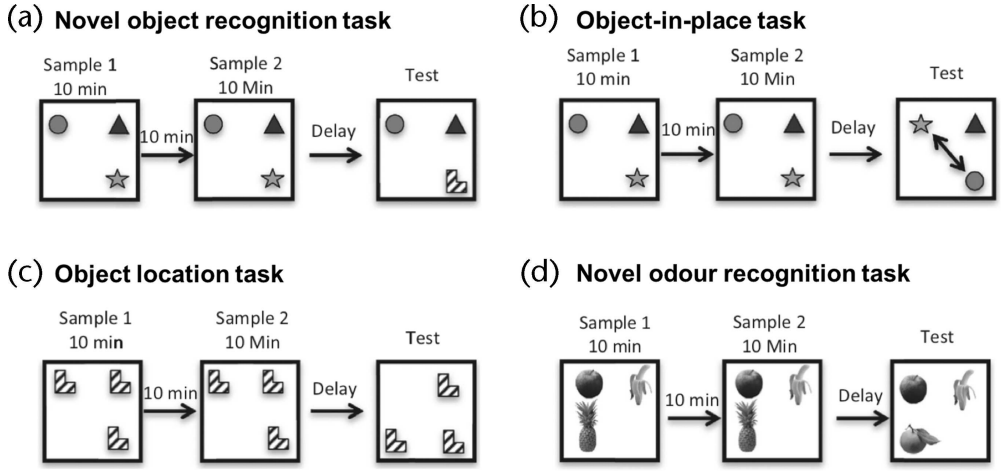

FIGURE 16.3 Design of memory tasks used with mice in Hall et al. (2016): (a) Novel object recognition task; (b) Object-in-place task; (c) Object location task; (d) Novel odour recognition task. Modified from Hall et al. (2016)

In the *novel object recognition task* (Figure 16.3a), a mouse was placed in the centre of the arena and presented with three different objects, each in a different corner. The mouse was allowed to explore for 10 minutes and was then removed from the arena for 10 minutes, followed by another 10-minute sample phase and removal from the arena. After these two sample phases, the mouse was returned to the arena either immediately (within approximately 30 seconds), after a short-term delay (10 minutes), or after a long-term delay (24 hours) for the test phase. In this test, one of the objects was replaced with a novel object. The objects and the arena were wiped down between sample phases and test phase to prevent a mouse from using olfactory cues. Increased exploration of the novel object in the test phase was taken as evidence of novel object recognition memory. Compared to non-genetically modified (wild type [WT]) mice, Tc1 mice showed intact immediate, impaired short-term, and intact long-term object recognition memory behaviour (for the summary of results, see Figure 16.4).

To test whether immediate and long-term memory behaviour were also intact in Tc1 mice for spatial organisation of objects, the *object-in-place memory task* was administered (Figure 16.3b). The procedure was identical to the novel object recognition task described above except that in the test phase, two of the objects swapped their spatial locations instead of a novel object appearing. As with the novel object recognition task, Tc1 mice showed sensitivity to the object-in-place change (i.e., increased exploration of the objects in a different location) in both immediate and long-term memory delay, although the short-term condition was not run here (for the summary of results, see Figure 16.4).

As described above, in the novel object recognition task, Tc1 mice demonstrated an impaired ability to detect the novel object after a 10-minute delay. However, it was unclear whether this impairment was specific to novel object detection only or broadly present across other types of memory. Therefore,

Mouse model – Memory task	Immediate memory	Short-term memory	Long-term memory
Tc1 – Novel object 	Intact	Impaired	Intact
Tc1 – Object-in-place 	Intact	NA	Intact
Tc1 – Object location 	NA	Intact	NA
Tc1 – Novel odour 	NA	Impaired	Intact
Tg2576 – Novel object 	NA	Intact	Impaired

FIGURE 16.4 Summary of results from Hall et al. (2016). Immediate memory = approximately 30-second delay; Short-term memory = 10-minute delay; Long-term memory = 24-hour delay; NA = not administered. Images modified from Hall et al. (2016)

sensitivity to change in location of a familiar object was tested in Tc1 mice after a 10-minute delay using the *object location memory task* (Figure 16.3c). In this task, the procedure was similar to the two tasks above, except that three identical objects were present in three corners of the arena during the sample phase. In the test phase, one of the three objects was moved to the previously empty corner of the arena. Increased exploration of the object in the novel location in the test phase was taken as evidence of memory for the locations occupied by objects in the sample phase. Tc1 mice showed sensitivity to the location change of the familiar object after a 10-minute delay (for the summary of results, see Figure 16.4). Therefore, the difficulty in detecting object novelty following a short-term delay was not due to a general problem with either detecting novelty or modifying exploratory behaviour after this delay.

To assess the extent to which the impairment in object recognition was part of a more general recognition memory deficit in Tc1 mice, an olfactory version of this task (the *novel odour recognition task*) was administered (Figure 16.3d). Here, a mouse was presented with three visually identical plastic cubes each containing a different scent, each in a different corner of the arena. After the sample phase, following a 10-minute or 24-hour delay, one of the odour cubes was replaced with a novel odour cube for the test phase. As with the novel object recognition task, the Tc1 mouse showed impaired short-term but intact long-term recognition memory behaviour for odours (for the summary of results, see Figure 16.4). Therefore, the short-term impairment in recognition memory was not specific to a single sensory modality, but generalisable across different sensory domains.

To ascertain whether the pattern of memory differences in Tc1 mice was specific to human chromosome 21 expression and not a general consequence of the expression of human genes, Tg2576 mice were also tested. Tg2576 mice express a human Swedish *APP* mutation associated with early onset AD (Hsiao

et al., 1996). The overexpression of *APP* is absent in Tc1 mice, unlike in individuals with DS, because the Tc1 mouse model does not contain an additional copy of *APP*. Therefore, it is theoretically interesting to compare these two mouse models. Tg2576 mice were administered the novel object recognition task. The pattern of results was opposite to what was found in Tc1 mice: Tg2576 showed intact performance following a short-term memory delay but impairment after long-term delay (for the summary of results, see Figure 16.4). This suggests that the short-term recognition deficit in the Tc1 mouse was not simply a non-specific consequence of the expression of human genes.

As summarised in Figure 16.4, Tc1 mice showed delay-dependent difficulties in recognition memory. Specifically, they showed intact immediate (30 seconds), impaired short-term (10 minutes), and intact long-term (24 hours) memory behaviour for novel object/odour recognition. No deficit was present in the ability to detect spatial novelty. The pattern suggests a memory consolidation rate deficit (such that memories are not consolidated by the end of the short-term delay, but are consolidated by the end of the long-term delay) in the object/odour memory system but not the spatial memory system.

Building analogues of mouse memory tasks for infants/toddlers with Down syndrome

The results from the Tc1 mice described above are broadly consistent with reports of short-term memory difficulties in individuals with DS (Godfrey, & Lee, 2018). However, any meaningful comparison would require a test of object and spatial memory using similar procedures in humans. There is currently only a limited number of memory studies on infants/toddlers with DS (for a review, see Godfrey, & Lee, 2018). Their designs are rather distant from the designs of the mouse memory tasks described above, predominantly focusing on imitation abilities (e.g., Milojevich, & Lukowski, 2016; Rast, & Meltzoff, 1995). Therefore, novel tasks are needed.

In Annette's presentations, she would emphasise several points important for establishing genotype/phenotype correlations across species when designing new tasks. Human/animal models must compare "like with like". We need to develop tasks that can be used across different species (mouse, human) and age groups (infants/toddlers, children, adults, elderly). This means that the tasks have to be non-verbal: they cannot rely on the ability to understand or produce language. Furthermore, the tasks must impose comparable cognitive and neural demands.

Clearly, when designing analogues of the mouse memory tasks for infants/toddlers with DS, a number of factors need to be considered. Unfortunately, many decisions have to be made with little empirical guidance, as there is a lack of systematic investigation of these factors across species (and often, even within species). Some of the factors will be discussed below.

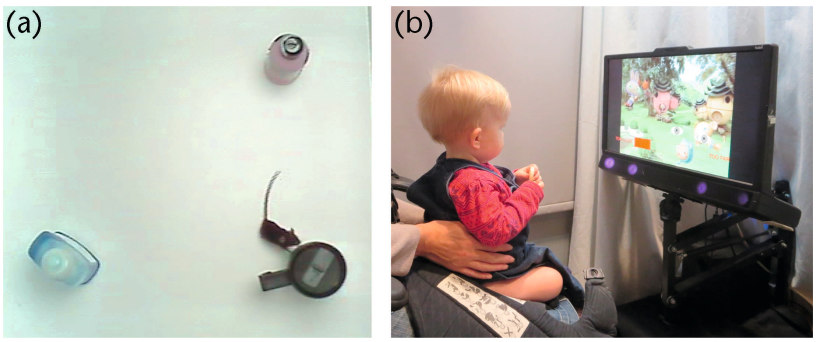

FIGURE 16.5 (a) Hall et al.'s procedure with mice relies on gross motor abilities during exploratory behaviour; (b) Remote eye-tracking as a data collection technique suitable across different ages in humans, minimising the need for whole-body or limb motor skills

Measure of exploration

Hall et al.'s procedure with mice relies on gross motor abilities (Figure 16.5a), a domain in which many infants/toddlers with DS show delays (D'Souza, Karmiloff-Smith, Mareschal, & Thomas, 2020; Fidler, Hepburn, & Rogers, 2006; Will, Caravella, Hahn, Fidler, & Roberts, 2018). Even though virtually all children with DS eventually learn to walk, some of them do not start walking until they are 5 years of age (Palisano et al., 2001). Therefore, any measure relying on locomotor abilities is unsuitable for infants/toddlers with DS. Similarly, manual exploration has been found to be delayed (de Campos, da Costa, Savelsbergh, & Rocha, 2013).

Thus, comparing "like with like" across species may not always mean using an identical method of exploration, but instead one should consider what the most efficient mode of exploration is for a particular species and age group. Visual exploration has been used in a number of studies with infants/toddlers with DS as an indicator of cognitive abilities (D'Souza, D'Souza, Johnson, & Karmiloff-Smith, 2015; D'Souza, D'Souza, Johnson, & Karmiloff-Smith, 2016; Fidler, Schworer, Will, Patel, & Daunhauer, 2019). In the past, it was necessary to video record an infant's eyes and tediously hand-code changes in eye gaze frame-by-frame. However, nowadays eye movements can be detected and coded automatically, using infant-friendly remote eye-tracking technology (see Figure 16.5b; Gredebäck, Johnson, & von Hofsten, 2009). Therefore, ease of administration and analysis made remote eye-tracking the method of choice for mouse task analogues in this study.

Stimuli

Another decision to be made when analogues of mouse and human studies are designed is what stimuli should be used. Here again, comparing "like with like" may not always mean using identical stimuli across species. Rather the concern

should be about using stimuli which engage interest (i.e., attentional processes) across species. The stimuli used by Hall et al. (2016) consisted of everyday objects made of non-porous materials, selected on the basis of differences in shape and pattern (based on pilot work), as mice do not have particularly good colour vision (Jacobs, 1993).

Selecting engaging stimuli for use in human infant/toddler studies is of utmost importance for retention of these participants in eye-tracking paradigms. The stimuli selected by the LonDownS Infant stream involved photos of everyday objects or child friendly pictures (see below in Section 4) that infants/toddlers showed interest in during the pilot study.

Number of trials and timing

Another set of considerations when aligning mouse and human studies relates to differences in the timing of cognitive processes across species. This is crucial to consider when thinking about the number of trials needed for learning, as well as the duration of familiarisation and test trials. What is the human infant/toddler equivalent of a 10-minute exposure to objects in the mouse? What is the human infant/toddler equivalent of a 10-minute delay in the mouse? Hall et al. (2016) presented mice with two familiarisation trials, each lasting 10 minutes. For any study with infants/toddlers relying on visual exploration, this would be a very long time frame. We know from widely used habituation designs in human infant testing, which rely on the infant's diminished interest in a stimulus over time, that human infants lose interest in novel visual stimuli after just a few seconds (e.g., Bremner, & Fogel, 2004). A lack of interest in experimental stimuli would increase the chance of premature termination of the testing paradigm before any test trials could be administered, as infants/toddlers are more likely to become bored and/or upset. Therefore, it is necessary to think carefully about the minimum exposure needed to tap into similar cognitive processes in the mouse and human. Again, there is a lack of systematic comparative studies on rate of learning in mice and human infants. Therefore, the convention was borrowed from human infant studies, where the length of trials used with human infants is usually in the order of seconds. The exact length of trials (see Section 4 below) was determined by piloting.

LonDownS memory task design for human infants/ toddlers with Down syndrome

In this section, we describe the specific task design constructed in an attempt to align mouse models of DS with human infant/toddler studies. The LonDownS Infant stream focused on two memory tasks administered by Hall et al. (2016): *novel object recognition* and *object-in-place* (described in detail above), with the aim of creating analogues in human infants/toddlers with DS for the immediate time point. In the human infant/toddler equivalent of the task, a number of objects were presented to the child on a screen. Each memory task consisted of two

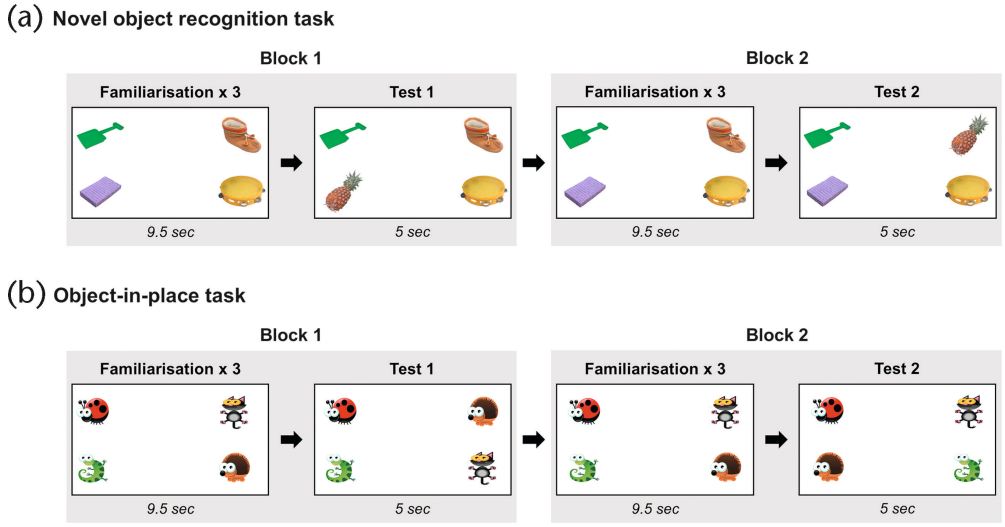

FIGURE 16.6 The layout of the stimuli on the screen in the human analogues of the mouse tasks: (a) Novel object recognition task; (b) Object-in-place task

blocks. In each block, three familiarisation trials were followed by a test trial. In both memory tasks, each familiarisation trial contained four stimuli onscreen. In the test trial of the *novel object recognition task* (Figure 16.6a), one of the familiar stimuli was replaced with a novel stimulus. In the test trial of the *object-in-place memory task* (Figure 16.6b), two of the familiar stimuli exchanged positions. To make the task more interesting for children, the images in the familiarisation trials expanded and contracted, and the tasks were accompanied by child-friendly music. Furthermore, to increase data quality, the pace of the stimuli presentation was tailored to each child, in that each trial was manually initiated by the experimenter only when the child was looking at the screen.

An eye-tracker was used to record eye gaze. Change in looking was computed by subtracting the proportion of looking at each quadrant in the familiarisation trial that immediately preceded the test trial, from the proportion of looking at each quadrant in the test trial. A positive change in looking indicates an increase in looking towards the quadrant after the change.

Participants

Data were collected from 85 infants and toddlers with a clinical diagnosis of DS and 63 typically developing (TD) children at Birkbeck, University of London as part of the LonDownS Consortium Infant stream protocol[1]. The DS group did not significantly differ from the TD group on chronological age or gender (see Table 16.1). A further 12 young children with DS and two TD children were tested but did not yield usable data for diverse reasons including fatigue, fussiness and technical difficulties.

TABLE 16.1 Participant characteristics

		Group		
		DS	*TD*	*Comparison*
Number		85	63	NA
Age (months)		6.13-51.50 M = 26.76, SD = 12.35	4.67-52.47 M = 24.11, SD = 12.93	$t(146) = 1.27$, $p = .207$
Gender	Females	39 (45.9%)	34 (54.0%)	$\chi^2(1) = 0.95$, $p = .331$
	Males	46 (54.1%)	29 (46.0%)	

Note. DS = children with Down syndrome; TD = typically developing children; NA = not applicable.

The participants were recruited via existing participant databases and support groups. Ethical approval was obtained from the North West Wales National Health Service (NHS) Research Ethics Committee (13/WA/0194) and Birkbeck Psychological Sciences Ethics Committee (121373). Prior to testing, informed consent was obtained from parents. Participants were given a small gift (e.g., a T-shirt) in return for their participation.

Equipment

A Tobii TX300 remote eye-tracker was used to capture information on moment-to-moment point of gaze, with measurement accuracy of approximately 0.5° and spatial resolution of approximately 0.05°. The tracking equipment and stimulus presentation were controlled using customised Matlab scripts (Mathworks Inc., Natick, MA, U.S.). The screen was 58.42 cm with a resolution of 1920 × 1080 pixels. Participants were seated approximately 65 cm from the screen. A camera mounted directly above the horizontal midpoint of the screen was used to monitor and record the child's behaviour. Auditory stimuli were delivered via two speakers centrally positioned below the screen.

Results

Novel object recognition task

As illustrated in Figure 16.7, both TD and DS groups showed an increase in looking to the quadrant in which the novel object appeared – for both the first test trial (bottom left quadrant, one sample t-test against 0, TD: $t(53) = 10.52$, $p < .001$, $d = 1.43$; DS: $t(77) = 6.50$, $p < .001$, $d = 0.74$) and second test trial (top right quadrant, one sample t-test against 0, TD: $t(53) = 4.90$, $p < .001$, $d = 0.67$; DS: $t(76) = 2.58$, $p = .012$, $d = 0.29$).

To compare looking behaviour in the quadrant of change across groups and blocks, as well as examine the effect of chronological age, we conducted a 2 × 2 × 2

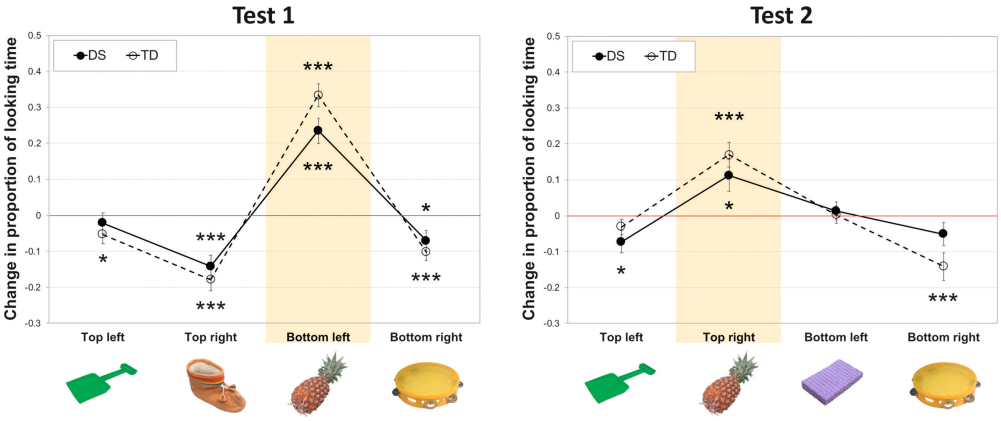

FIGURE 16.7 Change in proportion of looking time from the last familiarisation trial to the test trial in the novel object recognition task in typically developing children (TD) and children with Down syndrome (DS). The quadrant in which a new object appeared is highlighted in yellow. Stars indicate that the values significantly differed from 0 (no change, red line), analysed using one-sample *t*-tests. Error bars show ±1 *SE*; ⋆$p < .050$, ⋆⋆$p < .010$, ⋆⋆⋆$p < .001$

mixed ANCOVA. This analysis included trial type (last familiarisation trial, test trial) and block (first, second) as the within-subject factors, group (TD, DS) as the between-subject factor, and chronological age as a covariate. There was a significant main effect of block, $F(1,126) = 15.82$, $p < .001$, $\eta_p^2 = .11$. This was unexpected and is consistent with a location/object bias. Importantly, there was a significant main effect of trial, $F(1,126) = 30.63$, $p < .001$, $\eta_p^2 = .20$. This suggests that irrespective of the group, children showed a significant increase in looking in the quadrant of change in the test trial compared to the last familiarisation trial. This pattern did not differ between blocks, as none of the interactions including trial x block were significant.

Object-in-place memory task

As illustrated in Figure 16.8, both TD and DS groups showed an increase in looking to the quadrants in which the objects changed their position in the first test trial. As indicated by one-sample *t*-tests against 0, the TD group increased their looking to the bottom right quadrant ($t(56) = 3.03$, $p = .004$, $d = 0.40$) and not to the top right quadrant ($t(56) = -1.57$, $p = .121$); the opposite was true for the DS group (bottom right quadrant: $t(75) = -1.84$, $p = .070$; top right quadrant: $t(75) = 2.35$, $p = .021$, $d = 0.27$). For the second test trial, both TD and DS increased their looking to the bottom left quadrant (TD: $t(54) = 2.12$, $p = .039$, $d = 0.29$; DS: $t(77) = 4.90$, $p < .001$, $d = 0.55$).

To compare looking behaviour in the quadrant of change across groups and blocks, and to examine the effect of chronological age, we conducted a 2 × 2 × 2 mixed ANCOVA. This analysis included trial type (last familiarisation trial, test trial) and block (first, second) as the within-subject factors, group (TD, DS) as the between-subject factor, and chronological age as a covariate. The quadrant of change for the analysis was selected based on the above presented one sample

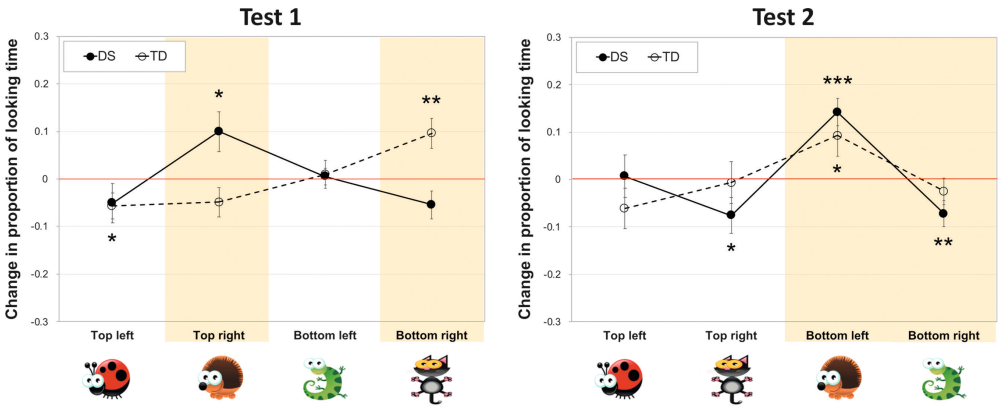

FIGURE 16.8 Change in proportion of looking time from the last familiarisation trial to the test trial in the object-in-place memory task in typically developing children (TD) and children with Down syndrome (DS). The quadrants in which the two objects changed location are highlighted in yellow. Stars indicate that the values significantly differed from 0 (no change, red line), analysed using one-sample *t*-tests. Error bars show ±1 *SE*; ⋆$p < .050$, ⋆⋆$p < .010$, ⋆⋆⋆$p < .001$

t-tests analysis (i.e., in the first block, the bottom right quadrant for TD and the top right quadrant for DS; for the second block, the bottom left quadrant for both groups). There was a significant main effect of block ($F(1,125) = 5.06$, $p = .026$, $\eta_p^2 = .04$) and a significant interaction between block and group ($F(1,125) = 16.55$, $p < .001$, $\eta_p^2 = .12$). This was unexpected and is consistent with a location/object bias. Importantly, there was a significant main effect of trial ($F(1,125) = 15.12$, $p < .001$, $\eta_p^2 = .11$). This suggests that irrespective of the group, children showed a significant increase in looking in the quadrant of change in the test trial compared to the last familiarisation trial. This pattern did not differ between blocks, as none of the interactions including trial x block were significant.

Summary

In sum, like TD children of the same chronological age, infants/toddlers with DS showed looking behaviour consistent with an ability to recognise the change in the novel object recognition task as well as in the object-in-place memory task at the immediate test. This is consistent with behaviour shown in the Tc1 mouse.

Improving alignment between mouse and human studies in the current study

More tasks, more delayed timepoints, and converging evidence

The current memory study with human infants/toddlers with DS focused only on the novel object recognition task and object-in-place memory task. We present the data here not because the design is complete, but because it illustrates the kinds of

decisions that need to be made in attempting to align human infant/toddler and mouse model paradigms. For a more comprehensive comparison, other memory tasks used with mice (as introduced above) need to be included. Furthermore, the current study tested only the immediate timepoint. Future studies need to investigate how infants/toddlers with DS perform after a short-term and long-term delay in order to ascertain the extent to which their performance parallels that of the Tc1 mouse. The short-term delayed time point can be easily built into the eye-tracking session, though it needs to be determined whether a 10-minute delayed time point in mice taps into similar cognitive processes as a 10-minute delay in humans. Here, combining behavioural and electrophysiological/neuroimaging measures could help ascertain the progression of various cognitive processes, as well as confirm that the underlying neural processes do not differ across species. Matching time courses of different cognitive processes is of importance as the crucial finding in the Tc1 mouse was a specific deficit in short-term memory delay (10 minutes), consistent with a consolidation rate deficit (the impairment was not manifested at either the immediate or long-term timepoints). Ideally, one would like to test infants/toddlers and mice at incrementally increasing delays to map and compare memory functioning across time. Of course, this would be a rather challenging endeavour, as a large sample size would be needed: different participants would have to be tested at different delays, because participants would be likely to habituate to the paradigm even if numerous interesting objects were included.

Re-centering infant/toddler attention and the use of a mask between trials

The test trials in the current study followed directly from the familiarisation trials, without re-centring the infant's attention in between. For future studies, the infant's attention needs to be brought back to the centre of the screen at the beginning of each test trial in parallel to the mice being placed in the centre of the arena. Furthermore, a mask between trials should be considered to flush out any retinal image in the human infants/toddlers, similar to the arena being wiped down between trials in the mouse task to control for potentially confounding factors.

Number and positioning of stimuli

In the mouse memory tasks, only three objects were used, while four images were presented in the case of human infants/toddlers. It is difficult to know whether these created a similar memory load across species. Furthermore, the duration of the familiarisation trials substantially differed across the two species (2 × 10 minutes for the mice, 2 × 28.5 seconds for the human infants/toddlers). The effects of the number of stimuli and duration of familiarisation on memory across species require systematic investigation. The current study with humans suggests that processing four stimuli within the familiarisation window is possible for both young children with DS and TD children of the same chronological age.

The stimuli across the human and mouse studies were positioned in the corners of their respective "arenas". In the case of the mouse, that meant in three corners of the square arena; in the case of the human infant/toddler, in four corners of the rectangular screen. This means that while objects were evenly distributed along the perimeter in the case of the mouse, this was not the case in the human study. Future studies should consider whether this could affect task performance. Generally, it is advisable to keep distances between objects constant in order to control for any confounding effect of object distance between object pairs. Furthermore, it is advisable to introduce counterbalancing in order to disentangle object bias from quadrant bias. Finally, using only three objects for future infant/toddler designs would allow researchers to more easily include an object location memory task which requires one of the corners to be kept empty (for one of the objects to move to a new location). Keeping the number of stimuli consistent across the different tasks would allow for a more direct comparison (at least within species).

Analogous developmental stages across species

One key challenge is how to match the ages of human participants to the animals in the model species. Hall et al. (2016) tested adult mice (4–7 months of age), while the LonDownS Infant stream focused on human infants/toddlers (4–52 months). Comparison of different developmental stages complicates comparisons across species, as some of the memory impairments may emerge later in development or be present early and then disappear. The cognitive profile in individuals with DS changes across the lifespan, showing not only general slowing over development, but also changes in strengths and weaknesses (D'Souza, Karmiloff-Smith, et al., 2020; Grieco, Pulsifer, Seligsohn, Skotko, & Schwartz, 2015). Therefore, taking a snapshot of development in infancy/toddlerhood in humans and in adulthood in mice is unlikely to provide us with insights about such developmental changes. As Annette ferociously argued throughout her career, we need to place development at the very core of our studies (D'Souza, D'Souza, & Karmiloff-Smith, 2017; D'Souza, & Karmiloff-Smith, 2011; D'Souza, & Karmiloff-Smith, 2017; Karmiloff-Smith, 1981, 1998, 2010; Karmiloff-Smith, Scerif, & Thomas, 2002). Therefore, future studies should conduct systematic longitudinal comparisons across species. Understanding the development of memory in infancy/toddlerhood in DS may help to identify precursors of memory decline in middle and later adulthood, which are of interest due to highly comorbid AD (Wiseman et al., 2015), as well as factors that exaggerate or attenuate the risk of memory decline (D'Souza, Mason, et al., 2020; Thomas et al., 2020).

Future focus: Understanding individual differences in mouse models and humans

Large individual differences have been described in DS (Karmiloff-Smith et al., 2016). For example, even though the median IQ in DS is around 50, some

individuals have an IQ as low as 30 or as high as around 90 (D'Ardhuy et al., 2015). Furthermore, although all individuals with DS exhibit the neuropathology associated with AD, the onset of AD is highly variable (McCarron et al., 2017; Zigman, & Lott, 2007). Individual differences are already present early in development, and understanding these individual differences across time has been a focus of the LonDownS Infant stream (e.g., D'Souza, Karmiloff-Smith, et al., 2020; D'Souza, Lathan, Karmiloff-Smith, & Mareschal, 2020; D'Souza, Mason, et al., 2020; Thomas et al., 2020). Following this line of research, it would be of interest to see whether individual differences on the eye-tracking memory tasks are predictive of developmental outcomes in DS. However, the current design is unlikely to permit such investigations. Although the low number of test trials in the current study was sufficient to analyse group performance, it may be psychometrically too weak to enable us to reliably measure individual performance. With the small number of trials, even though the mean performance of the groups was above chance, there was large variability in looking responses. Furthermore, no relationship with chronological age was observed either in infants/toddlers with DS or TD infants/toddlers (for illustration, see Figure 16.9). This may reflect lack of predictive validity due to the low number of trials (Siegelman, Bogaerts, & Frost, 2017).

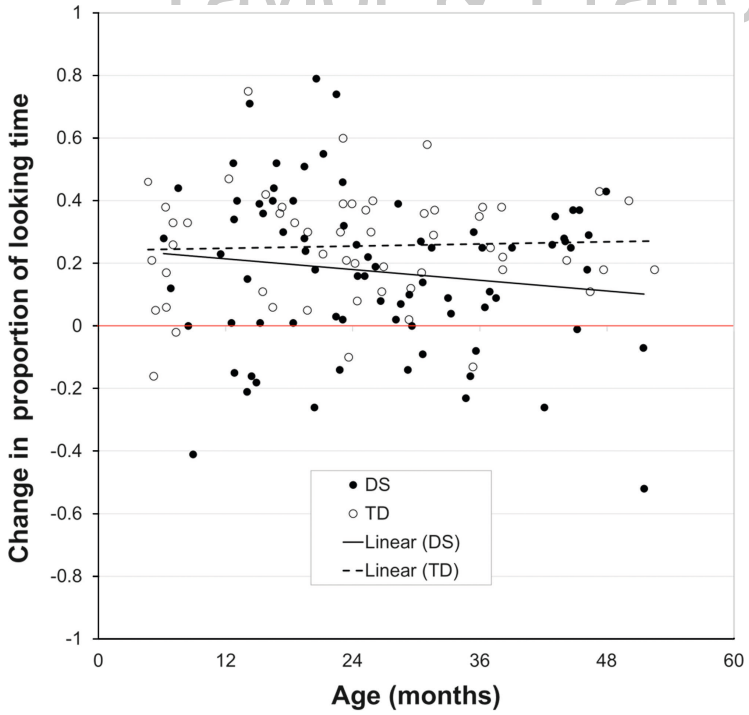

FIGURE 16.9 Change in proportion of looking time from the last familiarisation trial to the test trial averaged over the two blocks of the novel object recognition task, plotted against chronological age in months. The red line indicates no change (0). There is no correlation with age in either typically developing children (TD; $r(55) = .04$, $p = .753$) or children with Down syndrome (DS; $r(79) = -.14$, $p = .230$)

Future studies need to increase the number of trials. There is in fact a need for systematic investigation of how many trials are sufficient to learn about individual differences. While the number of trials can be easily increased in adult participants who are compliant, in infants/toddlers an increase of trials will increase the risk of boredom. This will have a negative impact on retention and limit how many different tasks the infants/toddlers can be administered within one testing session. Therefore, in order to reliably capture individual differences in infants/toddlers across a range of tasks, future projects will likely have to take place over multiple sessions. Developing tasks that can reliably measure individual differences is of utmost importance as there is an interest in pharmacological interventions early in development (even prenatally, Guidi et al., 2014), yet there is a lack of outcome measures which could be reliably used at this stage. Keeping the designs across mice and humans consistent would make interventions developed in mice easier to translate to humans.

The individual differences in infants/toddlers with DS are likely to be an outcome of different genetic backgrounds interacting with various environments (Karmiloff-Smith et al., 2016). These complex interactions between genes and environment are difficult to disentangle in humans, as there is limited scope for experimentally controlling factors contributing to development – something which is possible to do more comprehensively in mice. However, even though mouse models have provided us with important insights into possible mechanistic pathways, their explanatory power for individual differences is rather limited. This is partly due to careful control of mouse research to limit unknown genetic variation by control of genetic background (Finlay, 2019; Herault et al., 2017). However, the choice of genetic background can have a drastic impact on the outcome, even determining the offspring's viability (Sanford, Kallapur, Ormsby, & Doetschman, 2001; Threadgill et al., 1995). The influence of variability in the genetic background can also be detected in phenotypic outcomes when modelling neurodevelopmental and psychiatric disorders (Arbogast et al., 2016; Sittig et al., 2016).

Control of the genetic background of mouse models, and hence the limited range of combination of alleles studied in research, is accompanied by lack of variability in the environment, since mice are raised in controlled, stable environments. Yet, it is known that environment can influence phenotypic outcomes. For example, variability in maternal care has been shown to impact the phenotype of the offspring in both rodents and humans (Watamura, & Roth, 2019; Weaver et al., 2004). Furthermore, environmental enrichment has been shown to affect a phenotypic outcome in a mouse model of DS (Ts65Dn;Martínez-Cué et al., 2002; Martínez-Cué et al., 2005).

In sum, it is challenging to untangle the complex interactions between genes and environment across development in humans as there is very little space for the experimental manipulation of human development. Here, mouse models present promising future avenues because genetic background and environment can be systematically manipulated and measured across developmental time.

Conclusions

Mouse models hold great promise for advancing our understanding of the aetiology of cognitive profiles in humans with DS and may pave the way for more targeted interventions. However, the validity of each mouse model critically depends on how well the tasks used with the mice map onto cognitive processes of interest in humans. In this chapter, we have outlined some of the first attempts, led by Annette, to design tasks for infants/toddlers with DS based on those used with mouse models. Going forward, it is essential to keep communication lines between different disciplines open in order to design tasks that would enable us to directly compare emerging phenotypes across species and developmental time.

Annette addressed head on the methodological challenges of employing mouse models to understand behavioural phenotypes in neurodevelopmental disorders, as was her style. It was part of her broader commitment to a multidisciplinary, multilevel approach to investigating cognitive development, where the very complexity of the problems she was facing seemed to inspire Annette to new levels of methodological and theoretical innovation. This pioneering spirit will be much missed.

Acknowledgements

We would like to thank all the families in this study for their time. This work would also not have been possible without the help of George Ball, Joanne Ball, Nazanin Biabani, Amelia Channon, Charlotte Dennison, Dean D'Souza, Maria Eriksson, Jennifer Glennon, Kate Hughes, Malin Karstens, Amanda Lathan, Esha Massand, Kate Mee, Beejal Mehta, Lidija Nikolova, Isabel Quiroz, Irati Rodriguez Saez de Urabain, Jessica Schulz, Laura Simmons, Rebecca Thoburn Pallant, Margaux Trombert, Kate Whitaker, and many others. We would particularly like to highlight the contribution of George Ball and Esha Massand in designing the eye-tracking tasks, and Irati Rodriguez Saez de Urabain for programming these. We would also like to thank Tamara Al-Janabi for managing the LonDownS Consortium as a whole. This work was funded by a Wellcome Trust Strategic Award (grant number: 098330/Z/12/Z) conferred upon the LonDownS Consortium. Our work was further supported by the Waterloo Foundation and the Baily Thomas Charitable Fund. HD is the Beatrice Mary Dale Research Fellow supported by Newnham College (University of Cambridge) and the Isaac Newton Trust. FKW was supported by the UK Dementia Research Institute which receives its funding from DRI Ltd, funded by the UK Medical Research Council, Alzheimer's Society and Alzheimer's Research UK, and by an Alzheimer's Research UK Senior Research Fellowship.

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Fisher, Department of Neurodegenerative Disease, Institute of Neurology, University College London, London, UK; Dean Nizetic, Blizard Institute, Barts and the London School of Medicine, Queen Mary University of London, London, UK, and Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore, Singapore; John Hardy, Reta Lila Weston Institute, Institute of Neurology, University College London, London, UK, and UK Dementia Research Institute at UCL, London, UK; Victor Tybulewicz, Francis Crick Institute, London, UK, and Department of Medicine, Imperial College London, London, UK; Annette Karmiloff-Smith, Birkbeck University of London, London, UK (deceased); Michael Thomas, Birkbeck University of London, London, UK; and Denis Mareschal, Birkbeck University of London, London, UK.

Notes

1 For detailed demographics and health information on the children with DS in the LonDownS Consortium, see Startin et al. (2020).

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