Literature Review Part 3: Annotated Bibliography

The following annotated bibliography is organized to give context to the reader before diving into a more specified topic (of mouse exploratory behaviour from a neurodevelopment perspective). As such, it will begin with articles that generalize the animal's behaviour and how it is best studied, following which, it will feature articles on the proximate (neurobiology) and more ultimate (genetics) influences on such a behaviour and how these studies reinforce an understanding of both mouse and human brain development/disorders.

1. Reference: Tanaka, S., Young, J. W., Halberstadt, A. L., Masten, V. L., & Geyer, M. A. (2012). Four factors underlying mouse behavior in an open field. *Behavioural Brain Research*, *233*(1), 55–61. https://doi.org/10.1016/j.bbr.2012.04.045

Summary: The open field analysis (OFA) is a widely used method in behavioural neuroscience as rodent exploration in a novel open field is fundamental to characterizing motor phenotypes. Tanaka (2012) et al makes a claim that a pitfall to these analyses is that animal behaviour is multifaceted and composed of several distinct domains (is not unitary in nature). Therefore, the researchers in this article propose a necessity for a more refined assessment of different exploratory characteristics. With this in mind, the authors constructed a model that uses a small number of factors to characterize mouse exploratory behaviour in an open field. The model was composed from data collected from male mice (n=268) that were placed in a chamber made of clear plexiglass (with holes present in the ground/walls), with their behaviour recorded by infrared photobeams. After one-hour in the open field, the behavioural data was transformed into nine variables (recorded in an ethogram), based on several mathematical models that were categorized into four overarching behavioural factors. These were described as activity, sequential organization (described as a decrease in time spent in the center of the field), diversive exploration (showing a small number of repeated exploratory behaviours such as visiting the same hole) and inspective exploration (described as a high number of repeated exploratory behaviours). This findings of Tanaka et al (2012) are significant as they simplify the extensive characteristics seen in rodent exploration into a four-factor model. This is substantial as this model allows for the ease of characterizing genetic manipulations, neurological diseases and even drug administration on mouse exploratory behaviour (previous thought difficult as it requires simultaneous assessment of multiple characteristics). However, despite the authors contributions, future studies should apply such a model when examining mouse exploratory behaviour in an open field to confirm its utility in such conditions.

Contribution: The model created by Tanaka et al (2012) is an outline to analyzing rodent exploration. Indeed, the authors advance the field of animal behaviour (and studying such behaviour in rodents), by creating a helpful model to characterize exploration. This is significant as it can allow for the ease of studying mouse exploration in experimental conditions. However, this study proposes a high potential for their refined assessment of rodent exploratory behaviour, without putting such a model to use. Therefore, future studies that utilize this four-factored model and its adequacy in characterizing mouse exploration would be beneficial.

2. Reference: Thompson, S. M., Berkowitz, L. E., & Clark, B. J. (2018). Behavioral and neural subsystems of rodent exploration. *Learning and Motivation*, *61*, 3–15. https://doi.org/10.1016/j.lmot.2017.03.009.

Summary: This article reviews a large body of work attempting to understand what influences rodent exploratory behaviour. Of particular importance, the authors review research pertaining to home base behaviour and its role in rodent exploration, and the neurobiological basis of rodent home base behaviour. Thomson et al (2018) describes home base behaviour as rats and mice establishing home bases to obtain security when placed in an open field, influencing their willingness to explore. They detail work that shows both mice and rats as having a preference in forming home bases (when provided the means to do so), and the exploratory movements from those home bases as minimal and direct, with a fast return to the home location. This contrasts data that in the absence of a home base, exploratory behaviour is more frequent and chaotic. The work surrounding home base behaviour is an attempt to determine the implication of anxiety in rodent exploration.

Furthermore, in reviewing research of this organized behaviour following manipulations to the brain, the neural subsystems behind rodent exploration become more evident. The organized pattern known as home base behaviour was analyzed following lesions to the rodent hippocampus. Thomson et al (2018) details such research, describing rodent exploration following hippocampal damage as hyperactive and above the threshold of normal exploratory behaviour. This hyperactive behaviour is described as a loss of home base behaviour and an excess in locomotion (such as an increase in walking or shorter pauses in overall movement). This suggests that the damage to the hippocampus does not abolish rodent exploratory behaviour but affects the type of exploratory behaviour performed (noted by the impairment in organized behavioural patterns). Furthermore, the research outlined in the article report that lesions to the hippocampus produce impairments in the tendency to return homeward when home bases are provided.

Contribution: This article summarizes the underpinnings of rodent exploration using home base behaviour as a baseline, while broadening the knowledge of the neurobiological mechanisms involved in rodent exploration. Focusing on hippocampal integrity and its effect on exploration, Thompson et al (2018) describes a neural subsystem involved in rodent exploration. Despite the information provided in this review, there remains many unanswered questions on what other circuits are involved in exploration. In its entirety, the article is a great starting point for a literature review on mouse exploratory behaviour, providing context to the role of such a behaviour when studying brain development.

3. Reference: Crusio, W. E., Schwegler, H., & van Abeelen, J. H. (1989). Behavioral responses to novelty and structural variation of the hippocampus in mice. I. Quantitative-genetic analysis of behavior in the open-field. *Behavioural Brain Research*, *32*(1), 75–80. https://doi.org/10.1016/s0166-4328(89)80074-9

Summary: This article is the first in a two-part study to determine the genetic variation in hippocampal structure and its implication in mouse exploratory behaviour. Crusio et al (1989), focused on ten components of exploratory behaviour when mice are placed in an open field. These included, but were not limited to, locomotion, rearing (standing on hind legs), leaning (placing forepaws on walls) and sniffing, with six additional exploratory behaviours analyzed. In effort to determine the genetic factors influencing mouse exploration, the authors performed a diallel cross (a mating scheme to investigate the genes involved in quantitative traits), between five different homozygous mouse strains. After breeding, one male from each litter was placed in an open field and its behaviour analyzed (following which, a diallel analysis was performed). The results of the analysis were three-fold: first, all components of mouse exploratory behaviour were influenced by one or more genes that displayed an additive effect on the mouse behaviour. The second was that significant dominance deviation was seen (described as an interaction between different alleles at the same locus), for most exploratory behaviours apart from both sniffing and grooming. The last observation recorded was that for the exploratory behaviour of object sniffing, no genetic variation was detected for all litters. The findings of Crusio et al., are significant as they begin to dissect the genetic underpinnings and heritability of mouse exploratory behaviour. This is a first step in an investigation of the role of the hippocampus in rodent exploration, as previous studies have confirmed a large genetic variance in the relative size in the hippocampus. Despite novel findings, future studies done by Crusio et al., should address the relationship of the hippocampus to the variation in behavioural responses when mice are placed in a novel open field.

Contribution: The work of Crusio et al (1989), provides insight into a genetic selection involved in mouse exploratory behaviour using a diallel cross on five strains of mice, each with their own unique genetic architecture. This is with relevance to the field of neurology as the authors attempt to expand on a previous finding of genetic variation and its implication on hippocampal size. This work provides follow-up questions on the role of the hippocampus in the variation of exploratory behaviour when mice respond to a novel open field, imploring the audience to read the latter part of this two-part study.

4. Reference: Crusio, W. E., Schwegler, H., & van Abeelen, J. H. (1991). Behavioural and neuroanatomical divergence between two sublines of C57BL/6J inbred mice. *Behavioural Brain Research*, *42*(1), 93–97. https://doi.org/10.1016/s0166-4328(05)80043-9

Summary: In this article, the researchers focused on sublines of inbred mice (C57BL/6J) to investigate the divergence of both neuroanatomy and exploratory behaviour. This paper succeeds an article by Crusio et al (1989), which aimed to determine the implication of the hippocampus in mouse exploratory behaviour with this present article attempting to further this understanding. In this paper (1991), the researchers subjected sublines of C57BL/6J mice to three experiments. Experiment I placed C57BL/6J males from different litters into an open field and recorded their behaviour (to determine if the behavioural change in C57BL/6J was due to chance or a true pattern of behaviour). Experiment II investigated the hippocampal anatomy of C57BL/6J sublines via sectioning, to establish if behavioural differences correlated with differences in hippocampal substructures. Lastly, experiment III sought to investigate the gene sequence of C57BL/6J mice with divergence in exploration by investigating the compatibility of C57BL/6J skin tissue, when transplanted to another C57BL/6J mouse (to determine if this divergence was due to mutations or allelic differences). The results of the three experiments determined that a divergence in exploratory behaviour of C57BL/6J mice (if seen), was reproducible and correlated to a divergence in neuroanatomy (such as differences in the size of the mossy fibers). Furthermore, upon investigating the compatibility of skin transplants between mice, no rejection was seen and thus it was concluded that spontaneous mutations, rather than allelic differences (such as heterozygosity), prompted the divergence in behaviour. The findings of Crusio et al (1991), are significant as they further determine the correlation of genetic architecture to changes in both neuroanatomy and exploratory behaviour. However, given the results of the histocompability test (skin transplant), this leads one to question how strongly mutations can influence both mouse exploration and neurodevelopment, and future studies that investigate this question would be beneficial.

Contribution: This article provides further insight into the implication of neuroanatomy (specifically, that of the hippocampus), in mouse exploration. Crusio et al (1991) expands on a previous theory that the hippocampus has a role in mouse exploration and provides compelling evidence in the present article that a change in the relative size of the mossy fibers will implicate mouse exploratory behaviour. This is significant to a study of exploratory behaviour and its relevance to the field neurodevelopment, as it displays that mouse exploration is not only influenced by environment but correlated to both anatomy and development.

5. Reference: Laghmouch, A., Bertholet, J. Y., & Crusio, W. E. (1997). Hippocampal morphology and open-field behavior in *Mus musculus domesticus* and *Mus spretus* inbred mice. *Behavior Genetics*, *27*(1), 67–73. https://doi.org/10.1023/a:1025667426222

Summary: Prior to the work provided by Laghmouch et al (1997), it had been established that inbred lines of mice often display large structural variation in their hippocampus. As mentioned in previous literature, this structural difference is determined in part by genetic variation and provides a means to analyze the hippocampal contribution to mouse behaviour. In this paper, the researchers crossed two lines of inbred mice (from two mouse species: *Mus spretus* (SEG) and *Mus musculus domesticus* (C57BL6), the latter which has known variations in hippocampal structure). The purpose of these crosses was to introduce a high level of DNA polymorphisms (different DNA sequences among individual mice) and allow for the ease of gene mapping. Males from the following crosses were placed in an open-field and ten exploratory behavioural components were analyzed (the same as in the Crusio et al (1989) study). Following an open field analysis (OFA), the hippocampal size and structure for each mouse was analyzed through histological staining. The results of the OFA revealed clear difference for all components of exploratory behaviour between the two strains of mice except for sniffing, grooming, gnawing and defecation (with the SEG mice scoring lower in all but two categories of exploratory behaviour). However, upon investigation of hippocampal morphology, the authors noted no significant interspecies difference in the neuroanatomy of the hippocampus, except for in the mossy fibers (an important input to the cerebellum). These were three times larger in the C57BL6 mice that displayed more exploratory behaviours. These findings are significant as they reveal a positive correlation between the size of the mossy fibers and mouse exploratory behaviour. However, despite the results provided by Laghmouch and colleagues, future studies should further address this brain-behaviour relationship to determine if other substructures in the hippocampus are implicated in mouse exploration.

Contribution: By crossing two inbred lines of mice, the work of Laghmouch et al (1997) analyzes the covariation of genetics and hippocampal structure and its implication in mouse exploratory behaviour. This advances the knowledge in the field of neuroscience as the mossy fibers of the hippocampus display a positive correlation to both the type and frequency of mouse exploration. However, this study begs the question as to what specific genes and gene regulatory networks are involved in hippocampal development, and whether hippocampal structural variation is influenced by environmental changes during mouse early development.

6. Reference: Crusio, W. E. (2001). Genetic dissection of mouse exploratory behaviour. *Behavioural Brain Research*, *125*(1), 127–132. https://doi.org/10.1016/S0166-4328(01)00280-7

Summary: This latest article by Crusio (2001) is a summary of previous research on the implication of both neuroanatomical variation (hippocampus) and genetics in mouse exploration. In this paper, the author briefly discusses the methods to studying mouse exploratory behaviour (with emphasis on the OFA), while conferring the biological significance of such a behaviour as an innate need to find necessities for survival. However, the author focusses most of the review on research of the genetic correlation and neuroanatomical implication in mouse exploration, citing their own research in part.

Crusio (2001) first details the work of a diallel cross of five inbred mouse lines (as outlined in the previous summary of Crusio et al., 1989). However, the author confirms the relevance of this work by citing another study which utilizes the mice of the same diallel cross, subjecting them to an OFA and subsequent histology/morphometry of their brains. Here, Crusio details the results as revealing a negative correlation to the size of hippocampal intra-and-infrapyramidal mossy fibers (IIPMF) to mouse exploration. The author hypothesizes that this result is due to an increased efficiency of mice with a larger IIPMF to process spatial information, decreasing the novelty of the open field. Moreover, Crusio specifies that when researchers investigated the genomic architecture of such mice, substantial genetic correlations were found between the size of the IIPMF and the exploratory activity in the OFA. Here, the author indicates that these two articles confirm the utility of the diallel cross as a tool for genetic dissection of both neural and behavioural phenotypes. In reviewing the following work, Crusio attempts to further elucidate a relationship between the brain and exploratory behaviour, a significant finding when challenging the paradigm that emotionality is the only driving force in mouse exploration, a behaviour which appears to be influenced by neurodevelopment.

Contribution: This article is a cohesive summary of the work of Crusio (and colleagues), and their investigation into the genetic and neuroanatomical underpinnings of mouse exploration. Citing their own published research and that of their successors, the author provides a compelling argument to the theory that mouse exploratory behaviour is multifaceted, that is, influenced by not only emotion (stress/fear), but both genetics and neurobiology. Notwithstanding the utility of this review, it contradicts previous information provided by Laghmouch et al (1997), and future studies that address whether the hippocampus acts in a positive or negative correlation to mouse exploration would be beneficial.

7. Reference: Caston, J., Chianale, C., Delhaye-Bouchaud, N., & Mariani, J. (1998). Role of the cerebellum in exploration behavior. *Brain Research*, *808*(2), 232–237. https://doi.org/10.1016/S0006-8993(98)00847-6

Summary: It is well-established that the cerebellum has a role in maintaining the locomotor capabilities of the mouse. However, Caston et al (1998) sought to investigate the role of the cerebellum in nonmotor functions such as mouse exploratory activity. The authors aimed to determine the relative contribution of both the cerebellar cortex and cerebellar nuclei on the motivation for mice to explore novel environments and stimuli. To explore this question, adult wild-type (+/+) mice and lurcher mutant mice (characterized as having a mutation that causes the degeneration of cerebellar structures), were subjected to three experiments that analyzed their spontaneous activity, exploration behaviour and habituation of exploration. The authors used four different mouse groups, +/+ and +/Lc with an intact cerebellum, and +/+ and +/Lc with their cerebellum removed. Experiment one found that +/Lc mice with an intact cerebellum had significantly higher spontaneous locomotor activity than intact +/+. The second experiment found that between the four groups of mice, the exploration (defined as walking around a novel arena or investigating holes in the floor of the arena) was significantly higher in non-cerebellectomized mice when compared to mice that had undergone a cerebellectomy. The last experiment investigated the role of the cerebellum in the habituation of exploratory behaviour and found that for mice lacking the cerebellum, habituation occurred for all exploratory behaviours but did not for non-cerebellectomized mice after several trials. The findings of Caston et al., are significant as they pinpoint a role for the cerebellum in mouse exploration, emphasizing the idea that the cerebellum is not only implicated in motor function. Despite their robust investigation, further research into why an increase in spontaneous activity is seen for in intact +/Lc when compared to intact +/+ mice might allude to what other brain structures are involved in mouse exploratory behaviour.

Contribution: This article provides insight into the role that neuroanatomy plays in the exploratory behaviour of mice, challenging the previous paradigm that exploration is only influenced by underlying stress and fear. This advances the knowledge in the field of neurodevelopment that discrete brain structures and the proper development of such structures, can significantly influence mouse behaviour. As such, the findings present a line of inquiry for the role of the nervous system in mouse behaviour. Thus, to explore the topic of mouse exploratory behaviour and how it reinforces an understanding of neurodevelopment, this article was chosen for my literature review.

8. Reference: Rosin, J. M., McAllister, B. B., Dyck, R. H., Percival, C. J., Kurrasch, D. M., & Cobb, J. (2015). Mice lacking the transcription factor SHOX2 display impaired cerebellar development and deficits in motor coordination. *Developmental Biology*, *399*(1), 54–67. https://doi.org/10.1016/j.ydbio.2014.12.013

Summary: It has been established that the proper development of the cerebellum is important in fine motor control and coordination (as evident by ataxia in individuals with abnormal cerebellar structures). However, the work of Rosin et al (2015) is an effort to determine the role of certain genes in mammalian brain architecture and subsequent behaviour (such as exploration). In the article, the researchers focused on an important developmental gene in mice known as *Shox2* and its function in the brain. To do so, Rosin and colleagues created conditional knock out mice (with both copies of the *Shox2* gene removed in the brain) and subjected the mice to a battery of behavioural tests, one being the open-field analysis (OFA). Furthermore, the researchers sectioned and stained the brains of conditional knock out (cKO) mice and determined the morphometry of brain structures using CT-imaging, comparing them to wild-type brains. The results of the OFA revealed that cKO mice travelled 1.5-times less than wild-type mice and 1.4-times slower. Upon sectioning/staining and CT-imaging the brains of such mice, it was found that the cerebellar folia were smaller in the cKO mice, and the overall size was reduced. Therefore, the authors concluded that *Shox2* is an important gene in the development of the cerebellum, which affects mouse exploratory behaviour. The findings of Rosin et al are significant as it highlights a genetic underpinning for not only mouse brain development, but exploratory behaviour. More specifically, the researchers determined that *Shox2* is important in neurodevelopment and is implicated in mouse exploration. With this information, this leads one to question what other genes may be involved in brain development, affecting mouse exploration. Future studies that seek to understand what other genes are important in proper neurodevelopment and whether these genes affect the behavioural profile of mice would be advantageous.

Contribution: By conditionally knocking-out *Shox2* in the central nervous system, Rosin et al (2015) determined that a properly developed cerebellum is important for mice to exhibit exploratory behaviour. This expands both the field of neurodevelopment and animal behaviour by connecting the two through the study of genetics/genomics. That is, Rosin and colleagues provide a compelling study on the importance of gene expression on both embryonic development and behaviour. Despite the intriguing results provided by this study, the question of what other genes may be involved in mouse embryonic development, and how these genes influence exploratory behaviour remains to be explored.

9. Reference: Walsh, J., Desbonnet, L., Clarke, N., Waddington, J. L., & O'Tuathaigh, C. M. P. (2012). Disruption of exploratory and habituation behavior in mice with mutation of DISC1: An ethologically based analysis. *Journal of Neuroscience Research*, *90*(7), 1445–1453. https://doi.org/10.1002/jnr.23024

Summary: Prior to the work of Walsh et al (2012), the gene know as disrupted-in-schizophrenia-1 (*DISC1*), was identified as having important neurodevelopmental roles such as the outgrowth of neurons and the differentiation of oligodendrocytes (myelin precursors). Furthermore, when mutated, this gene causes hyperdopaminergia (known as too much dopamine in the brain). Given its implication in neurodevelopment, the researchers in this article used mice to model the effects of mutating *DISC1* on exploratory behaviour in an open field (OF). Walsh and colleagues evaluated mouse exploration based on ethologically based behaviours (such as the ten components described in Crusio et al., 1989) of homozygous mutant (HOM), wild-type (WT) and heterozygous (HET) mice (for the *DISC1* gene), over 60-minutes in an OF. Initially, the results revealed that total locomotion in the HOM and HET was significantly higher than in WT, which was pronounced in males (consistent with previous research that a mutation in *DISC1* causes hyperactive behaviour). However, as time elapsed in the OF only certain exploratory behaviours were increased in the HOM, such as total rearing or seated rearing (reaching forepaws upward while standing on hindlimbs). Furthermore, both HOM and HET mice displayed a delay in habituation to the OF and exploratory behaviours. The results of Walsh et al., are significant as they show that the inactivation of one or both copies of the *DISC1* gene is enough to modify exploratory behaviour of mice. Furthermore, the authors reference previous human clinical studies that have identified abnormal exploratory activity in patients suffering from schizophrenia and therefore, their results show the validity in using rodent models to complement studies on neuropsychiatric disorders and resulting behaviour. However, future studies that address the sex-specific influence on mouse exploration (when important neurodevelopmental genes are mutated), would benefit a study of rodent behaviour from a developmental perspective.

Contribution: The article of Walsh et al (2012) displays the utility in studying rodent exploratory behaviour to determine the function of important developmental genes (such as *DISC1).* Moreover, through their analysis of *DISC1*-mutant mice in an OF, the authors show the relevance of using animal models to expand the knowledge of common human disorders that exist at the forefront of neurology or neuropsychology (such as schizophrenia and depression). This paper therefore presents a line of inquiry as to what other genes (implicated in human disorders) exist in mice that influence the development of the brain, and innate behavioural patterns.