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Environmental Enrichment and the Striatum:

The influence of environment on inhibitory circuitry within the striatum of environmentally enriched animals and behavioural consequences

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All procedures were approved by the Animal Ethics Committee of the University of Sydney and conformed to National Health and Medical Research Council of Australia guidelines under the ethics numbers K22/09-09/3/5128 and K22/11-12/3/5838.

The work presented within this thesis is, to the best of my knowledge and belief, original except as acknowledged within the text. I hereby declare that I have not submitted this material, either in full or in part, for a degree at this or any other institution.

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Angela May O'Connor

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Abstract

Environmental enrichment (EE) provides animals with sensory, motor and social stimulation above that usually experienced within a laboratory setting, and has been shown to have a variety of impacts upon the nervous system and animal behaviours. There has recently been an increase in the number of studies investigating the impact of environmental manipulations such as EE.

The striatum is the entry nucleus for the basal ganglia, receiving afferent projections from the cerebral cortex, thalamus and brainstem, and in turn projecting to other nuclei within the basal ganglia, controlling activity within, and output of, this system. The striatum contains a population of fast-spiking inhibitory interneurons expressing the calcium-binding protein Parvalbumin (PV) that receive direct input from corticostriatal afferents, project to the medium spiny output neurons of the striatum, and take part in feed-forward inhibition and modulation of the activity within this nucleus.

PV+ inhibitory interneurons within the striatum have been shown to correlate with Perineuronal Nets (PNNs), an extracellular matrix structure, in determined ratios. In adult animals, PV+ inhibitory neurons within other areas of the brain have been shown to regulate parvalbumin expression in response to EE and neural activity levels. I found that EE increased the number of PV+ inhibitory interneurons present within the striatum of adult animals, resulting in a change in the correlation ratio of these neurons with PNNs, providing evidence that EE can continue to influence neural circuitry beyond the critical period.

The development of PV+ inhibitory neurons in other areas of the brain has been shown to control the onset of the critical period, a time in early life when plasticity within a neural system is at its peak. Maturation of these neurons is known to be regulated by a neurotrophic growth factor, Brain-Derived Neurotrophic Factor (BDNF), both within the striatum and other regions of the brain. EE accelerates cellular and functional maturation of sensory systems within the brain, resulting in earlier onset of system-specific critical periods. EE has also been shown to accelerate the maturation

of sensorimotor behaviours potentially mediated by the striatum. I found that young animals raised within EE demonstrate accelerated maturation of PV+ inhibitory interneurons and elevated levels of BDNF protein within the striatum. Exposure to EE also resulted in an alteration to the ultrasonic vocalisation (USV) call profile of juvenile mice, providing evidence of a change in a striatally-mediated behaviour. Together, these results suggest that a putative early-life striatal critical period regulated by the maturation of PV+ inhibitory interneurons is able to be influenced by environmental factors.

Different laboratory groups may utilise different EE paradigms depending upon the animal species, area of the brain, and particular issue under investigation. Thus, the efficacy of a particular environmental paradigm must be determined when undertaking research. Most techniques used to determine whether EE paradigms are effective are either post-mortem anatomical, molecular and physiological metrics, or behavioural tests requiring a degree of training and exposure to testing arenas prior to conducting experiments. I found that the Puzzle-Box, a test of goal-orientated learning, problem solving and memory, successfully and consistently returned evidence of the impact of EE upon animal behaviours.

Animals exposed to EE demonstrate improved performance within behavioural tasks assessing cognitive capacities and sensorimotor coordination. The striatum is known to mediate a variety of behaviours, including cognitive processes and coordination of sensory and motor behaviours. I found that animals raised within EE demonstrated improved problem-solving and goal seeking within the Puzzle-Box, and slightly improved task acquisition upon a rotarod motor learning task. Behavioural patterns of movement within the Puzzle-Box differed in EE animals compared to those raised within standard laboratory housing. PNNs within the brain are able to be dissolved by use of the bacterial enzyme Chondroitinase ABC (ChABC), increasing neuroplasticity and resulting in an “immature” state within neural circuitry. I found that striatal ChABC treatment exerted an opposite effect to EE, impairing problem-solving behaviour within the Puzzle-Box. Enrichment is also known to increase

plasticity levels within neural circuitry; however, animals raised within EE did not show the same degree of ChABC-induced behavioural changes as mice raised within standard laboratory housing. Together, these results suggest that plasticity mechanisms and striatal effects of EE and ChABC treatment work via differing pathways.

The striatum is known to play a role in decision-making and choice of action behaviours. One behavioural task used to assess decision-making is the Iowa Gambling Task (IGT), where participants make choices between overall disadvantageous and overall advantageous decisions. There are several rodent versions of the IGT (RGT) that have shown similar results to human testing, and demonstrate that rats are capable of performing this task. Impaired decision-making within the IGT is thought to result from an underactive ability to inhibit behaviours, suggesting that the striatum is likely involved in mediating decision-making during this behavioural task. I successfully developed a murine version of the RGT using the IntelliCage, a novel behavioural testing arena, and found that exposure to EE had little effect upon reward-based decision making within this task. Despite little effect of EE upon RGT performance, the development of a murine gambling task is potentially of great use for the assessment of animal models of human diseases known to interfere with decision-making during the IGT.

The basal ganglia is integral to the healthy and whole functioning of an organism, mediating interactions with and responses to an organism's surroundings. The effects of environmental enrichment upon the striatum and animal behaviours documented within this thesis provide evidence for the significant impact that an organism's surroundings may exert upon this important part of the brain. Determining the manner in which environmental enrichment influences specific cognitive neural networks may assist in the development of early education and intervention programs targeted at young children, or environmentally-based therapies for individuals suffering from neurological disease or injury.

Publications resulting from this thesis:

The use of the Puzzle Box as a Means of Assessing the Efficacy of Environmental Enrichment, A.M. O'Connor, T. Burton, C.A. Leamey, A. Sawatari, The Journal of Visualised Experiments (in press at time of initial submission, published on 29/12/2014)

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Invited/Oral:

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2013: *Bosch Institute Young Investigators Symposium, University of Sydney, NSW, Australia*, Environmental Enrichment affects Striatal Maturation

2013: *University of Colorado, Boulder, USA,* Environmental Enrichment, Striatal Circuitry and Behaviours

2013: *University of Oregon, Eugene, USA*, Environmental Enrichment, the Striatum and Behaviour

2013: *Bosch Institute Facilities Users Meeting, University of Sydney, NSW, Australia*, Environmental Enrichment and Behaviour

2012: *Bosch Institute Facilities Users Meeting, University of Sydney, NSW, Australia,* The Effect of Environmental Enrichment on Decision Making in the IntelliCage

Poster Conference Abstracts:

2015: *British Neuroscience Association, Edinburgh, Scotland,* **Angela M O'Connor,** Catherine A Leamey, & Atomu Sawatari, Environmental enrichment accelerates the maturation of murine striatal inhibitory circuitry and impacts juvenile striatally-mediated behaviours

2015: *British Neuroscience Association, Edinburgh, Scotland,* **Angela M O'Connor,** Catherine A Leamey, & Atomu Sawatari, Environmental enrichment and striatal perineuronal net dissolution exert opposing effects upon the performance of mice in a cognitive behavioural task

2015: *Biodynamics 2015, Edinburgh, Scotland,* **Angela M O'Connor,** Catherine A Leamey, & Atomu Sawatari, Environmental enrichment and the striatum

2014: *Australasian Neuroscience Society, Adelaide, SA, Australia,* **Angela M O'Connor**, Catherine A. Leamey, & Atomu Sawatari, Environmental Enrichment Influences both Development and Function of Striatal Circuitry Within the Mouse

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2013: *Australasian Neuroscience Society, Melbourne, Vic, Australia,* **Angela M O'Connor**, Catherine A. Leamey, & Atomu Sawatari, Early Environmental Enrichment Influences the Rate of Parvalbumin-Positive Inhibitory Interneuron Development within the Striatum

2012: *Australasian Neuroscience Society, Gold Coast, Qld, Australia*, **Angela M O'Connor**, Catherine A. Leamey, & Atomu Sawatari, Early Environmental Enrichment Influences the Rate of Parvalbumin-Positive Inhibitory Interneuron Development within the Striatum

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Abbreviations used throughout thesis

ACh – Acetylcholinesterase

ANS – Autonomic Nervous System

BDNF – Brain-derived Neurotrophic Factor

ChABC – Chondroitinase ABC

CNS – Central Nervous System

CPu – Caudate Putamen

CR – Calretinin

CSPG – Chondroitinase sulphate proteoglycan

DAPI - 4', 6-diamidino-2-phenylindole

DSA – Drinking Session Adaptation

E – Enriched

ED – Embryonic Day

EE – Environmental Enrichment

ELISA - Enzyme-linked Immunosorbant Assay

FA – Free Adaptation

GABA – Gamma Amino Butyric Acid

GPe – Globus Pallidus external (also Lateral Globus Pallidus)

GPi – Globus Pallidus internal (also Medial Globus Pallidus)

HA - Hyaluronan

IGF-1 – Insulin-like Growth Factor-1

IGT – Iowa Gambling Task

LGP – Lateral Globus Pallidus

MPTP - 1-methyl-4 phenyl-1,2,3,6-tetrahydropyridine

mRNA – messenger Ribonucleic Acid

MSN – medium spiny neurons

NGF – Neurotrophic Growth Factor

NOS – Nitric Oxide Synthase

NPA – Nose-Poke Adaptation

NPY – Neuropeptide Y

OD – Ocular Dominance

P – Postnatal Day

PB – 0.1M Phosphate Buffer

PNN – Perineuronal Net

PV – Parvalbumin

PV+ – Parvalbumin-expressing

RFID – Radio Frequency Identification

RGT – Rodent Gambling Task

S – Standard

S.E.M. – Standard error of the mean

SE – Standard housing followed by Enriched housing

SNpc – Substantia Nigra pars compacta

SNpr – Substantia Nigra pars reticulate

SS – Somatostatin

SS – Standard housing followed by Standard housing

TN-R – Tenascin-R

TSA – Tyramide Signal Amplification

USV – Ultrasonic Vocalisation

WFA – Wisteria Floribunda Agglutinin

Chapter 1: Literature Review

"How can a three-pound mass of jelly that you can hold in your palm imagine angels, contemplate the meaning of infinity, and even question its own place in the cosmos? Especially awe-inspiring is the fact that any single brain, including yours, is made up of atoms that were forged in the hearts of countless, far-flung stars billions of years ago. These particles drifted for eons and light-years until gravity and change brought them together here, now. These atoms now form a conglomerate- your brain- that can not only ponder the very stars that gave it birth but can also think about its own ability to think and wonder about its own ability to wonder. With the arrival of humans, it has been said, the universe has suddenly become conscious of itself. This, truly, is the greatest mystery of all."

- Vilayanur S. Ramachandran, *The Tell-Tale Brain: A Neuroscientist's Quest for What Makes Us Human*

Investigating the influence an organism's environment may exert upon its nervous system is a growing field of study, with manipulations such as environmental enrichment used to assess the way surroundings can impact both the brain and behaviours of laboratory animals. This thesis focuses on the entry nucleus of the basal ganglia, the striatum, responsible for gating input to this region of the brain [1-3]. The basal ganglia and striatum are highly important components of the brain, connecting the cerebral cortex with older areas such as the thalamus and brainstem. Integrating sensory [4-6], motor [7-9], and cognitive [10-13] information, the striatum is placed in an ideal position to initiate behaviours and actions based on external stimuli, mediating the interaction between an organism and its surroundings.

The striatum is a highly plastic system with the hallmarks of undergoing a critical period, and its placement within the brain makes it particularly susceptible to environmental manipulations. Environmental enrichment provides animals with sensory input, motor experience and social interaction above and beyond that usually experienced within standard laboratory housing. Enrichment is known to effect behaviours mediated by the basal ganglia and striatum [9, 14-17], and affect the anatomy [16], protein levels [15, 18] and gene expression [19] of this nucleus. The full extent of environmental influences upon the striatum, however, is still unknown. It is not clear how environmental factors impact striatal function or the development of circuits contained within this nucleus.

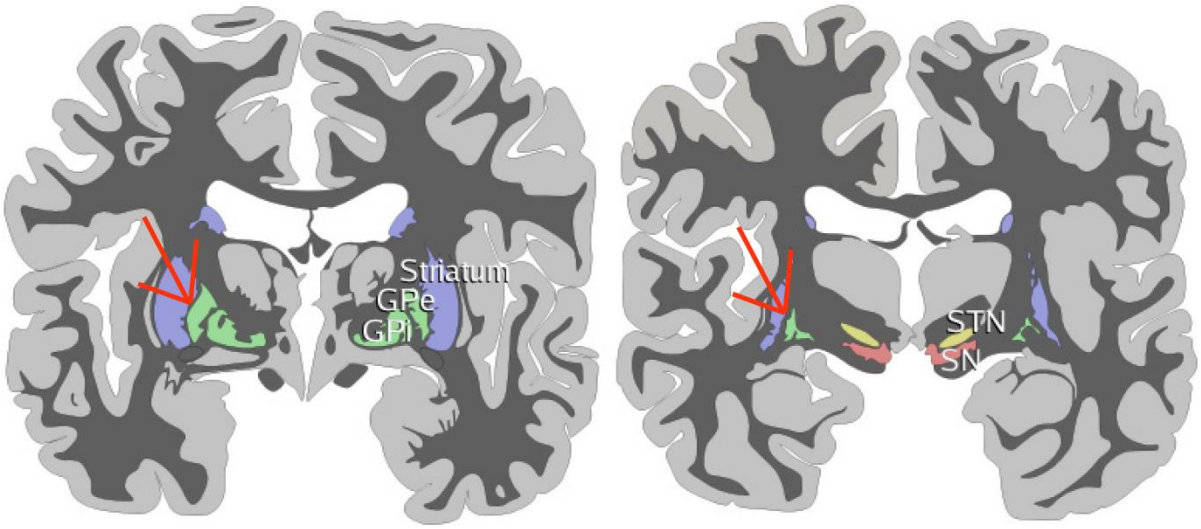
The following review will touch on these issues. I will first provide an overview of the striatum and its connections and composition, followed by an explanation of critical periods and the role that inhibitory circuitry, extracellular matrix and neurotrophic factors play in determining the timing of these important developmental epochs. Finally, I will cover the concept of environmental enrichment and previous work that has been conducted regarding this manipulation, as well providing an overview of the research contained within this thesis.

1. The Striatum

The basal ganglia help to regulate motor behaviours, habit learning and action selection [1-3] and is comprised of the caudate and putamen nuclei (together known as the striatum), the globus pallidus, the substantia nigra, the nucleus accumbens, and subthalamic nuclei (Fig. 1.1). Located around the third ventricle and cerebral aqueduct, the basal ganglia takes part in sensory, motor, and cognitive behaviours, including the coordination of sensorimotor function, habit learning, reward and goal-orientated learning, action selection, and limbic function [1-3].

This literature review will focus on the striatum, the main input nucleus of the basal ganglia [1-3] and an important regulator of sensory [4-6], motor [7-9] and cognitive [10-13] functions and behaviours. The following provides an outline of the circuitry and composition of this nucleus, with a particular emphasis on behavioural functions mediated by the striatum. The striatum is composed of the caudate and putamen nuclei: in the primate striatum, these nuclei are separated by the internal capsule, a tract of white matter connecting the thalamus to various cortical areas (Fig. 1.1A). In the rodent striatum however, located ventral and medial to the corpus callosum in the caudal plane, the caudate and putamen nuclei are intermingled with one another and the internal capsule (Fig. 1.1B) [5, 20-22].

A


Striatum
GPe
GPl
STN
SN

B

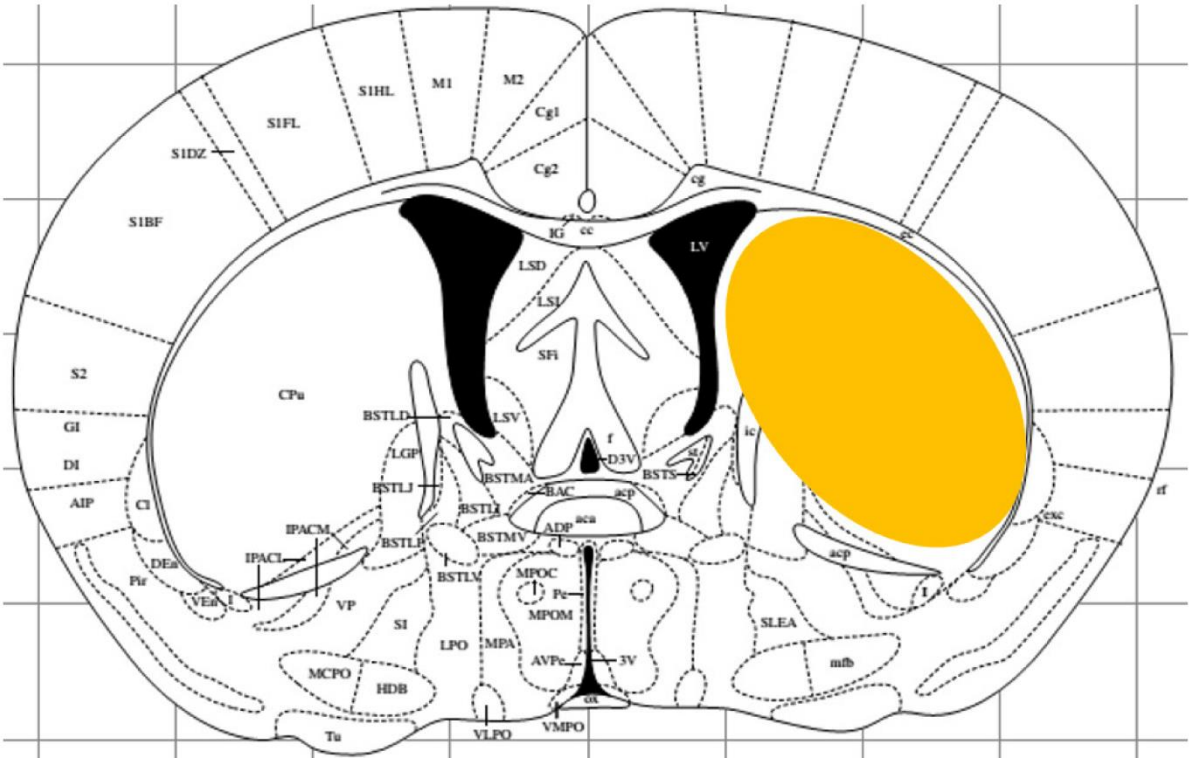

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Figure 1.1 Diagram of the basal ganglia

(A) A diagram representing the structure of the basal ganglia, as it appears within a coronal section of the human brain. The basal ganglia is composed of the caudate nucleus, putamen nucleus, globus pallidus internal and external, the substantia nigra, and the subthalamic nuclei. These structures are located around the third ventricle and cerebral aqueduct within the brain. Within the human brain, the internal capsule (red arrow) separates the caudate and putamen nuclei from the globus pallidus. Striatum: caudate + putamen; GPe: globus pallidus external; GPi: globus pallidus internal; STN: subthalamic nuclei; SN: substantia nigra. (B) A diagram representing the structure of the basal ganglia, as it appears within a coronal section of the rodent brain. Within the rodent brain, the caudate and putamen nuclei are not separate (striatum, highlighted in yellow) and are intermingled with the internal capsule. Image taken from Paxinos & Watson, 2004 [22]. CPu: caudate-putamen (striatum); LGP: lateral/external globus pallidus.

1.1 Striatal Circuitry

Afferent input to the striatum comes from various regions within the brain: glutamatergic input is received from the thalamus and the cerebral cortex; serotonergic input from the raphe nucleus within the brainstem; and dopaminergic input from the substantia nigra pars compacta within the midbrain [1, 20, 23, 24]. Recently, it has been demonstrated that the striatum receives input from the cerebellum via a disynaptic pathway passing through the thalamus [25, 26].

Output from the striatum is inhibitory: within the primate brain the two output nuclei of the basal ganglia, the globus pallidus internal (GPi) and substantia nigra pars reticulate (SNpr), tonically inhibit projection target activity, and are themselves modulated by afferent input received from the striatum via the rest of the basal ganglia [1, 27]. Two pathways – the direct and indirect pathways – provide the link between the striatum and the GPi and SNpr (Fig. 1.2A). The direct pathway routes through the GPi, whilst the indirect pathway courses through the globus pallidus external (GPe), and subthalamic nuclei [1, 27]. Stimulation of the direct pathway enables activation of the thalamus and ultimately the cerebral cortex. Activation of the indirect pathway briefly increases inhibition of the thalamus, preventing the activity of thalamocortical neurons [1, 27]. The direct and indirect pathways may be considered as "closed loop" circuits, following the well-characterised basal ganglia-thalamocortical course of connectivity [28]. There are also "open loop" circuits connecting the basal ganglia to regions within the cerebral cortex via currently unknown pathways [28]. These connections form the neural circuitry of the basal ganglia and its links with the cerebral cortex and thalamus, enabling behaviours mediated by the striatum and other basal ganglia nuclei to take place.

Within the rodent brain, there also exist direct and indirect pathways within the basal ganglia (Fig. 1.2B). The direct pathway consists of striatal projections to the SNpr and endopunducular nucleus (the equivalent of GPi), whilst the indirect pathway passes through the GPe [29]. Distinct populations of efferent projection neurons within the striatum take part in each of these pathways [30]: the direct pathway consists of axon projections from substance P-containing neurons that express the D1 dopamine receptor, whilst the indirect pathway involves axon projections from enkephalin-containing neurons expressing the D2 dopamine receptor [31-33] (Fig. 1.2B).

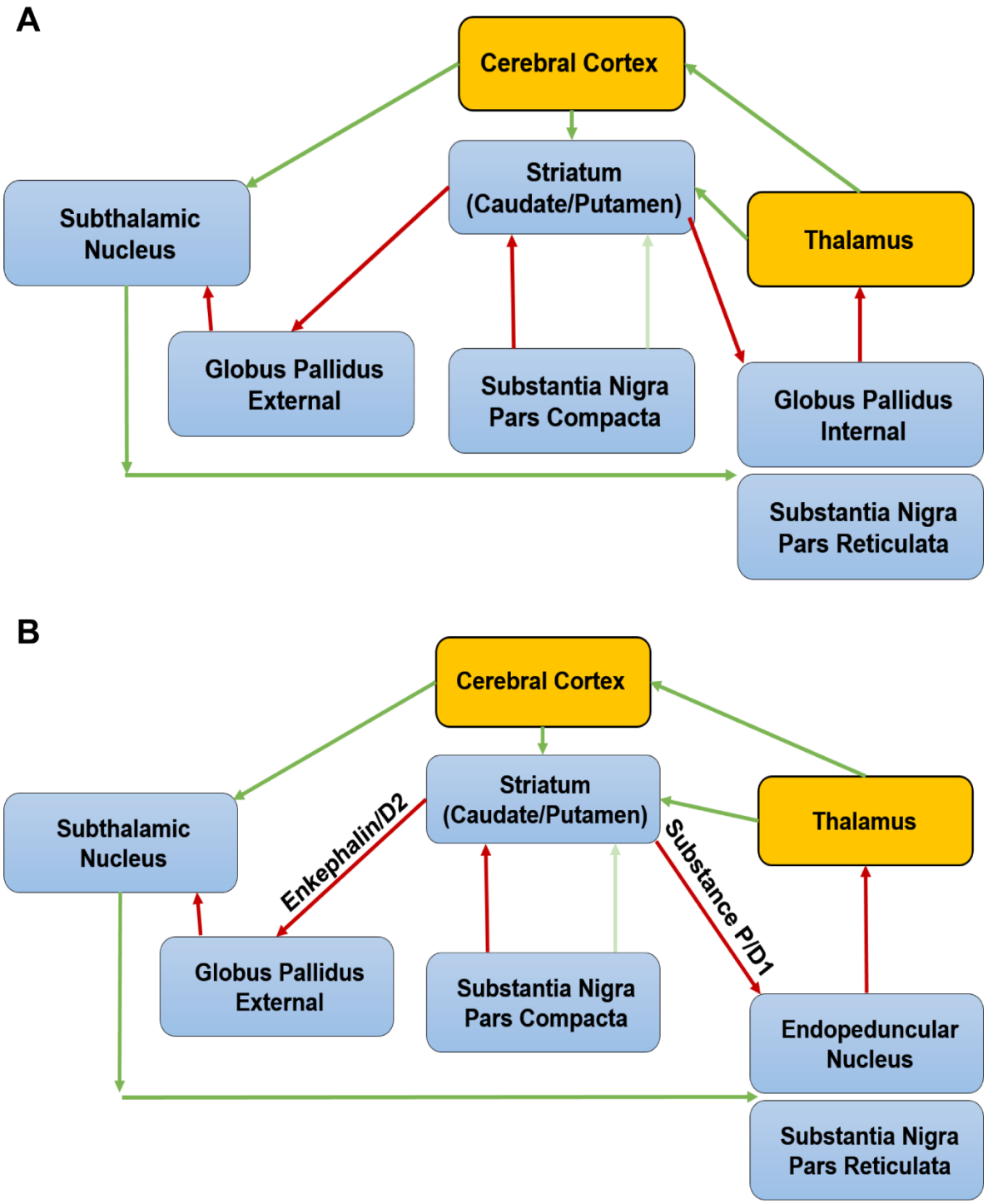
A
Cerebral Cortex
Striatum
(Caudate/Putamen)
Subthalamic
Nucleus
Thalamus
Globus Pallidus
External
Substantia Nigra
Pars Compacta
Globus Pallidus
Internal
Substantia Nigra
Pars Reticulata
B
Cerebral Cortex
Striatum
(Caudate/Putamen)
Subthalamic
Nucleus
Enkephalin/D2
Thalamus
Substance P/D1
Globus Pallidus
External
Substantia Nigra
Pars Compacta
Endopeduncular
Nucleus
Substantia Nigra
Pars Reticulata
Excitatory/Dopaminergic
Excitatory/Glutamatergic
Inhibitory/GABAergic

Figure 1.2 Diagram of the basal ganglia circuitry within the primate and rodent brain

A) A diagram representing the connections between the cerebral cortex, thalamus, and nuclei within the primate basal ganglia. Excitatory glutamatergic afferent input from the cerebral cortex and thalamus, and dopaminergic afferent input from the substantia nigra pars compacta project to the striatum. Inhibitory projections from the striatum form the "direct" pathway with the globus pallidus internal and the "indirect" pathway with the globus pallidus external. Both direct and indirect pathways eventually project to the thalamus, which in turn projects back to the striatum and the cerebral cortex. B) A diagram representing the connections between the cerebral cortex, thalamus, and nuclei within the rodent basal ganglia. The circuitry is similar to that seen within the primate basal ganglia, with the exception of the endopeduncular nucleus in place of the globus pallidus internal. Striatal projections to the direct and indirect pathways originate from distinct populations of neurons: the direct pathway from substance P-containing neurons that express the D1 dopamine receptor; and the indirect pathway from enkephalin-containing neurons that express the D2 dopamine receptor [31-33].

1.2 Striatal Composition and Input

Unlike the cerebral cortex the striatum is not arranged in cellular layers; despite its apparent homogeneity, there are histochemically and functionally distinct zones within this nucleus [34, 35], similar to other brain regions.

A formation of acetylcholinesterase-poor regions, described as “striosomes”, along with the acetylcholinesterase-rich region known as the “matrix” make up the mammalian striatum [36, 37]. Striosomes and matrix differ both histochemically and in the projections they receive (Fig. 1.3). Within the rodent striatum, striosomes are rich in mu-opioid binding sites [38], whilst the matrix contains both calbindin$_{D28kD}$ immunoreactive neurons and a rich plexus of somatostatin fibres and dopamine islands [34, 35].

Afferent input to striosome and matrix regions of then rodent striatum originate from distinct areas of the brain. Striosomes principally receive projections from deep layer V and layer VI neurons within the cerebral cortex, and the majority of projections from allocortical areas and prelimbic cortex [20]. The matrix principally receives projections from layers II, III and superficial layer V of the cerebral cortex, and the majority of projections from neocortical areas, agranular motor cortex, cingulate cortex, somatic sensory cortex, and visual cortex [20]. Both striosomes and matrix receive corticostriatal projections from prefrontal and motor areas and nigrostriatal projections [5, 6, 20, 24], whilst input from the thalamus and raphe nucleus terminate within the matrix compartment [37, 39].

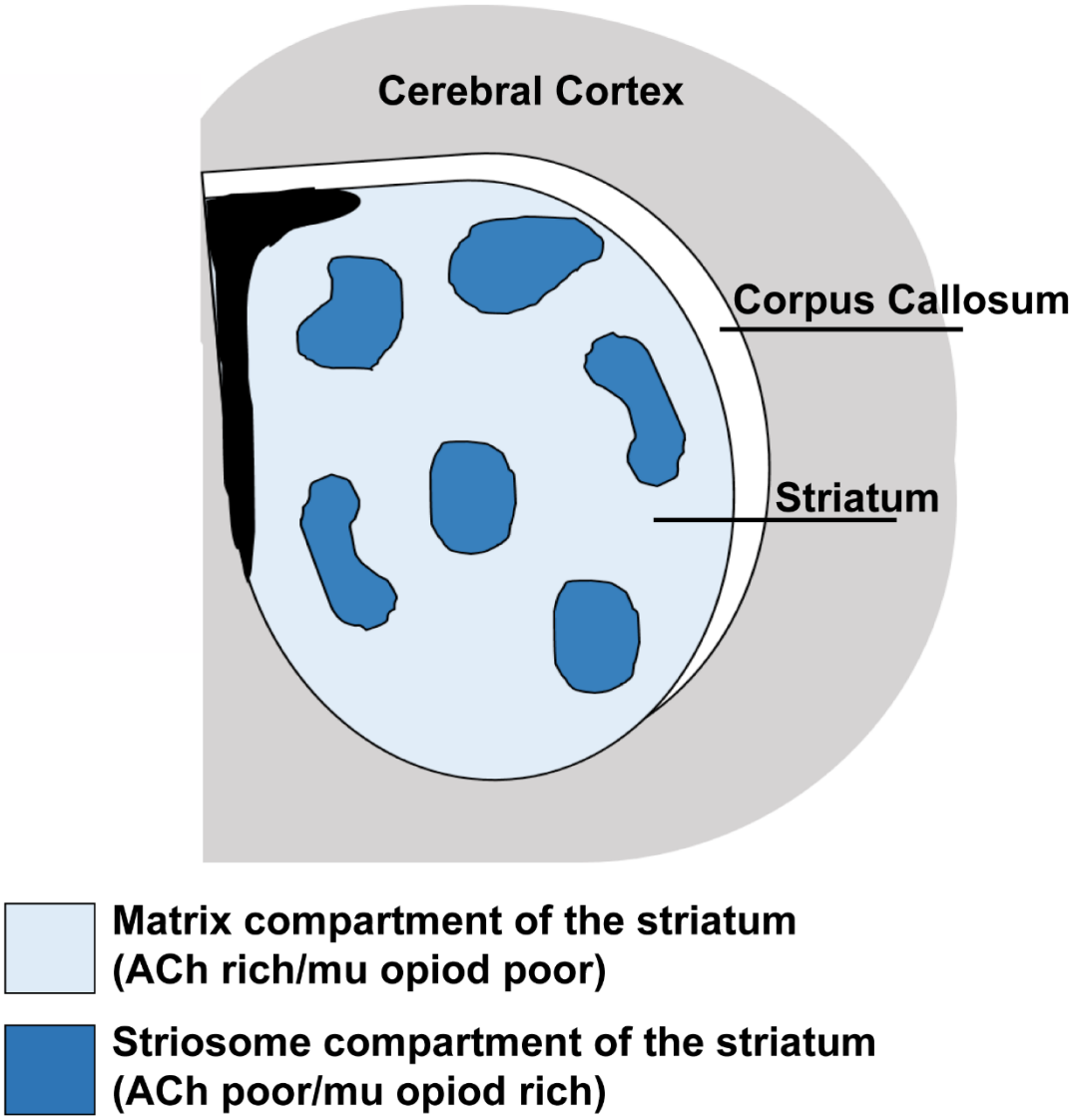

Figure 1.3 Diagram of the striosome/matrix compartments within the striatum

A diagram representing the appearance of the acetylcholinesterase (ACh) poor/mu opioid rich striosome and ACh rich/mu opioid poor matrix compartments of the striatum. Striosomes appear as "patches" within the matrix, and are sometimes referred to as such.

1.3 Striatal function

Given the wide variety of input received by the striatum[5, 6, 20, 24], it is not unexpected that this nucleus mediates a range of behaviours [8, 9,10, 13, 52, 53, 54, 55, 56, 57].

The striatum is involved in cognitive processes such as decision-making [13, 40], choice of action [40], goal-orientated learning [41], rule-based learning [10], and task acquisition [42]; as well as sensorimotor behaviours, including motor coordination [9, 43], balance [9, 44], motor skill acquisition [8, 45], and synchronisation of sensory and motor input [9, 43, 44]. Functions carried out by the rodent striatum are, similar to afferent input, localised within certain regions of this nucleus.

Some regions of the striatum are known to carry out both cognitive and sensorimotor functions: dorsolateral striatum mediates the transition from flexible outcome-based actions to habitual decision-making processes [8, 40, 46], whilst also being involved in the coordination of limbs during locomotion [44], and the maintenance of balance and sensorimotor coordination [9, 43, 44].

More specifically, the dorsomedial striatum is involved with the acquisition of goal-orientated learning [41], dorsal striatum in the acquisition of rule-based learning [10]. Medial striatum in turn has been shown to mediate general task acquisition [42], position discrimination and reversal learning [47]. Ventral and medial striatum are known to carry out sensorimotor behaviours, with both regions involved in the maintenance of balance and sensorimotor coordination [9, 43, 44]. Ventral striatum is also involved in the coordinated movement of forelimbs [9].

1.4 Cell types within the Striatum

The rodent striatum is composed of a number of different cell types (Fig. 1.4) [48-51]. The following provides a brief description of the main neurons present, with a particular emphasis on Parvalbumin-expressing interneurons, a key regulator of striatal circuitry [50, 52], and a focus of the work presented in this thesis.

The striatum not only differs from the cerebral cortex in its cytoarchitecture, but also in the types of neurons present within this brain region. Nearly all known neurons within the striatum contain the inhibitory neurotransmitter Gama Amino Butyric Acid (GABA), with the sole exception of the population of interneurons containing the neurotransmitter Acetylcholine (Ach) [49-54] (Fig. 1.4). The vast majority of neurons present within the striatum are GABA-ergic medium spiny projection neurons, which constitute up to 90% of striatal neurons [49, 54]. These cells receive nearly all input projection to the striatum, and provide all of the efferent output from this nucleus. Medium spiny neurons are alike in morphology, unlike the interneurons that make up the remaining 10% of the striatal neuronal population [48, 49, 54]. Four classes of interneurons have been identified within the striatum: large cholinergic neurons as well as three types of GABA-ergic inhibitory interneurons, containing the calcium-binding protein calretinin (CR), the neuropeptide somatostatin, or the calcium-binding protein parvalbumin (PV), [48-51].

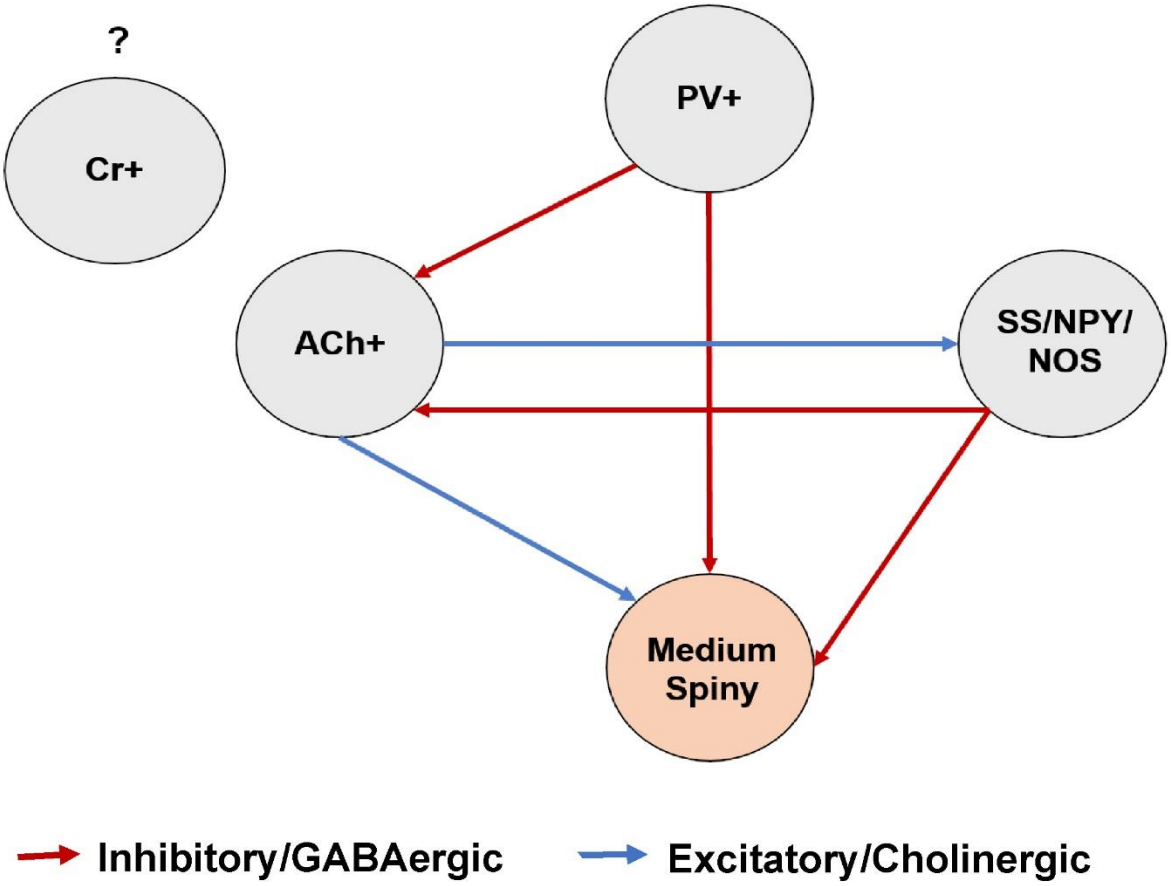

Figure 1.4 Diagram of cell types and their connections within the striatum

A diagram representing the five known types of neurons present within the striatum, and their synaptic connections with one another. There are four types of interneurons found within the striatum, represented here in grey, three of which are GABA-ergic inhibitory interneurons and the fourth of which contains the neurotransmitter acetylcholine. Striatal interneurons form synaptic connections with one another and with the efferent neurons of the striatum, medium spiny projection neurons, shown here in pink. The connections of calretinin-containing neurons are not yet determined. CR+: calretinin-expressing, ACh+: acetylcholine-expressing, PV+: parvalbumin-expressing, SS: somatostatin-expressing, NPY: neuropeptide Y-expressing, NOS: nitric oxide synthase-expressing.

1.4.1 Medium Spiny Neurons

Medium spiny neurons have a round or polygonal soma with a diameter of around 15μm and four to eight primary dendrites that develop in a roughly spherical pattern of arborisation over an area around 500μm in diameter, and are covered in dense dendritic spines. The axons of these neurons originate from the cell body, and form a dense local axon collateral plexus [49, 54, 55].

Maturation of medium spiny neurons begins around embryonic day (ED) 12 with differentiation, and continues until several weeks after birth [54]. Medium spiny neurons do not display adult electrophysiological properties until well into the third and fourth postnatal weeks, during which time the morphology of these cells continues to mature [54]. The dendrites of these neurons do not develop adult levels of spine density until the third or fourth postnatal week [54]. Many morphological characteristic such as somatic area, number of primary dendrites, distance to first dendritic branch, and dendritic or axonal field diameter remain unchanged throughout the development of medium spiny neurons [54].

Medium spiny neurons develop along caudorostral and mediolateral gradients within the rodent striatum [54]. The majority (approximately 85%) of corticostriatal projections to these neurons terminate upon dendritic spine heads, with the remainder synapsing upon dendritic shafts, necks of dendritic spines and other unknown targets [54]. As animals mature, there is an increase in the density of asymmetric (likely excitatory corticostriatal) axospinous synapses upon striatal medium spiny neurons [54]. Symmetric synapses (likely inhibitory) upon medium spiny neurons undergo little developmental change and are relatively mature around 10 days postnatal [54]. The majority of symmetric synapses terminate upon spine necks, and occasionally onto spine heads. There appears to be no synaptic interaction between medium spiny neurons within the rodent striatum, although there is some suggestion of gap junction enabled connectivity between these neurons in young animals [54]. Medium spiny neurons fire phasically following activation by excitatory post-synaptic potentials received from extra-striatal projections originating within the cerebral cortex [56-59], with

the activity of this neural population modulated within the striatum by GABA-ergic and cholinergic interneurons [60, 61]. Medium spiny neurons taking part in the direct pathway that express the D1 dopamine receptor are less excitable than neurons taking part in the indirect pathway that express the D2 dopamine receptor [62].

1.4.2 Cholinergic Interneurons

Cholinergic interneurons within the striatum are large, with a 20 – 50 μm diameter soma that may be spindle-like, oval, triangular or multipolar [48, 50]. These neurons have a relatively small number of primary dendrites (2 – 5) but display a widespread dendritic arbour that can reach over 600μm, and be 200μm distant from the cell body of origin [48, 50]. There is little cortical innervation upon both the soma and dendrites of cholinergic interneurons [63], although they have been shown to receive thalamic and dopaminergic inputs along with axodendritic input from other striatal cholinergic cells [49, 52, 63]. Cholinergic interneurons are located within the matrix compartment of the striatum [45]. Given the extensive dendritic arbour and synaptic contacts made with medium spiny projection neurons and other striatal interneuron populations, it seems that cholinergic interneurons act as associative interneurons within the striatum, taking the place of associative fibre systems seen in other areas of the brain [56, 63, 64].

Cholinergic interneurons constitute a population of tonically active neurons within the rodent striatum that display constant, irregular firing patterns [59, 65].When activated, these neurons demonstrate long duration action potentials, with a long-lasting after hyperpolarisation effect [48]. These neurons are thought to modulate thalamic input to the striatum, and integration of the direct and indirect pathways within the basal ganglia, facilitated by their connectivity [49, 50, 61, 63].

Cholinergic interneurons are observed within the lateral region of the rodent striatum on postnatal day one, and reach adult numbers during the second month of life [50]. Before postnatal day 20

(P20), a greater number of these neurons is present in the lateral portion of the striatum. Afterwards, cholinergic interneurons are distributed evenly throughout the striatum [50]. It is thought that this trajectory of maturation is due to a change in function from Acetylcholine acting as a developmental factor to becoming a mature neurotransmitter regulating striatal activity [50].

1.4.3 Calretinin Inhibitory Interneurons

Calretinin is a calcium-binding protein, found in small aspiny neurons within the rodent striatum [49, 50, 52]. Calcium-binding proteins are thought to buffer the effects of fast calcium ion movement within a cell, preventing excitotoxicity and enabling the occurrence of fast-spiking action potentials [66, 67]. Striatal calretinin (Cr+) inhibitory interneurons are GABA-ergic and have a medium sized oval or polygonal soma 7 – 20μm in diameter [49, 50, 52]. A low number of primary dendrites (2 – 3) originate from this soma, and in turn branch to give two or three secondary and tertiary dendrites, tapering to very thin processes that may extend to beyond 200μm from the cell body [50]. Large numbers of Cr+ inhibitory interneurons are present within the striatum at birth, and display a mediolateral gradient of maturation until the third postnatal week, when distribution becomes more homogeneous [50]. The number of these neurons present within the rodent striatum increases until P5; during the following two weeks there is a decline in number until adult levels of expression are reached [50]. This transient expression of a calcium-binding protein is thought to protect neurons against calcium toxicity during the early developmental period [50]. Cr+ inhibitory interneurons make up a very small proportion of the total neuronal population within the striatum, and as such their physiological properties are less well characterised than those of other neurons within this nucleus.

Table 1.1 Summary of striatal neurons and their connections.

	Neurotransmitter	Soma dimensions	Afferent input	Efferent output
Medium spiny projection neurons	GABA	15μm, round/polygonal	Cortex, PV+, ACh+ & SS+ striatal neurons	Globus Pallidus External, Endopeduncular nucleus, Substantia Nigra pars reticulata
Cholinergic Interneurons	Acetylcholine	20 – 50μm, spindle-like/ triangular/oval/ multipolar	Thalamus, Substantia Nigra, PV+ & SS+ striatal neurons.	Striatal medium spiny projection neurons & SS+ neurons
Calretinin Interneurons	GABA	7 – 20μm, oval/polygonal	Not yet determined	Not yet determined
Somatostatin Interneurons	Nitric oxide synthase (NOS), somatostatin, neuropeptide Y & GABA	12 – 25μm	Cortex, Substantia Nigra & striatal ACh+ neurons.	Striatal medium spiny projection neurons & Ach+ neurons
Parvalbumin Interneurons	GABA	10 – 25μm, oval	Cortex & Thalamus.	Striatal medium spiny projection neurons & ACh+ neurons

Table 1.1 Summary of striatal neurons and their connections.

Cr+: calretinin, ACh+: acetylcholine, PV+: parvalbumin, SS: somatostatin, NPY: neuropeptide Y, NOS: nitric oxide synthase, GABA: Gamma Amino Butyric Acid.

1.4.4 Somatostatin Inhibitory Interneurons

Somatostatin is a neuropeptide, derived from secretory proteins formed within the cell body of neurons, and acts as a neurotransmitter at short range [68-70]. Somatostatin (SS+) neurons within the rodent striatum are aspiny, with a soma 12 – 25μm in diameter, relatively few dendrites and extensive axonal arborisations [48]. This neural population expresses a greater range of neurotransmitters and co-transmitters than any other striatal neurons, containing nitric oxide synthase (NOS), somatostatin (SS), neuropeptide Y, and low levels of GABA [70-75]. Approximately 20% of these neurons also contain the calcium-binding protein calbindin D, thought to protect against calcium toxicity during fast-spiking action potentials [67, 76].

SS+ interneurons within the striatum receive direct cortical input, along with dopaminergic innervation from the SNpc and cholinergic innervation from ACh+ interneurons within the striatum [24, 64, 77, 78]. In turn, the terminals of these neurons synapse upon the cell bodies and dendrites of other striatal neurons, including medium spiny projection neurons [79]. It is thought that SS+ interneurons take part in feed-forward processing and that their unusually long axons allow for an influence on neurotransmission over a great area [24, 52, 79]. SS+ neurons are visible within the rodent striatum prior to birth, appearing around ED 18 and do not display a clear anatomical gradient of development, suggesting that there are multiple subtypes of these interneurons present within this nucleus [80-82].

1.4.5 Parvalbumin Inhibitory Interneurons

Similar to other populations of inhibitory interneurons found within the rodent striatum, parvalbumin-expressing interneurons also contain a calcium-binding protein, in this case parvalbumin (PV), to protect against calcium toxicity during fast-spiking action potentials [48, 66, 67, 83-85]. PV+ interneurons have a medium, oval shaped soma 10-25μm in diameter, with three to five

thick primary dendrites originating from the cell body. These dendrites are aspiny, branch into thin processes a short distance from the cell body, and form elaborate, dense dendritic arbours over 200 – 300μm [48, 50, 86]. These neurons possess dense axonal branches very near to the dendritic arbour, with a great deal of collateral branching [48].

PV+ interneurons within the rodent striatum form axosomatic and axodendritic synapses with other PV+ neurons and medium spiny projection neurons within this nucleus [54, 63]. The vast majority of input to PV+ interneurons originates from the cerebral cortex, although these neurons also receive direct asymmetrical (likely excitatory) synaptic input from the parafascicular nucleus of the thalamus [60, 87, 88]. The connections of striatal PV+ neurons thus provide a link between the cerebral cortex and medium-spiny projection neurons, enabling them to regulate activity levels, integrate synaptic inputs over large areas, and provide feed-forward modulation of medium spiny projection neurons [50, 52].

Along with synaptic connections, PV+ neurons within the rodent striatum are also connected to one another electrotonically through gap junctions formed between membranes of adjacent cells [86, 89]. This electrical coupling allows PV+ interneurons to act as a syncytium within the striatum, coordinating action potential firing and activity within the microcircuitry formed between these neurons [90-94]. Striatal PV+ neurons are GABA-ergic, express the fast activating Shaw-like potassium channel, Kv3.1 [48, 87], and are accordingly able to fire fast action potentials: these neurons display short duration spikes, with short after-hyperpolarisations, and can fire at a high rate with little adaptation of spike frequency [48]. The electrophysiological properties of PV+ interneurons, together with their high levels of electrotonic coupling, indicates that these neurons are capable of generating fast action potential responses, coordinated over a large field within the striatum.

PV+ inhibitory interneurons emerge during the embryonic period, and continue to mature throughout early postnatal life. The peak period of production for these neurons are ED 14 to 17,

with a greater density present within the lateral part of the rodent striatum [95]. This lateral to medial gradient is maintained throughout development, with mature animals having a greater density of PV+ neurons within lateral striatum [50, 53, 96]. Striatal PV+ inhibitory interneurons have also been revealed to mature along a caudorostral gradient [50, 53, 95].

Despite the presence of these neurons within the rodent striatum from an early age, they do not fully mature until around the fourth postnatal week. Striatal PV+ inhibitory interneurons are first able to be visualised using immunohistochemical techniques around P9, when neurons become visible in the lateral portion of the striatum. The visible expression of PV spreads throughout the striatum until reaching adult levels around P28 [50]. This apparent increase in striatal PV+ inhibitory interneurons during early life is thought to result from an up-regulation of the parvalbumin calcium-binding protein in response to afferent innervation of these neurons [50, 87]. Corticostriatal connections are established towards the end of the first postnatal week, shortly before when PV is first able to be visualised within the striatum [21, 50], with electrophysiological maturation and increased expression of Kv3.1 channels occurring during the same time period [87]. With a high level of cortical innervation received by the end of the second postnatal week, it is thought that the maturation of PV-expression within the striatum is driven by corticostriatal afferents synapsing and becoming active throughout the striatum [87].

2. Perineuronal Nets (PNNs)

The early postnatal period is a time of intense development within the rodent striatum and its associated circuitry. In the striatum itself there are alterations in cellular morphology [50]*, ongoing innervation from extra-striatal brain regions [87], and the growth of Perineuronal Nets (PNNs) in the extracellular matrix [97, 98]. PNN composition, function and the cell types with which they are known to be associated play a role in their relevance to striatal circuitry.*

Perineuronal nets (PNNs) are extracellular matrix structures found within the CNS, first described by Camillo Golgi as an “endocellular reticular apparatus” enveloping the cell body and dendrites of various neurons [99, 100]. Thanks to improved histochemical and microscopy techniques, we now know that PNNs are found throughout the CNS and surround a variety of neurons within the brain and spinal cord [84, 96, 101-106]. PNNs are thought to play several roles in the CNS, ranging from consolidation of synaptic connections between neurons [107, 108], protecting against oxidative or other damage [109-114], and maintenance of ionic homeostasis around highly active neurons [84, 104].

PNNs have been shown to play an important role in the consolidation of maturing neural circuitry, and in the maintenance of strong synaptic connections between neurons within the adult rodent brain [105, 107, 108, 115]. PNN formation correlates with the development of mature neuronal properties [108] and function [96, 97]. The temporary removal of these structures within the adult brain using a bacterial enzyme, Chondroitinase ABC (ChABC), leads to an immature phenotype and increases the neural plasticity within a circuit [101, 102, 105]. PNN digestion has been shown to result in an increase in axon sprouting from Purkinje cells within the cerebellum [107], as well as encouraging collateral sprouting by afferent projections to target nuclei following partial lesion [101]. It appears that the dissolution of PNNs within the adult brain allows the neurons they surround to form new synaptic connections by “freeing up” their axons. Together, these findings suggest that PNNs are important for the development and maintenance of strong synaptic connections, and their removal may impact upon neural circuitry and function.

PNNs are composed of highly charged Chondroitin Sulfate Proteoglycans (CSPGs), hyaluronan, tenascin and link proteins. CSPGs are comprised of a wide variety of proteins and covalently connected chondroitin sulphate glycosaminoglycans [116]. The protein components of CSPGs are what regulates integration into the extracellular matrix, and are thought to play a role in attachment and signalling functions [115]. Hyaluronan is a simple glycosaminoglycan; link proteins are small

glycoproteins that provide connections between hyaluronan and the aggrecan component of CSPGs; and tenascin is an adhesive molecule that interacts with CSPGs and provides connections with other components of PNNS [115, 117-120]. CSPGs and hyaluronan are both highly negatively charged, making PNNs polyanionic, enabling them to maintain local ion homeostasis within the brain by scavenging and binding redox-active ions, minimising oxidative damage [112-114]. PNNs have previously been shown to protect against neurodegeneration caused by both ageing and Alzheimer's disease [109, 111]. The presence of PNNs is therefore highly important for the maintenance of both individual neurons and the circuits they make up.

2.1 Perineuronal Nets and Parvalbumin-expressing neurons

PNNs are widespread throughout the rodent CNS, having been identified in the cerebellum [107], cerebral cortex [121], brainstem [101, 104, 121], spinal cord [102], subcortical regions [121], and the striatum [96-98]. While these extracellular matrix structures are known to associate with a variety of neurons, the cell type most commonly linked with the presence of PNNs is that of fast-spiking neurons [84, 96, 103, 104, 106]. It is thought that the highly anionic microenvironment provided by the presence of PNNs allows for the fast transference of cations, aiding fast-spiking action potentials [104].

PNNs within the cerebral cortex, hippocampus and brainstem are seen to surround neurons expressing the Shaw-like potassium channel, Kv3.1 [84, 104], found within fast-spiking PV+ inhibitory interneurons [84, 122]. Direct immunohistochemical staining for PV+ neurons and PNNs has revealed that within the cerebral cortex, visual cortex, reticular nucleus of the thalamus, and the medial trapezoid nucleus of the brainstem, a large proportion of GABA-ergic inhibitory neurons expressing PV are surrounded by PNNs [103, 104, 106, 123]. The association between PNNs and PV+ inhibitory interneurons is maintained within the striatum, but is not exclusive [96].

2.2 Perineuronal Nets within the striatum

There are no PNNs present within the rodent striatum prior to birth or during the first week of life; instead, there are large, amorphous “clouds” containing CSPGs that are associated with the striosome compartment of the striatum [97]. As animals mature, these CSPG-containing clouds disperse and are no longer detectable by the end of the second postnatal week [97]. Mature striatal PNNs make their first appearance, around the same time [50, 97]. Curiously, this is also the period during which PV+ inhibitory interneurons are first able to be immunohistochemically visualised within this nucleus [44, 71]. Mature PNNs surround the soma of associated neurons within the striatum, with extended enmeshment of dendritic processes (Fig. 1.5). PNNs are restricted to the matrix subregion of the striatum, exhibiting a reversal of the association between diffuse CSPG clouds and striosomes during the early postnatal period [97].

As in other areas of the brain, striatal PNNs demonstrate an overlap with PV+ neurons [96, 103, 104, 106, 123]. Within the mouse striatum this association is not exclusive – approximately 50% of PNNs within the striatum are associated with PV+ inhibitory interneurons, with a similar proportion of PV+ inhibitory interneurons surrounded by PNNs [96]. This is not unexpected, given the mismatch in overlap of anatomical position displayed by these two populations [50, 96]. In other areas of the rodent brain, PNNs have been shown to associate with a variety of neurons expressing other calcium binding proteins [104]. Thus far, neither cholinergic nor calretinin expressing interneurons have been shown to overlap with PNNs within the mouse striatum [96]. Given that PV expression levels within interneuron populations can vary in a given brain region [124], it is possible that some of these unidentified cells associated with striatal PNNs may be interneurons with low PV concentration.

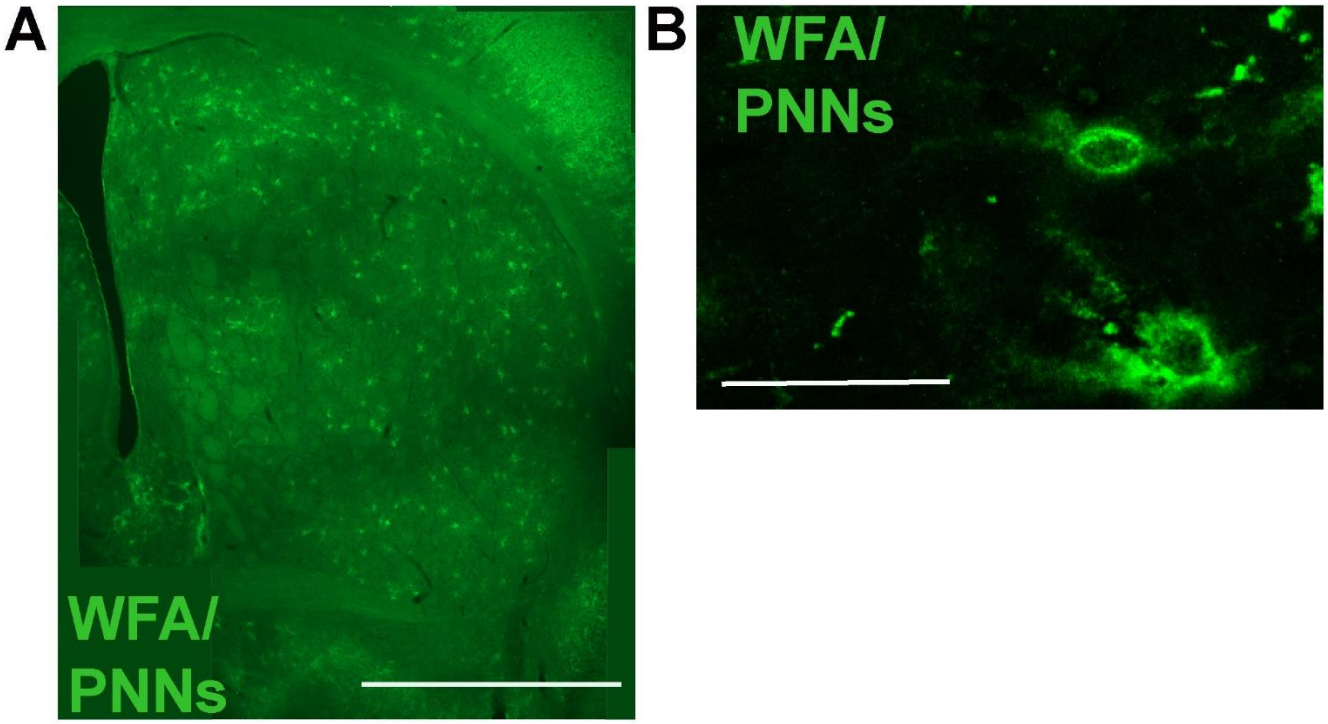

Figure 1.5 Perineuronal Nets (PNNs) within the striatum.

(A) Perineuronal nets (PNNs) within the striatum, visualised using the plant lectin Wisteria Floribunda Agglutinin (WFA), imaged at 5,000X magnification and photo-merged using Adobe Photoshop. Scale bar=1000μm. (B) A similar image, taken at 40,000X magnification. Note the ring-like structure surrounding the soma of the PNN-associated cells, and the extended staining observable upon the neurites of these cells. Scale bar=50μm.

3. Growth Factors

Maturation of PV+ neurons within the striatum and other areas of the rodent brain has been shown to depend upon appropriate levels of growth factors, proteins that are also capable of influencing the expression of mature PNNs [106, 123, 125, 126].

Growth factors are proteins secreted by cells that act locally to promote cell development, growth and repair. Neurotrophic factors are the growth factors found within the nervous system, and primarily promote the survival of neurons, along with facilitating appropriate neuronal maturation [127, 128]. Neurotrophins are thought to be released by the axonal target of a neuron, and are taken up via specific receptors [127, 128]. An inadequate supply of neurotrophic factors may have a variety of effects, ranging from lack of maturation and development of particular types of neurons or entire neural circuits [125, 126], to being the potential cause of neuropathologies [129, 130], and may even result in cellular death [128, 131]. There are a variety of neurotrophic factors – this review will focus on one specific type: Brain-Derived Neurotrophic Factor (BDNF).

3.1 Brain-Derived Neurotrophic Factor (BDNF)

Brain-Derived Neurotrophic Factor is related to Neurotrophic Growth Factor (NGF), and primarily interacts with the trkB tyrosine kinase receptor [127, 132]. BDNF is composed of a non-covalently linked homodimer with a signal peptide and a pro-region containing an N-linked glycosylation site, with two binding sites per BDNF molecule for trkB receptors [127, 133, 134]. BDNF, like other growth factors, promotes growth, repair and development of neurons, but is not limited to this: BDNF has been shown to regulate the synthesis, metabolism and release of neurotransmitters at synapses, and is also capable of modulating ion channel flux in postsynaptic neurons [135-137]. BDNF also has the ability to regulate synaptic efficiency by altering neuronal firing rates and inducing long term synaptic potentiation between neurons within the rodent CNS [128, 135-137], suggesting that this

neurotrophic factor plays an important role in the maintenance of neurons and the circuitry they compose.

BDNF is distributed throughout the rodent CNS, with the protein previously found within the cerebellum [18, 138], retina [126, 139-141], cerebral cortex [142, 143], hippocampus [15, 142, 143], and the striatum [15, 125, 130]. Neurotrophic factors influence various neurons within the brain and BDNF is no exception [125, 126, 144]; the neuron type that appears to be most dependent upon the presence of BDNF are PV+ GABA-ergic inhibitory neurons [125, 126]. Within the rodent visual system, BDNF is responsible for the maturation of inhibitory circuitry containing PV+ interneurons [126], and within the rodent striatum a lack of BDNF results in fewer visible PV+ cells [125].

3.2 BDNF within the striatum

Despite the well documented presence of BDNF protein within the rodent striatum [15, 125, 130], very little BDNF mRNA is expressed in this nucleus [142]. Areas that contribute afferent projections to the striatum – particularly the cerebral cortex, thalamus and SNpc – do exhibit high levels of BDNF mRNA expression [125, 142]. It is thought that BDNF protein within the rodent striatum is transported in an anterograde fashion from other areas of the brain projecting to this nucleus [125]. Animals that receive lesions to the cerebral cortex and SNpc demonstrate a decrease in the level of BDNF protein present in the striatum; a similar effect is observed with the use of axon transport inhibitors [125]. In contrast, lesions within the striatum that do not damage afferent projections have little effect on the level of BDNF protein found within this nucleus [125]. PV+ inhibitory interneurons within the striatum appear to be dependent upon the presence of BDNF protein for normal cellular processes: rodents both heterozygous and homozygous for a null mutation in the gene encoding BDNF have a reduced incidence of PV+ inhibitory interneurons within the striatum as measured by immunohistochemistry [125]. It is not known whether PV+ cells within the rodent

striatum die off in response to lack of BDNF protein, or if they merely do not develop a mature profile of PV expression.

Expression of PV is able to be regulated by activity levels within individual neurons in response to afferent input [124]. At the same time, a mature neurochemical profile of PV+ neurons appears to be dependent upon the presence of BDNF protein [125, 126]. Based on physiological data, the innervation of striatal PV+ inhibitory interneurons by corticostriatal afferents is thought to begin after the first postnatal week [87]. These afferents not only provide activity-dependent drive to PV+ cells, they also transport BDNF protein into this area of the brain [125], suggesting that BDNF, along with corticostriatal afferent activity, drives the maturation of PV expression in the striatum.

4. The Critical Period

The time during early life when PV, PNNs and BDNF are undergoing changes within the striatum may represent an early-life critical period for rodent striatal circuitry. This section will explain the concept of the critical period, and the role PV, PNNs and BDNF play in regulating this developmental epoch.

The nervous system is not fully mature at birth; in humans it is thought that the nervous system does not reach full maturity until the early twenties, and whilst rodents have less drawn-out development, there is ongoing neural maturation after birth. A fully mature nervous system may be defined as having restricted levels of neuroplasticity, relative to the peak neuroplasticity observed during development [145, 146]. Neuroplasticity is the ability of the brain to re-wire itself in response to the input it receives, and has been observed throughout the lifespan of laboratory animal subjects [15, 16, 18, 145, 147, 148], but never appears to again reach the levels observed during early maturation in a developmental epoch that may be referred to as the critical period [149]. During the critical period, neuroplasticity, malleability and the vulnerability of neurons are at their peak;

individual systems and areas within the brain are known to undergo their own particular critical periods at various times throughout development [146, 150-152].

Neuroplasticity may be dependent on coincident activation of linked cells within circuits during the critical period. This concept was first posited as important by Donald Hebb in 1949, whose idea of more efficient firing between cells as a result of repeated activation has become known as "Hebbian theory" [153]. This theory states that *"when an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased"* [153]. Today, this theory is best known as the aphorism *"cells that fire together, wire together"*. Hebb's theory has since been expanded to apply to most neural structures, ranging from the synapses formed between individual neurons, to microcircuits formed within particular brain regions, to circuits involving several areas of the brain [154]. Neuroplasticity allows the nervous system to reshape itself in response to the stimuli it receives, with changes in firing of neurons leading to changes in wiring between cells.

The best-characterised circuit within the nervous system with regards to the role of cellular activity during the critical period is the visual system; the well-defined timing of the critical period within this system has been demonstrated in several species [149, 155-157]. During the critical period, the activity level of neurons within the visual system is thought to play a role in strengthening cellular connections [149, 158]. Another way of phrasing Hebbian theory with regards to the critical period might be *"use it or lose it"*, as neurons that do not receive appropriate levels of activation and trophic support during this time tend to die off [128, 159, 160]. Through the use of mechanisms such as monocular deprivation [149, 156, 158], which leads to a decline in the number of cells within the primary visual cortex able to be activated by the deprived eye [149, 158], neural activity levels have been demonstrated to impact cellular and whole system development. Spontaneous action potential firing by retinal ganglion cells is another example of this mechanism at action in the visual system

[161, 162]. Blocking this spontaneous firing leads to greater levels of cellular death than normally would occur within the projection targets of the retina [161]. Further, abnormal firing patterns and rates within retinal ganglion cells lead to a disruption in the precise retinotopic mapping usually observed within the visual system. This suggests that the spatiotemporal patterning of activity can be just as important for establishment and maintenance of functioning neural circuitry as the presence or absence of activity [163]. Together, these findings highlight the importance of activity-dependent mechanisms on the development of early visual pathways.

There is also evidence of an activity-dependent period of plasticity for motor behaviours in the rodent striatum from P30 to P37 [164-169]. Curiously, the manifestation of this period of heightened plasticity appears to be dependent on cholinergic and dopaminergic systems within this region of the brain [164, 165, 167]. Circle training - in which animals are taught to turn in a particular direction for a set number of times – during this time has been shown to permanently reduce the expression levels of muscarinic ACh receptors and D2 dopamine receptors within the rodent striatum [164, 165, 167].

4.1 The role of inhibitory interneurons in the critical period

The critical period is thought to come about due to a disparity in the maturation rates of excitatory and inhibitory neurons. Inhibitory interneurons are known to be strong modulators of neuroplasticity at the level of synaptic connections between cells, taking part in cellular interactions such as feed-forward inhibition that modulate the activity of downstream neurons within circuitry [151, 170-173].

The possibility of inhibitory neurons playing an important role in learning and development was first put forward by Hebb in 1976, who believed that these cells functioned to “promptly shut off” activity of excitatory neurons once they had fired [174]. Inhibitory circuitry has been shown to

mature more slowly than its excitatory counterpart in several cortical areas [126, 175] (for review see Micheva, 1997 [146]), potentially creating a “gap” in the timing of circuitry development during which it is possible for life experiences to influence neural maturation.

The delayed maturation of inhibitory circuitry that potentially allows for critical periods to occur has been observed in a variety of brain areas [150, 151, 170, 172, 175-181]. Much of the work explicitly investigating the effects of this mismatch in excitatory and inhibitory network development has again been performed within the visual system. It is thought that the end of the visual critical period is brought about via the full maturation of PV+ GABA-ergic inhibitory interneurons within the visual cortex [126, 170, 177, 178]. It is speculated that later maturing inhibitory interneurons consolidate circuits formed by earlier maturing excitatory neurons, preventing ongoing high levels of neuroplasticity [126, 170, 177, 178]. Both accelerated maturation of PV+ GABA-ergic neurons [126] and increased inhibitory tone [177, 178] within the rodent primary visual cortex have been shown to result in an earlier onset and termination of the visual critical period, as indicated by the earlier termination of both prolonged response following visual stimulation and decline in cortical long-term potentiation induced by white matter stimulation [126, 177, 178].

Similarly to cortical areas, the rodent striatum contains a population of PV+ inhibitory interneurons that have been shown to undergo a protracted postnatal development [50] during the time that these neurons begin to receive cortical input [87] and striatally-mediated behaviours begin to emerge [182-184]. PV+ inhibitory interneurons in the striatum are thought to play an integral role in modulating the activity within this important nucleus [50, 54, 87]. Unlike the cortical areas investigated with regards to the critical period, PV+ inhibitory interneurons within the striatum develop earlier than the neural population they exert control over, demonstrating both mature morphology [50, 54] and electrophysiological properties earlier than do medium spiny projection neurons [54, 87]. PV+ inhibitory interneurons also show a response to corticostriatal afferent activation earlier than do medium spiny neurons [87], and generate inhibitory postsynaptic

potentials within medium spiny neurons [54, 185] from P14 that decrease in amplitude as medium spiny neurons mature [54]. Given the correlation of striatal PV+ inhibitory interneuron maturation with the emergence of striatally-mediated behaviours and the ability of these neurons to exert control over striatal activity once mature, it is possible that a "critical period" within the rodent striatum exists that is mediated by the development of this neural population.

4.2 The role of extracellular matrix in the critical period

The extracellular matrix within the CNS provides support to the neurons contained within it, and plays an important role in the maintenance of synaptic connections, ionic homeostasis, and prevention of cellular damage [84, 104, 107-114]. PNNs in particular are thought to be vital for the healthy functioning of a mature CNS, and are associated with PV+ inhibitory neurons within several areas of the rodent brain [103, 104, 106, 123], including the striatum [96]. Similar to the role of inhibitory neurons within the critical period, the most researched brain area with regards to PNNs and the critical period is that of the visual system, and particularly the primary visual cortex. PNNs within the primary visual cortex are associated with GABA-ergic PV+ inhibitory neurons [123, 126]. Animals that have been dark reared from birth in an exclusively light free environment exhibit a visual critical period that initiates upon exposure to ambient light, effectively delaying the epoch of maximal plasticity beyond the age at which it usually occurs [105]. Dark rearing results in a comparable delay in the formation of mature PNNs within mammalian primary visual cortex, suggesting that these extracellular matrix structures play a role in "closing off" the visual critical period [105, 108, 186]. Digestion of PNNs within the rodent visual cortex has been shown to "re-open" this critical period, reactivating ocular dominance (OD) plasticity [105] and reversing the effects of monocular deprivation [187], suggesting that PNNs within the rodent visual system are involved in the consolidation of circuitry leading to the end of the visual critical period.

Similar to the cortical areas that have been more thoroughly investigated, PNNs within the rodent striatum form during the postnatal period when striatally mediated behaviours are emerging [97]. Motor behaviours in particular develop during the first two weeks of life, as striatal PNNs are forming and the PV+ inhibitory interneuron population is maturing [50, 97]. Dissolution of PNNs within the striatum of adult mice leads to an immature gait, with animals adopting a crawling style of locomotion rather than adult walking [96]. The development of PNNs within the mouse striatum is able to be influenced by the environment an organism inhabits, with accelerated appearance of mature PNN structures in the striatum of animals raised within an enriched environment [98]. This is correlated with an accelerated onset of striatally-mediated motor behaviours [98], providing further evidence of an early postnatal critical period within the rodent striatum, the timing of which is likely mediated by the maturation of PV+ inhibitory interneurons and the formation of PNNs.

4.3 The role of neurotrophic factors in the critical period

Neurotrophic factors provide trophic support to cells within the nervous system [127, 159], with BDNF in particular associated with the full maturation of PV+ inhibitory neurons in several regions of the rodent brain including the striatum [125, 126]. Again, the most thoroughly researched area of the brain with regards to the effect of BDNF upon the critical period is that of the visual system. Rodents that overexpress BDNF such that the postnatal increase in the levels of this protein is accelerated also demonstrate both accelerated maturation of PV+ GABA-ergic interneurons [126] and decline of white-matter evoked long-term potentiation only observed within juvenile animals [126, 173]. Along with these circuit-level changes, BDNF overexpression within the rodent primary visual cortex also influences system function, accelerating the onset of ocular dominance plasticity and provoking precocious development of visual acuity [126]. Thus, BDNF within the rodent visual system regulates the maturation of PV+ inhibitory interneurons and is involved in determining the timing of the visual critical period.

The rodent striatum undergoes a critical period at the end of the first month of life, the timing of which is influenced by the neurotrophic factor NGF. During this critical period, the cholinergic and dopaminergic systems within the striatum exhibit sensitivity to motor experience [164, 165, 167]. The application of exogenous NGF maintains high levels of neuroplasticity beyond the age at which it normally decreases [168, 169], suggesting that timing of a late motor critical period within the striatum can be influenced by the presence of a particular neurotrophic factor. Despite the lack of BNDF mRNA within the rodent striatum, there is still a large amount of the protein found within this nucleus, the presence of which is vital for the full maturation of striatal PV+ inhibitory interneurons [125, 188]. The pattern of maturation observed within the rodent striatum with regards to development of PV+ inhibitory interneurons, PNNs within the extracellular matrix, and the influence BDNF has on PV expression suggests that there may be another, earlier, critical period occurring within the rodent striatum regulated by these three neural components [50, 97, 98, 125].

5. Animal Behaviours

The behaviours of animals provide insight into neural function [42, 189], cognitive [98, 190, 191], motor [97, 148] and emotional [192-194] capacity and state. The following section provides an overview of some of the most frequently used behavioural assays, along with tasks that have been designed to specifically test striatal function.

Assessing the cognitive capacity of animals can be a difficult process – there is no easy way to communicate with animal experimental subjects directly, and as a result much of the behaviour manifested during assessment is subject to interpretation. Despite these difficulties, a number of tests have been developed to evaluate explicit cognitive abilities of rodent subjects. These behavioural tasks include the Morris Water Maze, designed to assess spatial learning and memory [98, 190, 191] and operant or instrumental conditioning tasks evaluating learning, memory and decision-making behaviours [46, 195, 196]. Unlearnt behaviours, such as novelty seeking [195, 197],

motor capacity [9, 160], social interactions [198, 199] and baseline anxiety [191, 193, 194, 200, 201] may also provide insight into an animal's cognitive capacity, emotional state or sensorimotor capabilities.

The striatum mediates a variety of behavioural functions: cognitive processes such as decision-making [13, 40], choice of action [40], goal-orientated learning [41], rule-based learning [10], and task acquisition [42]; as well as sensorimotor behaviours, including motor coordination [9, 43], balance [9, 44], motor skill acquisition [8, 45], and synchronisation of sensory and motor input [9, 43, 44]. The following will describe and critique tasks designed to assess both developing and mature striatally mediated behaviours.

5.1 Tests for striatally mediated juvenile behaviours

The majority of tasks assessing striatally mediated behaviours has focused on the performance of adult animals [42, 44, 46, 202]. Less has been done to explore developing or emergent behaviours associated with this particular nucleus.

Assessing the developing behaviours of juvenile animals can be a difficult task – young mice are unable to interact with their surroundings and manipulate objects to the same degree as adult animals. Previous work from our laboratory has demonstrated the successful assessment of juvenile mouse sensorimotor coordination and exploratory behaviours using a forced-swim and open-field task, respectively [98]. Recently, the production of Ultrasonic Vocalisations (USVs) has garnered interest as an indicator of rodent behaviours such as anxiety status [203-206], social [198, 207, 208] and maternal-infant interactions [209-211]. USV production by rodents may provide a mammalian model for vocal learning similar to that observed in songbirds [7], and can be used to assess environmental influences on a highly ethologically relevant, naturally occurring behaviour [203, 212, 213]. The striatum has been shown to undergo motor-driven activation related to vocalisation

during the production of USVs [7], making the assessment of these calls a potential assay for striatal function.

5.2 Tests for striatally mediated adult behaviours

The many functions carried out by the striatum [8, 9,10, 13, 52, 53, 54, 55, 56, 57] means that assessing behaviour mediated by this nucleus in adult animals must cover a variety of tasks assessing sensorimotor and cognitive capabilities.

Assessing the motor capacity and sensorimotor coordination of rodents quite often overlaps, although the two can be distinctly measured. Motor capacity refers to the ability of animals to perform a particular motor task, and may be evaluated by such apparatus as a grip strength meter, used to assess maximum limb muscle strength and animal models of neuromuscular disorders [214]. Sensorimotor coordination refers to the synchronization between sensory input and motor output, and may particularly be considered a striatally mediated function. Sensorimotor coordination may be assessed by tests such as the balance beam [9, 160], consisting of an elevated beam leading to a sheltered box, and gait analysis [215, 216], where the locomotion of animals is evaluated.

Another task used to assess sensorimotor coordination is the rotarod behavioural task, consisting of a rotating rod large enough for rodents to comfortably sit upon when not in motion. The speed and direction of rotation may be controlled so that abilities such as balance [217, 218], coordination [217, 218], and motor learning [8, 45] may be assessed using this apparatus. A once-off test using the rotarod may shed light upon the baseline sensorimotor coordination abilities of animals [218], whilst testing over a period of days allows assessment of motor learning [8, 45]. Injury to the rodent striatum, either due to a neurodegenerative condition [130, 219, 220] or induced damage [189, 202], has been shown to impair performance within this test of sensorimotor coordination [130, 189, 202, 219, 220], whilst knock-out animals with altered thalamostriatal projections also display impaired

performance in motor skill acquisition [45]. Together with work demonstrating that the rodent striatum undergoes direct activation during the rotarod task [8], these studies suggest that the striatum is involved in generating behaviours needed to perform the rotarod task, making it a suitable test for assessing striatally mediated sensorimotor behaviours.

One behavioural apparatus that may be used to assess the cognitive capacity of rodents is the operant conditioning chamber. This arena generally consists of a lever that animals can press in response to a cue in order to receive a reward, usually food. Variants on the operant conditioning chamber may have two or more "choice" stations to assess goal-orientated learning and decision-making [46, 195, 196], behaviours known to be mediated by the striatum [13, 40].

Goal-orientated learning mediated by the rodent striatum may also be assessed using a Puzzle-Box behavioural test, an arena that offers a more naturalistic context for evaluating goal-seeking and learning behaviours [221-223]. This apparatus consists of an open-field start zone, and a dark goal-box zone. Similar to balance beam behavioural tasks, this test utilises the natural preference of rodents for small, dark, enclosed spaces over brightly lit open-fields [221-223]. Animals are required to solve obstruction puzzles placed between the two zones within the Puzzle-Box in order to access the goal-box [221-223]. This arena tests the ability of animals to convert goal-directed intention into sensorimotor behaviours, whilst retention of solutions are also able to be assessed by repetition of obstruction puzzles [221-223]. Given the interaction between goal-orientated learning and sensorimotor behaviours that occurs within the Puzzle-Box behavioural test, it is likely that striatal circuitry is involved in this task.

Decision-making behaviours have been shown to involve the dorsal rodent striatum (see Balleine et al, 2007 for review [13]). This nucleus is known to mediate executive function such as task acquisition [42], rule-based learning [10], and the transition from flexible outcome-based action selection to habitual decision-making [40, 46]. A decision-making task that examines specific reward-learning behaviours is the Iowa Gambling Task, a test traditionally used in human psychological

assessment [224-226] that has of late been adapted for rats [196, 227, 228]. Recently developed flexible behavioural testing systems such as the IntelliCage, that allow for continual performance monitoring of a number of mice simultaneously [229], may provide an ideal platform for the development of tasks to assess decision-making and other striatally mediated behaviours. To date, the IntelliCage has been used to assess a number of cognitive processes, including place learning [230], exploratory behaviours [229, 231, 232], and spatial learning [233, 234], but not the reward guided choice behaviour associated with striatal function.

6. Environmental Enrichment

An enriched environment may be defined as surroundings that provide increased opportunity for social interaction and motor activity and greater sensory stimulation than that experienced in standard laboratory housing (Figure 1.6). Animals raised or housed in environmental enrichment display a myriad of cellular, molecular, and behavioural differences when compared to animals raised or housed within a standard laboratory environment (for review, see Sale, 2009 or van Praag, 2000 [154, 235]).

Environmental enrichment (EE) provides animals with increased opportunities for stimulation and activity within their home cages, in turn increasing the levels of stimulation and activity experienced within the nervous system. Cellular activity levels are postulated to influence both the strength of connections between neurons and the level of trophic support present within a neural circuit [153, 174]. Historically, Donald Hebb first noted that an organism's environment may impact neural functioning, after observing that rats he had taken home behaved differently to those still housed within the laboratory. The opportunity provided by EE for extra stimulation of the nervous system, particularly during the critical period, may result in a greater number of neurons within the brain arranged in more strongly connected, and possibly more complex, circuitry.

Similar to research investigating the critical period, the region of the mammalian nervous system that has been most thoroughly investigated at a cellular level with regards to the influence of environment is the visual system. Hubel and Wiesel's seminal work illustrating that lack of sensory input is able to impact the maturing visual system was amongst the first indicators of how important an organism's environment can be for neural development and function [149, 158]. It has since been well-established that early life experience is capable of influencing the anatomy and physiology of visual circuitry. It is not just deprivation of stimuli that can impact the visual system; EE is also capable of influencing this circuitry.

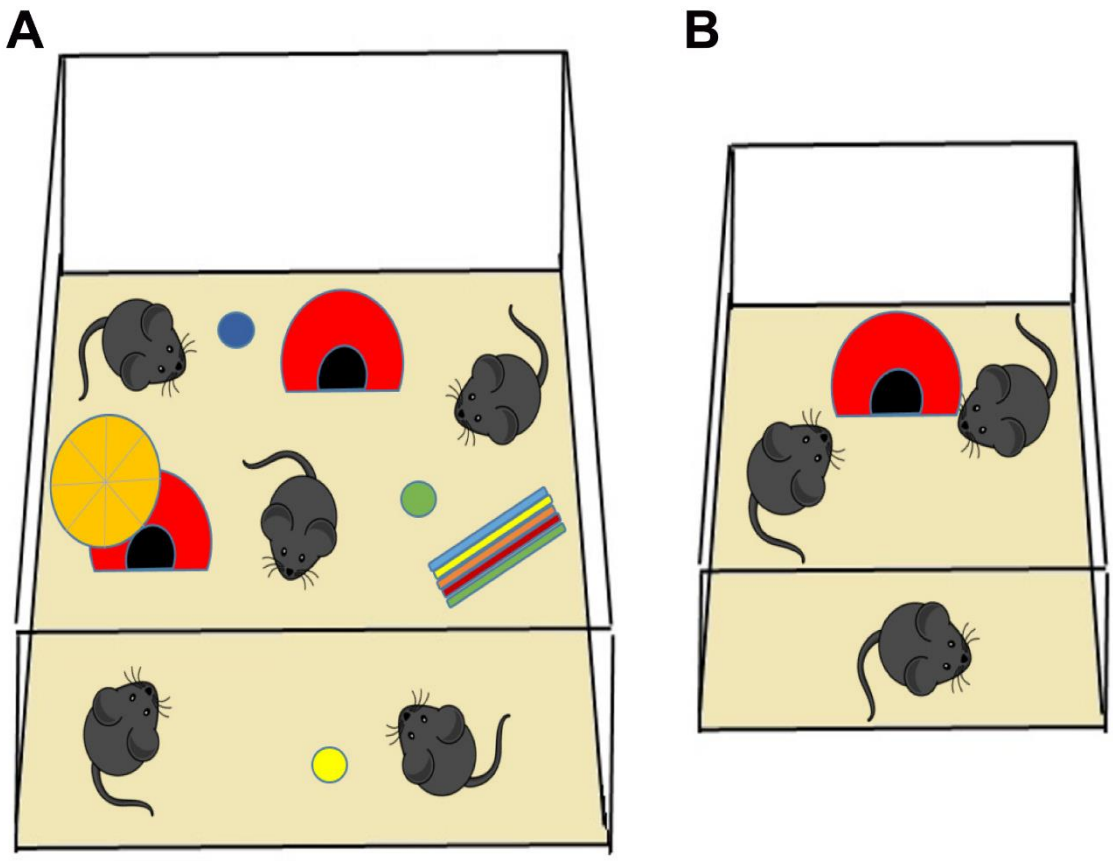

Figure 1.6 Variable housing environments.

(A) A diagram illustrating environmentally enriched housing. Animals are housed in a larger cage, with extra shelter, a running wheel and sensory stimuli such as balls and coloured objects. (B) A diagram depicting standard environment housing. Animals are placed in smaller cages with a limited number of objects such as a red mouse igloo and bedding materials. There are generally fewer animals placed within standard environments compared to enriched cages.

6.1 The effect of environmental enrichment upon the visual system

Exposure to EE during the prenatal or postnatal period has been shown to have a great effect upon neural development, whilst EE during adulthood is capable of influencing neural function. Prenatal, postnatal and adulthood EE have all been shown to impact the anatomy and function of the visual system [98, 105, 139-141, 144, 187].

Prenatal enrichment occurs when pregnant rodent dams are placed into enriched housing during their pregnancy, impacting their offspring. This form of EE has been shown to increase levels of the neurotrophin Insulin-like Growth Factor-1 (IGF-1) within the brains and breast milk of dams [236]. Increased levels of IGF-1 are also present within the retina of fetuses taken from enriched dams, along with an acceleration of programmed cell death and migration of differentiating neural cell precursors [236], suggesting that prenatal EE accelerates the development of a component within the visual system.

Postnatal EE occurs when late-pregnancy rodent dams are placed into enriched environments immediately prior to giving birth, and offspring raised within enriched housing. This form of EE has also been shown to accelerate the maturation of several components within the rodent visual system, and is known to impact upon visual function [123, 139-141, 144, 237]. Postnatal enrichment leads to a precocious increase in levels of BDNF, IGF-1 and GABA within the primary visual cortex [123, 139, 237]. These effects are accompanied by an accelerated maturation in the development of visual acuity, earlier termination of the visual critical period, and a lifelong improvement in visual acuity compared to standard housed age cohorts [123, 139, 237]. Postnatal enrichment also impacts the retina, increasing levels IGF-1 and BDNF within this structure, accompanied by a precocious development of retinal acuity [140, 144]. Taken together, these results suggest that postnatal EE accelerates neural development within the rodent visual system.

Prescribed periods of EE in adult animals can also affect neural circuitry and function, despite subjects being past the age of critical period when neuroplasticity is at its peak [106]. Animals that

have undergone long term monocular deprivation develop what is known as amblyopia. Amblyopia occurs when one eye loses visual acuity and function due to a shift of ocular dominance towards the non-deprived eye [106, 149, 158]. Amblyopic rodents that undergo reverse-suturing and are then placed into enriched environments show a full recovery of visual acuity in originally deprived eyes, accompanied by an increase in BDNF and a decrease in GABA and density of PNNs within the primary visual cortex [106]. In contrast, animals that undergo the same treatment but are not placed into enriched environments show no such recovery of visual function [106], suggesting that adulthood EE is capable of influencing inhibitory circuitry and function within the rodent visual system.

As studies investigating the rodent visual system have demonstrated, EE has the ability to accelerate neural development, promote recovery of normal function in pathological conditions, improve the function of the visual system under normal conditions, and may impact the visual system during both the critical period and in later life when neuroplasticity is no longer at its peak [106, 140, 141, 144, 236]. Overall, the visual system has provided researchers with an excellent model to examine the impacts of EE. Other areas of the brain are also able to be influenced by the environment an organism inhabits; the following will provide a brief summary of this research, with a particular focus on the effect EE has on the hippocampus, frontal/motor cortices, and the cerebellum.

6.2 The effect of environmental enrichment upon other neural systems

The visual system may offer an ideal model for investigating the impact of environmental enrichment upon brain function, but there are many other areas within the brain that have demonstrated a response to EE. This widespread impact of EE is not unexpected, given that most enriched environments contain objects that allow for a broad spectrum of stimulation, likely influencing more areas of the brain that those solely concerned with immediate sensory processing. Other areas of the

brain previously shown to be affected by EE include the hippocampus [17, 238] , 41] , prefrontal cortex [248] , motor cortex [16, 17], and cerebellum [18, 144, 252].

The hippocampus is the region of the brain involved in formation and recall of declarative memories [239], and also plays a vital role in cognitive functions such as learning [240]. Exposure to EE leads to increased levels of BDNF and NGF [143, 241-243] and neural generation and metabolic activity [17, 238] within the rodent hippocampus. Morphological features of hippocampal neurons are also impacted by EE, with an increase in dendritic branching and total dendritic length observed in two populations of hippocampal neurons in enriched mice [244], suggesting that EE is capable of inducing both molecular and anatomical changes within this brain region.

Non-sensory cortical areas such as prefrontal and motor cortex involved in executive function [221, 245, 246] and motor planning [8, 247], respectively, have been less well-investigated with respect to EE than sensory cortices. Levels of the excitatory neurotransmitter serotonin are increased within the rodent prefrontal cortex in response to EE, along with an increase in the overall weight of this area of the brain [248]. Enrichment increases dendritic branching, density of dendritic spines of layer V pyramidal neurons, and neural metabolic activity within the rodent motor cortex [16, 17]. More generally, there is an increase in levels of the neurotrophic factors BDNF, NGF and neurotrophin-3 within the rodent cerebral cortex following exposure to EE [18, 143].

Another motor area that has been investigated with regards to EE is the cerebellum. The cerebellum is involved in the coordination, precision control and timing of motor activities [249-251], and so is likely to be stimulated by the extra motor activities available to animals within enriched environments. Similar to other previously investigated brain areas, the rodent cerebellum displays an increase in levels of BDNF and NGF in response to EE [18, 138], along with an increase in levels of the excitatory neurotransmitter noradrenaline [252].

As demonstrated by studies mentioned above, EE has the ability to impact many areas of the rodent brain that are not primary sensory areas. Of all the brain regions other than the visual system that

have been investigated with regards to enrichment, the striatum is amongst the more thoroughly researched.

6.3 The effect of environmental enrichment upon the striatum

The purported functions carried out by the striatum – sensorimotor coordination [9, 43, 44], goal-orientated learning [41] and decision-making [13, 40]– suggest the possibility that the sensory, motor and social aspects of EE would all impact upon this area of the brain.

Within the rodent striatum, adulthood EE influences protein levels of BDNF and NGF [15, 18, 253], and increases levels of the excitatory neurotransmitter norepinephrine [248], whilst decreasing the levels of dopamine transporter [253]. Postnatal exposure to EE is capable of modulating the expression of many genes and proteins within the striatum, including those involved in cell proliferation, differentiation, structure and metabolism, as well as signal transduction, transcription and translation [19]. Thus, an enriched environment is capable of inducing molecular changes within the rodent striatum at various ages.

Anatomical features within the rodent striatum are also impacted by exposure to enriched environments. Medium spiny projection neurons display a greater level of dendritic branching and an increase in the density of dendritic spines in response to EE [16, 254, 255]. The effects of enrichment have also been observed during a putative early life critical period within the mouse striatum; recent work from our laboratory has demonstrated accelerated maturation of striatal PNNs in response to early life EE [98]. EE induces anatomical and morphological changes in the striatum, and potentially impacts critical period timing within this nucleus, given its effect on striatal extracellular matrix structures associated with PV+ inhibitory interneurons [98].

Previous studies have shown that the effects of EE are not always consistent. For example, both decreases [18], as well as increases [15], in striatal BDNF levels in response to enrichment have been

described, albeit in different rodent species. Differences in animal species and breeds, along with the EE paradigms used [15, 18], can account for at least some of the discrepancies in experimental findings. It is not currently known whether BDNF levels during a putative early striatal critical period are affected by exposure to EE.

These examples do highlight a key problem associated with EE research: that laboratories use different EE paradigms, with different time lengths of enrichment, and different ages and types of animals as experimental subjects. These factors can make it difficult to draw consistent and coherent conclusions about the effects of EE upon specific regions of the brain. Further, given that engaging in or acquiring standard behavioural tasks can rely on a number of neural processes and/or areas, changes in behaviours resulting from enrichment can be extremely difficult to interpret. Careful task selection, however, can provide substantial insight into the effects of enrichment on specific circuits. Despite the level of previous investigations into the effect of EE upon the striatum, the full effects of enrichment upon this nucleus are still not known.

6.4 Environmental enrichment and behaviour

Given the impact of EE upon the anatomy and biochemistry of the brain, it is not surprising that it has been shown to also affect behaviours of animals that have undergone both postnatal and adulthood enrichment. Animals raised or housed in enriched environments demonstrate behavioural changes such as a decrease in stress and anxiety-related behaviours [15-17, 191], improved performance in learning and memory tasks [98], accelerated motor development [98], changes in maternal behaviours [139, 141], and resistance to addictive substances [14, 253, 256].

Enrichment has been shown to decrease the incidence of rodent stress and anxiety-related behaviours such as thigmotaxis and stereotypies [15-17, 191]. Thigmotaxis is the tendency of animals to remain close to the periphery or walls when placed into a behavioural apparatus [193,

194], and is observable when animals undergo behavioural testing; EE begun in adulthood has been shown to reduce the occurrence of thigmotactic behaviours during testing within the Morris Water Maze apparatus [191]. Stereotypies are repetitive, pointless motor activities, such as pacing, flipping or jumping that are thought to result from basal ganglia dysfunction, in particular a lack of disinhibition of response selection from the striatum [200, 201]. A series of studies has demonstrated that enrichment begun at weaning prevents the development of jumping and back-flipping stereotypies, accompanied by alterations in dendritic morphology, BDNF levels, and neuronal metabolic activity within the mouse striatum, providing evidence that stereotypies may be mediated by this area of the brain [15-17]. EE has also been shown to improve learning and memory of rodents within the Morris Water Maze apparatus: animals exposed to EE take less time to complete the Morris Water Maze [98] and demonstrate a greater number of entries to the quadrant where a platform was previously located when undergoing probe testing [191].

Along with affecting anxiety and cognitive behaviours, EE accelerates the maturation of exploratory and motor behaviours in young animals. A previous study from our laboratory revealed that juvenile mice raised within enriched housing explore a greater area within an open-field and demonstrate more mature swimming behaviour than standard housed counterparts [98]. Exposure to EE has been shown to improve balance and sensorimotor coordination in rodents that have received lesions to the striatum [9], and to improve performance in a one-off test of sensorimotor coordination [218], demonstrating that enrichment can accelerate the development of motor behaviours, and ameliorate motor deficits caused by striatal damage.

As evidenced by previous studies, the impact of EE is able to be observed in very young animals that are not yet capable of directly interacting with their environment [98, 139, 141]. This effect of very early EE is thought to come about due to an enrichment-induced change in maternal behaviours. Previous studies have confirmed this to be the case: rodent mothers within an enriched environment groom their pups more than those within standard housing, despite spending less total

time on the nest [139, 141]. Grooming behaviour provides a strong tactile stimulation to pups; effects similar to those resulting from EE-induced increased grooming have been emulated using manual manipulation of pups [237], suggesting that such sensation is important for the maturation of neural systems within young rodents.

Along with effects on the cognitive, motor and sensory systems within the brain, EE also appears to be capable of impacting the "limbic" or reward system. Previous studies have demonstrated that rodents housed within an enriched environment display markedly different reactions to addictive substances than those housed within standard environments. Animals raised in EE demonstrate behavioural resistance to the rewarding effects of both cocaine and heroin when exposed to these drugs [14, 253, 256], despite the administration of these substances still having the ability to activate the limbic system and enriched animals demonstrating "high" behaviours [14, 253, 256]. Along with this protective function against developing addiction, EE has been shown to reverse addiction to cocaine. Animals displaying addiction-induced behaviours placed into enriched environments no longer demonstrate behavioural sensitization or conditioned place preference to cocaine and do not relapse into addictive behavioural patterns [257]. This effect of EE has also been demonstrated using sugar; animals housed within enriched cages following weaning demonstrate lower preference for sucrose consumption than those housed within a standard environment [258]. It seems that EE is capable of reducing the incidence of addictive behaviours mediated by the limbic system within the rodent brain, one component of which is the basal ganglia [2, 41].

Raising or housing animals within enriched environments appears to have a beneficial effect on many behaviours that are mediated by various systems within the brain: stress and anxiety-related behaviours, sensorimotor coordination, cognitive abilities and reward systems. These behavioural changes likely result from the environmentally-induced anatomical and molecular alterations observed in many areas of the brain highlighted above. Another method used for elucidating the mechanism by which EE works is to investigate animal models of neurological diseases or disorders.

6.5 Environmental enrichment and neurological deficits

The ability of EE to reverse an induced sensory deficit within the visual system was discussed previously: adult animals that undergo monocular deprivation followed by reverse suturing and enrichment demonstrate a complete recovery of visual acuity within the originally deprived eye [106]. Other induced deficits, along with more serious and global neurological disorders, have been shown to benefit from enrichment. Exposure to EE improves recovery of function following lesions to the brain [190], and reduces the severity of symptoms in autistic [148, 259], Huntington's [130, 219, 220], Parkinson's [253, 260], and Alzheimer's [261, 262] disease models.

Surgically or chemically induced lesions within the brain are used to characterize the functionality of particular regions within the CNS and model neurological damage states such as traumatic brain injury or the occurrence of stroke. The combination of these techniques with enriched housing has revealed that EE is capable of reducing the impact of neurological injuries within rodent models. Animals housed in an enriched environment following a controlled cortical injury demonstrate improved sensorimotor coordination and cognitive ability when compared to standard housed injured cohorts [190]. The effects of chemically induced lesions are also reduced by exposure to EE: animals raised within an enriched environment demonstrate fewer detrimental effects on behavioural flexibility following a lesion to the basal forebrain [263], nor do they show the same level of motor and balance deficits following a striatal chemical lesion [9]. It appears that EE is capable of ameliorating injury-induced behavioural impairments, attenuating both cognitive and sensorimotor deficiencies.

Autism is a neurodevelopmental disorder, characterized by stereotypic behaviours, difficulty in social engagement and communication, and cognitive and sensorimotor deficits [264]. The *Mecp2*^{1lox} mouse models an autism spectrum disorder known as Rett Syndrome, associated with mutations in a gene that codes for methyl CpG binding protein 2 [148, 265]. EE from weaning has been shown to decrease the severity of locomotor deficits and motor learning difficulties observed in *Mecp2*^{1lox}

mice [148, 259], reduce ventricular volume, and decrease severity of a cerebellar BDNF deficit in these animals [259]. Volumetric changes within the basal ganglia, specifically the caudate nucleus of the striatum, are associated with autism spectrum disorders and the presentation of stereotypic behaviours in human patients [266]. A reduction in stereotypic behaviours observed within enriched mice is known to be accompanied by a variety of anatomical and molecular changes within the striatum [15-17], suggesting EE may reduce the severity of autistic symptoms such as stereotypies through an effect on the basal ganglia.

Huntington's disease is a genetic neurodegenerative disorder that manifests around the fourth decade of human life. Symptoms begin with psychiatric and cognitive decline; patients eventually experience movement disorders, with a decline in motor function and involuntary writhing [267]. This disease state may last for decades, and is thought to result from dysfunction within the cerebral cortex and striatum [267]. The R6/1 and R6/2 mouse models of Huntington's replicate many symptoms of this disease, with onset occurring around three to four months of age followed by rapid progression of the disease state [130, 219, 220, 268, 269]. Motor symptoms common to Huntington's disease animal models include reduced motor coordination, reflexive rear paw clasping, and a reduction in activity and rearing behaviour within an open field [130, 219, 220]. EE begun at weaning improves performance of R6/1 and R6/2 animals on the accelerating rotarod behavioural task, delays the onset of rear paw clasping in R6/1 mice, and increases exploratory and rearing behaviours in R6/1 animals [130, 219, 220]. EE also prevents the loss of BDNF within the striatum and a dopaminergic signal regulator within the cerebral cortex of R6/1 disease model mice [130], suggesting that enrichment slows the onset of symptoms in a disease associated with striatal dysfunction.

Huntington's disease is not the only neurodegenerative disease whose effects are reduced due to EE. Parkinson's disease, a condition in which dopaminergic neurons within the SNpc of the basal ganglia undergo cell death [1, 3], begins with motor deficits and progresses to include cognitive and

psychiatric issues such as dementia and depression. Parkinson's disease affects two percent of adults over the age of 50, and manifests in the fifth or sixth decade of human life [1, 3]. Animal models of Parkinson's disease are chemically induced by the administration of a drug, 1-methyl-4 phenyl-1,2,3,6-tetrahydropyridine (MPTP), resulting in a loss of dopaminergic neurons within the SNpc similar to that observed in human Parkinson's disease [253, 260]. Mice raised within enriched housing from weaning demonstrate double the level of cell survival within the SNpc and ongoing reduction in the incidence of cell death within this nucleus following MPTP treatment, suggesting that enrichment protects against the effects of this drug [253, 260] and is capable of reducing the severity of a neurodegenerative disease situated within the basal ganglia.

Another neurodegenerative disease that is not typically associated with the basal ganglia but has also been shown to benefit from exposure to EE is Alzheimer's disease. Symptoms of this disease include memory loss, psychological issues, and cognitive and language deficits [270-272]. Alzheimer's disease manifests around the fifth to sixth decade of human life and leads to the formation of plaques within neurons causing neuronal death [270-272] A commonly used mouse model of Alzheimer's disease is the TgCRND8 strain of mice, which express high levels of proteins involved in the formation of the plaques so characteristic of this disorder [192, 261, 262, 273]. TgCRND8 animals housed in enriched environments from weaning show greater exploration of novel objects than those housed within standard environments [192], a reduction in the prevalence of plaques within both neurons and blood vessels, and an increase in angiogenesis within the brain [261, 262]. Exposure to enrichment has also been shown to increase the survival and proliferation of hippocampal neurons within TgCRND8 mice [273], suggesting that EE can reduce the severity of a neurodegenerative disease situated within brain regions other than the basal ganglia.

Neurological disorders are not the only disease states that have been shown to benefit from enriched environments: mice exposed to EE have decreased tumour size in models of melanoma

[274], colon [274] and breast cancer [275], demonstrating that EE is able to ameliorate disease states other than neurological disorders.

Raising or housing animals within enriched environments appears to have a beneficial effect upon several neurological disorders. The way in which an organism's surroundings influence the health and functioning of its nervous (and other) systems has only just begun to be fully understood. Whilst animal models provide an excellent example of the ability of environment to influence neural circuitry and function, there have been some investigations into enrichment and its impact upon the function of the human brain.

6.6 Environmental enrichment and humans

It is not just animals within a laboratory that may experience benefits from EE – humans have also been shown to derive positive benefit from exposure to "enriched environments", with effects on both neurologically normative children [276, 277] and those with neurodevelopmental issues [278].

It is not entirely clear what form environmental enrichment for humans might take: however, assuming that exposure to a variety of sensory stimuli, motor exercises, social interaction, and enhanced nutrition and education are enriching, then a handful of studies have shown an effect of these factors upon human children.

One study demonstrating the effect of an "enriching" nursery school began on the island of Mauritius in 1972. Funded by the World Health Organisation, the study was originally intended to bring together psychologically at-risk children within nursery schools where "drugs could be used to bring their autonomic function back into normal range" [276, 277]. Instead, 100 children were drawn from the local population at random to undergo an enriching and stimulating nursery school from the ages of three to five, with the goal to assess what effects an early enriching experience may have on psychophysiological orienting and arousal [276, 277]. The enriched nursery schools employed

highly trained staff and ran on a structured schedule with activities providing cognitive, verbal, conceptual, memory and visuospatial skills, along with visuomotor coordination and sensation and perception experiences [276, 277]. Children within these nurseries were provided with nutritious meals, taught general hygiene, and underwent health inspections and exercise sessions. Parents were encouraged to become active participants in the programs run by the enriched nursery school, and children were prepared for the transition to primary school during their last term at the school [276]. In contrast, the control group consisting of 100 children undergoing the usual nursery care available on the island were under the supervision of untrained staff, in large children to adult ratios (30:1 versus the 5-10:1 within the enriched nursery schools), with only around half of the children receiving any sort of rudimentary education, and no provisions made for food or napping [276].

All children underwent psychophysiological testing at age eleven: arousal and attention were tested using skin conductance and electroencephalograms. Recordings were made during both an orientation task and continuous performance task, and during rest periods between testing [276, 277]. During testing, children that attended the enriching nursery school demonstrated increased skin conductance orienting, with a faster time rise. Orienting and timing of skin conductance are thought to be indicative of attention allocation, and thus information processing ability [276]. Children that attended the enriching nursery school also demonstrated increased electroencephalogram arousal compared to children that attended the usual nursery school on the island [276]. Exposure to EE during early life appears to be capable of influencing neural functioning within humans many years later. Given that such an effect is demonstrable in neurologically normal subjects, it is possible that enrichment therapy could be of benefit to people suffering from neurological disorders.

Exposure to EE is capable of preventing the occurrence of autism-like behaviours that occur spontaneously within wild type mice [15-17] and reducing the severity of behavioural symptoms and brain changes in an animal model of autism [148, 259, 265], suggesting that EE may be a potentially

powerful treatment for human autism. One study has investigated the effect of an enrichment treatment protocol on the severity of autistic symptoms within male children between three to twelve years of age [278]. Twenty eight subjects previously diagnosed with autism were randomly divided into a treatment group receiving sensorimotor enrichment therapy, and a group without any extra stimulation above ongoing therapies [278]. Subjects within the sensorimotor enrichment group experienced 34 different sensorimotor tasks, four to seven of which were undertaken twice a day whilst listening to classical music, and were exposed to four to seven different scents on a daily basis [278]. The severity of autistic symptoms was assessed at the beginning of the study, and again after six months of therapy. Subjects that had experienced the sensorimotor enrichment demonstrated an improvement in both severity of autistic symptoms and cognitive performance, as assessed by the Childhood Autism Rating Scale and the Leiter-R Visualisation and Reasoning score [278], suggesting that exposure to a form of EE is capable of reducing the severity of a neurodevelopmental disorder within humans.

Exposure to EE is capable of influencing many areas of the brain, with effects ranging from accelerated maturation to amelioration of disease states, impacting upon anatomical, molecular, and behavioural measurements. It would seem that benefits derived from EE are not restricted to rodents – a program of enrichment can also be beneficial for humans. EE may offer a relatively inexpensive, non-invasive, and straightforward treatment option for individuals with neurological disorders such as autism spectrum disorder and Huntington's, Parkinson's, and Alzheimer's disease.

7. Project Outline

The effect of EE upon maturation of inhibitory circuitry within the rodent striatum has not yet been thoroughly investigated: previous work from our laboratory has demonstrated that early postnatal enrichment accelerates maturation of PNNs within the mouse striatum [98]; whether there is a similar impact upon the PV+ inhibitory interneurons associated with PNNs in this nucleus [96] has

yet to be determined. Striatal BDNF protein is modulated in adult rodents in response to EE [15, 18, 130], and is vital for the visible presence of mature striatal PV+ inhibitory interneurons [125]. Any effect of early postnatal EE upon the maturation of striatal BDNF protein levels has not been evaluated. It is known that early postnatal EE accelerates the development of sensorimotor coordination behaviours thought to be mediated by the mouse striatum [98]: whether EE influences the production of ultrasonic vocalisations, another striatally-mediated behaviour [7] displayed by young rodents [209, 210], has yet to be ascertained.

The difficulties associated with the use of differing EE paradigms between laboratories and ascertaining the effectiveness of particular enriched environments may be lessened by the use of a behavioural task providing consistent and reliable feedback about the effects of EE paradigms. Such a task has yet to be developed. Exposure to EE and dissolution of PNNs are both known to increase plasticity within neural circuitry [101, 106, 107, 115, 187, 273, 279], and impact upon cognitive [96, 98, 191] and sensorimotor [9, 96, 148, 218] behavioural performance of rodents. It is not known whether there is any interaction between the two treatments during either type of behaviour, or if the increase in plasticity induced by both enrichment and PNN digestion share underlying mechanisms.

Decision-making behaviours may provide an indication of cognitive capacity, and reflect ability to integrate sensorimotor, cognitive and motivational behaviours when choosing a course of action [13, 280]. The striatum is involved in such behaviours [13], whilst early experience of maternal care is known to influence decision-making in later life [228]. Postnatal EE has been shown to influence levels of maternal care received by rodent pups during early life [139, 191]; whether adulthood enrichment also influences the decision-making behaviour of adult animals has yet to be determined.

7.1 Determining the effect of environmental enrichment upon striatal PV+ inhibitory interneurons (Chapter 2)

Striatal PV+ inhibitory interneurons provide a link between the cerebral cortex and the output neurons of the rodent striatum, receiving direct afferent input from both the cerebral cortex and the thalamus [60, 87, 88] and synapsing upon medium spiny projection neurons [54, 63]. This configuration of synaptic connections means that the activity of striatal PV+ inhibitory interneurons is modulated by input from corticostriatal afferents [60, 87, 88]. The expression of calcium-binding proteins within neurons is able to be influenced by cellular activity levels [66, 67], as has been demonstrated by modulation of parvalbumin in PV+ inhibitory neurons within the rodent hippocampus and primary motor cortex in response to behavioural training and environmental experience [124]. EE has been shown to enhance transmission, long-term depression, and long-term potentiation at synapses between neurons within both the hippocampus and cerebral cortex [281-283], and increase both weight of the cerebral cortex and dendritic complexity of cortical neurons [16, 284, 285]. It may be that exposure to EE results in a change in both the manner and level of afferent activation experienced by striatal PV+ inhibitory interneurons.

PV+ inhibitory interneurons are associated with PNNs in various areas of the rodent brain, including the striatum [84, 96, 103, 104, 106, 123]. Within this nucleus, PV+ inhibitory interneurons and PNNs have been revealed to overlap in a known ratio [96]. I aim to assess the correlation of these neurons with PNNs to ascertain whether EE influences the association between these two markers within the adult striatum. I propose that determining the effect of lifelong EE upon the PV+ inhibitory interneuron population within the striatum will provide preliminary insight into how ongoing enrichment affects mature striatal circuitry.

The timeline of PV+ inhibitory interneuron maturation within the rodent striatum synchronises with the innervation of ipsilateral corticostriatal afferents into this region of the brain [50, 87]. The majority of extra-striatal input received by PV+ inhibitory interneurons originates from corticostriatal

afferents [60, 87, 88], providing activation to striatal PV+ inhibitory interneurons [87], and the neurotrophic factor BDNF to the striatum at large [125]. BDNF is responsible for the maturation of PV+ inhibitory neurons in other areas of the brain [126], and lack of this neurotrophic factor within the striatum results in a decrease in immunohistochemically detectable PV+ inhibitory interneurons within the rodent striatum [125].

The maturation of PV+ neurons and BDNF in the visual cortex heralds the onset of the visual critical period [126]. I hypothesise that the PV+ inhibitory interneurons within the rodent striatum play a similar role in determining the timing of an early life critical period within this region of the brain, and that BDNF is likely to influence the maturation of this neuronal population. I aim to characterise the development of PV+ inhibitory interneurons and to profile the levels of BDNF protein present within the murine striatum during this putative critical period.

Within the striatum, early exposure to EE results in the accelerated formation of PNNs, an extracellular matrix structure associated with PV+ inhibitory interneurons [96-98]. EE is a means by which the timing of critical periods within the brain may be manipulated, providing an insight into the effect an organism's surroundings have on neural development. I propose that early life enrichment begun immediately prior to birth is capable of accelerating the maturation of PV+ inhibitory interneurons within the striatum, and that this will likely impact upon the timing of a putative early critical period within this region of the brain. I aim to establish the effect of early EE upon the development of PV+ inhibitory interneurons within the striatum, and whether levels of the neurotrophic factor BDNF are impacted, providing a possible mechanism for any changes in the maturation of striatal PV+ inhibitory interneurons.

7.2 Assessing the impact of environmental enrichment upon juvenile animal behaviour (Chapter 2)

There are a number of behavioural tasks that may be used to assess striatal function in mature animals; however, assessing striatally-mediated behaviours in juvenile animals is more difficult. Young mice do not begin to show coordinated sensorimotor behaviours until towards the end of the second week of life [182, 183], making it difficult to assess the early development of either cognitive or sensorimotor processes mediated by the murine striatum.

One behaviour produced by the striatum that does not require the performance and/or acquisition of complex tasks to assess is Ultrasonic Vocalisation (USV). USVs are produced by mouse pups as early as P3, and the striatum is involved in the motor components of generating these vocalisations [7, 286, 287]. Young animals produce distinctive USVs in response to social isolation, to induce their retrieval by dams; the incidence of these calls decreases as animals age and become independently mobile [182, 183, 209, 210, 288, 289], displaying a clear developmental trajectory [209, 210, 288] that takes place over the same period as striatal PV+ inhibitory circuitry maturation [50, 209, 210, 288].

There are many factors that may influence the production of USVs induced by maternal separation, including temperature, the presence of a strange male, tactile stimulation, and maternal behaviours [210, 213, 289-291]. Dams housed within enriched environments display differing maternal behaviours to those housed within standard cages: pups experience greater levels of grooming despite mothers spending less time within the nest [139, 141]. I propose that ascertaining the number and type of USV calls produced by juvenile animals may be used as a behavioural measure of striatal maturation in very young mice. I aim to determine any effects of early life EE upon the call profile of USVs produced by juvenile animals in response to social isolation.

It is known that an activity-dependent critical period of neuroplasticity for motor behaviours modulated by dopaminergic and cholinergic systems within the rodent striatum begins on P30 and

continues through until P37 [164-169]. Coordinated sensorimotor behaviours such as locomotion and swimming emerge earlier, around the same time as PV+ inhibitory circuitry within the rodent striatum begins to mature, with both behaviours and PV+ neurons fully developed around the same time [50, 182, 183]. Early life EE has been shown to accelerate the development of coordinated swimming behaviours of young mice [98]. It may be that a putative early critical period within the striatum modulates an aspect of sensorimotor circuitry, and that the earlier appearance of coordinated sensorimotor behaviours resulting from EE is due to an effect upon inhibitory circuitry within the striatum [98]. Locomotion behaviours continue to mature and undergo refinement during the third and fourth weeks of life [182, 183]. I propose the use of the rotarod apparatus to assess sensorimotor coordination behaviours of young animals from ages P21 to P26. I aim to determine whether EE may accelerate the development of refined sensorimotor coordination, resulting in an earlier appearance of mature locomotion behaviours.

7.3 Assessing the effect of environmental enrichment upon goal-orientated learning and sensorimotor behaviours mediated by the striatum (Chapters 3 & 4)

Adult rodents that have experienced EE demonstrate improved learning and memory in cognition-based behavioural tasks such as the Morris Water Maze [98, 191], and do not display the same degree of sensorimotor deficit as standard housed counterparts when investigating animal models of neurological disorders [9, 219, 220, 292]. The striatum is known to be involved in both cognitive and sensorimotor processes [5, 8-10, 40-44, 46, 47], and is likely to play a part in the improvement observed in these behaviours when animals are exposed to an enriched environment.

Cognitive processes mediated by the striatum include goal-orientated learning [41], rule-based learning [10], and task acquisition [42]. One behavioural test that may be used to assess goal-orientated problem-solving and learning is the Puzzle-Box behavioural task. This task tests the ability of animals to convert goal-directed intention into sensorimotor behaviours and utilises the natural

preference of rodents for small, dark, enclosed spaces over brightly lit open-fields [221-223]. I propose the use of the Puzzle-Box behavioural task as a suitable test for the effects of EE upon a number of cognitive processes. Specifically, I aim to determine how EE impacts upon goal-orientated problem-solving by monitoring the way in which mice approach the Puzzle-Box behavioural task.

Along with the impact of EE upon cognitive behaviours, I will also investigate its effect upon sensorimotor behaviours. Exposure to EE has been shown to improve the performance of adult rodents during a once-off test upon the rotarod behavioural task [218], along with preventing motor deficits specific to this task in Huntington's disease animal models [130, 219]. Along with testing native sensorimotor coordination ability, the rotarod may also be used to assess motor learning across a period of time [8, 45]. I propose the use of the rotarod behavioural task as a suitable test for the effects of EE upon baseline sensorimotor coordination and motor learning, and aim to determine how EE impacts these behaviours.

Both enrichment and PNN digestion increase plasticity within neural circuitry [101, 106, 107, 115, 187, 273, 279], and impact upon cognitive [96, 98, 191] and sensorimotor [9, 96, 148, 218] behaviours. Previous work from our laboratory has demonstrated the ability of the bacterial enzyme Chondroitinase ABC (ChABC) to affect striatally-mediated sensorimotor and cognitive behaviours of mice [96]. I propose the use of ChABC within the striatum to determine the role of this nucleus in both the Puzzle-Box and rotarod behavioural tasks. The use of ChABC, rather than pharmacological or electrolytic lesions, will prevent the occurrence of cell death within this nucleus and minimize the chance of damage to other areas of the brain that may be involved in the behaviours under investigation [96]. My aim is to determine whether striatal ChABC application impacts goal-orientated and motor learning of mice; and ascertain the manner in which EE interacts with this treatment to gain insight into whether neuroplasticity induced by both enrichment and PNN digestion share underlying mechanisms.

7.4 Determining the impact of environmental enrichment upon decision-making behaviours (Chapter 5)

Along with goal-orientated cognitive processes and sensorimotor coordination, the striatum is also involved in decision-making and choice of action behaviours [13, 40]. Rodents with lesions to the striatum demonstrate impaired decision-making, suggesting that this area of the brain is involved in expressing the association between action and outcome [11, 12, 41]. Reward-based learning behavioural paradigms can be used to assess decision-making processes. One such paradigm is the Iowa Gambling Task (IGT), a test traditionally used in human psychological evaluation to assess how sensitivity to reward may impact choices made during testing [226, 280]. Subjects are given a choice between two high-reward/high-loss (high risk/overall disadvantageous) groups and two low-reward/low-loss (low risk/overall advantageous) groups. More reward is gained over time by consistently choosing from the low risk/overall advantageous groups [226, 280]. Factors such as sex, age, weight, anxiety status, and neuropsychological conditions are all capable of impacting subjects' performance within the IGT [224-226, 266, 293-297].

There have been several studies investigating a rodent version of the Iowa Gambling Task (RGT), using food and its withdrawal as reward and loss [196, 227, 228]. These studies have made use of rats as subjects and have revealed that – similar to humans – there is a proportion of the rodent population that consistently makes risky decisions, both within the RGT and other behavioural tasks [196]. Decision-making within the RGT is influenced by early life levels of maternal care, with animals receiving greater care choosing low risk options more often than those receiving less [228]. Animals raised in enriched environments have been shown to receive a greater level of maternal care than those raised in standard environments [139, 141], whilst animals that have experienced EE during adulthood demonstrate improved cognitive ability [191]. It is not known how EE from birth or during adulthood may impact upon decision-making behaviours in a paradigm such as the RGT. I propose to develop a murine version of the RGT in order to ascertain whether EE both from birth and in

adulthood has any impact upon decision-making behaviours, using the IntelliCage, a novel automated behavioural testing and tracking system.

The IntelliCage system was designed to mimic a more naturalistic setting for rodent test subjects. The testing arena also serves as the home cage in this system, with the movement and water consumption of individual animals tracked through the use of radio frequency identification (RFID) chip implants [229]. Access to water within conditioning corners of the IntelliCage can be regulated and used as a reward to train animals to complete tasks or behaviours [229, 234, 298-300]. The automated nature of the system limits animal handling, removing a potential experimental confound [123, 237]. Previous work has demonstrated that aspects of EE added to an IntelliCage decreases exploratory behaviours within the arena, but improves performance in a spatial learning task [299]. The effect of home cage EE upon behaviours within the IntelliCage testing arena has not yet been established. I propose the use of the IntelliCage to conduct a mouse version of the IGT, using water access and restriction as the reward and loss components of this RGT. I aim to determine whether EE from birth and during adulthood has an impact upon decision-making behaviours during a RGT within the IntelliCage. To the best of my knowledge, this is the first time that the IntelliCage system has been used to develop a RGT, and the first time that the effects of EE upon decision-making using a rodent version of the IGT has been assessed.

7.5 Overall Aims

This thesis aims to shed light upon the mechanisms by which environmental enrichment affects the striatum.

Characterising the maturation of PV+ inhibitory interneuron maturation and BDNF protein levels within the striatum during early development, and how early EE may impact these measurements, will aid in establishing the presence of a putative early life striatal critical period. The assessment of

both USV production and locomotion of juvenile animals, and whether EE impacts the development of these behaviours, will also provide an insight into the presence of an early life striatal critical period. Ascertaining the correlation of PV+ inhibitory interneurons and PNNs within the striatum of adult animals will allow me to determine whether EE continues to have any impact upon striatal inhibitory circuitry beyond the early postnatal period.

The development of a Puzzle-Box behavioural task to allow consistent assessment of EE effects upon cognitive behaviours will provide researchers with a useful tool when determining the efficacy of enrichment paradigms. Assessing the impact of EE upon animal behaviours within the Puzzle-Box and upon the rotarod grants an overview of the ways in which enrichment can influence both cognitive and sensorimotor processes. The use of striatal ChABC injection in combination with EE during these tasks gives an insight into the mechanisms underlying neuroplasticity effects of both these treatments, and the role the striatum may play in mediating goal-orientated learning and sensorimotor coordination behaviours.

Finally, determining the effects of EE upon reward-based decision-making within a novel testing environment will provide researchers with a new behavioural task that may be used to emulate the human psychological IGT assessment; grant insight into the impact of EE upon decision-making behaviours based on non-addictive reward substances; and supply information about the behavioural patterns of enriched animals within automated testing apparatus.

The nervous system both mediates an organism's external interactions and maintains internal homeostasis. This system enables sensory experience, motor movement, cognitive processes, and ultimately controls everything an organism does. Determining the impact of environmental factors upon this system and the behaviours it mediates highlights the importance of surroundings when it comes to neurological health and function, and may aid in the development of early education or intervention programs.

8. References

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Chapter 2:

Environmental Enrichment from Birth Accelerates the Maturation of Parvalbumin-Expressing Neurons within the Striatum of the Mouse

Abstract

The consolidation of networks composed of parvalbumin-positive inhibitory interneurons is a crucial step in the maturation of neural circuits. Enriching the environment of developing animals can affect both the timing and rate of this process. Although the influence of environmental enrichment on parvalbumin-positive cells have been well characterized in sensory cortex, less is known about the role environmental factors play on the maturation and maintenance of inhibitory circuits involved in motor control and action. Accordingly, I examined the effect of enriching mice from birth on the development of parvalbumin-positive neurons in the striatum, the input nucleus of the basal ganglia, and a key structure in the regulation of motor control and goal directed learning. I find that perineuronal nets, extracellular matrix structures associated with the maturation of inhibitory networks, show increased overlap with parvalbumin-positive interneurons in the striatum of enriched adult animals and that early enrichment leads to an acceleration of striatal parvalbumin expression. As a first step in establishing a potential mechanism underlying the changes in the striatum induced by environmental enrichment, the level of brain derived neurotrophic factor (BDNF), an important regulator of inhibitory network maturation, was measured across early postnatal development in enriched and standard housed pups. I find that BDNF protein levels exhibit accelerated postnatal increases in the striatum of enriched cohorts. Finally, enrichment leads to changes in the dynamics of juvenile ultrasonic isolation calls, a behaviour regulated by striatal function. Together, these findings indicate that environmental enrichment can profoundly affect early striatal development and function and provide preliminary evidence that an organism's surroundings are capable of influencing striatal circuitry throughout life.

Keywords: Striatum; Development; Parvalbumin Inhibitory Interneuron; Brain-Derived Neurotrophic Factor; Behaviour.

1. Introduction

In many organisms, there exists an early postnatal "critical period" during which their nervous systems become particularly susceptible to activity-dependent changes derived from interactions with the external environment [1-4]. The onset and offset of this critical period is dependent on the shifting balance of excitatory and inhibitory drive within neural circuits, with the maturation of inhibitory networks (consisting of parvalbumin positive (PV+), fast spiking interneurons) signalling the end of this epoch of peak plasticity [5-8]. The encapsulation of PV+ neurons by perineuronal nets (PNNs; extracellular matrix structures composed of chondroitin sulphate proteoglycans (CSPGs)) [9-13] occurs concurrently, suggesting that these structures contribute to the consolidation of associated circuitry [11, 14-16]. At a mechanistic level, the maturation of inhibitory networks has been shown to be dependent on the expression of Brain-derived neurotrophic factor (BDNF) [7].

The influence of environmental attributes on neural development has been explored by systematically manipulating an animal's surroundings. One particularly effective approach has been to expose subjects to environmental enrichment (EE) by increasing sensory, motor and social stimulation (for reviews, see Sale et al 2009 and van Praag et al 2000) [17, 18]. Animals raised in enriched environments exhibit an accelerated maturation of neural circuitry, especially in regard to inhibitory networks, and the coincident formation of perineuronal nets (PNNs) [9, 10, 12, 19].

Much of the work examining the influence of EE on neural circuitry has focused on primary sensory cortex; its role in the development and function of motor areas, particularly the striatum (the rodent equivalent of the caudate/putamen) has been less well characterised. This vitally important front-end nucleus of the basal ganglia receives, filters and integrates input from various cortical and sub-cortical areas, and projects this information to other circuits within and across associated networks [20-26]. GABA-ergic medium spiny projection neurons (MSNs), the main striatal output cells, comprise over 90% of the neuronal population within the striatum [26, 27]. PV+ cells are also present, although they constitute less than 3% of neurons within the nucleus [27, 28]. Despite these

low numbers, PV+ cells receive direct cortical as well as nigral input, and play a vital role in modulating the activity of striatal circuits [28-31].

Previous work from our laboratory has demonstrated the accelerated maturation of PNNs within the striatum of young mice raised in enriched housing [19], with recent findings showing that exposure to EE modulates PV expression within the hippocampus of adult animals [32]. PV+ neurons and PNNs within the striatum of adult animals correlate in a known ratio [16], but it has not been determined whether enrichment continues to impact striatal PNNs beyond early development, or similarly modulates the expression of parvalbumin within the adult striatum.

Striatal PV+ interneurons begin to emerge developmentally between the first and third postnatal weeks [33, 34]. This is also the period during which a host of other changes, including a second postnatal wave of innervations by dopaminergic afferents [23, 35] as well as the formation of PNNs, occur in the striatum [15, 19]. Within the visual cortex, the maturation of PV+ neurons is dependent on BDNF expression [7]. Although there is very little mRNA of the trophic factor expressed within the striatum [36], lesion studies have revealed that BDNF protein reaches the nucleus via anterograde transport along cortical afferents [37]. Previous work has demonstrated that EE accelerates the formation of striatal PNNs [19], and can also affect striatal BDNF levels of adult animals [38-40]. Whether the maturation of PV+ cells in the striatum is similarly influenced by EE, and how this relates to BDNF levels in early postnatal development has yet to be determined.

Functionally, the striatum has been revealed to regulate both the emergence and maintenance of a number of behaviours in developing mice [19, 41, 42]. Amongst these, the generation of ultrasonic vocalizations (USVs) is of particular importance due to its ethological relevance [43]. Juvenile mice begin emitting USVs in response to social isolation during the first postnatal week of development [44-46]. They cease to make these vocalizations a week later (2nd postnatal week), when pups become independently mobile [45-48], further reinforcing the notion that the circuitry regulating this behaviour is undergoing key maturational changes during this period. Simple locomotion begins

to appear around the same time, on postnatal day 9 [41, 42], whilst more complex, coordinated sensorimotor behaviours mature during the third week of life [41, 42]. One such behaviour is the ability to negotiate a narrow rotating surface; rats younger than 20 days postnatal (P20) experience difficulty when traversing a slowly rotating surface, whilst animals younger than P25 are generally unable to traverse a surface rotating at a faster speed [41].

Previous work from our laboratory has indicated that pups raised within an enriched environment demonstrate accelerated maturation of sensorimotor coordination behaviours at 10 days postnatal [19], during what is known as the postnatal transition stage of sensorimotor development [42]. Together, these findings suggest the possibility that this epoch serves as a developmental "critical" period for the striatum.

Accordingly, I have examined the overlap between PV+ cells and PNNs in adult striatum to determine whether lifelong enrichment impacts PNN or PV expression within the mature nucleus. I observe an EE dependent effect on the degree to which these two entities interact with one another, suggesting that enrichment may contribute to an activity-dependent impact on PV expression within the adult striatum. Given this effect, I then investigated the impact of early EE upon maturation of the PV+ population of striatal neurons, and how this is related to changes in BDNF expression. I find that enrichment from birth accelerates the maturation of this population of inhibitory interneurons, and show that EE increases striatal levels of BDNF protein in early postnatal mice, suggesting a possible mechanistic link between trophic factor levels and PV+ maturation. In order to identify potential behavioural consequences of the striatal changes wrought by early enrichment, I assessed juvenile mice in the time course and characteristics of ultrasonic vocalisations (USVs) [43] and locomotor behaviour [16]. I found that EE influences the call-profile and duration of USVs as the animals develop, and has no impact upon locomotion. Together, these findings indicate that EE can continue to influence striatal circuitry beyond the critical period, and that exposure to

enrichment can profoundly affect early striatal development, influencing PV and BDNF expression and contributing to changes in a juvenile striatally-mediated behaviour.

2. Materials and Methods

2.1 Ethics Statement

All procedures were approved by the Animal Ethics Committee of the University of Sydney and conformed to National Health and Medical Research Council of Australia guidelines (Protocol number K22/11-12/3/5838). Experiments were performed on C57/BL6J mice which were reared at the University of Sydney animal house facility. All mice were housed in a single adequately-ventilated room in 21^{o}C ambient temperature on a 12-hour light-dark cycle with *ad libitum* access to dry food and water.

2.2 Housing of animals in standard and enriched environments

On arrival, half of the pregnant dams were randomly assigned to standard home cages (dimensions: 30cmx13cmx13cm), and the other half to enriched housing (dimensions: 45cmx30cmx13cm), adapted from Simonetti et al, 2009 [19]. Standard cages contained a translucent, red plastic mouse "igloo"/shelter and extra material for bedding. Enriched cages contained these same items as well as extra sheltering material (paper tubing, spare igloo), a running wheel for voluntary exercise, objects to provide extra tactile stimulation including Velcro strips, marbles and rubber balls, olfactory stimuli such as scented plush balls (vanilla, strawberry and cinnamon), and two high-contrast visual stimuli. These items were moved around the cage every two to three days. Enriched housing also contained two litters to enable greater social interaction between dams and pups.

At 21 days postnatal (P21), pups were weaned and placed into gender segregated housing of the same environmental conditions in which they were born.

2.3 Measuring PV+ cell density and correlation with PNNs in fixed tissue

P10, P15 and adult (12 – 14 weeks of age) mice were euthanised with >100mg/kg of sodium pentobarbitone injected intraperitoneally, and transcardially perfused using 0.9% saline followed by 4% paraformaldehyde in 0.1M phosphate buffer (PB). The brain was dissected out, post-fixed overnight in 4% paraformaldehyde in 0.1M PB, and cryoprotected in 30% sucrose in 0.1M PB.

Brains were then embedded in gelatin-albumin hardened by 25% glutaraldehyde in 0.1M PB, and sectioned coronally at 60μm on a freezing microtome.

Double-staining for parvalbumin-positive (PV+) neurons, and perineuronal nets (PNNs) was adapted from Fukuda & Kosaka, 2003 and Lee et al, 2012 with some slight modifications [16, 49, 50]. Sections were cryoprotected in 30% sucrose 0.1M PB and put through a rapid freeze-thaw procedure using liquid nitrogen [49, 50]. These sections were then labelled for CSPGs using Wisteria Floribunda Agglutinin (WFA), a plant lectin used to visualize CSPGs, as described previously [16] (Vector Labs, Burlingame, CA, USA). Following this, sections were incubated 48-72 hours at 4^{o}C in a rabbit polyclonal antibody against parvalbumin (dilution 1:500; Abcam (catalog number: ab11427; Antibody Registry ID: AB_298032), Cambridge, England, UK))[51-53], followed by three hours of incubation in goat anti-rabbit AlexaFluor 594 (dilution 1:200; Life Technologies, NY, USA), then mounted in 50/50 glycerol-0.1M PB with 1:1000 DAPI and imaged.

Sections were digitally imaged at low power using a Zeiss deconvolution microscope with AxioCamHR camera and Axiovision software (Carl Zeiss Microscopy GmbH, Jena, Germany) and at high power using a Zeiss LSM 510 META confocal laser scanning microscope and Zeiss LSM software (Carl Zeiss). In each imaged slice, PNNs and PV+ cells were manually marked (Photoshop; Adobe

Systems Inc., San Jose, CA, USA) and both PNN and PV+ density measurements obtained, along with co-expression information. Measurements were imported into SPSS (SPSS Inc., Chicago, IL, USA) for statistical analysis with the degree of freedom calculated according to the number of brain sections analysed. Multifactorial ANOVAs (housing condition and age as factors) were used to compare PNN and PV+ densities and co-expression across groups and developmental time points. Univariate ANOVAs were used to compare density and co-expression values between housing condition groups at each age point.

2.4 Fresh tissue and protein analysis

P8, P10, and P15 standard and enriched mice were euthanized with >100mg/kg of sodium pentobarbitone injected intraperitoneally. Animals were then decapitated and the brain immediately removed and placed on ice. The striatum (caudate and putamen nuclei) was rapidly dissected out using ice-cold instruments as demonstrated in Chiu et al, 2007 [54]. All care was taken to avoid including other brain structures or white matter from the corpus callosum. All samples were weighed, then snap-frozen in liquid nitrogen and stored at –80^{o}C until processed.

Striatum were suspended in 1000μl of lysis buffer [137mM sodium chloride (NaCl); 20mM Tris-hydrogen chloride (Tris-HCl) (pH 8.0); 1% triton-x 100; 10% glycerol; 1mM phenylmethylsulfonyl fluoride (PMSF); 10μg/mL aprotonin; 1μg/mL leupeptin; 0.5mM sodium vanadate]. Samples were then sonicated, vortexed, and centrifuged at 1500Xg for 20 minutes at room temperature. The concentration of BDNF was determined using the E-max ImmunoAssay system (Promega, WI, USA). Standard 96-well flat-bottom ELISA (Enzyme linked Immuno-Sorbent Assay) plates were incubated overnight at 4^{o}C with anti-BDNF monoclonal antibody. The next day, plates were blocked with 1XB&S Buffer for 1 hour at room temperature. Serial dilutions of known amounts of BDNF ranging in concentration from 500pg to 0pg were performed in duplicate for the standard curve of each set of mouse tissue. 100μl of sample was added to each well in triplicate and incubated at room

temperature for 2 hours. The wells were then incubated with an antihuman BDNF polyclonal antibody for 1 hour at room temperature, followed by anti-IgY conjugated to HRP for 1 hour at room temperature. A 3, 3', 5, 5'-tetramethylbenzidine (TMB) solution was used to develop colour in the wells for 10 minutes at room temperature. The reaction was stopped with the addition of 1N hydrochloric acid (HCl) to the wells and the absorbance read at A450 within 30 minutes (BMG POLARstar Galaxy microplate reader; MTX Lab Systems, VA, USA).

A multifactorial ANOVA was used to assess protein levels across age groups, using age and housing condition as between-subjects factors. A univariate ANOVA was used to compare protein levels between housing condition groups at each age point. The degree of freedom was calculated according to the number of animals assessed.

2.5 Behavioural analyses

2.5.1 Recording of ultrasonic vocalisations

The ultrasonic vocalisations (USVs) of juvenile mice were recorded every second day at 50kHz $\pm$ 10kHz, from 5 to 15 days postnatal (P5 to P15). Vocalisations were recorded using a Magenta Bat 5 bat-detector (NHBS, Devon, UK) and Audacity sound recording software (sourceforge.net). Animals were placed in a clean standard housing cage on top of a surgical heating pad maintained at 28^{o}C. The cage contained no bedding, and was cleaned between animals with 70% ethanol. Individual mice were recorded within this apparatus for five minutes (per session).

2.5.2 Maternal potentiation

For each recording day, pups were placed within the recording arena for a single five minute session. They were then returned to their home cage for five minutes, before being recorded again in the

same arena for another five minutes (second session) [55]. All animals were weighed at the conclusion of each recording session.

2.5.3 Analysis of ultrasonic vocalisations

Spectrograms of audio recordings were produced and the properties of vocalisations extracted using AviSoft SAS Lab Pro (AviSoft Bioacoustics, Glienicke, Germany). The numbers of total USVs, and the average time length (duration) of USVs were tallied. Calls were grouped by type according to peak frequency at the beginning, maximum, and end amplitude of each call. Repeated measures ANOVA was used to assess the number and duration of total USVs as well as for each call type across animal ages, using age as the within-subject factor, and housing condition as the between-subjects factor. A univariate ANOVA was used to compare differences between enrichment cohorts at each age tested.

To assess potentiation, an index comparing the number of USVs generated between the two sessions per recording day was calculated: $R_i = (S2_i – S1_i) / (S2_i + S1_i)$; where S2 and S1 are the number of vocalizations made during recording day i. Indices were calculated for both the number and duration of call types, and a repeated measures ANOVA was used to compare these values with age as a within-subject factor and housing condition as the between-subject factor. A univariate ANOVA was used to detect differences between enrichment cohorts at each age assessed. The degree of freedom was calculated according to the number of animals assessed.

2.5.4 Rotarod behaviour

Young animals (P21 – P26) underwent testing on a rotarod apparatus for 5 consecutive days. All animals were weaned on the first day of testing, and separate by sex after the last day of testing; they were housed in original home-cages for the duration of testing. Each day, animals were placed onto the Rotarod (IITC Inc., Woodland Hills, CA, USA) for five minutes of habituation prior to

undertaking five runs of three minutes duration, starting at the lowest speed (1RPM) and ramping up to the highest speed (45RPM) at the three minute mark. Five animals were tested at a time, and runs were started when all animals were orientated in the same direction. Note was made of the time and distance run before an animal fell off the apparatus. A repeated measures ANOVA was used to compare performance of the sum of time and distance run within a testing day, using day of testing as the within-subjects factor and housing condition as the between-subjects factor.

3. Results

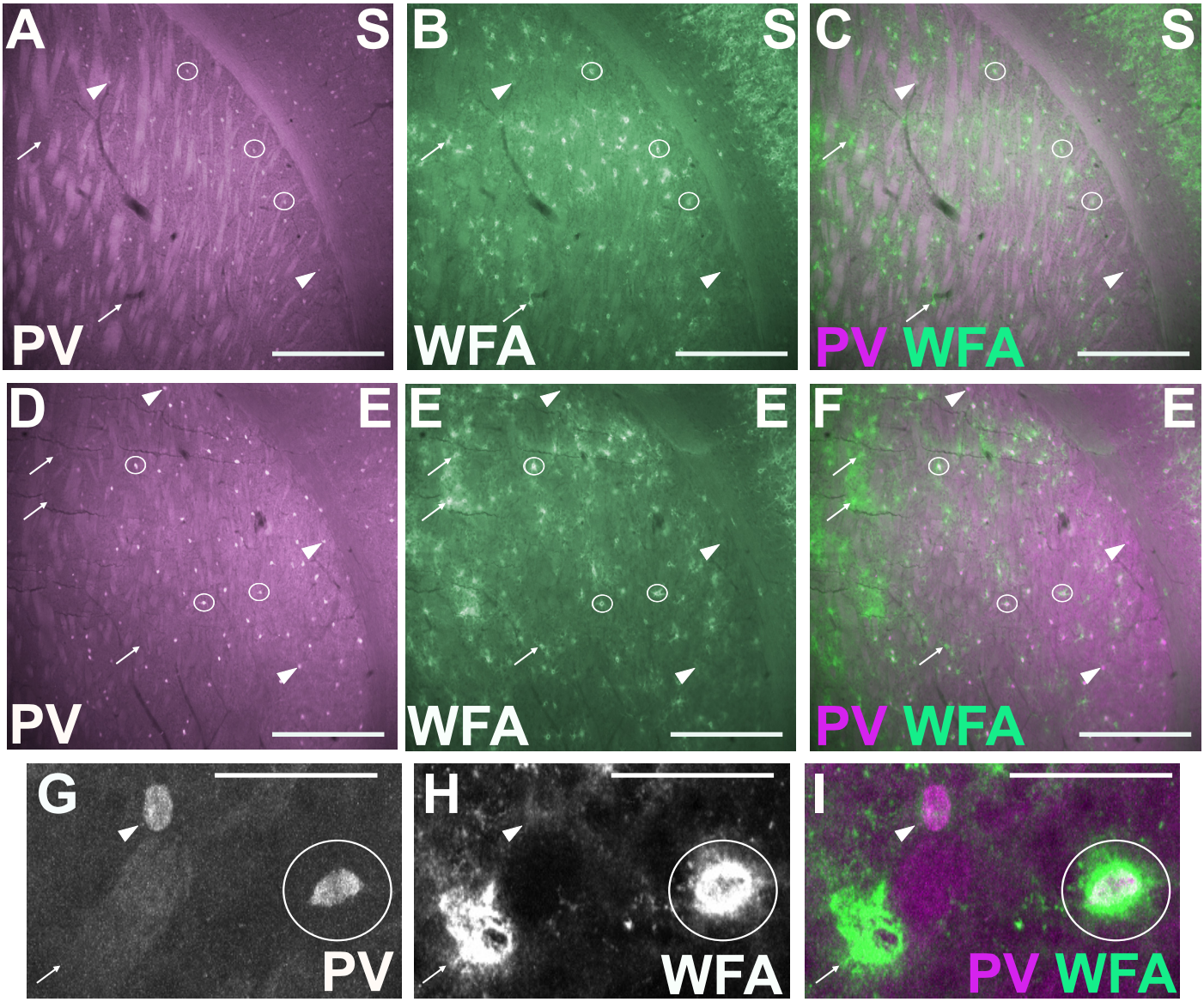
3.1 Environmental enrichment influences the number and PNN overlap of PV+ cells in adult striatum

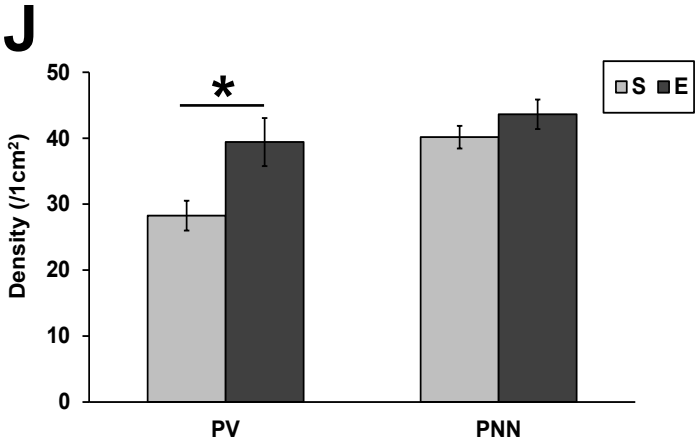
Both experience and environmental factors are capable of modulating PV expression within inhibitory circuits [32]. Further, although a close association between PNNs and PV+ interneurons has been well documented in sensory cortex [9-13], roughly only half of all PV+ cells are ensheathed by these CSPG structures within the striatum [16]. Moreover, even though the rate of increase in striatal PNN density across early postnatal development is greater in enriched compared to standard raised cohorts [19], whether this difference persists into adulthood has also yet to be determined. I therefore asked if EE influenced the density of PV+ cells as well as PNNs in adult murine striatum, and whether enrichment affected the degree to which they associated with one another.

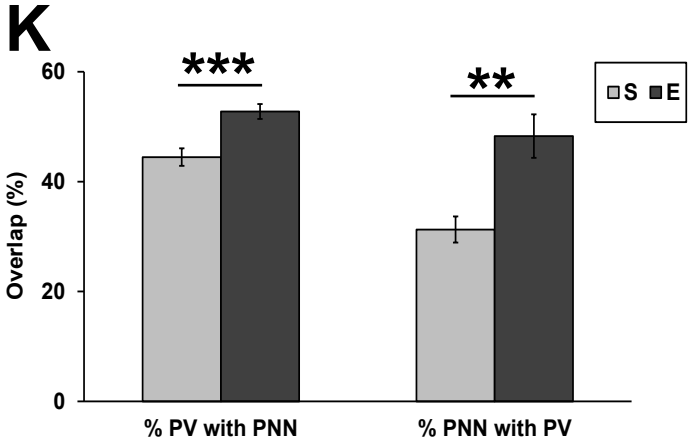
Qualitatively, I observed an increase in the number of striatal PV+ neurons (Fig. 2.1A, C, G, arrowheads) of enriched adult mice (n=3). Quantitative analysis confirmed that EE significantly increased the density of striatal PV+ cells within the striatum (univariate ANOVA, housing condition as between-subjects factor, $F(1, 34)=6.407$, $P=0.016$) (Fig. 2.1J).

Surprisingly, EE also affected the association between striatal PV+ interneurons and PNNs (Fig. 2.1B, E, H, arrows). Although EE did not impact the density of PNNs within the striatum (univariate

ANOVA, housing condition as between-subjects factor, $F(1, 34)=1.455$, $P=0.236$) (Fig. 2.1J), analysis of the percentage of PV+ neurons encapsulated by PNNs, as well as the percentage of PNNs surrounding PV+ neurons, revealed that enrichment significantly increased the degree of overlap between the two (univariate ANOVA, housing condition as between-subjects factor: % PV with PNNs, $F(1, 34)=15.831$, $P<0.001$; % PNNs with PV, $F(1, 34)=12.802$, $P=0.001$) (Fig. 2.1K). Conversely, both the proportion of PV+ neurons not encapsulated by PNNs and the percentage of PNNs not surrounding PV+ neurons were decreased to the same degree in enriched animals when compared to standard mice (univariate ANOVA, housing condition as between-subjects factor: % PV without PNNs, $F(1, 34)=15.831$, $P<0.001$; % PNNs without PV, $F(1, 34)=12.802$, $P=0.001$) (Fig. 2.1L). Together, these findings reveal that not only is the increased density of PV+ neurons in the striatum of enriched mice maintained into adulthood, but the degree to which these cells are encapsulated by PNNs is also dramatically influenced by EE.


A
S
PV
B
S
WFA
C
S
PV WFA
D
E
PV
E
E
WFA
F
E
PV WFA
G
PV
H
WFA
I
PV WFA


J
Density (/1cm²)
50
40
30
20
10
0
*
S E
PV
PNN


K
Overlap (%)
60
40
20
0
***
**
S E
% PV with PNN
% PNN with PV

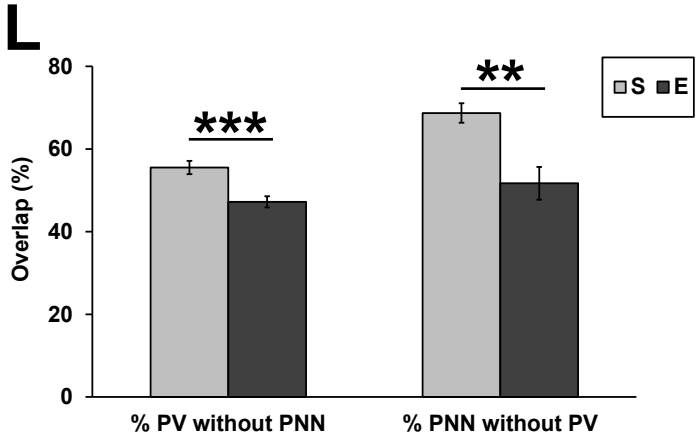

L
Overlap (%)
80
60
40
20
0
***
**
S E
% PV without PNN
% PNN without PV

Figure: 2.1 Environmental enrichment increases PV+ cell density and overlap with PNNs in adult animals.

(A – C) Sample sections showing the pattern of PV (A), PNN (WFA) staining (B), and overlap (C) in the striatum of adult mice raised in standard (S) housing. PV staining is most prominent in the lateral part of the striatum (arrowheads), whilst PNN straining is distributed more evenly throughout the nucleus (arrows). Unlike in cortex, only a partial overlap between PV+ cells and PNNs is observed in the striatum (C: non-overlapping samples represented by arrows and arrowheads; circles highlight some examples of PV+ cells encapsulated by PNNs). (D–F) Corresponding sample striatal sections for enriched mice. Figure conventions are identical to (A-C). The number of PV+ cell appears to have increased (compare D to A), while little or no difference is observable for PNNs (compare E to B). (G – I) Higher-power micrographs showing overlapping (circles) and non-overlapping PV+ cells (arrowheads) and PNNs (arrows). Scale bars: 1000μm in A – E, 50μm in G – I. (J) Mean density of PV+ cells and PNNs for adult enriched (E) and standard (S) animals. While a significant increase was detected in enriched PV+ densities compared to standard cohorts (univariate ANOVA, $F=6.407$, $P=0.016$), no difference was observed for PNNs. (K – L) Mean overlap between PV+ cells and PNNs in the striatum of adult animals. A significant increase in overlap between PV+ cells and PNNs was detected in enriched (E) compared to standard (S) adult mice (univariate ANOVA: % PV with PNNs & % PV without PNNs, $F=15.831$, $P<0.001$; % PNNs with PV & % PNNs without PV, $F=12.802$, $P=0.001$). Six sections from 3 animals were quantified for each group. **: $P<0.01$, ***: $P<0.001$. Error bars= Standard Error of the Mean (SEM).

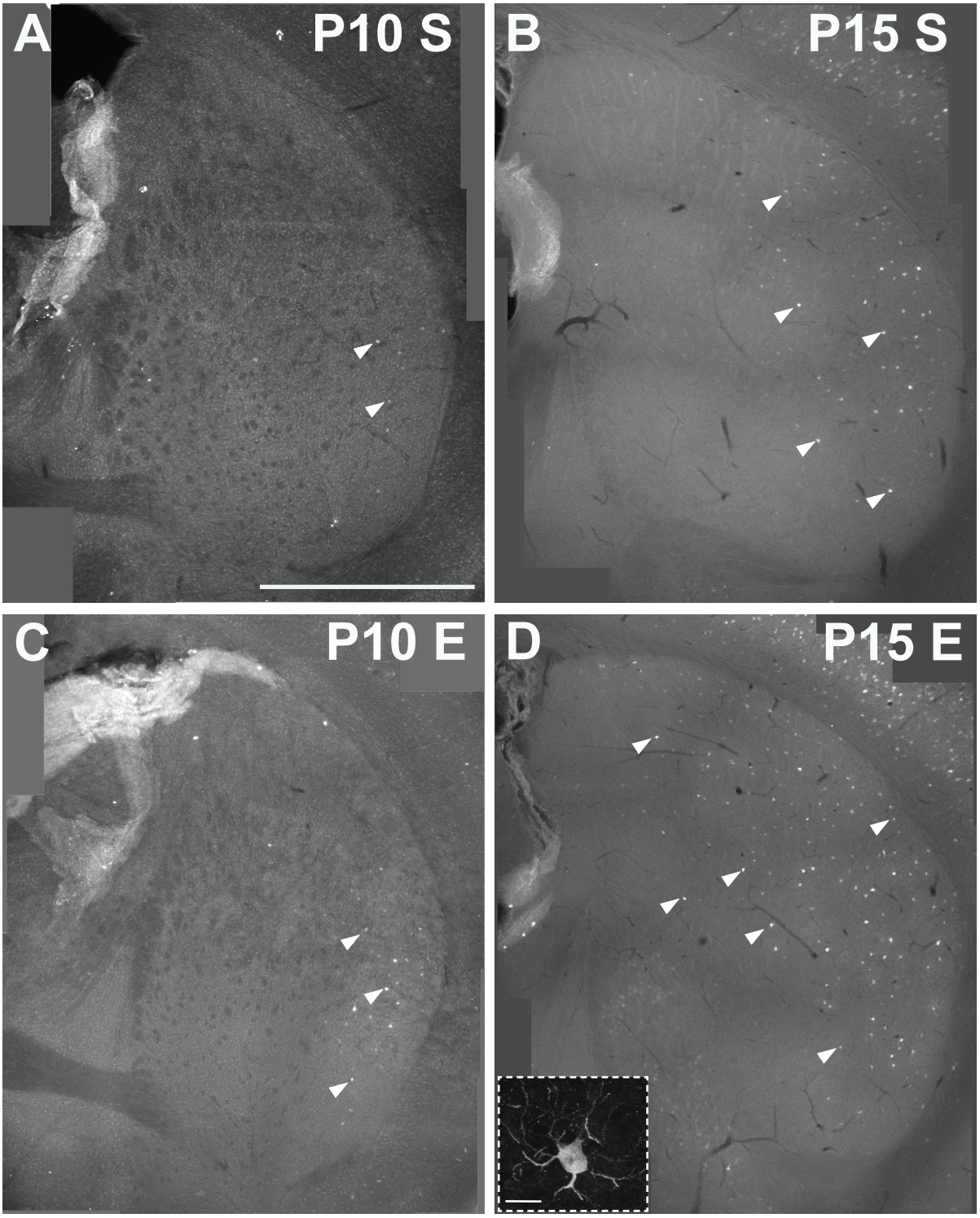
3.2 Environmental enrichment affects the maturation of PV+ cells

Previous work has established a link between PNN formation and the maturation of PV+ inhibitory interneurons [10-13]. Striatal PNNs begin consolidating at around postnatal day 10 (P10) [15], increasing in density with age. Further, EE accelerates the formation of these structures within the nucleus [19]. Although striatal PV expression is also first observed around the same age point [33, 34], whether the maturation of PV+ cells is similarly affected by enrichment is not known. Accordingly, I compared PV expression within the developing striatum of enriched and standard housed mice pups at two ages (P10 and P15, n=4-6) corresponding to when PV+ cells are first appearing.

I found that enrichment accelerated the age-dependent increase in PV expression within the developing murine striatum. At P10, striatal PV+ cells were already present, albeit at low numbers, predominantly within the lateral striatum, in both standard and enriched pups [33] (Fig. 2.2A, C; arrowheads), with expression increasing by P15 for both cohorts (Fig. 2.2B, D, arrowheads). In comparison to the standard-housed animals, pups raised in enriched environments exhibited a greater number of PV-expressing neurons in all sections of the striatum at each age-point (Fig 2.2A – D, arrowheads).

Quantitative analysis across these two developmental time points confirmed that density of PV+ neurons within the striatum increased significantly with age for both housing conditions (univariate ANOVA, age as between-subjects factor: S, $F(1, 128)= 123.681$, $P<0.001$; E, $F(1, 128)=162.513$, $P<0.001$) (Fig. 2.2E). EE also greatly affected PV expression, with enriched pups showing significantly higher densities of PV+ cells compared to standard housed cohorts (univariate ANOVA, housing condition as between-subjects factor: At P10, $F(1, 197)=11.445$, $P=0.001$; at P15, $F(1, 59)=8.612$, $P=0.005$) (Fig. 2.2E). The significant interaction between age and housing condition suggests that EE accelerated the expression of PV within striatal inhibitory interneurons (univariate ANOVA, housing condition and age as between-subjects factors, $F(1, 256)=13.782$, $P<0.001$). Together, these findings

suggest that EE can accelerate the maturation of PV+ inhibitory networks within the developing striatum.


A
P10 S
B
P15 S
C
P10 E
D
P15 E

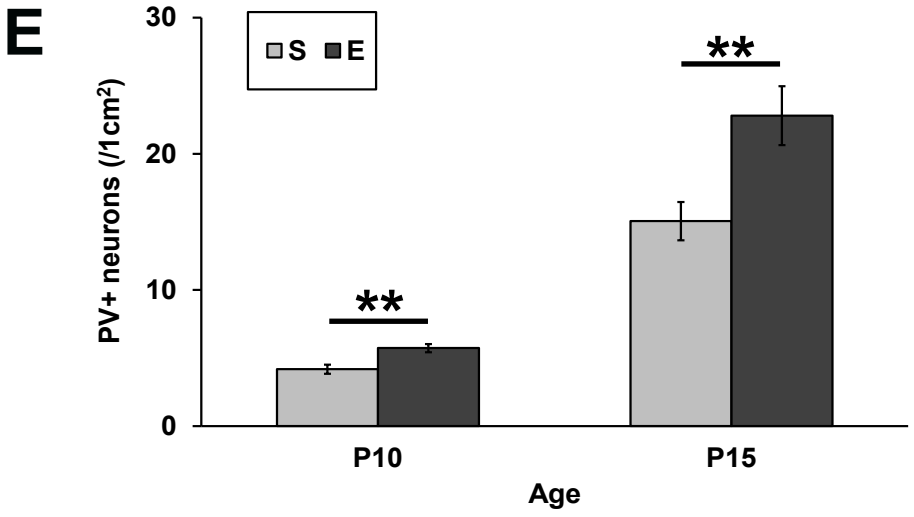

E
S
E
PV+ neurons (/1cm²)
30
20
10
0
**
**
P10
P15
Age

Figure: 2.2 Environmental enrichment accelerates the emergence of striatal PV expression.

Sample striatal sections showing the pattern of PV staining at P10 (A) and P15 (B) of standard (S) housed mice. PV staining (arrowheads) in juvenile mice is most prominent in the lateral part of the striatum, spreading medially with age. Very few positively stained cells are present at P10, but they become more numerous by P15. (C, D) Comparable sample striatal sections for enriched mice. Note the greater number of positively stained PV cells at both P10 (C) and P15 (D) compared to standard housed mice. The inset in (D) shows the appearance of a sample PV+ neuron at higher power. Scale bars: 1000μm in A – D, 100μm for the inset in D. (E) Striatal PV+ cell density in standard (S) and enriched (E) mice at the two age points assessed. Enriched mice exhibited significantly greater densities at both P10 (univariate ANOVA, $F=11.445$, $P=0.001$) and P15 (univariate ANOVA, $F=8.612$, $P=0.005$). Six sections from 4 – 6 animals were quantified for each group. **: $P<0.01$. Error bars=SEM.

3.3 Environmental enrichment increases levels of striatal brain-derived neurotrophic factor in juvenile mice

Previous work has demonstrated an up-regulation of BDNF within various areas of the brain in response to EE [9, 12, 38-40, 56-59]. Further, this enrichment-induced increase in trophic factor levels has been shown to accelerate the maturation of PV positive interneurons [9, 12]. Interestingly, unlike in many other cortical and subcortical regions, BDNF is not synthesised in the stratum; instead it is delivered to the nucleus via anterograde transport along afferent axons [37]. I reasoned that if BDNF is responsible for the impact of EE on PV expression in the striatum, then this should be reflected in increased levels of the trophic factor detected within the striatum at key age-points. To assess this possibility, I compared the presence of BDNF protein within the striatum of enriched and standard housed pups at three early postnatal ages: P8 (n = 5), P10 (n = 8) and P15 (n = 8), time points just prior to and inclusive of the early stages of PV expression.

I found that EE accelerated the developmental regulation of BDNF protein levels within the striatum. Quantitative analysis across these three early postnatal time points confirmed that striatal BDNF levels increased with age (univariate ANOVA, age as between-subjects factor, $F(2, 35)=17.190$, $P<0.001$). Although striatal BDNF levels exhibited no difference between standard and enriched pups at P8, the earliest age tested (univariate ANOVA, housing condition as between-subjects factor, $F(1, 7)=0.93$, $P=0.769$) (Fig. 2.3), by P10 the level of BDNF protein had at least doubled in both housing conditions, and was significantly greater in the striatum of enriched pups (univariate ANOVA, housing condition as between-subjects factor, $F(1, 14)=8.223$, $P=0.009$) (Fig. 2.3). By P15, striatal BDNF levels had peaked in both standard and enriched pups, and there was no longer any effect of housing condition (univariate ANOVA, housing condition as between-subjects factor, $F(1, 14)=0.32$, $P=0.860$). Together, these findings suggest that, as in other brain areas, the effect of EE on the maturation of inhibitory circuits within the striatum may be mediated by BDNF.

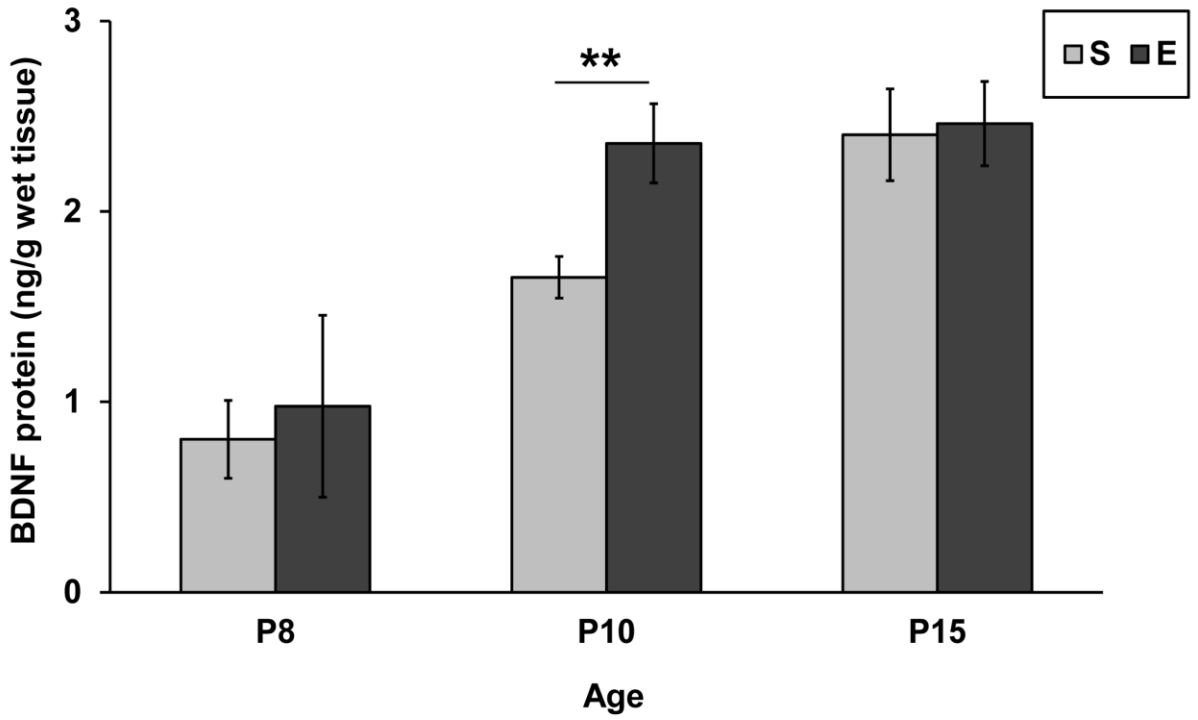


Figure: 2.3 Environmental enrichment increases levels of BDNF protein in early postnatal striatum

Mean BDNF protein concentration (ng/g wet weight tissue) for standard (S), and enriched (E) housed mice at ages P8 (n=4-5), P10 (n=8) and P15 (n=8). There is a significantly greater level of BDNF protein present in the striatum of enriched mice at P10 compared to standard housed mice of the same age (univariate ANOVA $F=8.223$, $P=0.009$). **: $P<0.01$. Error bars=SEM.

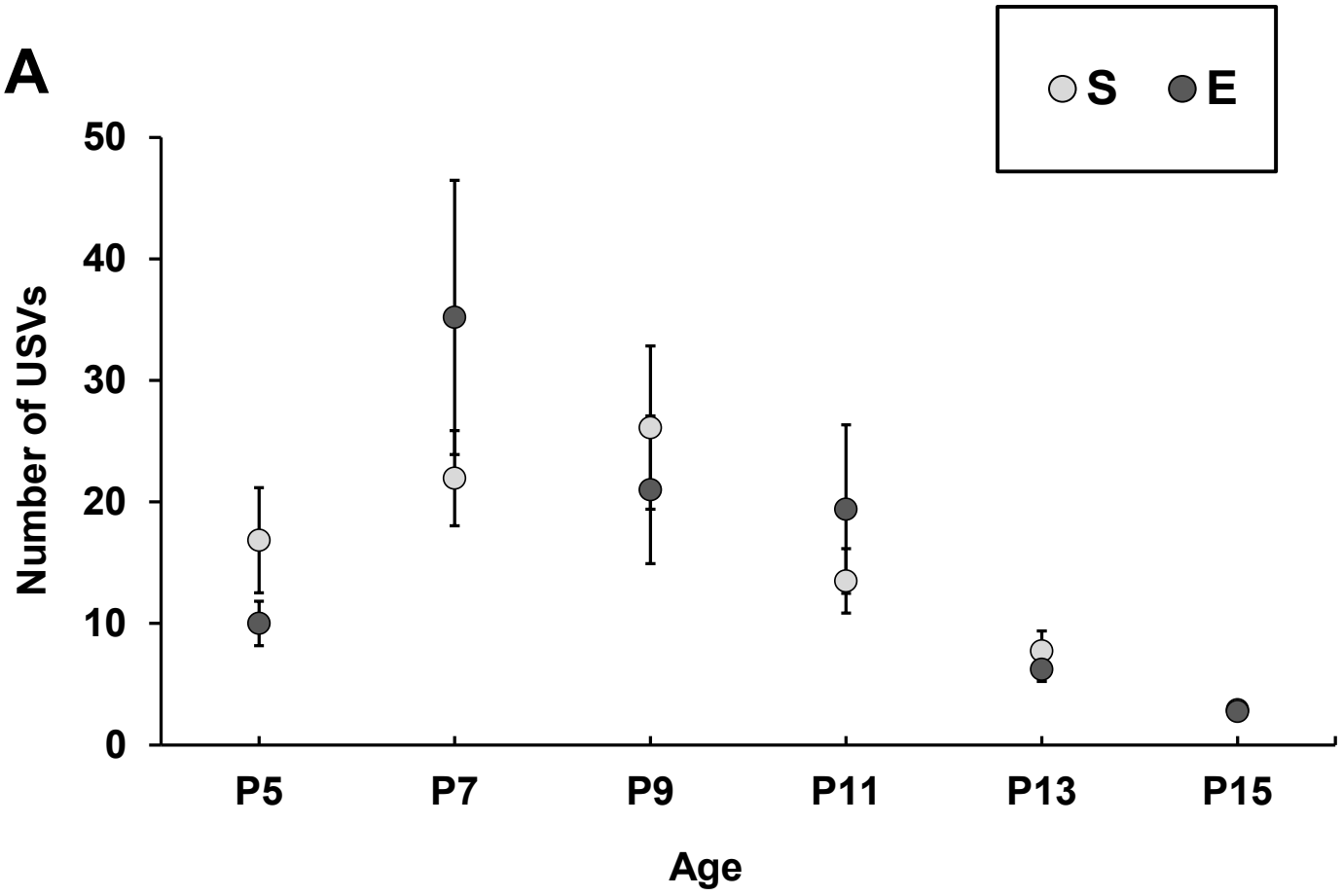
3.4 Enrichment affects ultrasonic vocalisations produced by juvenile mice

The emission of USVs by juvenile rodents in response to social isolation is a key ethologically relevant behaviour regulated by the striatum [44-46]. The period during which these calls peak corresponds well with other key developmental events occurring in postnatal striatum, including PV+ cell maturation [33] and PNN formation [15]. Given the current findings that show enrichment can accelerate the maturation of striatal PV+ neurons, along with previous work that has revealed that environmental enrichment can affect striatal PNN formation [19], and early life environmental experience can impact rodent USV production [60-62], I asked if the number and duration of social isolation USVs produced by juvenile animals were also impacted by EE.

The number of USVs emitted by individual pups peaked and tapered off as the animals aged, with less than half a dozen calls being produced at the oldest time point tested (P15). Although no difference was observed between housing cohorts (repeated measures ANOVA, housing condition as between-subjects effect, $F(1, 46)=0.082$, $P=0.776$), quantitative analysis confirmed that the age of recording had a significant effect on the number of USVs produced for both enriched (repeated measures ANOVA, age as within-subject effect, $F(5, 105)=6.704$, $P=0.001$) as well as standard cohorts (repeated measures ANOVA, age as within-subject effect, $F(5, 125)=6.747$, $P=0.001$) (Fig. 2.4A). The greatest number of USVs were generated at P7 for enriched, and at P9 for standard cohorts.

The duration of individual calls was also observed to increase as the animals aged in both standard and enriched pups (Fig. 2.4B). Quantitative analysis confirmed that age of recording had a significant effect on the duration of individual USV calls (repeated measures ANOVA, age as within-subjects factor, $F(5, 230)=19.211$, $P<0.001$), whilst housing condition did not (repeated measures ANOVA, housing condition as between-subjects factor $F(1, 46)=4.019$, $P=0.051$). Comparisons between standard and enriched cohorts at each date assessed, however, did reveal a difference in USV durations at the two oldest ages recorded (P13 and P15), with enriched pups exhibiting significantly

shorter calls compared to standard cohorts at these time points (univariate ANOVA, housing condition as between-subjects factor: P13, $F(1, 46)=11.736$, $P=0.001$; P15, $F(1, 46)=7.084$, $P=0.011$).


A
S
E
Number of USVs
50
40
30
20
10
0
P5
P7
P9
P11
P13
P15
Age

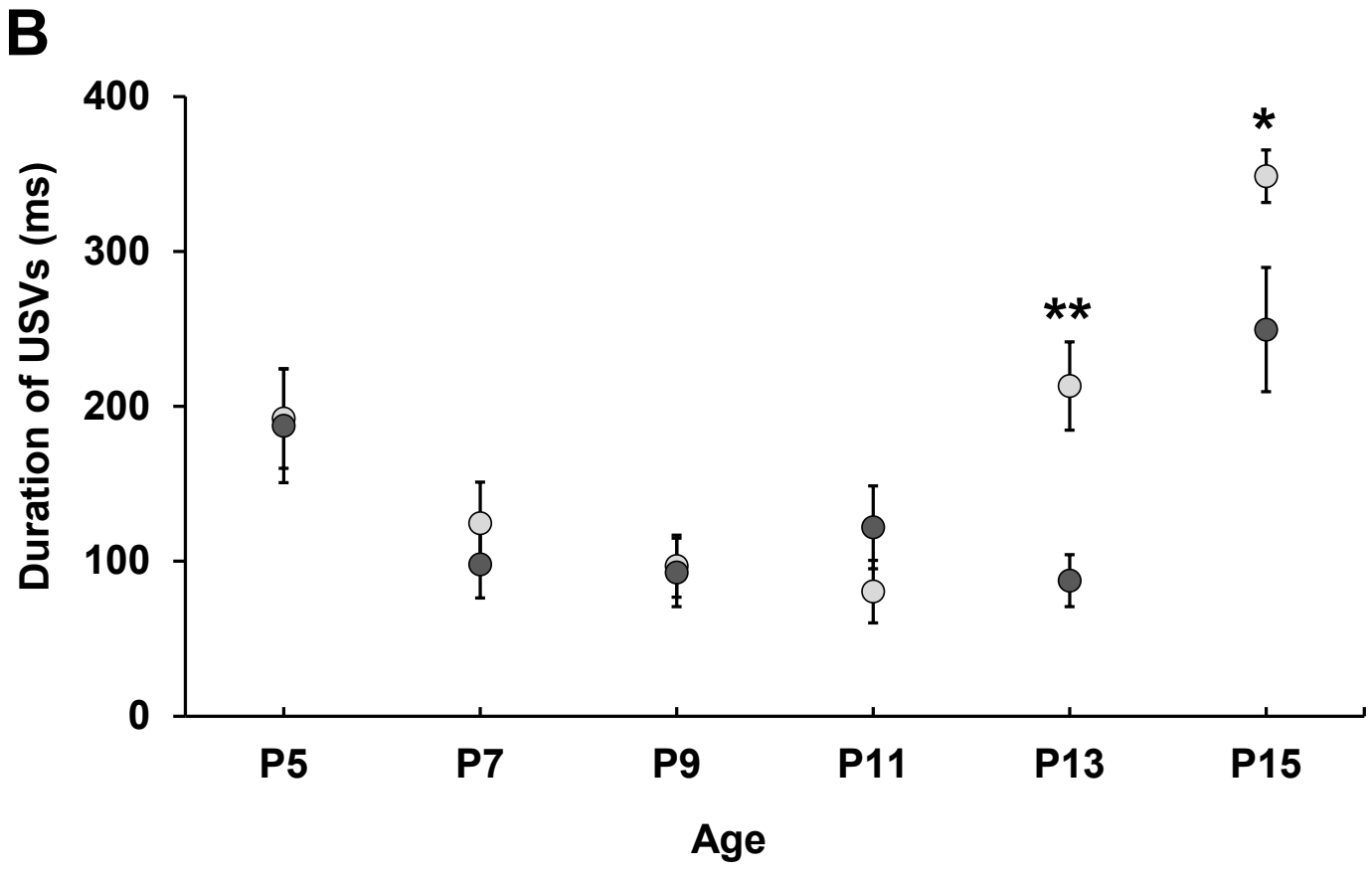

B
Duration of USVs (ms)
400
300
200
100
0
*
**
P5
P7
P9
P11
P13
P15
Age

Figure: 2.4 Environmental enrichment affects the duration of juvenile USVs

(A) Mean number of USVs produced during each day of recording. There was no effect of housing condition upon this metric (repeated measures ANOVA, F=0.082, p=0.776). Age significantly affected the number of USVs produced for both enriched (E) (repeated measures ANOVA, F=8.680, P<0.001) and standard pups (S) (repeated measures ANOVA, F=9.125, P<0.001). (B) Mean duration of USV calls produced during each day analysed. Age (repeated measures ANOVA, F=19.211, P<0.001), but not housing condition (repeated measures ANOVA, F=4.019, P=0.051), had a significant effect upon the duration of USV calls. Comparisons at each age point revealed that housing condition did impact mean duration of USVs at the two oldest ages recorded – enriched animals had significantly shorter calls at both P13 (multivariate ANOVA, F=11.736, P=0.001) and P15 (multivariate ANOVA, 7.084, p=0.011) than did standard housed mice. Standard n=26. Enriched n=22. *: P<0.05 **: P<0.01. Error bars=SEM.

In order to further examine the potential influence of housing conditions on USVs, calls emitted by pups were categorized into separate types based on their peak frequency at beginning, maximum and end amplitudes. USVs with a generally monotonic increase or decrease in peak frequency between beginning and end were classed as call type 1; calls with maximum peak frequencies that were either greater or less than beginning and end peak frequencies were classed as call type 2; and calls with equal peak frequency at beginning, maximum and end were classed as call type 3 (Fig. 2.5A-C, left panels).

When each call type was considered separately, further differences in the number of USVs emitted by housing condition were observed (Fig. 2.5A-C). The more numerous type 1 (Fig. 2.5A) and type 2 (Fig. 2.5B) USVs exhibited a call number profile that closely resembled the total vocalisation distribution. For both types, peak call numbers occurred at the same ages observed for the combined total (P7 for enriched and P9 for standard housed pups). A small, but significant distinction in call numbers with respect to housing condition was observed for type 2 calls at age P13, when standard pups exhibiting a slightly greater number relative to enriched cohorts (univariate ANOVA, housing condition as between-subjects factor, $F(1, 46)=6.389$, $P=0.015$).

For the least frequent calls, type 3, peak call number occurred at later age points relative to the combined total (Fig. 2.5C). This varied with housing condition, as standard pups exhibited the greatest number of this USV type at P11, while enriched juveniles revealed a further delay, with peak call numbers occurring at P13. Enriched pups maintained a relatively high type 3 call rate through P15, resulting in an overall significantly greater number of these USVs for these last two days tested (univariate ANOVA, housing condition as between-subjects factor: P13, $F(1, 46)=18.183$, $P<0.001$; P15, $F(1, 46)=9.672$, $P=0.003$) (Fig. 2.5C).

Examination by individual call type also revealed that the observed age-dependent duration effect was due primarily to changes observed in USVs type 1 and 2 (Table 2.1). Both these call types

exhibited significant increases in duration as the pups aged (repeated measures ANOVA, age as within-subjects factor: call type 1, $F(5, 100)=10.158$, $P<0.001$; call type 2, $F(5, 140)=14.697$, $P<0.001$).

Together, these findings suggest that EE can influence both the number and duration of USVs generated by developing mouse pups.

Table 2.1: Duration of USV calls by type

Call type	P5	P7	P9	P11	P13	P15
Call type 1	151.27 ± 28.4	105.49 ± 19.47	93.7 ± 19.02	61.21 ± 19.22	182.83 ± 34.61	369.85 ± 30.78
Call type 2	195.55 ± 28.96	100.53 ± 18.6	74.34 ± 14.71	142.03 ± 33.4	222.87 ±.30.45	384.48 ± 18.26
Call type 3	7.38 ± 2.04	7.91 ± 2.88	31.48 ± 7.24	30.61 ± 13.23	18.01 ± 2.87	25.69 ± 4.81

Table 2.1: Duration of USV calls by type

Average duration of USV calls in milliseconds with ± SEM. There is a significant effect of age of recording upon the duration of calls of type 1 and 2 as animals mature (repeated measures ANOVA: type 1, $F=10.158$, $p<0.001$; type 2, $F=14.697$, $P<0.001$).

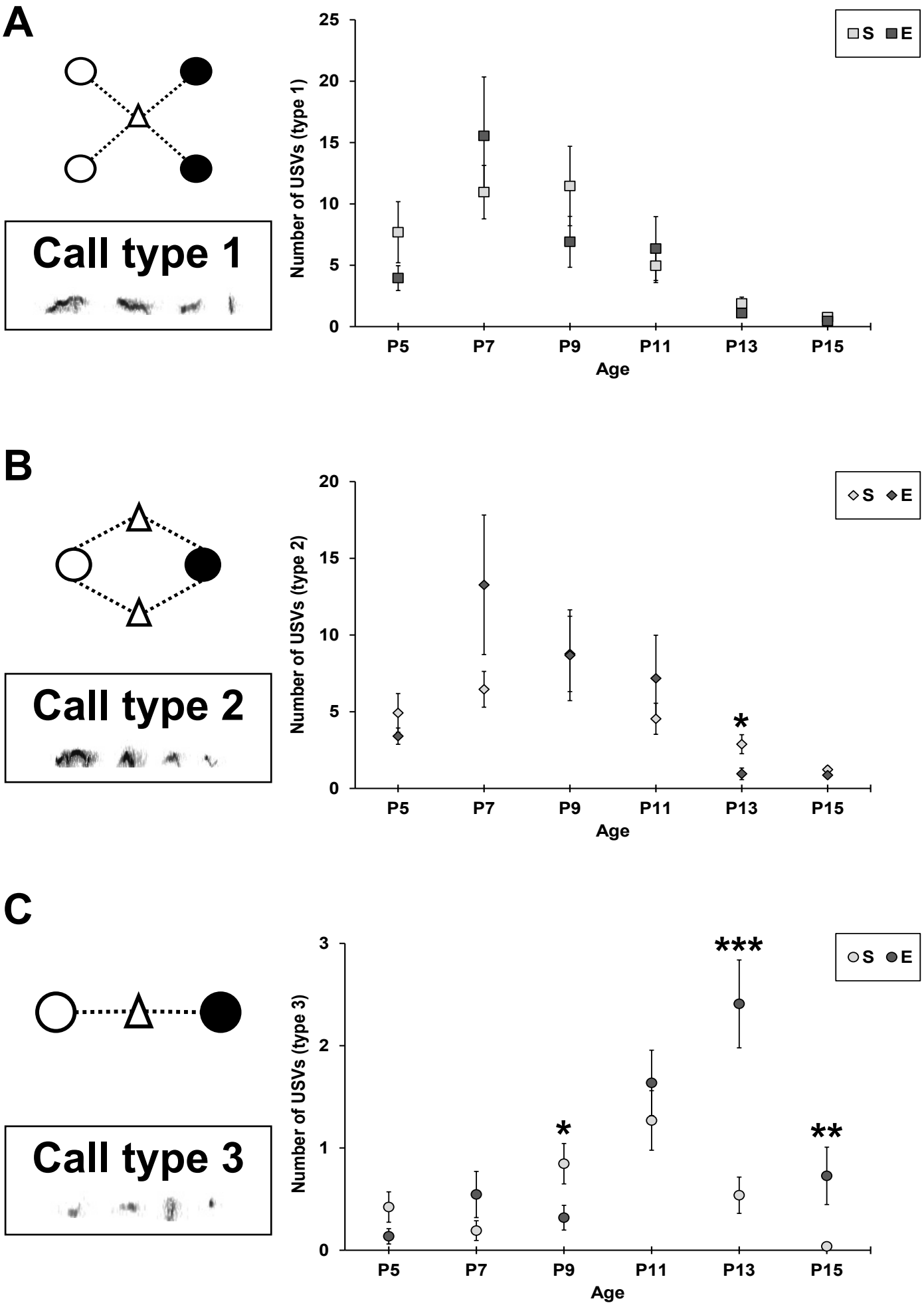

A
Call type 1
Number of USVs (type 1)
S
E
25
20
15
10
5
0
P5
P7
P9
P11
P13
P15
Age
B
Call type 2
Number of USVs (type 2)
S
E
20
15
10
5
0
*
P5
P7
P9
P11
P13
P15
Age
C
Call type 3
Number of USVs (type 3)
S
E
3
2
1
0
*
***
**
P5
P7
P9
P11
P13
P15
Age

Figure: 2.5 Environmental enrichment exerts a call type specific effect on juvenile USVs

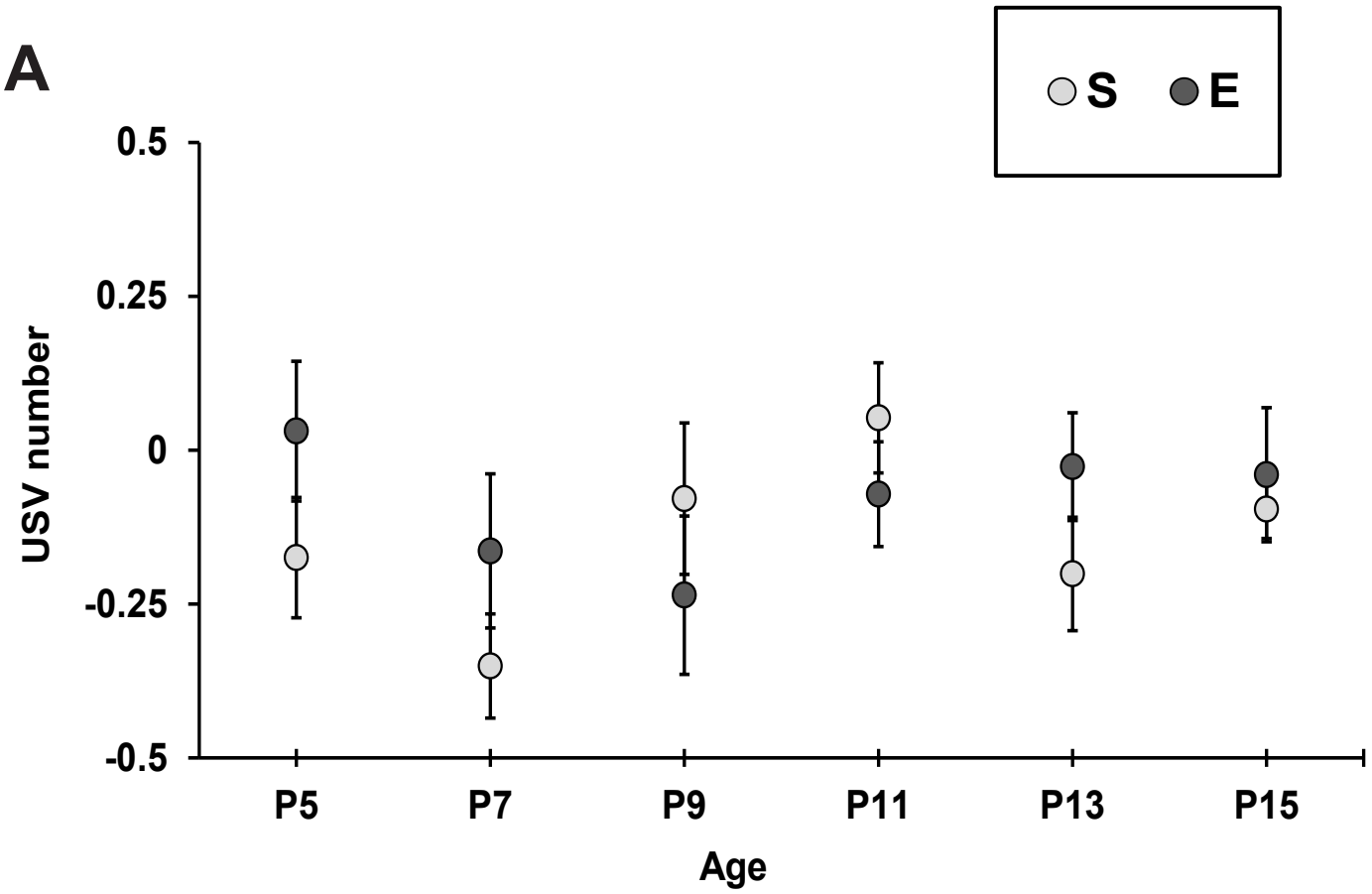
(A – C) Schematic representation of the 3 call types classified within this study as they appeared in spectrograms. Circles represent peak frequency at beginning (open) and end (closed) amplitudes, and triangles represent peak frequency at maximum amplitude. Dotted lines are possible call configurations. USVs with monotonically increasing or decreasing peak frequency were classed as call type 1; calls with maximum peak frequencies that were either greater or less than beginning and end peak frequencies were classed as call type 2; and calls with equal peak frequency at beginning, maximum and end were classed as call type 3. Below each schematic are four examples of calls recorded for that category. (A – C) Mean number of type 1 (A), type 2 (B), and type 3 (C) USV calls by housing condition for all ages assessed. No significant effect of housing condition upon the number of type 1 calls was detected (A; repeated measures ANOVA, $F=0.162$, $P=0.689$). Although no significant differences between housing cohorts was observed across all ages considered for type 2 calls (B; repeated measures ANOVA, $F=0.518$, $P=0.475$), a difference was revealed at P13 when enriched and standard call numbers were compared at individual age points (univariate ANOVA, $F=6.389$, $P=0.015$). A significant effect of housing condition was detected for type 3 calls, with enriched cohorts emitting more of these USVs, especially on the final two days assessed (C; P13, univariate ANOVA, $F=18.134$, $p<0.001$, and P15, univariate ANOVA, $F=6.968$, $P=0.011$) as well as at P9 (C; univariate ANOVA, $F=4.752$, $P=0.034$). Standard n=26. Enriched n=22. *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$. Error bars=SEM.

3.5 Enrichment has no significant effect on "maternal potentiation" of ultrasonic vocalisations produced by juvenile mice

USVs emitted by developing rodents have been shown to exhibit increases in incidence across two successive periods of social isolation separated by a stint within the home cage. Experimentally induced "maternal potentiation" has been well characterized in rat pups [61, 63, 64], and has also been demonstrated to occur in mice [55]. The degree to which environmental factors can influence this particular USV attribute is not well characterized. Accordingly, I assessed whether housing conditions affected the potentiation of either call number or duration across all ages tested.

There were no significant differences in the number of USVs potentiated across the two recording session observed between enrichment cohorts at the ages measured (repeated measures ANOVA, housing condition as between-subjects factor, $F(1, 46)=1.171$, $P=0.285$) (Fig. 2.6A). Similarly, there were no significant differences in the average duration of USVs potentiated across the two recording sessions when comparing enriched and standard housed mice (repeated measures ANOVA, housing condition as between-subjects factor, $F(1, 46)=0.037$, $P=0.849$) (Fig. 2.6B).

Together, these findings suggest that although early enrichment appears to exert little influence on the degree to which maternal potentiation can affect USV production, EE does exhibit a small but significant impact on other characteristics of social isolation calls.


A
S
E
0.5
0.25
0
-0.25
-0.5
USV number
P5
P7
P9
P11
P13
P15
Age

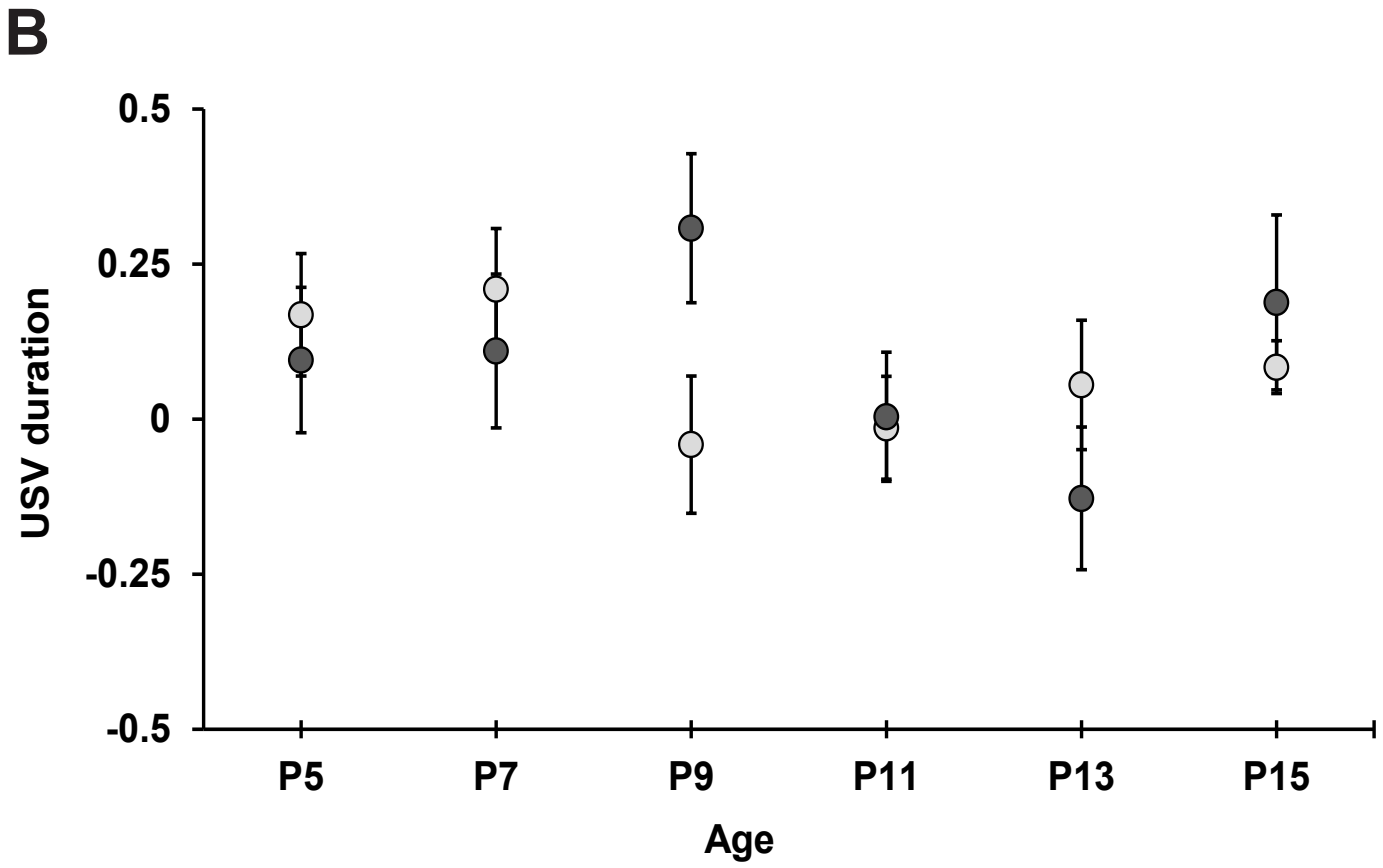

B
0.5
0.25
0
-0.25
-0.5
USV duration
P5
P7
P9
P11
P13
P15
Age

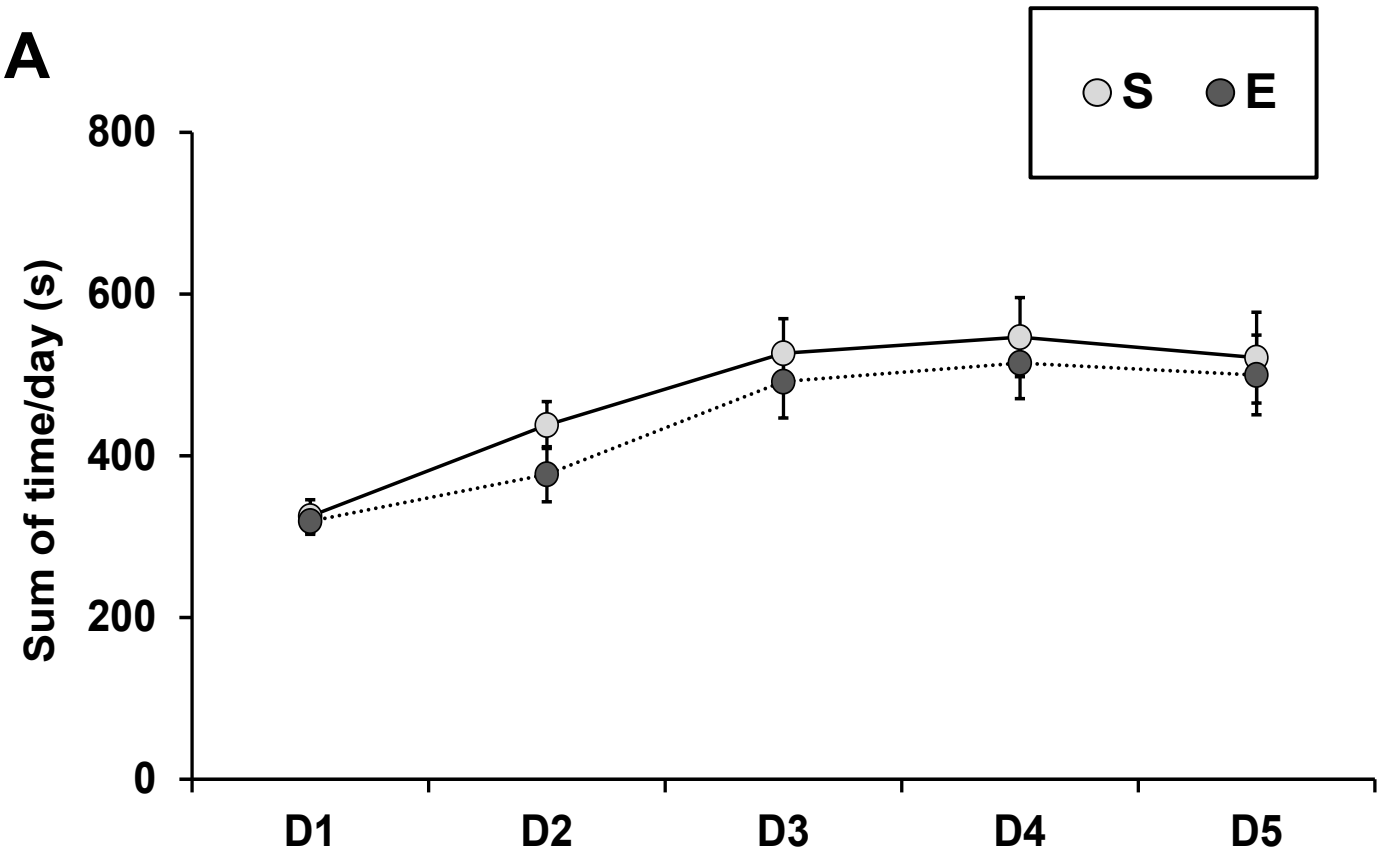
Figure: 2.6 Environmental enrichment had no significant effect on maternal potentiation

(A) Mean potentiation index ($R_i = (S2_i – S1_i) / (S2_i + S1_i)$; where S2 and S1 are the number of vocalizations made during recording day i for the number of USV calls produced as animals aged. There was no significant effect of enrichment upon this metric (repeated measures ANOVA, F=1.171, P=0.285) (B) Mean potentiation index for the average duration of USV calls produced as animals aged. No significant effect of enrichment was detected for this parameter (repeated measures ANOVA, F= 0.037, P=0.849). Standard n=26. Enriched n=22. Error bars=SEM.

3.6 Environmental enrichment does not affect performance of juvenile mice in a motor coordination task

Previous work from our laboratory has demonstrated the effect of EE on coordinated motor activity within juvenile animals. At P10, animals raised in an enriched environment demonstrate greater prowess and coordination in an open swim task than standard housed animals at the same age [19]. At this age, PV+ inhibitory circuitry within the striatum is not yet fully mature, and will not be for another two weeks [33]. It is not known whether improved motor coordination is sustained within enriched animals during this maturation, or if standard pups "catch up" to their performance at a later age. The impact of enrichment on coordinated ambulatory behaviours within older juvenile animals has yet to be analysed. Accordingly, I compared the motor ability of animals aged P21 – P26 (n=18 – 22) using a rotarod behaviour task.

Performance on the rotarod task was measured by assessing total time spent on the spinning rotor (Fig. 2.7A), and total distance run (Fig. 2.7B) each day of testing. I found that performance on the rotarod task was not impacted by housing condition (repeated measures ANOVA, housing condition as between-subjects factor, $F(1, 36)=0.209$, $P=0.650$) (Fig. 2.7A, B). Quantitative analysis across the day of testing demonstrated that both enriched and standard pups showed significant improvement in their ability to stay on the spinning rotor over the duration of testing (repeated measures ANOVA, day as between-subjects effect: E, $F=89.182$, $P<0.001$; S, $F=101.904$, $P<0.001$). The fact that both groups of animals showed effectively identical levels of improvement over the duration of testing suggests that the differences in motor coordination previously observed in very young enriched animals [19] may not be sustained.


A
S
E
800
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Sum of time/day (s)
D1
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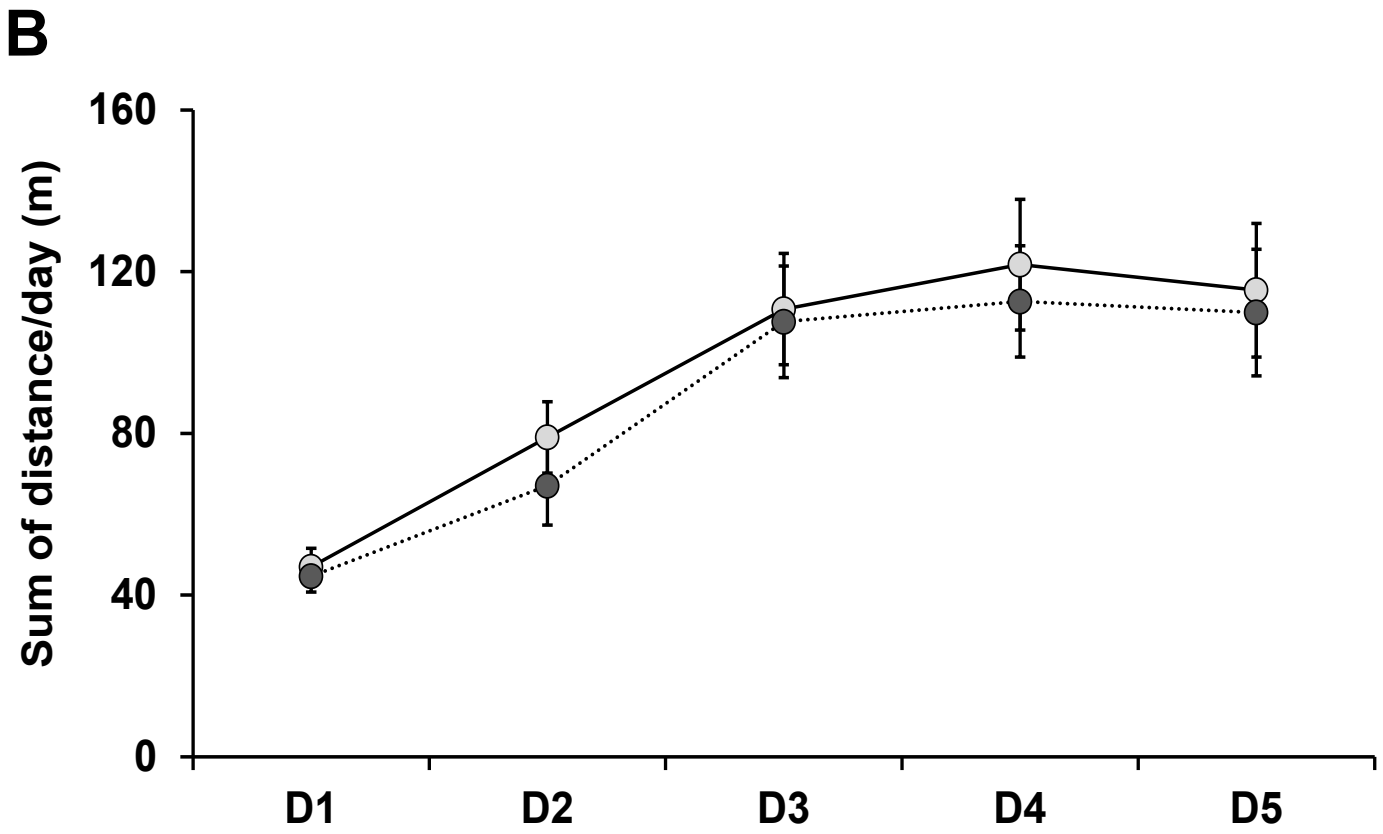
D5


B
160
120
80
40
0
Sum of distance/day (m)
D1
D2
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D4
D5

Figure: 2.7 Environmental enrichment does not affect performance of juvenile animals in a sensorimotor coordination task.

(A) A graph plotting the mean of total time spent on the rotorod each day (s) of standard housed (S) and enriched housed (E) mice aged P21 at the start of testing. (B) A similar graph to that in A but showing performance in mean of total distance run each day. There was no effect of housing condition on animals' performance (repeated measures ANOVA, $F=0.209$, $P=0.650$). Day of testing significantly effected both total time and distance achieved by both housing conditions (repeated measures ANOVA, $P<0.001$ for both groups). Standard n=18. Enriched n=22. Error bars=SEM.

4. Discussion

This study demonstrates that ongoing enrichment is capable of influencing striatal circuitry. Striatal PV+ cell density was higher for adult mice raised in enriched environments compared to standard cohorts, while PNN levels, previously shown to also exhibit accelerated formation in EE pups [19], were indistinguishable between mature enriched and standard cohorts. This difference was reflected in the association between these two entities, with adult animals raised in enriched environments exhibiting greater numbers of striatal PV+ cells encapsulated by PNNs. Assessment of the PV+ population of striatal neurons in young animals confirms that early EE is capable of dramatically affecting postnatal striatal development. Pups raised in enriched environments displayed a significantly accelerated maturation of PV+ cells within the nucleus. EE also had an influence on BDNF expression in early postnatal development, significantly accelerating the increase in levels of this protein within the striatum, providing a possible mechanism underlying enrichment dependent precocious PV+ expression. Finally, an ethologically relevant behaviour mediated by the striatum was also affected by EE, with enriched pups exhibiting a different developmental profile of ultrasonic vocalisations than that of standard cohorts. Together, these results have important implications for the role of environmental influences on the maturation and maintenance of striatal circuitry, and the development of ethologically relevant behaviours.

4.1 Environmental enrichment from birth increases Parvalbumin, but not PNN expression in adult mice

Striatal PV+ fast spiking interneurons play a key regulatory role in striatal function [16, 28, 34]: receiving afferent input from the cortex and thalamus [25, 28, 29, 34], these neurons form synapses upon, and regulate the activity of, medium spiny projection neurons, the sole efferent population of the striatum [29, 65]. Adult mice raised from birth in an enriched environment revealed a significantly higher density of PV+ cells within the striatum than did animals raised in standard

housing. This is consistent with the notion that EE continues to influence PV levels within elements of striatal circuitry, even after the rapid onset of expression observed during the first few postnatal weeks. In contrast, there was no effect of housing condition on the density of striatal PNNs of adult animals, despite the fact that EE has previously been shown to also accelerate the initial formation of these structures in early postnatal pups [19]. It would appear that early EE is capable of accelerating the maturation of both striatal PNNs [19] and PV+ neurons, but that lifelong EE only impacts PV+ cells, not PNNs, beyond the early postnatal period. Interestingly, this discrepancy is reflected in a change in association between these two entities, with enriched mice exhibiting a higher overlap between PV+ cells and PNNs compared to standard cohorts.

How this increase in PV expression comes about is not clear. Previous studies have demonstrated that increased activity resulting from EE in adulthood is capable of modulating the intensity of PV expression within the hippocampus [32]. PNN expression within the visual cortex has also been shown to be susceptible to modulation in response to EE in adult mice, presumably contributing to an improved ability to correct artificially induced strabismus by enriched animals [66]. Curiously, in both of these cases, EE appears to either decrease the intensity of PV expression, or PNN density, having an almost opposite effect to the enrichment-induced changes in the current study.

At a functional level, the increase in PV expression may be reflecting the inherent role striatal circuits subserve in the behaving animal. Corticostriatal pathways contribute to procedural learning and skill acquisition [67]. Hippocampal interneurons exhibiting low PV levels in adult mice exposed to EE express high levels of PV once they have acquired cognitive learning tasks [32]. It is possible that the higher levels of striatal PV expression observed in the current study reflect a greater range of skills EE animals have acquired and/or are continually acquiring and consolidating from their comparatively enriched surroundings.

Moreover, striatal circuitry differs from both the hippocampus and neocortex in that most of the local constituents are non-glutamatergic: excitatory drive is sourced primarily from cortical and/or

thalamic input [21-23, 25, 68]. Dopamine also plays a requisite role in regulating synaptic level plasticity in this nucleus [69-71]. It is possible that the differences in local connectivity may be, at least in part, contributing to the manner in which EE affects the development and maintenance of PV+ networks in the striatum. Further studies will be required to determine the mechanism underlying the influence of EE on the development and maintenance of striatal regulatory circuits.

4.2 The association between Parvalbumin-expressing cells and PNNs is increased in enriched adult mice

Remarkably, striatal PV+ cells and PNNs exhibited a greater degree of overlap in adult mice raised in enriched environments from birth compared to standard raised cohorts. The link between PV+ neurons and PNNs is well established [9-14], with recent findings suggesting that the formation of these extracellular matrix structures contribute to the maturation of PV+ basket cells by potentially facilitating the association of these interneurons with the homeoprotein Otx2 [72].

Unlike in other brain regions, however, there is only a partial overlap between PV+ cells and PNNs within the striatum [16]. Further, the identity of non-PV+, PNN associated cells has yet to be determined [16]. The current findings that EE induces an increase in PV+ cells, but not PNNs, within the adult striatum suggests that at least some PNN encapsulated, non-PV+ neurons are low PV-expressing interneurons akin to low differentiation state basket cells described in the hippocampus [32]. This greater degree of overlap in enriched animals suggests that the increased numbers of striatal PV+ neurons resulting from EE occurs in a usually quiescent population of PV+ cells surrounded by PNNs. The increased potential for activity and skill acquisition provided by EE over time may lead to more circuits consolidating, effectively converting low PV-expressing cells to mature high PV+ interneurons. Further work will be required to assess this possibility.

4.3 Accelerated maturation of Parvalbumin-expressing inhibitory circuitry in enriched pups

Previous work has revealed that the maturation of networks comprised of PV+ cells is vital for the opening and closing of developmental critical periods [5, 7, 73, 74], with evidence that EE can accelerate the maturation of PV+ networks in primary visual cortex (V1), thus shifting the timing of the developmental critical period [9, 12]. In the striatum, PV + cells do not begin to mature until around postnatal day 9-10 [33, 34]. PV expression itself exhibits a developmental gradient: it is first expressed in the lateral striatum, and gradually spreads across to more medial regions until reaching an adult-like expression profile in the fourth week of life [33]. The timing of postnatal PV expression overlaps with a number of key developmental changes associated with striatal circuitry: the innervation of the matrix compartment (one of two major immunohistochemically defined subregions within the striatum) by dopaminergic afferents [23, 35], the emergence or maturation of a host of striatum dependent behaviours including quadrupedal ambulation [41, 42], swimming, and exploration [19, 42], as well as the formation of PNNs [15, 19].

The current finding that enrichment leads to precocious expression of PV in the striatum, together with recent studies showing that EE can expedite the emergence of exploratory behaviour and motor control, as well as the formation of striatal PNNs [19], strongly suggest that this postnatal epoch shares hallmark features characteristic of cortical critical periods.

4.4 Changes in BDNF levels due to enrichment may underlie the accelerated maturation of PV+ cells

The striatum is involved in a number of functions including the regulation of coordinated movement, motivation drive and volitional action. These roles require the precise integration of input from a variety of cortical and sub-cortical sources [21, 23-25]. Previous work has demonstrated that EE can

dramatically affect both the anatomy and function of sensory cortex [9, 75]. Given that the enrichment paradigm used in the study was designed to provide animals with a host of sensory, motor and social stimulation, it is possible that the changes induced by EE in input areas are indirectly driving accelerated maturation of striatal PV+ interneurons.

Neurotrophins play a role in the development, maintenance and repair of neural circuits. In particular, BDNF has been linked with the maturation of GABA-ergic PV-expressing inhibitory interneurons within both the visual system and the striatum [7, 37]. In V1, excess expression of BDNF has been shown to accelerate the onset of the developmental critical period [7]. PV-expressing neurons within the striatum receive the bulk of their excitatory synaptic inputs from cortical afferents [29]. Previous work has shown that the maturation of PV expression in other brain areas is dependent on afferent drive [73, 76] with BDNF revealed to be a key contributor to this process [7].

There is very little BDNF mRNA found within the striatum: instead, the trophic factor is “ported in” to the striatum on afferent fibres projecting from the cortex [36]. Lesion studies have shown that cortical ablations lead to a lack of BDNF protein and decrease in PV-expressing neurons within the striatum [37]. Given the temporal alignment of corticostriatal terminal formation with the emergence of PV positive immunoreactivity within the striatum [33, 34], it is likely that BDNF may also contribute to the maturation of striatal PV+ neurons.

Enriched pups showed a significantly higher level of BDNF protein within the striatum at P10, around the same time that PV begins to be expressed. It may be that the observed increase in BDNF protein levels comes about as a result of cortical afferents forming functional striatal connections earlier due to EE. The BDNF protein provided by these afferents may in turn contribute to the maturation of PV+ inhibitory interneurons within the striatum of enriched pups. Interestingly, there was no difference in striatal BDNF protein levels at the older age tested (P15), suggesting that enrichment caused animals to reach a developmental peak in striatal BDNF protein earlier, but that the effect of EE upon striatal BDNF levels has an upper threshold. Given that BDNF protein is transported into the

striatum by cortical afferents [36, 37], the number and maturity of these projections may limit the amount of BDNF available within the juvenile striatum. Further experiments would be required to confirm whether cortical afferents form functional synapses on striatal PV + neurons earlier due to EE, and whether there are a greater number of these connections formed within the striatum of enriched animals.

4.5 Early enrichment can influence a striatally mediated behaviour

Ultrasonic vocalisations (USVs) are a large part of the range of utterances made by mice: these sounds are produced throughout an animals' lifespan, but their use differs with animal age, gender and context [46, 61, 77]. The striatum has been shown to undergo motor-driven, vocalisation-related activation during the production of USVs, suggesting it is a part of the pathway responsible for generating these sounds [43].

Social isolation USVs emitted by rodent pups can take on a variety of forms [78, 79]. Although a thorough acoustical analysis of USVs was beyond the scope of the current study, based on my simple categorization scheme, I was still able to detect differences in the USV emissions of juvenile mice raised in enriched and standard housing conditions. Most notably, enriched pups exhibited a significantly greater number of type 3 calls and standard animals emitted significantly longer calls at the two oldest age points assessed. It is not entirely clear why EE would result in older pups producing a greater number of shorter, unmodulated calls than standard counterparts; it may be a reflection of altered maternal behaviours, or of accelerated maturation within the enriched pups.

Recent work has revealed that the Fmr1 mouse model for Fragile X syndrome exhibit call type specific deficits in USV emissions [80]. As the mutation is associated with abnormal dendritic spines and altered synaptic plasticity (for review see, He & Portera-Cailliau, 2012 [81]), it is intriguing to note that environmental enrichment, which has been characterised as increasing plasticity [82, 83]

and dendritic density [84, 85], is contributing to an increase in utterance of a call type with similar properties to the one affected in the KO [80]. Whether this can be attributed directly to EE dependent changes in PV+ cells has yet to be determined. Both dendritic spine density [86] and arborisation [87, 88] are known to be decreased in animals showing higher levels of anxiety-like behaviours. Interestingly, BDNF protein levels are also known to be decreased in animals with higher anxiety and reduced dendritic spine density [86]. Conditional BDNF knockout animals display a smaller striatal volume and cultured medium spiny neurons taken from these mice show reduced dendritic complexity [89]. Dendritic analyses of striatal PV+ inhibitory interneurons were conducted in this study, but there were no detectable significant differences between enriched and standard animals at the ages assessed. This may be due to technical considerations, such as limited penetration of the anti-PV antibody into dendritic arbours, or it may that another cellular population within the striatum regulates anxiety-induced behaviours such as USVs.

Further, treatment with anxiolytics has recently been shown to decrease total call times in rat pups [90]. Accordingly, the longer USVs emitted by standard housed pups are consistent with increased anxiety levels exhibited by these mice. Indeed, previous work has revealed that early exposure to enriched environments as well as high levels of maternal care can reduce anxiety-like behaviour in rodents [91-93]. Evidence demonstrating that EE can also increase dam-pup interactions [9, 12], further supports the notion that the influence of enrichment on call duration may be at least in part attributable to changes in stress response. USVs induced by maternal separation are intended to attract the attention of dams for retrieval back to the nest, and this behaviour changes as pups age and become independently mobile [46, 78]. Given that enrichment has been shown to improve sensorimotor coordination and increase exploratory behaviours in animals as young as 10 days postnatal [19], it may be that pups raised in EE are less distressed by maternal separation at the older ages tested as they are more capable of conducting themselves back to the nest without assistance.

In light of this, it is curious that the degree of isolation potentiation, another attribute associated with juvenile USVs, did not exhibit a marked difference between enriched and standard housed animals. Previous studies have indicated that the rate of maternal potentiation is dependent on the balance of dopaminergic (DA) receptor expression in the nucleus accumbens [94], a different network from the circuits responsible for the generation of USVs [43, 95]. It is possible that the postnatal enrichment utilized here did not overlap enough with the relatively early prenatal establishment of this pathway [96-99] to affect its development. Further work will be required to determine if this is the case.

The changes observed in both the dynamics and characteristics of isolation USVs as a result of enrichment, along with the slightly earlier age at which the greatest number of isolation USVs were produced by enriched animals, is consistent with an overall acceleration of striatal development due to EE described in the current as well as previous studies [19]. Together, these findings suggest that the striatum may play a role in the emergence, regulation, and maintenance of this ethologically relevant behaviour.

4.6 Enrichment does not influence sensorimotor coordination of juvenile animals.

Previously, the development of a coordinated motor activity has been shown to undergo acceleration in young mice (postnatal day 10) as a result of EE [19]. Mouse pups begin to develop independent locomotion around this time, with locomotor activity further refined until reaching an adult-like state around P26 [42]. I examined the effect of enrichment on slightly older mice using a rotarod behavioural task to assess coordinated locomotor activity from the ages of P21 until P26. Both groups of animals demonstrated the same level of improvement over the duration of testing, and there was no noted effect of housing condition on the ability of animals to perform this activity. However, the lack of any significant effect of enrichment upon behavioural performance is noteworthy; previous work has demonstrated an observable difference in the sensorimotor

coordination behaviour of enriched and standard housed pups, occurring earlier in the postnatal period [19]. It is possible that the rotarod behavioural task may not be capable of distinguishing fine differences in sensorimotor coordination resulting from EE. More likely is that the impact of enrichment is only able to be observed during the onset of locomotion, possibly having more of an effect upon ontogeny, rather than refinement, of behaviours. It would be of interest to perform a detailed gait analysis on late-juvenile animals [42] to ascertain the exact effects of environmental enrichment upon locomotion behaviours.

4.7 Conclusions

The current results provide evidence that the effects of enrichment are not just restricted to early life: I demonstrate that on-going enrichment into adulthood is still capable of inducing cellular changes within the striatum. I also show that EE from birth can accelerate the maturation of motor control and motivational circuits, at the cellular, molecular and functional levels. Together, these results suggest that the development of this vital brain area is highly sensitive to environmental factors, and that an organism's surroundings are capable of influencing striatal circuitry throughout life.

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Chapter 3:

The Use of the Puzzle Box as a Means of Assessing the Efficacy of Environmental Enrichment

The Journal of Visualized Experiments (JoVE)

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- Angela May O'Connor – experimental set up, conducting experiments, analysis of data, manuscript preparation
- Thomas Burton – experimental set up, manuscript preparation
- Catherine Anne Leamey – manuscript preparation
- Atomu Sawatari – experimental set up, manuscript preparation

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18-12-2014

TITLE: The Use of the Puzzle Box as a Means of Assessing the Efficacy of Environmental Enrichment

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SHORT ABSTRACT:

Environmental enrichment provides a potential protective effect against neurodegenerative disorders. Currently, however, there is no easy way of determining the efficacy of enrichment procedures. This protocol describes a simple "Puzzle Box" method for assessing an animal's cognitive function, in order to reveal the effectiveness of environmental enrichment.

LONG ABSTRACT:

Environmental enrichment can dramatically influence the development and function of neural circuits. Further, enrichment has been shown to successfully delay the onset of symptoms in models of Huntington's disease[1-4], suggesting environmental factors can evoke a neuroprotective effect against the progressive, cellular level damage observed in neurodegenerative disorders. The ways in which an animal can be environmentally enriched, however, can vary considerably. Further, there is no straightforward manner in which the effects of environmental enrichment can be assessed: most methods require either fairly complicated behavioral paradigms and/or postmortem anatomical/physiological analyses. This protocol describes the use of a simple and inexpensive behavioral assay, the Puzzle Box[5-7] as a robust means of determining the efficacy of increased social, sensory and motor stimulation on mice compared to cohorts raised in standard laboratory conditions. This simple problem solving task takes advantage of a rodent's innate desire to avoid open enclosures by seeking shelter. Cognitive ability is assessed by adding increasingly complex impediments to the shelter's entrance. The time a given subject takes to successfully remove the obstructions and enter the shelter serves as the primary metric for task performance. This method could provide a reliable

means of rapidly assessing the efficacy of different enrichment protocols on cognitive function, thus paving the way for systematically determining the role specific environmental factors play in delaying the onset of neurodevelopmental and neurodegenerative disease.

INTRODUCTION:

Environmental enrichment (EE) may be defined as surroundings that provide animals with increased opportunity for social interaction, motor activity, and greater sensory stimulation than usually experienced in a standard laboratory environment. EE has been shown to consistently affect the behavior of animals, bringing about changes such as reduction of stress and anxiety-related activity[8-11], improved performance in learning and memory tasks[8,12], early onset of motor coordination and exploratory activity[12], changes in maternal care[8] as well as resistance to addictive substances[13-16]. Further, EE has been revealed to ameliorate the effects of neurodegenerative disorders, delaying the onset and decreasing the severity of symptoms in animal models of Huntington's[1-4,17], Parkinson's[18] and Alzheimer's disease[19].

These changes correlate with the anatomical and molecular alterations EE is known to induce throughout the brain. Animals raised in enriched environments from early stages of development show a myriad of neural changes, including increased brain weight and cortical thickness[20-22], dendritic branching[10,23-25] and synaptic density[26]. EE can alter both the level and timing of growth factor expression[9,27-34], which has been shown to contribute to accelerated development of sensory[28,29,31,32,35,36], mnemonic[34], as well as motor circuits[37,38].

Previous work has revealed at times contradictory findings when investigating the impact of EE, without taking into account the different types of animals and environments used within individual studies[9,27,30,33,34]. Currently, there is no consistent and simple behavioral task that can be used to measure the effectiveness of various EE paradigms in different strains and species of animals.

The Puzzle Box task was designed as a simple test to determine an animal's native problem solving ability[7]. Animals placed in the open area are required to remove obstructing materials situated within a small opening in order to access a covered region/shelter. Each subject is given three trials with the same obstruction in order to assess three different cognitive attributes. The first trial yields a baseline indication of inherent or native problem solving ability. The second trial, run on the same day, gives some indication of the animal's ability to improve upon and thus reinforce strategies for removing the specific obstruction. The third trial, conducted on the following day, provides insight into the ability of the subject to retain and recall the learned solution to the task.

The motivation for solving these "obstruction puzzles" by the animals can be varied, potentially evoking an innate desire to avoid open fields and seek shelter, as well as an inherent drive to explore their surroundings[6,7]. The multitude of potential behavioral drivers underlying the desire to solve the Puzzle Box suggests that various areas of the brain are involved in mediating task performance. Previous work has shown that in murine models of schizophrenia, the prefrontal cortex as well as the hippocampus are involved in the acquisition of this task[5]. A

lesion study in rats has also revealed a large number of brain regions involved in Puzzle Box performance, including various thalamic nuclei, the hypothalamus, the cerebellum, and limbic structures[39]. Together, these findings indicate that engaging in this problem solving task involves a host of neural structures associated with cognitive function.

The Puzzle Box has been used successfully to assess the problem solving ability of mice, as well as cognitive deficits exhibited by murine models of schizophrenia[5-7]. Performance on the task has been shown to be highly consistent, and correlate well with outcomes of other cognitive behavioral tests[6]. The goal of this work was thus to adapt the Puzzle Box task to become a simple and reliable means of determining the effectiveness of EE.

PROTOCOL:

Ethics statement: All procedures were approved by the Animal Ethics Committee of the University of Sydney and conformed to National Health and Medical Research Council of Australia guidelines. Procedures were performed on C57/BL6J mice which were reared at the University of Sydney Bosch Rodent Facility. All mice were housed in a single adequately-ventilated room in 21^{o}C ambient temperature on a 12-hour light-dark cycle with lights on at 0600 hours in individually ventilated cages with *ad libitum* access to dry food and water. Late-pregnancy females were randomly assigned to standard or environmentally enriched housing conditions.

1. Housing (Enrichment levels):

1.1) Obtain 4 late-pregnancy adult female mice. Randomly assign 2 to the standard condition and place each of them into a clean standard mouse cage (overall dimensions 391 x 199 x 160 mm) containing one red mouse igloo. For the enriched condition, place the remaining 2 mice into a single clean rat-sized cage (overall dimensions 462 x 403 x 404 mm).

1.2) Into the enrichment cage, place a variety of objects designed to increase sensory and motor stimulation (e.g. running wheels, visual stimuli, scented cotton balls, Velcro strips).

1.3) Every 2 to 3 days move these objects about within the cage; refresh any that have been destroyed.

1.4) Upon weaning at 21 days postnatal, sex animals and place into male-female segregated housing consistent with the environmental condition they were raised. For the enriched condition ensure that there are between 3 and 10 mice per cage with 2 and 5 mice for standard. Commence behavioral testing once animals reach adulthood (12 – 14 weeks of age).

2. Construction of the Puzzle Box

2.1) Obtain 6 pieces of white acrylic (or other non-porous material): one 750 x 280 mm, two 280 x 250 mm, two 750 x 250 mm, and one 150 x 280 mm (see Figure 3.1).

2.2) Obtain one piece of black acrylic 280 x 250 mm, with a 40 x 40 mm square opening cut into one side of this piece (see Figure 3.1).

2.3) Assemble the Puzzle Box as follows: use the 750 x 280 mm piece as the bottom of the box, use 280 x 250 mm pieces as the ends of the box, and the 750 x 250 mm pieces as the sides of the box.

2.4) Measure 150 mm into the box from one end, and place the black piece of acrylic across the box so that it splits it into two compartments (one large, one small) with the opening flush with the bottom of the box.

2.5) Take the 150 x 280 mm piece of white acrylic and place it atop the smaller compartment of the box making sure it covers this area completely, providing a dark “goal-box” chamber. Affix this piece of acrylic to the body of the main box by hinges, or leave free to be completely removed during behavioral testing.

2.6) Take 3 pieces of acrylic (three 4 x 120 mm) and join to make a “u-shaped” channel.

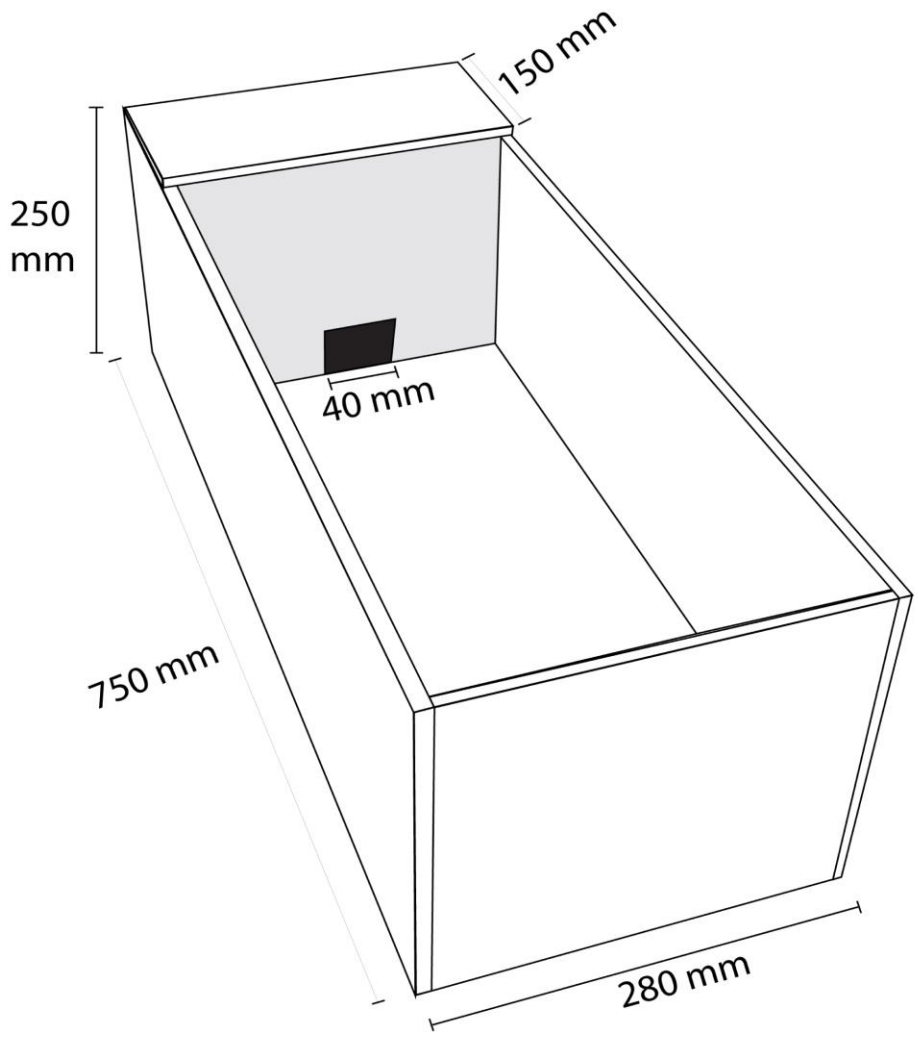

Figure 3.1 Schematic diagram of the Puzzle Box task.

The Puzzle Box is an acrylic box consisting of an open-field arena (600 x 280 mm) and a sheltered goal-box area (150 x 280 mm), measuring 750 x 280 mm in all. A 40 x 40 mm opening within the partition (grey) separating the two areas allows animals access to the covered goal-box area from the open-field. This opening is blocked with obstructions that are increasingly difficult to remove as testing progresses. Animals undergo a five-day protocol, consisting of four obstruction conditions with three trials for each condition.

3. Running of the Puzzle Box Task

3.1) Thoroughly clean the Puzzle Box with 70% alcohol. Repeat this step between each animal tested.

3.2) Place a clean red mouse igloo within the goal-box area of the Puzzle Box, and replace the lid on the goal-box.

3.3) If there is an obstruction condition being tested, place the obstruction within the doorway of the goal-box (see Figure 3.2).

3.4) Place the mouse being tested into the open-field section of the Puzzle Box, oriented towards the goal-box, and at the end furthest from the goal-box.

3.5) Record the time taken for all four paws of the animal to enter the goal-box section of the Puzzle Box.

3.6) If an animal does not enter the goal-box, terminate the trial once the set time limit is reached (see Table 3.1).

3.7) Once a trial is finished, remove the animal from the Puzzle Box and place it into a separate holding cage until the next trial begins. Keep a 60 to 180 sec gap between trials for

each animal.

3.8) For each animal, perform three trials per day for five consecutive days of testing, with four obstruction conditions and three trials of each condition. The third trial of a given obstruction condition was always administered on a subsequent day (see Table 3.1).

Day	Condition	Trial	Obstruction	Time limit (seconds)
1	0	1	Open door with no obstructions	180
	1	1	Open channel within doorway	180
	1	2	Open channel within doorway	180
2	1	3	Open channel within doorway	180
	2	1	Channel filled with bedding	180
	2	2	Channel filled with bedding	180
3	2	3	Channel filled with bedding	180
	3	1	Tissue plug within doorway	240
	3	2	Tissue plug within doorway	240
4	3	3	Tissue plug within doorway	240
	4	1	Foam plug within doorway	240
	4	2	Foam plug within doorway	240
5	4	3	Foam plug within doorway	240

Table 3.1: Scheme of the Puzzle Box task

The Puzzle Box task is run for five days, and consists of four obstruction conditions. There are three trials on each of the first four days, and one on the fifth day. Each obstruction condition has three trials; the first two on one day, and the third the day immediately following. The first trial of an obstruction condition aims to test native problem solving ability, the second trial examines task acquisition and reinforcement, and the third trial is used as an assay for solution retention and recall.

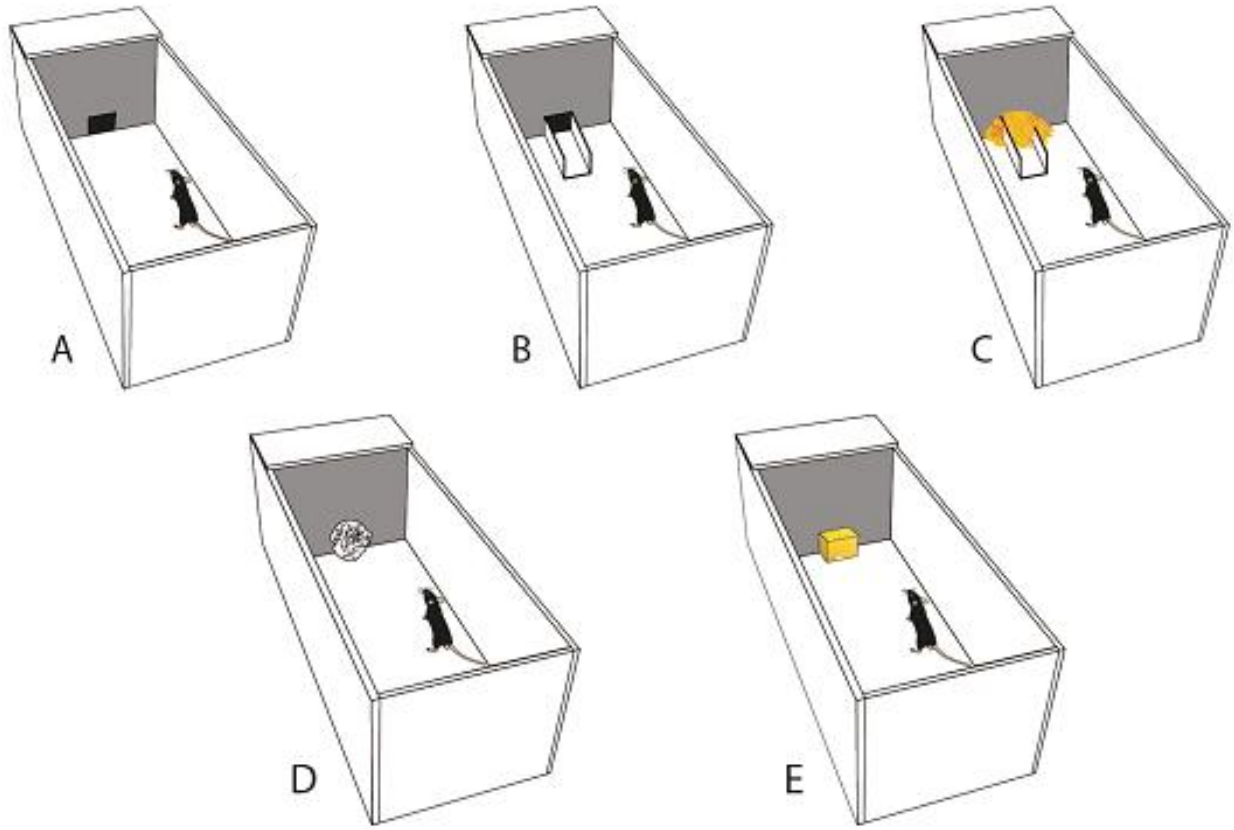

Figure 3.2: Obstruction conditions within the Puzzle Box task

Schematic diagrams of the Puzzle Box arena and the obstruction conditions used within this study. (A) Condition 0 (C0), with no obstruction present within the doorway between the open-field and goal-box areas. (B) Condition 1 (C1), with a “u shaped” chanenl present within the doorway between the open-field and goal-box areas. (C) Condition 2 (C2), where the channel is filled with clean bedding material. (D) Condition 3 (C3), with a “tissue plug” present within the doorway between the open-field and goal-box areas. (E) Condition 4 (C4) with a “foam plug” present within the doorway between the open-field and goal-box areas. Dimensions of the arena are as per listed in Methods and Figure 3.1.

4. Interpretation of data

4.1) Represent data as either time taken to complete the trial (including null trials where animals did not complete the task within the specified time limit) or as the number of null trials.

4.2) Use a repeated measures ANOVA to assess the effect of housing condition upon performance within the Puzzle Box, with obstruction type/condition (C) and task number (T) as within-subjects factors and enrichment level (standard versus enriched) as between-subjects factor.

REPRESENTATIVE RESULTS:

The results described here are a representative sample, with data taken from several cohorts consisting of different litters. All behavioral testing was conducted between 0700 and 1100 hours, with randomized testing order of animals within a cohort. Animals raised in an enriched environment (n=14, 7 female and 7 male) took significantly less time to solve the obstruction tasks within the Puzzle Box than those raised within a standard environment (n=15, 7 female and 8 male) (see Figure 3.3) (Repeated measures ANOVA with environment as between-subjects factor, $F=19.525$, $p<0.001$). This effect of EE on performance was observed within individual trials during the Puzzle Box, where enriched mice required significantly less time to solve each of the individual obstruction puzzles, and was particularly marked during the first trial of each condition (Univariate ANOVA with environment as between-subjects factor: Condition 1-Trial 1 (C1T1), $F=4.308$, $p=0.048$; C1T3, $F=4.317$, $p=0.047$; C2T1, $F=9.466$, $p=0.005$;

C2T2, F=5.164, p=0.031; C2T3, F=7.031, p=0.013; C3T1, F=19.979, p=0.000; C3T2, F=5.788, p=0.023; C3T3, F=4.711, p=0.039; C4T1, F=5.094, p=0.032). No effect of gender (repeated measures ANOVA with gender as between-subjects factor, F=1.827, p=0.188), nor any significant interaction between housing environment and gender was observed (repeated measures ANOVA with environment and gender as between-subjects factors, environment*sex, F=0.395, p=0.535).

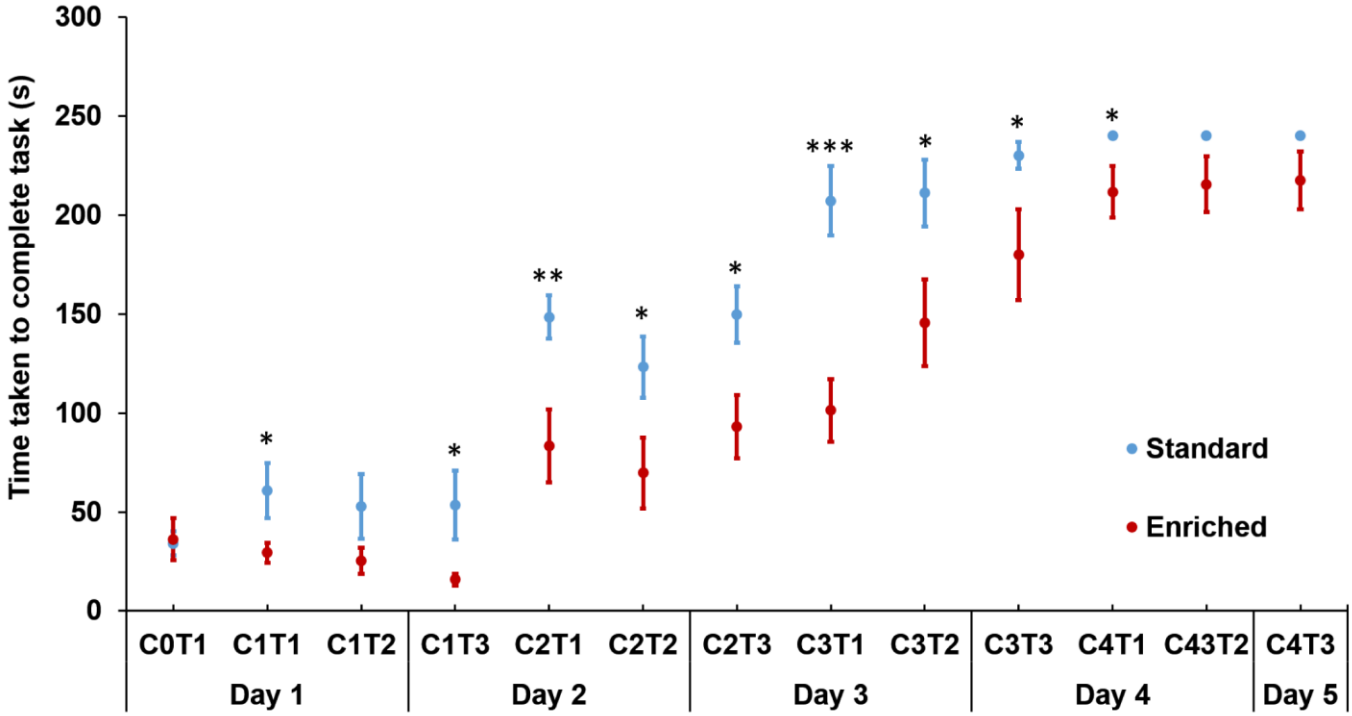

Figure 3.3 Environmentally enriched mice solve the Puzzle Box faster than standard housed animals.

Puzzle Box task performance of adult animals raised from birth in either enriched or standard environments, as measured by the time taken for all four paws to enter the goal-box area in seconds. Animals raised in an enriched environment (red) solved the obstruction tasks within the Puzzle Box significantly faster than those raised in a standard environment (blue) (repeated measures ANOVA with environment as between-subjects factor, $F=19.525$, $P<0.001$). Improved performance within individual obstruction puzzle tasks was observed for enriched cohorts (univariate ANOVA with environment as between-subjects factor: C1T1, $F=4.308$, $P=0.048$; C1T3, $F=4.317$, $P=0.047$; C2T1, $F=9.466$, $P=0.005$; C2T2, $F=5.164$, $P=0.031$; C2T3, $F=7.031$, $P=0.013$; C3T1, $F=19.979$, $P=0.000$; C3T2, $F=5.788$, $P=0.023$; C3T3, $F=4.711$, $P=0.039$; C4T1, $F=5.094$, $P=0.032$). C0: no obstruction; C1: U-shaped channel; C2: channel filled with bedding

material; C3: tissue plug; C4: foam plug. C1T1 refers to condition 1, trial 1 etc. (see text). Error bars: Standard error of the mean (SEM), enriched n=14 (7 female, 7 male), standard n=15 (7 female, 8 male). *: P<0.05, **: P<0.01, ***: P<0.001.

DISCUSSION:

The data presented demonstrate that the Puzzle Box can be used effectively to assess the impact of EE. Mice raised in enriched environments consistently took significantly less time to solve obstruction puzzles within this behavioral assay than did animals raised within standard laboratory conditions. Moreover, this difference was most prominent in the first trial for three of the four conditions tested, suggesting EE has a greater influence on an animal's native problem-solving ability, relative to their capacity to reinforce or retain solutions to the problems presented by the task.

The major advantages of the Puzzle Box are its inexpensive material cost, simplicity in terms of construction and implementation, as well as a lack of need for prior training of the subjects to be tested. Further, the method can be adapted to utilize a variety of obstruction materials and

conditions. For example, the protocol used here was adapted from previous studies that employed the Puzzle Box to assess cognitive ability in a variety of murine disease state models[5-7]. Other studies have previously utilized variations of the Puzzle Box to assess the impact of cognitive-enhancing antipsychotics[40] and observational learning within this testing arena[41]. The Puzzle Box therefore offers a behavioral task capable of assessing a wide variety of environmental, genetic and pharmacological manipulations, whilst being relatively time and cost effective.

This inherent flexibility, however, highlights the need for several key steps to successfully implement the task. As the method involves the physical removal of obstacles from a specific opening within the test arena, preliminary trials to determine which obstruction conditions are suitable and solvable within defined time limits by the animals to be assessed is critically important. This is particularly relevant when applying the task to determine the potential role of enrichment on animal models of neurodegenerative disorders whose motor abilities may be severely compromised[1-4,17,18]. Moreover, multiple trials across different time intervals are required to thoroughly assess the cognitive abilities of the subjects being tested. Although task acquisition and retention are related, they can be considered as separate processes[42-44]. As the findings in this study reveal, significant differences in performance can be greater within one of the factors assessed.

Although latency was the main metric used to monitor animal performance in this study, since the protocol includes video recording of all mice engaged in the task, it is also possible to

perform a more detailed analysis of behavior within the testing apparatus. The way subjects from enriched and standard raised groups behave within the arena, including the manner in which they approach obstacles at each phase of the task may reveal further, more subtle differences in performance between the two cohorts[45-50] Combined with the capacity to adjust obstacles to accommodate the animals being tested, the Puzzle Box has the potential to provide a rapid and straightforward means of gaining insight into the influence of environmental factors on a range of cognitive behaviors.

Although simple and powerful, the Puzzle Box is not a substitute for a more thorough analysis of cognitive function. Instead, it provides a rapid and reliable first-pass assessment of problem solving, as well as task acquisition and recall that should then be examined more thoroughly using conventional learning tasks. The importance of such a method cannot be overstated. Traditional learning tasks can require a considerable amount of familiarizing and training of subjects before they can yield interpretable results, which may itself impact upon performance[51,52]. Thus, an efficient and reliable method for obtaining a preliminary assessment of cognitive function that can be easily modified to the needs of individual experiments, such as the Puzzle Box, is highly advantageous.

Given that methods to alleviate and reverse the deleterious symptoms exhibited by transgenic models of disease states are being continually developed[53,54], a rapid and reliable means of assessing the effectiveness of the interventions from a behavioral perspective is critical. The data presented here suggests that this Puzzle Box is a useful tool that will enable such assessments.

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DISCLOSURES:

The authors declare they have no competing financial interests.

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Chapter 4:

Environmental Enrichment Improves Problem-Solving and Goal Seeking and Ameliorates the Effects of Striatal PNN Dissolution upon these Behaviours

Abstract

Although I have revealed that enrichment from birth can have a profound effect on the development of crucial elements within striatal circuitry, the degree to which lifelong enrichment can influence adult behaviour mediated by the striatum is not known. Further, whether the digestion of striatal perineuronal nets (PNNs), an important constituent of mature consolidated circuitry, affects functions regulated by this nucleus has yet to be determined. Accordingly, in the present study, I evaluated the effect of environmental enrichment upon performance of C57/BL6J mice within two behavioural tasks: the Puzzle-Box, assessing problem-solving and goal-orientated learning behaviours; and the rotarod, assessing sensorimotor coordination and motor learning. Animals raised in an enriched environment took significantly less time to solve obstacles in the Puzzle-Box, and showed minor improvement in the acquisition of a rotarod task, compared to standard housed counterparts. The potential involvement of the striatum within these behaviours was assessed by bilateral dissolution of perineuronal nets (PNNs) within the striatal extracellular matrix. Striatal PNN digestion impacted behaviours within the Puzzle-Box, but had little effect upon behavioural performance during the rotarod task. Environmental enrichment protected against the full behavioural effects of striatal PNN dissolution. These results provide evidence that the striatum is directly involved in problem-solving and goal-orientated learning behaviours within the Puzzle-Box, and that environmental enrichment is capable of reducing behavioural changes induced by dissolution of PNNs within the striatum.

Keywords: Environmental Enrichment; Striatum; Behaviour; PNN

1. Introduction

Environmental enrichment (EE) is known to have a range of anatomical, physiological and molecular effects on the brain. Exposure to EE has been shown to increase cortical thickness and brain weight [1-3], angiogenesis [4], synaptic density [5], and branching and spine density of dendrites [6-11]. EE is capable of accelerating the physiological maturation of the visual system [12-14], and modulating the levels of several growth factors during early development [12-19]. EE also impacts the behaviour of animals. Exposure to EE reduces the occurrence of anxiety-related behaviours [11, 19-21]; improves performance in cognitive tasks [15, 20, 22, 23]; and accelerates the development of both sensory [12, 24] and motor behaviours [22] within juvenile animals.

Our laboratory has previously revealed that enrichment from birth leads to the precocious maturation of circuit elements within the striatum, the input nucleus of the basal ganglia [25] and a key regulator of sensorimotor coordination [26, 27] and cognitive behaviours [28-31]. The formation of striatal perineuronal nets (PNNs), extracellular matrix structures composed of chondroitin sulphate proteoglycans (CSPGs), tenascin-R (TN-R) and hyaluronan (HA), with link proteins [32-34], is accelerated in animals raised within enriched environments [22]. These structures are thought to play a role in consolidating neural circuitry during development, by contributing to the formation of stable, mature synapses between neurons [35-37]. The formation of PNNs is associated with the maturation of GABA-ergic inhibitory interneurons [38-41], a key step in the timing of developmental critical periods [18, 24, 36, 42-45]. Previous work has shown that enrichment can influence the onset of these important developmental epochs [12, 18, 24, 42], and I have now revealed that the maturation of striatal GABA-ergic Parvalbumin-positive inhibitory interneurons, much like PNNs, is accelerated by exposure to EE (see Chapter 2: Environmental Enrichment from Birth Accelerates the Maturation of Parvalbumin Expressing Neurons within the Striatum of the Mouse).

Although the onset of PNN formation is affected by enrichment, the density of these structures in adult striatum does not differ between standard and enriched cohorts (see Chapter 2:

Environmental Enrichment from Birth Accelerates the Maturation of Parvalbumin Expressing Neurons within the Striatum of the Mouse). Curiously, in a mouse model of amblyopia, animals housed in enriched environments as adults have been shown to exhibit a reduction in PNN density within the visual cortex [46]. PNNs within the mature nervous system can also be removed by the application of an enzyme, Chondroitinase ABC (ChABC) [36, 37, 47-49], which reduces PNNs to their constituent molecules [36, 50]. Digestion of PNNs within adult visual cortex reactivates the cortical critical period [36, 37]. Together, these findings suggest that these extracellular matrix structures are at least partially responsible for regulating experience-dependent plasticity.

The characterisation of the manner in which EE influences behaviours dependent on striatal function is in its infancy. Although previous work has revealed a correlation between enrichment from birth and the emergence of motor coordination and exploratory behaviour [22], less is known about how EE influences striatum regulated behaviours in mature animals. Early enrichment can have a lasting influence on cognitive function, as measured by performance on the Morris Water Maze [22], although striatal circuits are considered to play only a minor role in the performance of this task [41]. The manner in which plasticity induced by EE influences striatum specific behaviour has yet to be determined.

Moreover, while the enzymatic removal of PNNs in visual cortex has been shown to re-establish high levels of activity dependent plasticity [36], how similar manipulations will influence the function of striatal circuits is not clear. Although previous work has shown that ChABC injections within the striatum can affect hind limb gait (a measure of motor coordination) [41], a more comprehensive analysis of the influence of PNN digestion upon striatally-mediated behaviours has not yet been done.

Further, while the decrease observed in PNN density due to both EE in amblyopic mice [46] and ChABC treatment [36] are interpreted as a reflection of an increase in network plasticity, it has yet to be determined whether these common changes are regulated by the same mechanisms. Whether

PNN digestion via ChABC injections will compliment or antagonise enrichment-dependent plasticity is not known.

Accordingly, in order to address these issues, I first examined whether EE from birth yields behavioural changes in adult animals performing two tasks designed to assess different aspects of striatal function: the Puzzle-Box [51-53] to examine goal-directed activity (see Chapter 3: The Use of the Puzzle Box as a Means of Assessing the Efficacy of Environmental Enrichment, O'Connor et al 2014, in press); and a modified version of the rotarod task to assess motor skill acquisition. I then determined the impact of striatal ChABC injections on the performance of both standard and enriched housed animals in these tasks. Finally, in order to assess how the effects of enrichment and ChABC interact, I compared task performances of treated and untreated, standard and enriched cohorts. I show that (1) exposure to EE results in decreased performance latencies in the Puzzle-Box task, (2) ChABC treatment increases latencies compared to vehicle controls in standard cohorts in the early stages of Puzzle-Box testing, and (3) ChABC treatment affects standard and enriched cohorts differently. Together, these findings suggest that the plasticity effects induced by EE and ChABC treatment are manifested at potentially different levels of striatal processing for enriched and standard mice.

2. Materials & Methods

2.1 Ethics Statement

All procedures were approved by the Animal Ethics Committee (AEC) of the University of Sydney (AEC protocols: K22/09-09/3/5128 and K22/11-12/3/5838) and conformed to National Health and Medical Research Council of Australia guidelines. Procedures were performed using C57/BL6J mice reared and housed at the University of Sydney/Bosch Institute animal house facility. All mice were

housed in individually ventilated cages at 21°C ambient temperature with *ad libitum* access to both food and water.

2.2 Housing of animals in standard and enriched environments

On arrival, half of the pregnant dams were randomly assigned to standard (30cm x 15cm x 13cm cage), and the other half to enriched cages (46cm x 40cm x 40cm cage) [22]. Standard (S) cages contained a translucent, red plastic mouse "igloo"/shelter and extra material for bedding. In addition to these materials, enriched (E) cages contained extra objects to encourage motor and sensory stimulation including a running wheel, marbles, tunnels, Velcro strips, scented plush balls (vanilla, strawberry and cinnamon), a rubber ball and two high-contrast visual stimuli. These items were moved around the cage every two to three days and refreshed once a month to maintain novelty. The enriched cage contained two dams and litters to enable greater social interaction between animals.

Young animals were weaned at 21 days postnatal (P21) and placed into male-female segregated cages with the same housing conditions into which they were born. Surgeries and behavioural testing were carried out once animals reached 12 weeks of age. All measurements were imported into SPSS (SPSS Inc., Chicago, IL, USA) for statistical analysis.

2.3 Surgeries

Animals within each cage were divided into approximately equal groups. Subjects from both standard (S) and enriched (E) cages received either bilateral Chondroitinase ABC injections (ChABC: SC n=8; EC n=13), or 0.9% saline vehicle injections within the striatum (SV n=7; EV n=13). Animals were anaesthetised with 2-4% isoflurane in oxygen and secured within a stereotaxic frame (Kopf Instruments, Tujunga, CA, USA). Bilateral craniotomies were performed at Anterior-Posterior 0

(Bregma) and Medial-Lateral ± 2.2mm [54], and either ChABC (protease free, 15 U/ml in 0.9% saline, Sigma, St Louis, MO, USA) or vehicle pressure injected into the striatum at a depth of 2.5mm from dura using a PicoPump (WPI, Sarasota, FL, USA). Following treatment, animals were returned to their home cages and allowed to recover for three days. During this time, water and food were available *ad libitum*.

2.4 Puzzle-Box behaviour

All animals (standard, S n=18; enriched, E n=21; vehicle, SV n=7, EV n=13; and ChABC treated, SC n=8, EC, n=13) underwent Puzzle-Box behavioural testing for five consecutive days. The protocol used within this study is a modified version of that previously published by other groups [51-53].

The Puzzle-Box arena consists of a white acrylic board box (750mm x 280cm x 250mm) split into two compartments: a brightly lit open-field start zone (600mm x 280mm x 250mm) and a dark goal-box zone (150mm x 280mm x 250mm) containing a red mouse igloo, divided by an opaque barrier with a single (40mm x 40mm) aperture or "entrance" abutting the floor (Fig. 4.1A). Animals were placed into the open-field portion of the Puzzle-Box, opposite the entrance. The subjects' task was to make their way through the entrance in order to escape the open-field. Animals were challenged with increasingly difficult to remove "obstruction conditions" placed within the entrance to the goal-box portion of the Puzzle-Box. Individual mice were subjected to a total of three trials of each obstruction condition, two on one day and one on the next, for a total of three trials per day (Table 4.1). This design allowed for the potential testing of three cognitive processes: the first trial provided an indication of inherent problem-solving ability; the second trial, a measure of the animal's ability to reinforce strategies for removing specific obstructions; and the third trial provided insight into the subject's ability to retain and recall the learned solution to a task over a longer period of time [51-53]. Mice were exposed to a total of five conditions: open passage in which no blockage was present; open passage with a "U" shaped channel effectively limiting passage size; channel filled

with bedding; passage blocked with a tissue plug; and passage blocked with a foam plug (Table 4.1). Performance was determined by the time taken for all four paws of an animal to enter the goal-box zone.

Video recordings of the Puzzle-Box behavioural task were made, and analysed using TopScan software (CleverSys Inc, Reston, VA, USA). The open-field start zone was divided into three areas: “wall”, an area 50mm wide flush to the walls of the arena; “obstruction”, centred upon and extending 25mm beyond the obstruction puzzle; and the “centre”, designated as the remainder of the open-field start zone (Fig. 4.1B). Individual animals were tracked throughout each trial, and the distance travelled and percentage of total time spent within each defined area was determined. A repeated measures ANOVA was used to assess performance, using condition and trial number as within-subjects factors, and housing condition and surgical treatment as between-subjects factors. A univariate ANOVA was also used to assess performance within individual trials, using housing condition and surgical treatment as between-subjects factors.

Day	Condition	Trial	Obstruction	Time limit (seconds)
1	0	1	Open doorway with no obstructions	180
	1	1	Open channel within doorway	180
	1	2	Open channel within doorway	180
2	1	3	Open channel within doorway	180
	2	1	Channel filled with bedding	180
	2	2	Channel filled with bedding	180
3	2	3	Channel filled with bedding	180
	3	1	Tissue plug within doorway	240
	3	2	Tissue plug within doorway	240
4	3	3	Tissue plug within doorway	240
	4	1	Foam plug within doorway	240
	4	2	Foam plug within doorway	240
5	4	3	Foam plug within doorway	240

Table 4.1 Schema outlining the Puzzle-Box Behavioural Task.

The Puzzle-Box task is run for five days, and consists of four obstruction conditions. There are three trials on each of the first four days, and one on the fifth day. Each obstruction condition has three trials; the first two on one day, and the third the day immediately following. The first trial of an obstruction condition aims to test native problem-solving ability, the second trial examines task acquisition and reinforcement, and the third trial is used as an assay for solution retention and recall [51-53].

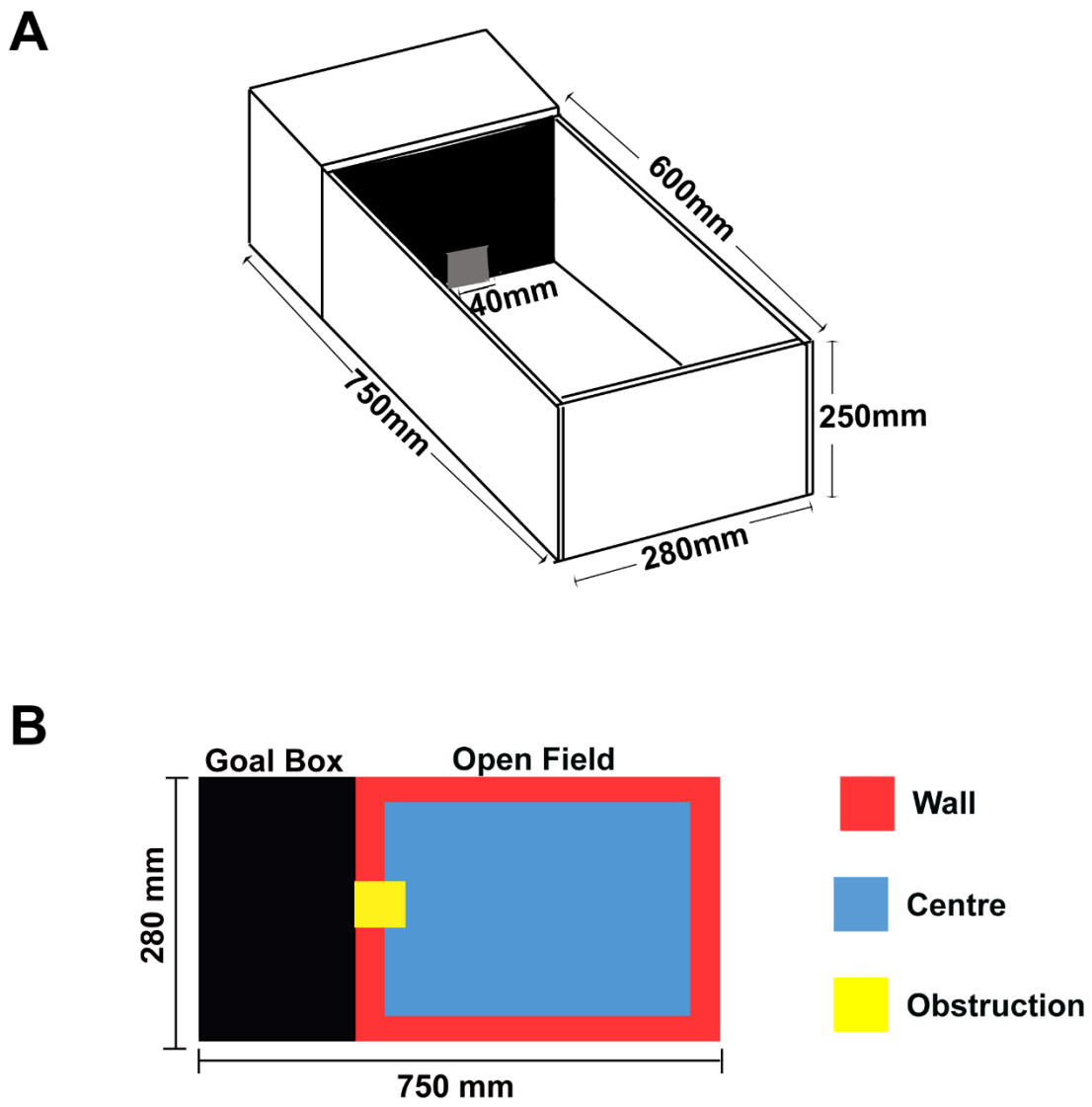


Figure 4.1: Schematic of the Puzzle-Box.

(A – B) A diagram of the Puzzle-Box behavioural task arena. (A) A white acrylic box, measuring 750 x 280mm, and split into two by a black dividing partition into which a door is cut. The start zone consists of an open field 600mm long x 280mm wide, and the goal box consists of a covered section 150mm long and 280mm wide, with a 40 x 40mm door cut into the separating partition between the two. (B) For video analysis of animal behaviour, the open field start zone was split into three areas: a "wall" section running flush to the arena wall and 50mm wide; an "obstruction" section surrounding the obstruction puzzle within the doorway; with the remainder of the open field designated as a "centre" section.

2.5 Rotarod behaviour

Animals underwent rotarod behavioural testing for five consecutive days. Non-surgery animals were tested as a control (S n=11, E n=13) to determine any effects of EE upon the performance of this behavioural task. Each day, animals were placed onto the Rotarod (IITC Inc., Woodland Hills, CA, USA) for five minutes of habituation prior to undertaking five runs of three minutes duration, starting at the lowest speed (1RPM) and ramping up to the highest speed (45RPM) at the three minute mark. Five animals were tested at a time, and runs were started when all animals were orientated in the same direction. The time and distance run before an animal fell off the apparatus were measured and recorded. A repeated measures ANOVA was used to compare performance of the daily sum of time spent upon the rotor, with testing day as within-subjects factor, and housing condition and surgery treatment as between-subjects factors.

2.6 Immunohistochemical staining for PNNs

Animals were euthanized on the final day of behavioural testing with intraperitoneal administration of >100mg/kg of sodium pentobarbitone, and transcardially perfused using 0.9% saline followed by 4% paraformaldehyde in 0.1M phosphate buffer (PB). The brain was dissected out, post-fixed overnight in 4% paraformaldehyde in 0.1M PB, and cryoprotected in 30% sucrose in 0.1M PB.

Brains were then embedded in gelatin-albumin hardened by 25% glutaraldehyde in 0.1M PB, and sectioned coronally at 60μm on a freezing microtome. Tissue was collected in such a way that sections within the same well were always at least 360μm apart from one another within the brain.

Staining for perineuronal nets (PNNs) was adapted from Lee et al, 2008 & 2012 with some slight modifications [41, 50]. Sections were labelled for CSPGs using Wisteria floribunda agglutinin (WFA), a plant lectin used to visualize CSPGs, as described previously [22, 41, 50]. Briefly, sections were washed in 0.1M PB prior to immunohistochemical procedures, then quenched in a mixture of 0.3%

hydrogen peroxide in 0.1M PB. After washing in 0.1M PB, sections were incubated overnight at 4°C in biotinylated WFA (Vector Laboratories, Burlingame, CA, USA) (10μg/ml). Bound WFA was visualized using a fluorescein-conjugated TSA kit (Perkin Elmer, Waltham, MA, USA), then washed several times in 0.1M PB and mounted onto gelatin coated slides with 50:50 glycerol:0.1M PB and 1:1000 DAPI.

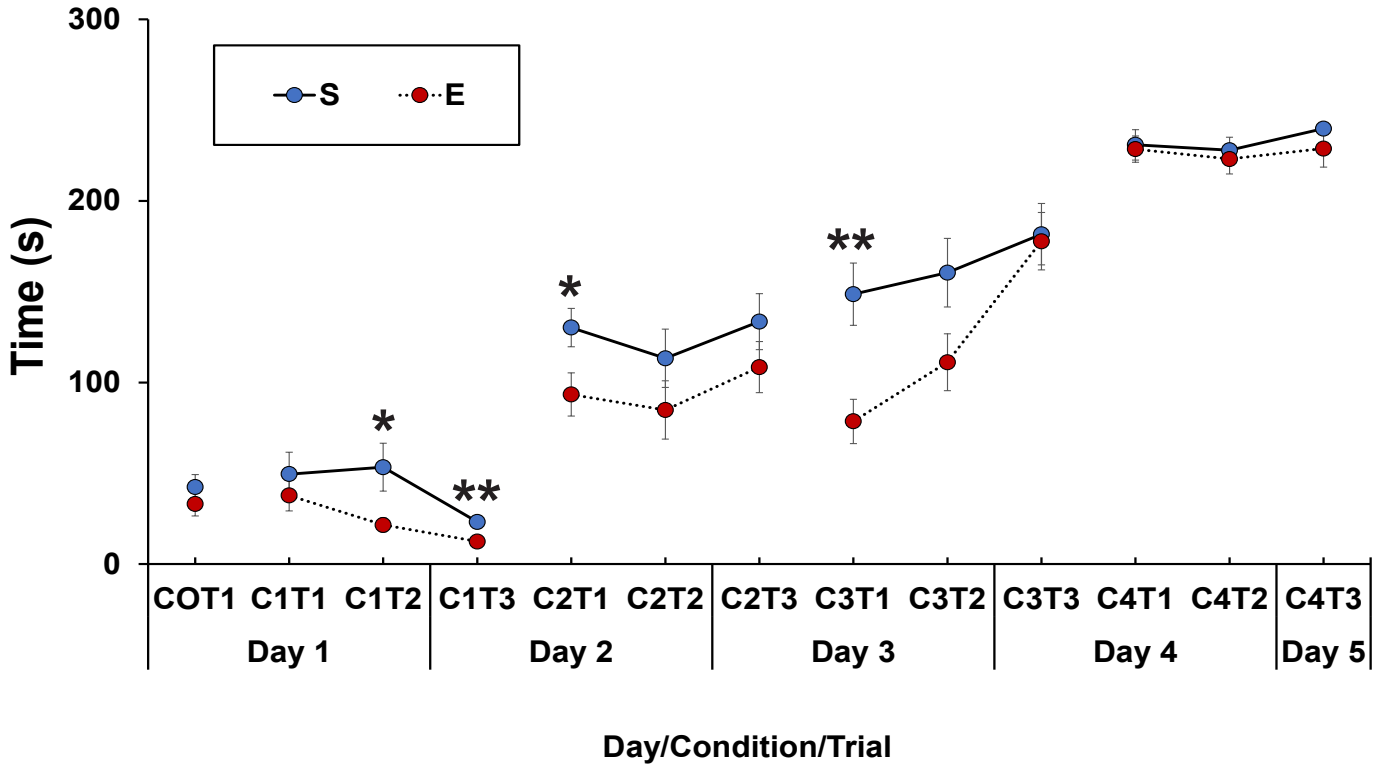
Sections were digitally imaged using a Zeiss deconvolution microscope with AxioCamHR camera and Axiovision software (Carl Zeiss Microscopy GmbH, Jena, Germany). Images were photomerged to produce a mosaic of the striatum, PNNs were manually marked (Photoshop, Adobe Systems Inc., San Jose, CA, USA), and density measurements obtained. A univariate ANOVA with housing condition as between-subjects factor was used to compare the density of PNNs across ChABC-treated experimental groups.

3. Results

3.1 Environmental enrichment from birth affects performance within the Puzzle-Box task

The striatum plays an important role in goal directed behaviours [28, 41, 55]. Although enriched mice have been shown to exhibit improved performance during the Morris Water Maze [15, 20, 22, 23, 56] compared to standard housed cohorts, the contribution of striatal function to this task is difficult to assess given the predominantly spatial, and thus hippocampus-dependent, nature of the task [56, 57]. In order to determine whether housing condition is capable of affecting performance on a goal seeking task that is independent of spatial acquisition, mice raised in either enriched (E, n=21) or standard (S, n=18) environments from birth were tested at 12 – 14 weeks of age on their performance in the Puzzle-Box behavioural task, a goal-seeking paradigm designed to test problem-solving capabilities by presenting incrementally difficult obstacles over five consecutive days.

I found that housing condition significantly affected the time taken to solve the obstruction puzzle tasks within the Puzzle-Box (Fig. 4.2). Specifically, animals that were raised within an enriched environment had significantly lower latencies to enter the goal-box when compared to mice raised within a standard environment (repeated measures ANOVA, housing condition as between-subjects factor, E vs S, $F(1, 37)=7.806$, $P=0.008$). This effect of housing condition was particularly notable in several individual trials within the task: enriched animals displayed significantly shorter latencies during the last two trials of the first open channel obstruction (univariate ANOVA, housing condition as between-subjects factor, E vs S: C1T2, $F(1, 37)=6.092$, $P=0.018$; C1T3, $F(1, 37)=7.848$, $P=0.008$); and during the first trial of the more difficult filled channel and tissue plug obstructions (univariate ANOVA, housing condition as between-subjects factor, E vs S: C2T1, $F(1, 37)=5.175$, $P=0.029$; C3T1, $F(1, 37)=11.547$, $P=0.002$) (Fig. 4.2). These findings indicate that EE appeared to have the greatest influence on the first exposure to a given obstruction condition.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COT1
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C4T1
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Day 1
Day 2
Day 3
Day 4
Day 5
Day/Condition/Trial

Figure 4.2: Enrichment decreases time taken to enter the goal-box zone of the Puzzle-Box.

Graph plotting the average time taken (s) for all four paws of an animal to enter the goal-box zone of the Puzzle-Box, upon which time a behavioural task was terminated, for mice raised from birth in enriched (E) and standard (S) housing. Obstruction conditions (C) 1 and 2 have an upper time limit of 180 seconds, and conditions 3 and 4 have an upper time limit of 240 seconds. Adult animals raised within an enriched environment (E) take significantly less time to enter the Goal Box than do those raised within a standard environment (S) (repeated measures ANOVA, $F=7.806$, $P=0.008$). This was particularly evident in several individual trials (T) within the task: C1T2 (univariate ANOVA, $F=6.092$, $P=0.018$); C1T3 (univariate ANOVA, $F=7.848$, $P=0.008$); C2T1 (univariate ANOVA, $F=5.175$, $P=0.029$); C3T1 (univariate ANOVA, $F=11.547$, $P=0.002$). *: $P<0.05$, **: $P<0.01$. E n=21, S n=18. Error bars = Standard Error of the Mean (SEM).

Latency to enter the goal-box may differ for a number of reasons, including anxiety, motivation or cognitive abilities [51-53]. Assessing the time taken to solve obstruction puzzles within the Puzzle-Box gives a metric of performance, but does not reveal complex behaviours that may be induced by exposure to a novel testing environment. To determine the means by which EE improved performance in the Puzzle-Box task, video recordings of this behavioural task were made and analysed. The open-field start zone was divided into three areas (Fig. 4.1B, "wall", "obstruction" and "centre", see Materials and Methods 2.4 Puzzle-Box Behaviour) and the distance travelled and percentage of total time spent in each of these areas analysed. I found that housing condition affected the way in which animals behaved within the Puzzle-Box (Fig. 4.3, 4.4). There were varying degrees of impact upon the distance travelled and proportion of time spent within the sections of the open-field start zone.

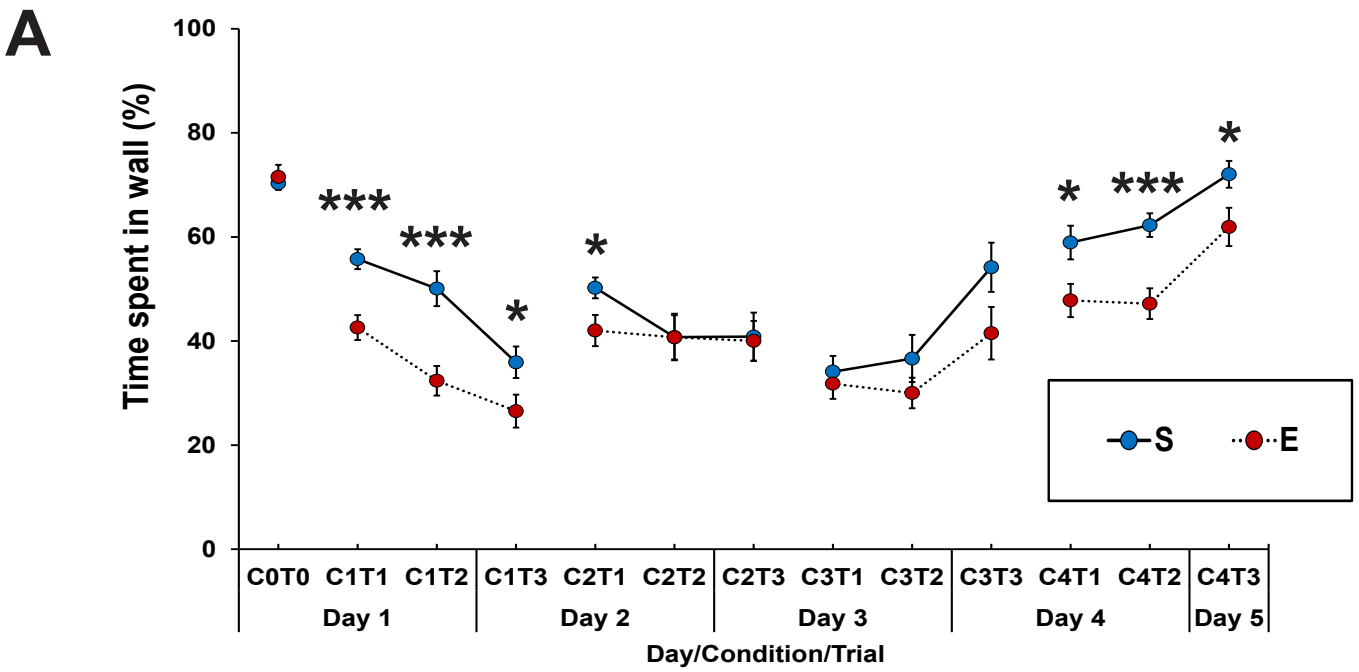
Quantitative analysis of the effect of housing condition revealed that animals raised within an enriched environment spent a significantly lesser proportion of time within the "wall" section of the open-field (repeated measures ANOVA, housing condition as between-subjects factor: E vs S, $F(1, 37)=15.306$, $P<0.001$) (Fig. 4.3A) and a significantly greater proportion of time within the "obstruction" section of the open-field (repeated measures ANOVA, housing condition as between-subjects factor: E vs S, $F(1, 37)=27.340$, $P<0.001$) (Fig. 4.3C) than did animals raised within standard laboratory housing.

The effect of housing condition was particularly evident at certain trials within the task: standard animals spent a significantly greater proportion of time within the "wall" during the first, open channel obstruction (univariate ANOVA, housing condition as between-subjects factor, E vs S: C1T1, $F(1, 37)=17.588$, $P<0.001$, C1T2, $F(1, 37)=16.326$, $P<0.001$; C1T3, $F(1, 37)=4.757$, $P=0.036$); during the first exposure to the more difficult filled channel obstruction (univariate ANOVA, housing condition as between-subjects factor, E vs S: C2T1 $F(1, 37)=4.857$, $P=0.034$); and during all trials of the most difficult foam plug obstruction (univariate ANOVA, housing condition as between-subjects factor, E

vs S: C4T1F(1, 37)=5.910, P=0.020; C4T2, F(1, 37)=15.564, P<0.001: C4T3, F(1, 37)=4.758, P=0.036) (Fig. 4.3A).

Although no overall significant differences were detected between housing groups when comparing the proportion of time spent in the centre zone (repeated measures ANOVA, F(1, 37)=2.074, P=0.159), a trial by trial analysis revealed that standard animals spend a greater proportion of time in the "centre" on the first trial of the open channel obstruction (univariate ANOVA, housing condition as between-subjects factor, E vs S: C1T1, F(1, 37)=7.143, P=0.011), as well as the first and third trials of the more difficult filled channel obstruction (univariate ANOVA, housing condition as between-subjects factor, E vs S: C2T1, F(1, 37)=9.272, P=0.004; C2T3, F(1, 37)=6.866, P=0.013) (Fig. 4.3B).

In turn, enriched animals spent a greater proportion of time within the "obstruction" during the first, open channel obstruction (univariate ANOVA, housing condition as between-subjects factor, E vs S: C1T1: F(1, 37)=24.144, P<0.001; C1T2, F(1, 37)=26.877, P<0.001); when first encountering the filled channel obstruction (univariate ANOVA, housing condition as between-subjects factor, E vs S, C2T1, F(1, 37)=9.952, P=0.003; C2T3, F(1, 37)=8.409, P=0.006); throughout the duration of the more difficult tissue plug obstruction (univariate ANOVA, housing condition as between-subjects factor, E vs S: C3T1 F(1, 37)=5.614, P=0.023; C3T2, F(1, 37)=4.683, P=0.037; C3T3, F(1, 37)=4.653, P=0.038); and upon first exposure to the most difficult foam plug (univariate ANOVA, housing condition as between-subjects factor, E vs S: C4T1, F(1, 37)=9.014, P=0.005; C4T3, F(1, 37)=4.280, P=0.046) (Fig. 4.3C).


A
Time spent in wall (%)
100
80
60
40
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0
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*
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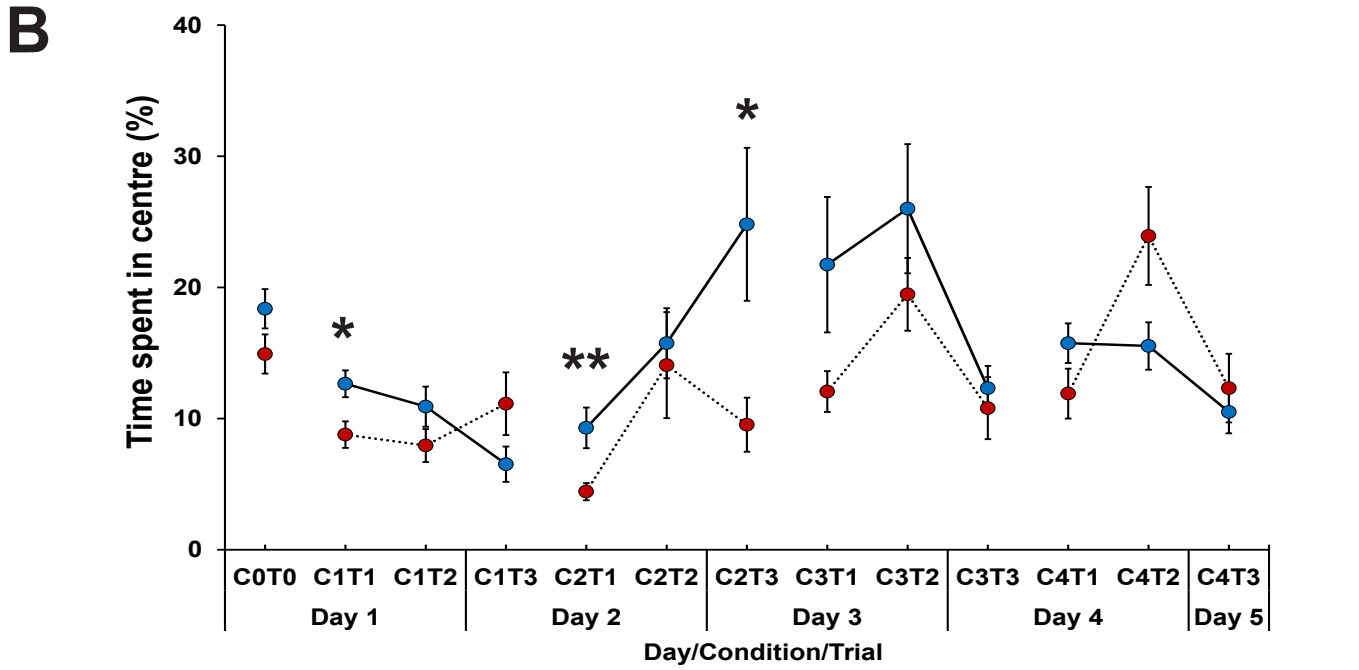
S
E
C0T0 C1T1 C1T2 C1T3 C2T1 C2T2 C2T3 C3T1 C3T2 C3T3 C4T1 C4T2 C4T3
Day 1
Day 2
Day 3
Day 4
Day 5
Day/Condition/Trial


B
Time spent in centre (%)
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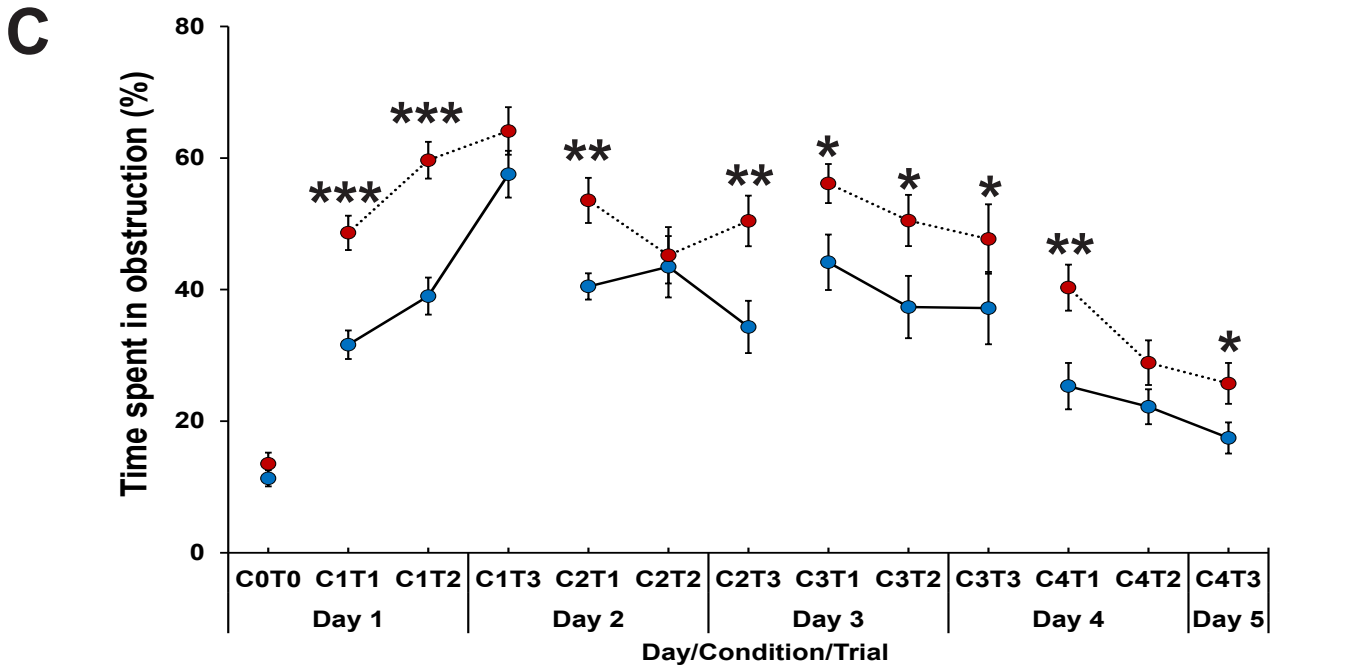
30
20
10
0
*
**
*
C0T0 C1T1 C1T2 C1T3 C2T1 C2T2 C2T3 C3T1 C3T2 C3T3 C4T1 C4T2 C4T3
Day 1
Day 2
Day 3
Day 4
Day 5
Day/Condition/Trial


C
Time spent in obstruction (%)
80
60
40
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0
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C0T0 C1T1 C1T2 C1T3 C2T1 C2T2 C2T3 C3T1 C3T2 C3T3 C4T1 C4T2 C4T3
Day 1
Day 2
Day 3
Day 4
Day 5
Day/Condition/Trial

Figure 4.3: Enriched animals spend less time in the wall zone and more time in the obstruction zone.

(A – C) Graphs plotting the average percentage of total time spent within the Puzzle-Box where an animal was within specified areas in the open-field start zone for mice raised from birth in enriched (E) and standard (S) housing. (A) Enriched animals spent a significantly lesser proportion of time within the "wall" zone than standard mice (repeated measures ANOVA, $F=15.306$, $P<0.001$), particularly at C1T1 (univariate ANOVA, $F=17.588$, $P<0.001$); C1T2 (univariate ANOVA, $F=16.326$, $P<0.001$); C1T3 (univariate ANOVA, $F=4.757$, $P=0.036$); C2T1 (univariate ANOVA, $F=4.857$, $P=0.034$); C4T1 (univariate ANOVA, $F=5.910$, $P=0.020$); C4T2 (univariate ANOVA, $F=15.564$, $P<0.001$); and C4T3 (univariate ANOVA, $F=4.758$, $P=0.036$). (B) There was no overall significant effect of housing condition on the proportion of time spent within the "centre" zone (repeated measures ANOVA, $F=2.074$, $P=0.159$). There were significant differences in some individual trials where enriched animals spent a lesser proportion of time within the "centre" zone than standard mice: C1T1 (univariate ANOVA, $F=7.143$, $P=0.011$); C2T1 (univariate ANOVA, $F=9.272$, $P=0.004$); and C2T3 (univariate ANOVA, $F=6.866$, $P=0.013$). (C) Enriched animals spent a significantly greater proportion of time within the "obstruction" zone than standard mice (repeated measures ANOVA, $F=27.340$, $P<0.001$), which again was particularly evident in certain trials: C1T1 (univariate ANOVA, $F=24.144$, $P<0.001$); C1T2 (univariate ANOVA, $F=26.877$, $P<0.001$); C2T1 (univariate ANOVA, $F=9.952$, $P=0.003$); C2T3 (univariate ANOVA, $F=8.409$, $P=0.006$); C3T1 (univariate ANOVA, $F=5.614$, $P=0.023$); C3T2 (univariate ANOVA, $F=4.683$, $P=0.037$); C3T3 (univariate ANOVA, $F=4.653$, $P=0.038$); C4T1 (univariate ANOVA, $F=9.014$, $P=0.005$); C4T3 (univariate ANOVA, $F=4.280$, $P=0.046$). *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$. E n=21, S n=18. Error bars = SEM.

When considering distances travelled within the open arena, quantitative analysis of the effect of housing condition upon distance travelled within the open-field portion of the Puzzle-Box revealed that animals raised within an enriched environment traversed a significantly lesser extent of the "wall" (repeated measures ANOVA, housing condition as between-subjects factor: E vs S, $F(1, 37)=72.492$, $P<0.001$) (Fig. 4.4A) and "centre" (repeated measures ANOVA, housing condition as between-subjects factor: E vs S, $F(1, 37)=20.723$, $P<0.001$) (Fig. 4.4B) sections of the open-field compared to standard-housed cohorts. Curiously, enriched animals also covered less distance than standard mice in the "obstruction" zone (repeated measures ANOVA, housing condition as between-subjects factor: E vs S, $F(1, 37)=9.175$, $P=0.004$) (Fig. 4.4C).

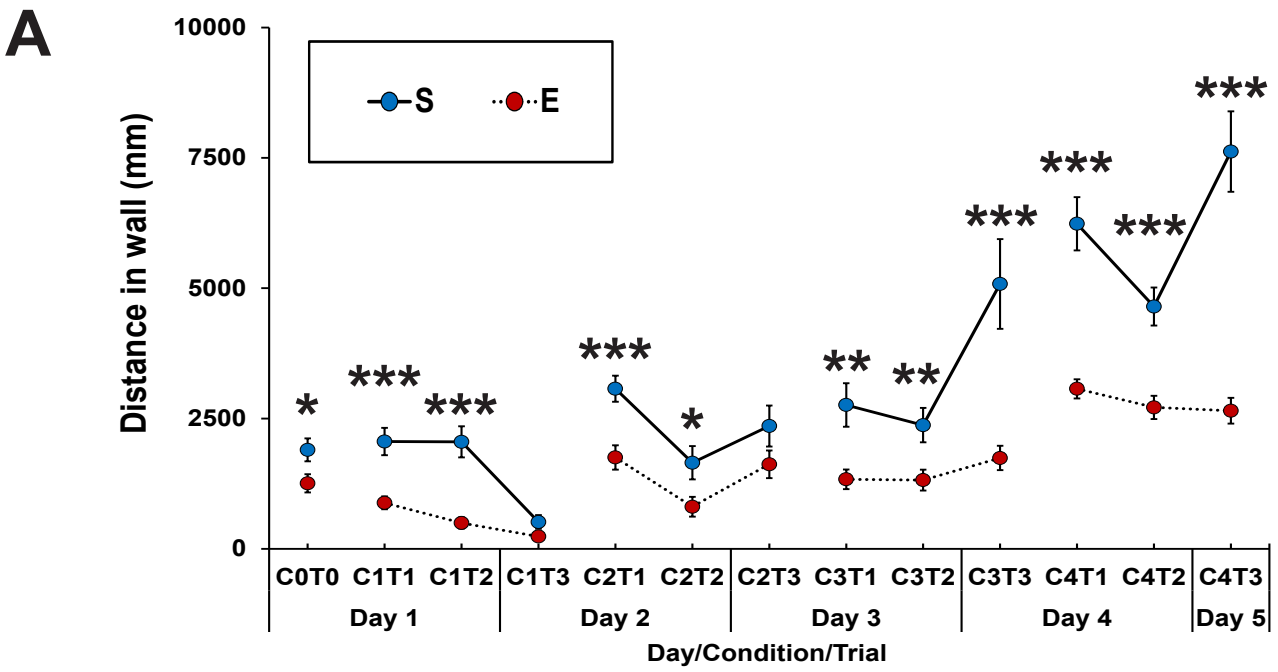
The effect of housing condition was notable in nearly all individual trials during testing. Enriched animals travelled significantly less distance within the "wall" than standard animals upon first exposure to the Puzzle-Box (univariate ANOVA, housing condition as between-subjects factor, E vs S: C0T1, $F(1, 37)=5.385$, $P=0.026$); during the first two trials of the empty and filled channel obstructions (univariate ANOVA, housing condition as between-subjects factor, E vs S: C1T1, $F(1, 37)=18.002$, $P<0.001$; C1T2, $F(1, 37)=27.119$, $P<0.001$; C2T1, $F(1, 37)=14.777$, $P<0.001$; C2T2, $F(1, 37)=5.539$, $P=0.024$); and throughout the duration of the more difficult tissue plug obstruction (univariate ANOVA, housing condition as between-subjects factor, E vs S: C3T1, $F(1, 37)=10.687$, $P=0.002$; C3T2 $F(1, 37)=7.959$, $P=0.008$; C3T3, $F(1, 37)=16.031$, $P<0.001$); and most difficult foam plug obstruction (univariate ANOVA, housing condition as between-subjects factor, E vs S: C4T1, $F(1, 37)=38.187$, $P<0.001$; C4T2, $F(1, 37)=21.822$, $P<0.001$; C4T3 $F(1, 37)=42.736$, $P<0.001$) (Fig. 4.4A).

Similarly, enriched animals traversed less distance within the "centre" than standard animals in particular trials: specifically, upon initial exposure to the Puzzle-Box (univariate ANOVA, housing condition as between-subjects factor, E vs S: C0T1, $F(1, 37)=8.736$, $P=0.005$); during the first two trials of the empty and first trial of the filled channel obstructions (univariate ANOVA, housing condition as between-subjects factor, E vs S: C1T1, $F(1, 37)=16.702$, $P<0.001$; C1T2, $F(1, 37)=15.363$,

P<0.001; C2T1, F(1, 37)=10.958, P=0.002); throughout the duration of the more difficult tissue plug obstruction (univariate ANOVA, housing condition as between-subjects factor, E vs S: C3T1, F(1, 37)=10.798, P=0.002; C3T2, F(1, 37)=5.580, P=0.024; C3T3, F(1, 37)=5.044, P=0.031) and in the first trial of the most difficult foam plug obstruction (univariate ANOVA, housing condition as between-subjects factor, E vs S: C4T1, F(1, 37)=14.100, P=0.001) (Fig. 4.4B).

The effect of housing condition upon distance travelled within the "obstruction" zone was also particularly notable, with S mice traversing further within this region upon first exposure to the Puzzle-Box apparatus (univariate ANOVA, housing condition as between-subjects factor, E vs S: C0T1, F(1, 37)=9.344, P=0.004); during the last trial of the open channel obstruction (univariate ANOVA, housing condition as between-subjects factor, E vs S: C1T3, F(1, 37)=9.832, P=0.003) and in the first trials of the more difficult filled channel and tissue plug obstructions (univariate ANOVA, housing condition as between-subjects factor, E vs S: C2T1, F(1, 37)=5.205, P=0.028; C3T1, F(1, 37)=6.329, P=0.016) (Fig. 4.4C).

Together, these results suggest that enriched animals solve obstruction puzzles and reach the goal/shelter more rapidly than standard cohorts. Performance differences were detectable in all but the most difficult obstruction condition. Curiously, no latency differences between E and S groups were observed in the initial habituation stage. Area analyses revealed that overall latency differences were due to S animals spending more time and traversing greater distances within the "wall" and "centre" zones. E mice consistently spent a greater proportion of time in the "obstruction" area. Trial by trial analyses did not reveal any dramatic trends, although performance differences appeared greatest during the first presentation of novel obstructions.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E
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Day 1
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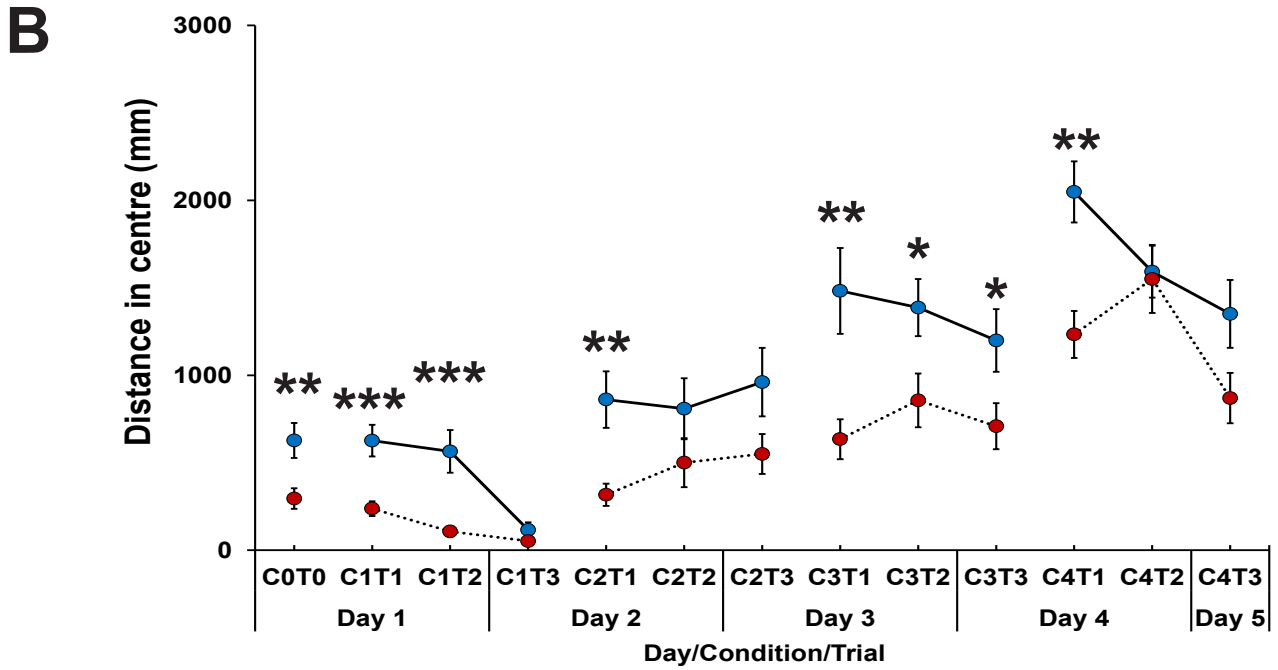
Day 5
Day/Condition/Trial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Day 1
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Day 4
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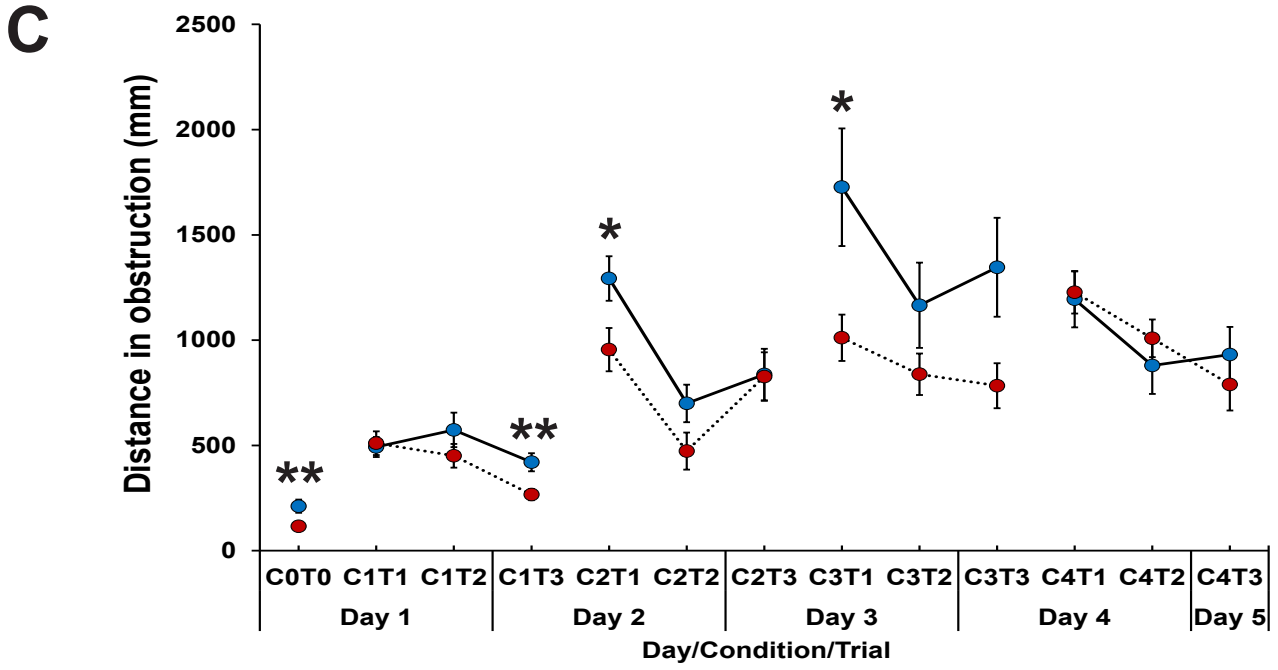
Day/Condition/Trial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Distance in obstruction (mm)
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2000
1500
1000
500
0
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Day 1
Day 2
Day 3
Day 4
Day 5
Day/Condition/Trial

Figure 4.4: Enriched animals show reduced locomotor activity within the Puzzle-Box.

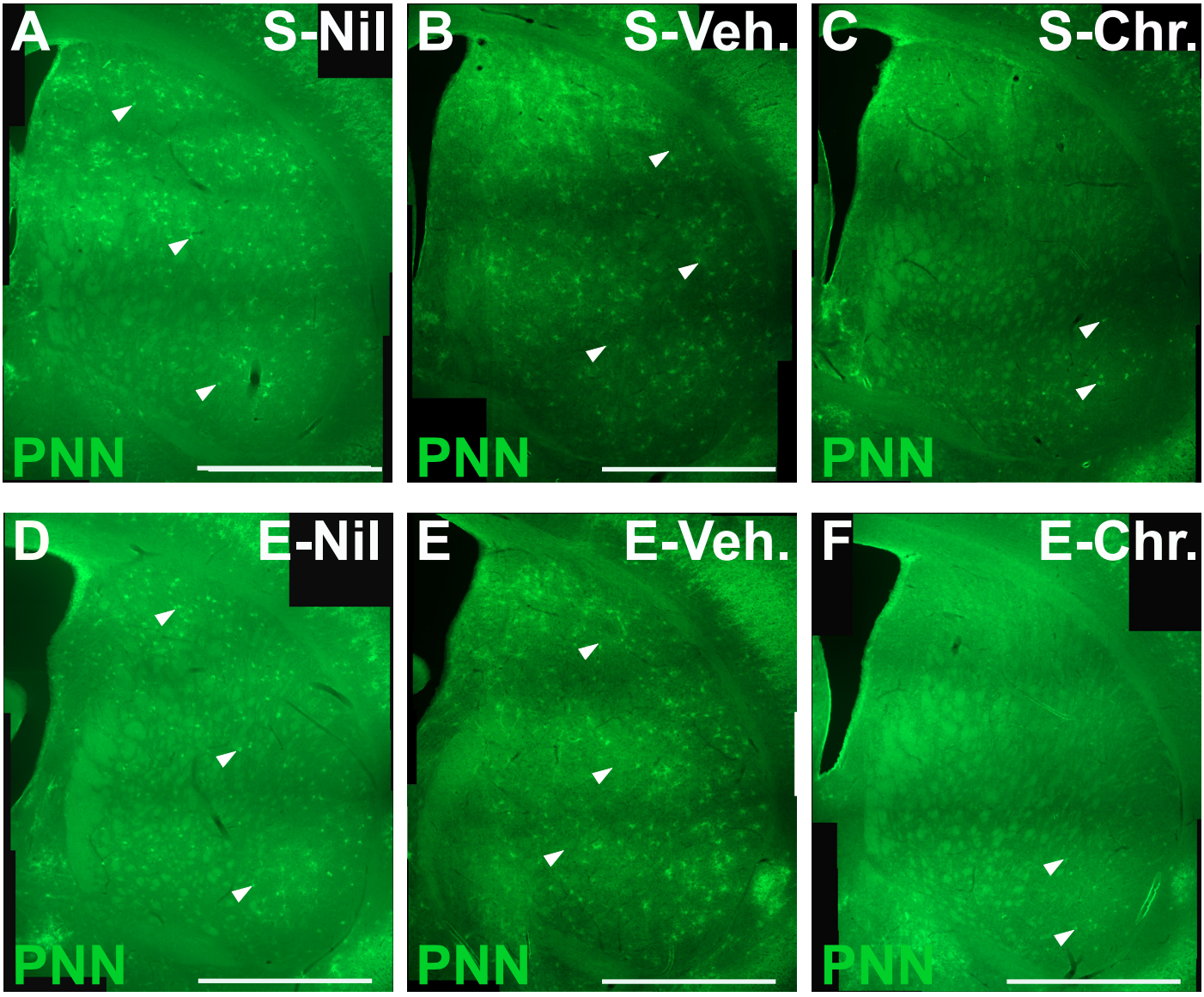
(A – C) Graphs plotting the average distance travelled within specified areas in the open-field start zone for mice raised from birth in enriched (E) and standard (S) housing. (A) Enriched animals traversed significantly less distance within the "wall" section of the open-field (repeated measures ANOVA, $F=72.492$, $P<0.001$) than standard mice, particularly at C0T1 (univariate ANOVA, $F=5.385$, $P=0.026$); C1T1 (univariate ANOVA, $F=18.002$, $P<0.001$); C1T2 (univariate ANOVA, $F=27.119$, $P<0.001$); C2T1 (univariate ANOVA, $F=14.777$, $P<0.001$); C2T2 (univariate ANOVA, $F=5.539$, $P=0.024$); C3T1 univariate ANOVA, $F=10.687$, $P=0.002$); C3T2 (univariate ANOVA, $F=7.959$, $P=0.008$); C3T3 (univariate ANOVA, $F=16.031$, $P<0.001$); C4T1 (univariate ANOVA, $F=38.187$, $P<0.001$); C4T2 (univariate ANOVA, $F=21.822$, $P<0.001$): and C4T3 (univariate ANOVA $F=42.736$, $P<0.001$). (B) Similarly, enriched mice travelled significantly less distance within the "centre" section of the open-field (repeated measures ANOVA, $F=20.723$, $P<0.001$) than their standard counterparts, particularly at C0T1 (univariate ANOVA, $F=8.736$, $P=0.005$); C1T1 (univariate ANOVA, $F=16.702$, $P<0.001$); C1T2 (univariate ANOVA, $F=15.363$, $P<0.001$); C2T1 (univariate ANOVA, $F=10.958$, $P=0.002$); C3T1 (univariate ANOVA, $F=10.798$, $P=0.002$); C3T2 (univariate ANOVA, $F=5.580$, $P=0.024$); C3T3 (univariate ANOVA, $F=5.044$, $P=0.031$); and C4T1 (univariate ANOVA, $F=14.100$, $P=0.001$). (C) Enriched mice also covered less distance within the "obstruction" zone of the open-field (repeated measures ANOVA, $F=9.175$, $P=0.004$) than standard animals, particularly at C0T1 (univariate ANOVA, $F=9.344$, $P=0.004$); C1T3 (univariate ANOVA, $F=9.832$, $P=0.003$); C2T1 (univariate ANOVA, $F=5.205$, $P=0.028$); and C3T1 (univariate ANOVA, $F=6.329$, $P=0.016$). *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$. E n=21, S n=18. Error bars = SEM.

3.2 ChABC treatment impacted performance within the Puzzle-Box task.

Previous work from our laboratory has demonstrated the presence of PNNs within the striatum [41, 50] and the accelerated maturation of these structures in response to early life enrichment [22]. PNNs play a role in maintaining strong synaptic connections within the adult brain [35, 36], and in the striatum are associated with Parvalbumin-expressing (PV+) GABA-ergic inhibitory interneurons [41], known to mediate striatal activity [58-61]. The dissolution of PNNs within the striatum improves task acquisition within the Morris Water Maze, and causes a reversion back to an "immature" gait style [41]. Whether striatal ChABC injection affects goal seeking behaviour has yet to be determined. Accordingly, I compared the performance of enriched and standard adult animals with striatal ChABC (EC, n=13 and SC, n=8) and 0.9% saline vehicle injection (EV, n=13 and SV, n=7) within the Puzzle-Box behavioural task.

The effectiveness of striatal ChABC injection during this study was confirmed: eight days post-injection, animals were sacrificed on the last day of behavioural testing and assessed for WFA labelling within the striatum. At this time, PNNs were almost completely removed from the treated region of striatum with ChABC injection (Fig. 4.5C, F). Similar to previous work [41], PNNs were present in the striatum of vehicle infused animals (Fig. 4.5B, E) and non-treated mice (Fig. 4.5A, D), as well as in the cortex and other regions surrounding the striatum of ChABC treated animals (Fig. 4.5G), suggesting that any effects resulting from this treatment are specific to the striatum. There was a significant effect of animal group upon the density of PNNs present within the striatum (univariate ANOVA, animal group as between-subjects factor, $F(5, 349)=47.824$, $P<0.001$) (Fig. 4.5H). Posthoc analysis revealed that both vehicle (multiple comparisons posthoc analysis: E vs EV, $P<0.001$; S vs SV, $P=0.001$; E vs SV, $P<0.001$; S vs EV, $P=0.017$) and ChABC treatment (multiple comparisons posthoc analysis: E vs EC, $P<0.001$; S vs SC, $P<0.001$; E vs SC, $P<0.001$; S vs EC, $P<0.001$) reduced striatal PNN density compared to untreated mice. ChABC treated animals had fewer PNNs present within the striatum than vehicle treated mice (multiple comparisons posthoc analysis: EV vs

EC, P<0.001; SV vs SC, P<0.001; EV vs SC, P<0.001; SV vs EC, P<0.001), suggesting that ChABC injection removed more PNNs than did vehicle treatment. There was no significant effect of enrichment upon PNN density within any of the animal groups (multiple comparisons posthoc analysis: E vs S, P=0.908; EV vs SV, P=0.607; EC vs SC, P=0.417) (Fig. 4.5H), suggesting that EE did not influence the recovery of PNNs within either vehicle or ChABC treated animals.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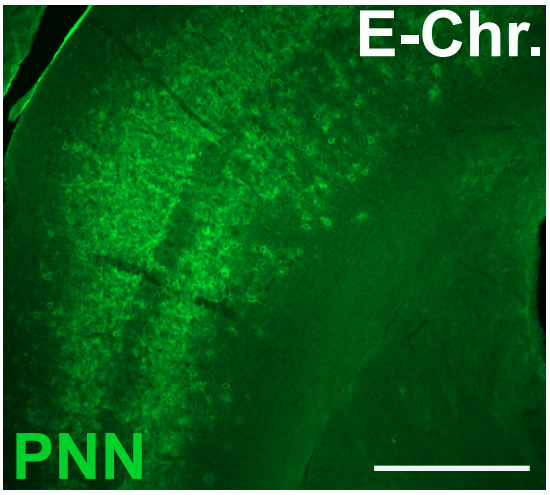
PNN
B
S-Veh.
PNN
C
S-Chr.
PNN
D
E-Nil
PNN
E
E-Veh.
PNN
F
E-Chr.
PNN

G


E-Chr.
PNN

H

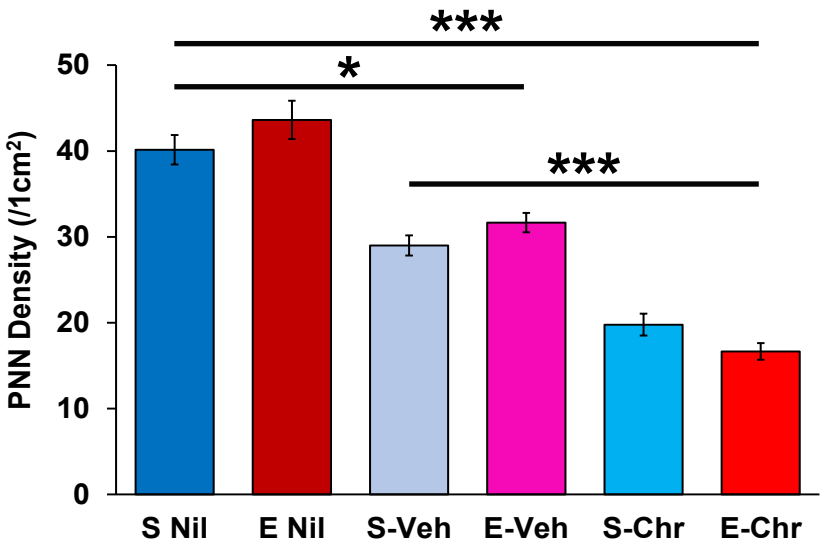

***
*
***
50
40
30
20
10
0
PNN Density (/1cm²)
S Nil
E Nil
S-Veh
E-Veh
S-Chr
E-Chr

Figure 4.5: Perineuronal net staining and density within differentially housed and treated animals.

(A – C) Sample hemispheres of striatum from animals that had completed behavioural testing, showing the pattern of perineuronal net (PNN) (arrowheads) staining within adult animals (12 – 14 weeks) raised in a standard environment and undergoing various surgical treatments. A lesser density of PNNs can be seen in ChABC treated animals (C) than is present in vehicle treated (B) and non-surgery (A) mice. (D – F) A similar series to that shown in A – C but for enriched mice. The appearance of PNNs within animals receiving ChABC (C, F) injection is less distinct than that of PNNs within non-surgery (A, D) and vehicle animals (B, E), regardless of housing condition. (G) Sample of cortex showing the pattern of PNN staining within an adult enriched animal that received striatal ChABC injection. (H) Graph comparing the average striatal PNN density of all animal groups. Vehicle and ChABC treatment both reduced the density of PNNs present within the striatum (univariate ANOVA, $F=47.824$, $P<0.001$). There was no significant effect of enrichment upon the density or recovery of PNNS throughout the striatum in any treatment group (multiple comparisons posthoc analysis: E vs S, $P=0.908$; EV vs SV, $P=0.607$; EC vs SC, $P=0.417$). Six sections from each animal were quantified. Scale bar = 1000μm Error bars=SEM. E n=3, S n=3, EV n=13, SV n=7, EC n=13, SC n=8. Chr: Chondroitinase ABC, Veh: Saline vehicle, Nil: non-surgery.

Quantitative analysis of performance within the Puzzle-Box revealed that treatment group significantly affected the time taken to solve obstruction puzzle tasks (repeated measures ANOVA, treatment group as between-subjects factor, $F(3, 37)=8.684$, $P<0.001$) (Fig. 4.6). Posthoc analysis showed that this was due to SC animals taking significantly longer to enter the goal-box than EC and EV cohorts (multiple comparisons posthoc analysis: SC vs EC $P<0.001$; SC vs EV $P=0.001$) (Fig. 4.6).

The effect of treatment group was particularly notable upon the first day of testing. During initial exposure to the Puzzle-Box arena (univariate ANOVA, treatment group as between-subjects factor: C0T1, $F(3, 37)=29.047$, $P<0.001$), SC animals took significantly longer to enter the goal-box than all other groups, and SV animals took significantly longer to enter the goal-box than both enriched cohorts (multiple comparisons posthoc analysis: SC vs EC $P<0.001$, SC vs EV $P<0.001$, SC vs SV $P=0.008$, SV vs EC $P=0.001$, SV vs EV $P=0.002$). Treatment group also impacted latency during the first two trials of the first, open channel obstruction (univariate ANOVA, treatment group as between-subjects factor: C1T1, $F(3, 37)=11.413$, $P<0.001$; C1T2, $F(3, 37)=20.879$, $P<0.001$) due to SC animals taking significantly longer to enter the goal-box than all other groups (multiple comparisons posthoc analysis: C1T1: SC vs EC $P<0.001$, SC vs EV $P<0.001$, SC vs SV $P=0.005$; C1T2: SC vs EC $P<0.001$, SC vs EV $P<0.001$, SC vs SV $P<0.001$). There were no significant differences observed between EC and EV groups, suggesting that striatal ChABC injection affects the performance of standard housed animals on the first day of testing within the Puzzle-Box more than that of enriched mice.

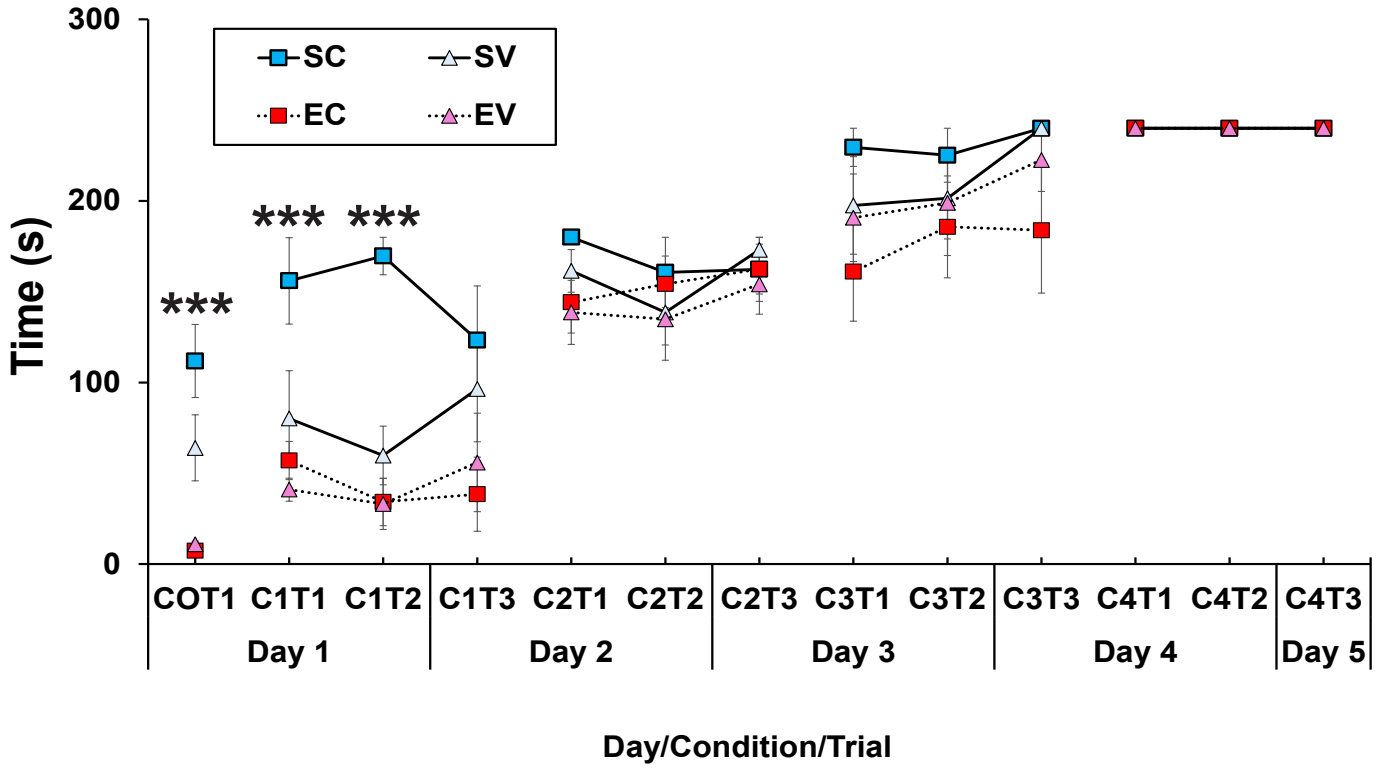

300
200
100
0
Time (s)
SC
SV
EC
EV
***
***
***
COT1
C1T1
C1T2
C1T3
C2T1
C2T2
C2T3
C3T1
C3T2
C3T3
C4T1
C4T2
C4T3
Day 1
Day 2
Day 3
Day 4
Day 5
Day/Condition/Trial

Figure 4.6: ChABC injection increases latency to goal-box zone during the Puzzle-Box test.

Graph plotting the average time taken (s) for all four paws of an animal to enter the goal-box zone of the Puzzle-Box, upon which time a behavioural task was terminated, for mice raised in enriched and standard housing receiving vehicle (EV, SV) or chondroitinase (EC, SC) striatal injection. Surgical treatment significantly impacted time taken to solve obstruction puzzles (repeated measures ANOVA, $F=8.684$, $P<0.001$), due to SC animals taking longer to enter the goal-box than either enriched group. The effect of treatment group was particularly evident during the first day of testing: C0T1 (univariate ANOVA, $F=29.047$, $P<0.001$); C1T1 (univariate ANOVA, $F=11.413$, $P<0.001$); and C1T2 (univariate ANOVA, $F=20.879$, $P<0.001$). **: $P<0.01$, ***: $P<0.001$. EV n=13, EC n=13, SV n=7, SC n=8. Error bars=SEM.

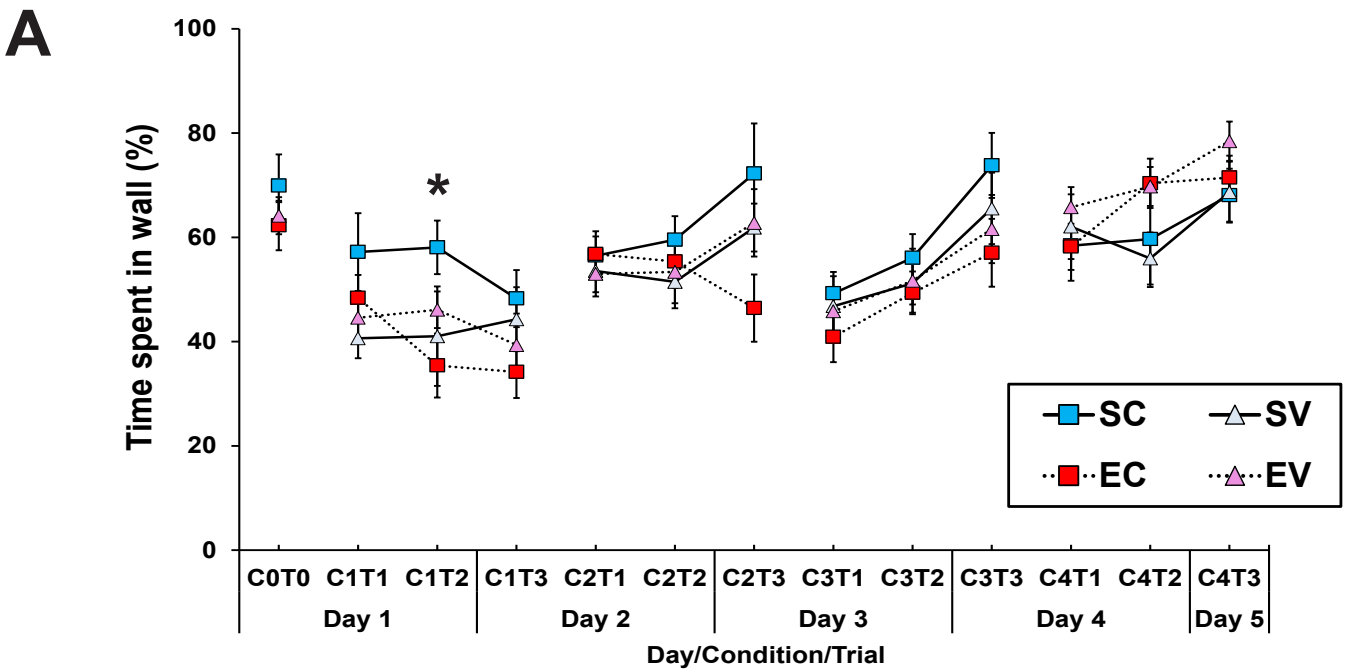
Similar to E and S cohorts, video recordings of EC, EV, SC and SV animals performing the Puzzle-Box behavioural task were made and time and distance within each area of the open-field start zone analysed. I found that treatment affected the way in which animals behaved within the Puzzle-Box (Fig. 4.7, 4.8). There were a variety of impacts upon the proportion of time and distance travelled within the sections of the open-field start zone.

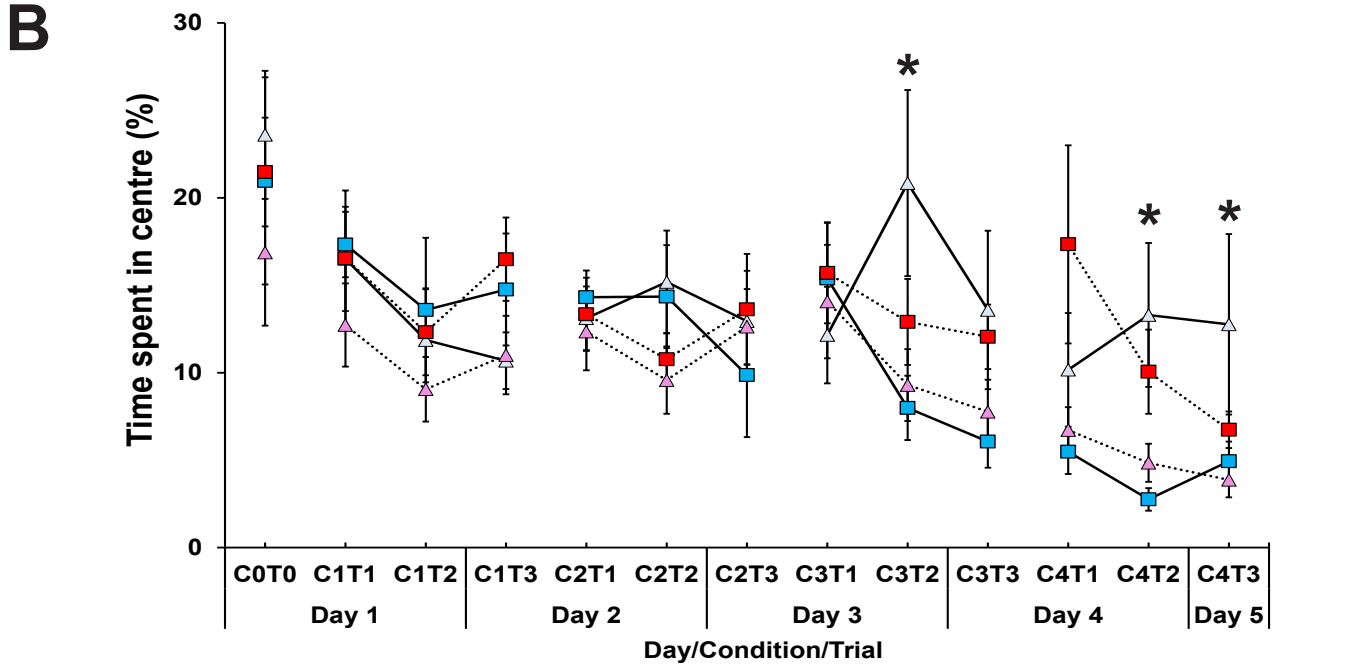
Quantitative analysis revealed that treatment group did not affect the proportion of time spent within the "wall" (repeated measures ANOVA, treatment group as between-subjects factor, $F(3, 37)=1.294$, $P=0.291$) (Fig. 4.7A) or "obstruction" sections of the open-field (repeated measures ANOVA, treatment group as between-subjects factor, $F(3, 37)=0.705$, $P=0.555$) (Fig. 4.7C). Treatment group did impact the proportion of time spent within the "centre" of the open-field (repeated measures ANOVA, treatment group as between-subjects factor, $F(3, 37)=4.052$, $P=0.017$) (Fig. 4.7B), which posthoc analysis revealed to be due to EV animals spending less time within this area than EC mice (repeated measures ANOVA, treatment group as between-subjects factor, multiple comparisons posthoc analysis, $F(3, 37)=4.052$: EV vs EC, $P=0.028$).

The effect of treatment group upon time spent in the "centre" was particularly evident at certain trials within the task: specifically, during the second trial of the tissue plug (univariate ANOVA, treatment group as between-subjects factor, C3T2: $F(3, 37)=3.230$, $P=0.033$), SV mice spent a greater proportion of time within the "centre" than EV and SC animals (multiple comparisons posthoc analysis: SV vs EV $P=0.044$, SV vs SC $P=0.042$). A similar difference was detected for the final two trials of the most difficult foam plug obstruction (univariate ANOVA, treatment group as between-subjects factor: C4T2, $F(3, 37)=4.186$, $P=0.012$; C4T3, $F(3, 37)=3.312$, $P=0.030$), with SV mice again spending a greater proportion of time within the "centre" than SC and EV animals (multiple comparisons posthoc analysis: C4T2: SV vs SC $P=0.023$; C4T3: SV vs EV $P=0.021$).

Despite there being no overall impact of treatment group upon the proportion of time spent within the "wall" section of the open-field, there was an effect in the second trial of the open channel

obstruction (univariate ANOVA, treatment group as between-subjects factor, $F(3, 37)=3.272$, $P=0.019$), which posthoc analysis revealed to be due to EC animals spending less time within this region than SC mice (multiple comparisons posthoc analysis: C1T2: EC vs SC $P=0.011$) (Fig. 4.7A). Similarly, there was a significant effect of treatment group on the proportion of time spent within "obstruction" during individual trials: specifically, upon first exposure to the testing arena (univariate ANOVA, treatment group as between-subjects factor, C0T1, $F(3, 37)=3.225$, $P=0.033$) due to EV animals spending a greater proportion of time within this region than SC mice (multiple comparisons posthoc analysis, C0T1: EV vs SC $P=0.030$); and during the first two trials of the open channel obstruction (univariate ANOVA, treatment group as between-subjects factor: C1T1, $F(3, 37)=3.709$, $P=0.020$; C1T2, $F(3, 37)=3.333$, $P=0.030$). Posthoc analysis revealed this effect to be due to EV and EC animals spending a greater proportion of time within this region than SC mice during C1T1 and C1T2, respectively (multiple comparisons posthoc analysis: C1T1: EV vs SC $P=0.026$; C1T2: EC vs SC $P=0.030$) (Fig. 4.7C).


A
Time spent in wall (%)
100
80
60
40
20
0
*
SC
SV
EC
EV
C0T0 C1T1 C1T2 C1T3 C2T1 C2T2 C2T3 C3T1 C3T2 C3T3 C4T1 C4T2 C4T3
Day 1
Day 2
Day 3
Day 4
Day 5
Day/Condition/Trial


B
Time spent in centre (%)
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20
10
0
*
*
*
C0T0 C1T1 C1T2 C1T3 C2T1 C2T2 C2T3 C3T1 C3T2 C3T3 C4T1 C4T2 C4T3
Day 1
Day 2
Day 3
Day 4
Day 5
Day/Condition/Trial

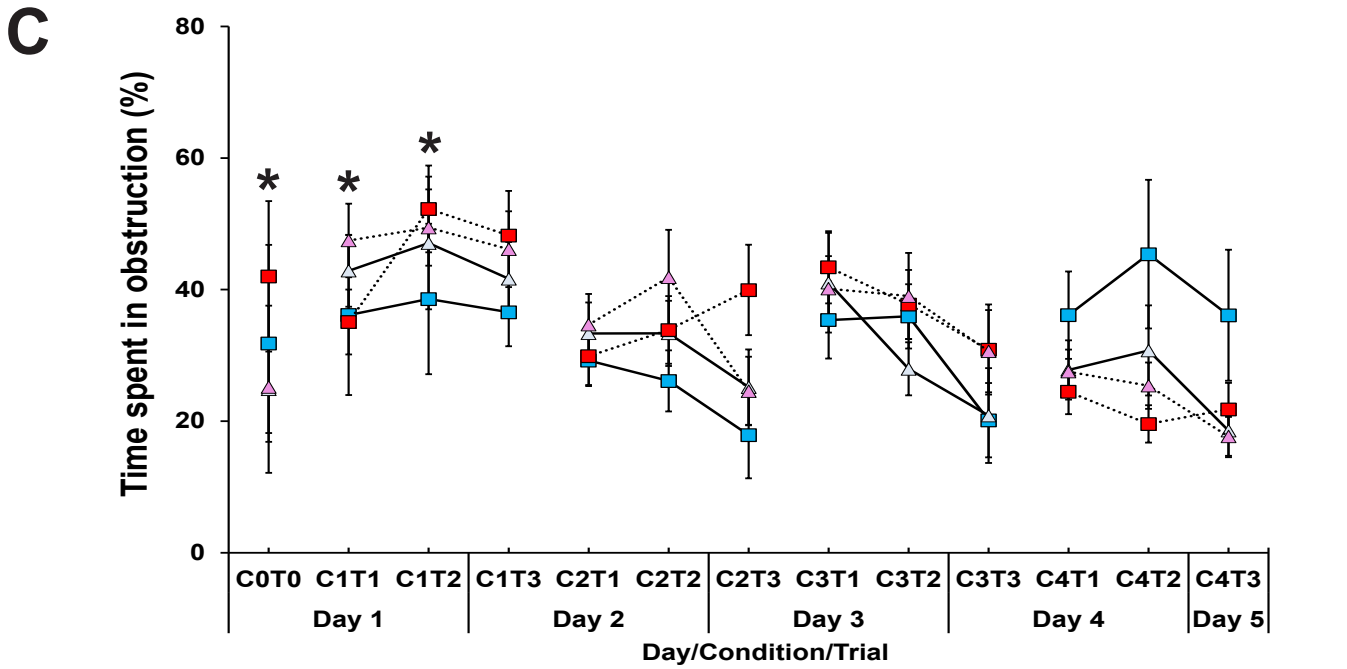

C
Time spent in obstruction (%)
80
60
40
20
0
*
*
*
C0T0 C1T1 C1T2 C1T3 C2T1 C2T2 C2T3 C3T1 C3T2 C3T3 C4T1 C4T2 C4T3
Day 1
Day 2
Day 3
Day 4
Day 5
Day/Condition/Trial

Figure 4.7: ChABC treatment impacts behavioural patterns within the Puzzle-Box apparatus.

(A – C) Graphs plotting the average percentage of total time spent within the Puzzle-Box where an animal was within specified areas in the open-field start zone for mice raised from birth in enriched and standard housing receiving vehicle (EV, SV) or chondroitinase (EC, SC) striatal injection. (A) There was no overall significant effect of treatment group on the proportion of time spent within the "wall" zone (repeated measures ANOVA, $F=1.294$, $P=0.291$). There was a significant difference in one individual trial: C1T2 (univariate ANOVA, $F=3.727$, $P=0.019$). (B) Treatment group significantly impacted the proportion of time spent within the "centre" section of the open-field (repeated measures ANOVA, $F=4.052$, $P=0.017$), due to EV animals spending less time within this zone than EC animals (see text for details). This effect of treatment group was particularly evident during individual trials: C3T2 (univariate ANOVA, $F=3.230$, $P=0.033$); C4T2 (univariate ANOVA, $F=4.186$, $P=0.012$); C4T3 (univariate ANOVA, $F=3.312$, $P=0.030$). (C) There was no overall significant effect of treatment group on the proportion of time spent within the "obstruction" area (repeated measures ANOVA, $F=0.705$, $P=0.555$). There were significant differences in some individual trials: C0T1 (univariate ANOVA, $F=3.225$, $P=0.033$); C1T1 (univariate ANOVA, $F=3.709$, $P=0.020$); and C1T2 (univariate ANOVA, $F=3.333$, $P=0.030$). *: $P<0.05$. EV n=13, EC n=13, SV n=7, SC n=8. Error bars=SEM.

Treatment group did not affect the distance traversed within the “wall” (repeated measures ANOVA, treatment group as between-subjects factor, $F(3, 37)=2.286$, $P=0.095$) (Fig. 4.8A) or “obstruction” sections of the open-field (repeated measures ANOVA, treatment group as between-subjects factor, $F(3, 37)=0.592$, $P=0.624$) (Fig. 4.8C). Treatment group did, however, impact the distance travelled within the “centre” of the open-field (repeated measures ANOVA, treatment group as between-subjects factor, $F(3, 37)=3.637$, $P=0.021$) (Fig. 4.8B). Posthoc analysis revealed that the detected difference was due to SV animals covering a greater distance than EV mice (multiple comparisons posthoc analysis: SV vs EV, $P=0.011$).

The effect of treatment group upon distance travelled in the “centre” was particularly evident at certain trials within the task: specifically, upon first exposure to the Puzzle-Box (univariate ANOVA, treatment group as between-subjects factor, C0T1 $F(3, 37)=6.544$, $P=0.001$) due to both enriched groups travelling less distance than standard animals receiving ChABC treatment (multiple comparisons posthoc analysis, C0T1: EC vs SC $P=0.007$, EV vs SC $P=0.007$). A similar difference was detected for the final two trials of the open channel obstruction (univariate ANOVA, treatment group as between-subjects factor: C1T2 $F(3, 37)=3.606$, $P=0.022$; C1T3, $F(3, 37)=5.088$, $P=0.005$), with enriched mice again covering less distance in the “centre” than SC animals (multiple comparisons posthoc analysis: C1T2: EC vs SC $P=0.017$, EV vs SC $P=0.048$; C1T3: EC vs SC $P=0.010$, EV vs SC $P=0.022$), and the first trial of the most difficult foam plug obstruction (univariate ANOVA, treatment group as between-subjects factor: C4T1 $F(3, 37)=3.003$, $P=0.043$), which posthoc analysis revealed to be due to SC animals travelling a greater distance than EC mice (multiple comparisons posthoc analysis, C4T1: EC vs SC $P=0.026$) (Fig. 4.8B).

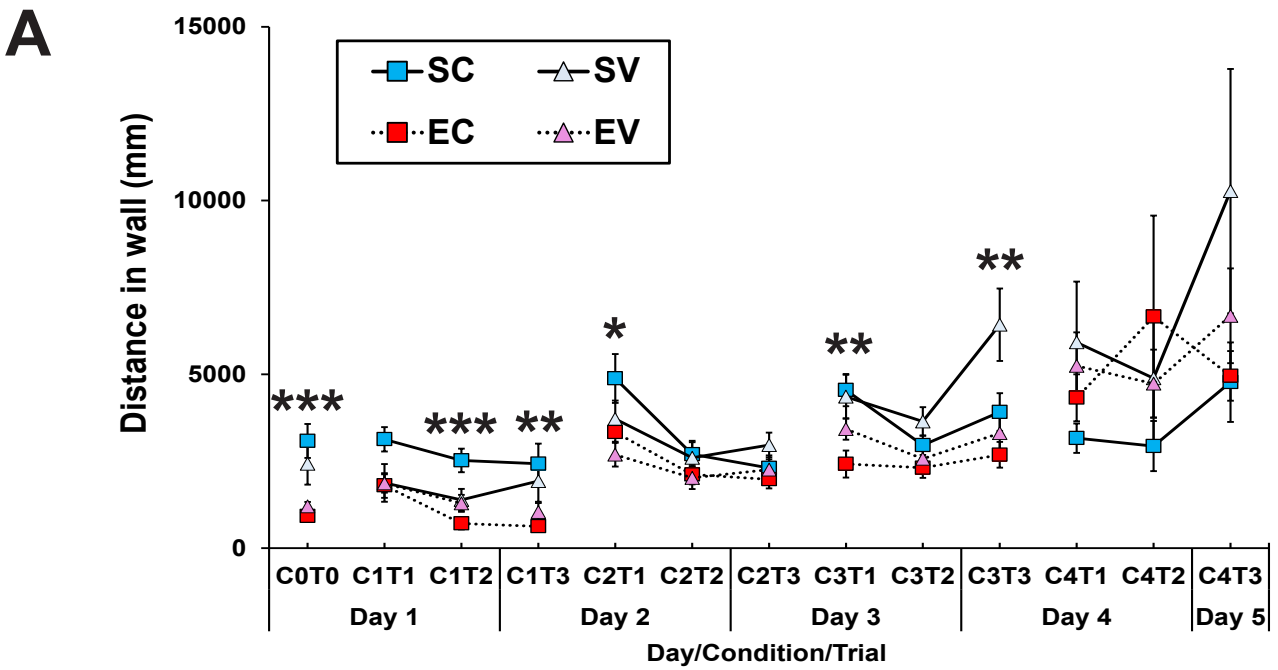
Despite there being no overall effect of treatment group upon distance travelled in the “wall” section of the open-field, there were significant differences in some individual trials: specifically, upon first exposure to the Puzzle-Box (univariate ANOVA, C0T1, $F(3, 37)=11.116$, $P<0.001$), due to enriched treatment mice travelling less distance within this region than standard treatment animals

(multiple comparisons posthoc analysis: C0T1: EC vs SC P<0.001, EC vs SV P=0.009, EV vs SC P<0.001, EV vs SV P=0.043); during the two last trials of the open channel obstruction (univariate ANOVA, treatment group as between-subjects factor: C1T2, F(3, 37)=8.202, P<0.001; C1T3, F(3, 37)=5.277, P=0.004) due to SC animals travelling further within this region than both enriched groups (multiple comparisons posthoc analysis: C1T2: EC vs SC P<0.001, EV vs SC P=0.009; C1T3: EC vs SC P=0.005, EV vs SC P=0.041); and during the first trials of the more difficult filled channel and tissue plug obstructions (univariate ANOVA, treatment group as between subjects factor: C2T1, F(3, 37)=4.376, P=0.010; C3T1, F(3, 37)=5.177, P=0.004); C3T3, F(3, 37)=6.637, P=0.001). Posthoc analysis revealed this effect to be due to EV animals traversing less distance in C2T1 than SC mice, EC animals travelling less than both standard groups during C3T1 and SV mice moving further than both enriched groups in C3T3 (multiple comparisons posthoc analysis: C2T1: EV vs SC P=0.005; C3T1: EC vs SC P=0.006, EC vs SV, P=0.020; C3T3: EC vs SV P=0.001, EV vs SV P=0.005) (Fig. 4.8A).

Similarly, there was an impact of treatment group upon the distance travelled within the "obstruction" region of the open-field during individual trials: specifically, upon the first trials of the open and filled channel obstructions (univariate ANOVA, treatment group as between-subjects factor: C1T1, F(3, 37)=3.055, P=0.040; C2T1, F(3, 37)=3.048, P=0.041). Posthoc analysis revealed this effect to be due to SV animals travelling further within this zone than EC mice during C1T1 and EV mice during C2T1 (multiple comparisons posthoc analysis: C1T1: EC vs SV P=0.044; C2T1: EV vs SV P=0.044) (Fig. 4.8C).

Together, these findings reveal that ChABC treatment affects S and E cohorts differently. S animals injected with the enzyme exhibited dramatically increased latencies to goal compared to all other groups, but only on the first day of testing Curiously, while no significant differences in goal seeking were detected between EV and EC mice, area analyses did show that vehicle treated enriched cohorts spent less time within the "centre" region of the open field. SC animals were also observed to traverse significantly greater distances within this same area, although this occurred across the

entire extent of the testing period, making it difficult to attribute the protracted latencies observed by these animals during the first few trials of this behavioural test to this performance difference. Nevertheless, the fact that the relative effect of vehicle and ChABC treatment on S and E cohorts varied suggests that pre-treatment conditions can influence how the removal of striatal PNNs can influence goal-seeking behaviour.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Day 1
Day 2
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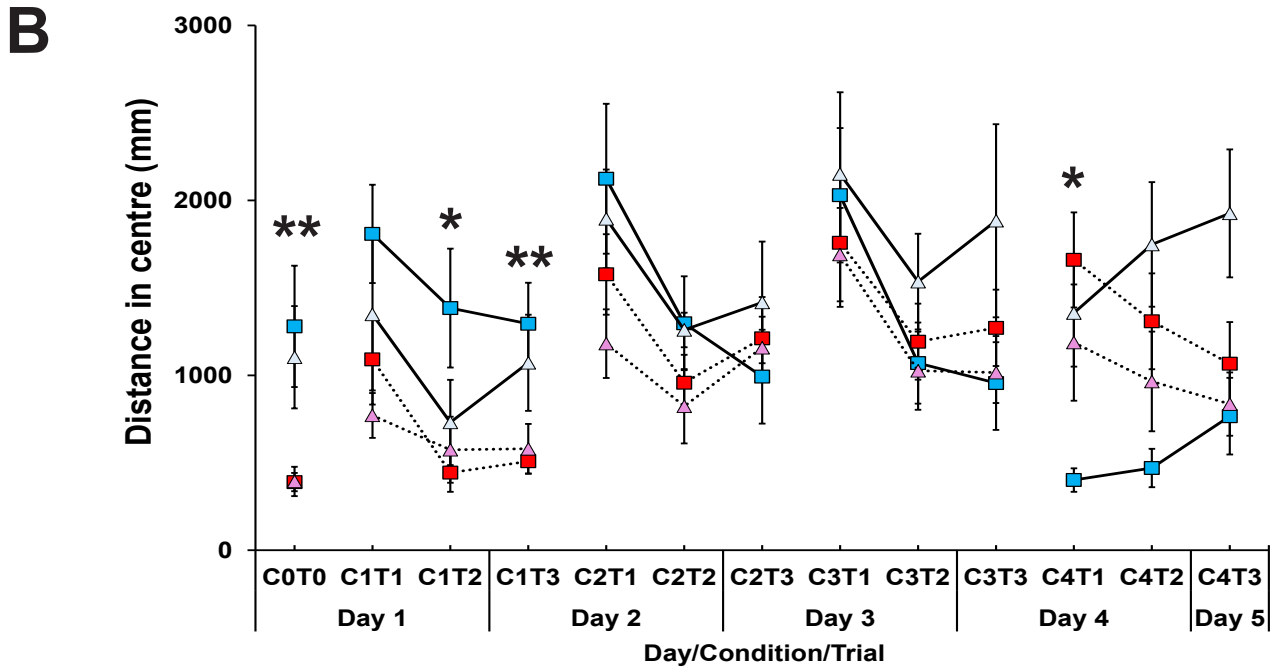
Day 4
Day 5
Day/Condition/Trial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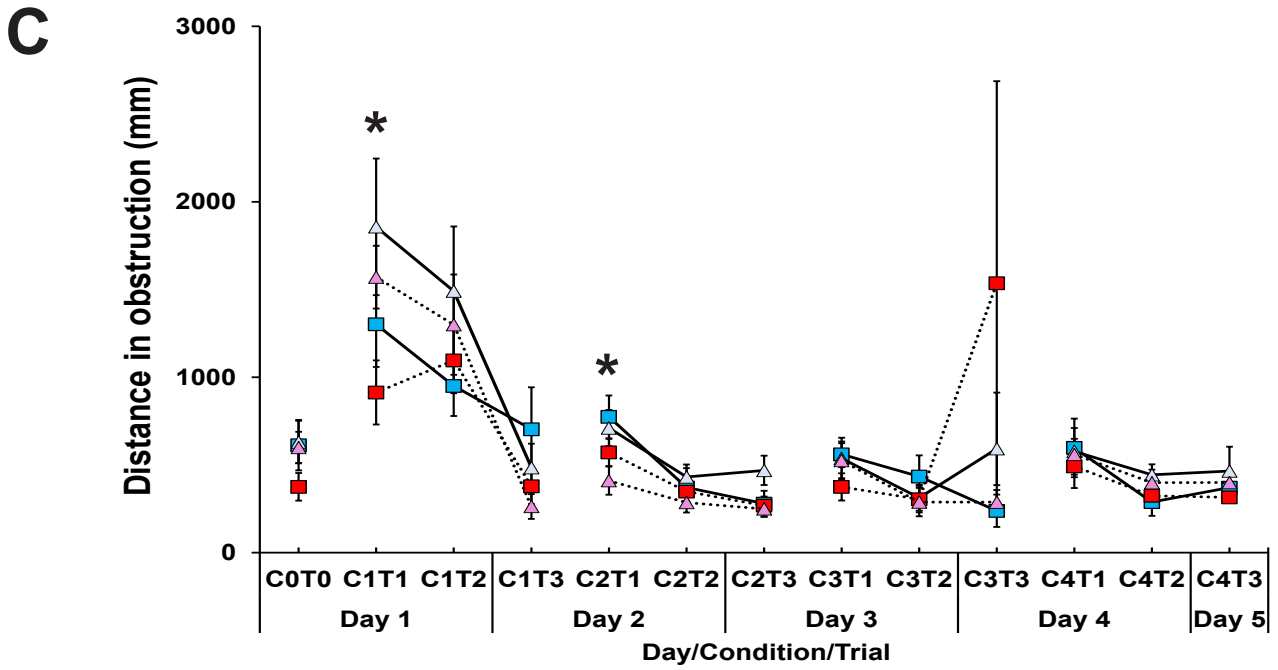
Day 1
Day 2
Day 3
Day 4
Day 5
Day/Condition/Trial


C
Distance in obstruction (mm)
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2000
1000
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C0T0 C1T1 C1T2 C1T3 C2T1 C2T2 C2T3 C3T1 C3T2 C3T3 C4T1 C4T2 C4T3
Day 1
Day 2
Day 3
Day 4
Day 5
Day/Condition/Trial

Figure 4.8: ChABC treatment has little impact on distance travelled within the Puzzle-Box apparatus.

(A – C) Graphs plotting the average distance travelled within specified areas in the open-field start zone for mice raised from birth in enriched and standard housing receiving vehicle (EV, SV) or chondroitinase (EC, SC) striatal injection. (A) There was no significant effect of treatment group upon the distance traversed within the "wall" section of the open-field (repeated measures ANOVA, $F=2.286$, $P=0.095$). There were, however, significant differences in some individual trials: C0T1 (univariate ANOVA, $F=11.116$, $P<0.001$); C1T2 (univariate ANOVA, $F=8.202$, $P<0.001$); C1T3 (univariate ANOVA, $F=5.277$, $P=0.004$); C2T1 (univariate ANOVA, $F=4.376$, $P=0.010$); C3T1 (univariate ANOVA, $F=5.339$, $P=0.004$); and C3T3 (univariate ANOVA, $F=6.637$, $P=0.001$). (B) Treatment group significantly impacted the distance travelled within the "centre" zone of the open-field (repeated measures ANOVA, $F=3.637$ $P=0.021$), due to SV animals covering a greater distance than EV animals (see text). The effect of treatment group was notable in some individual trials: C0T1 (univariate ANOVA, $F=6.544$, $P=0.001$); C1T2 (univariate ANOVA, $F=3.606$, $P=0.022$); C1T3 (univariate ANOVA, $F=5.088$, $P=0.005$); and C4T1 (univariate ANOVA, $F=3.003$, $P=0.043$). (C) There was no significant effect of treatment group upon the distance traversed within the "obstruction" section of the open-field (repeated measures ANOVA, $F=0.592$, $P=0.624$). There were, however, significant differences in some individual trials: C1T1 (univariate ANOVA, $F=3.055$, $P=0.040$); and C2T1 (univariate ANOVA, $F=3.048$, $P=0.041$). *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$.. EV n=13, EC n=13, SV n=7, SC n=8. Error bars=SEM.

3.3 Striatal injection of both vehicle and ChABC impacted performance within the Puzzle-Box task

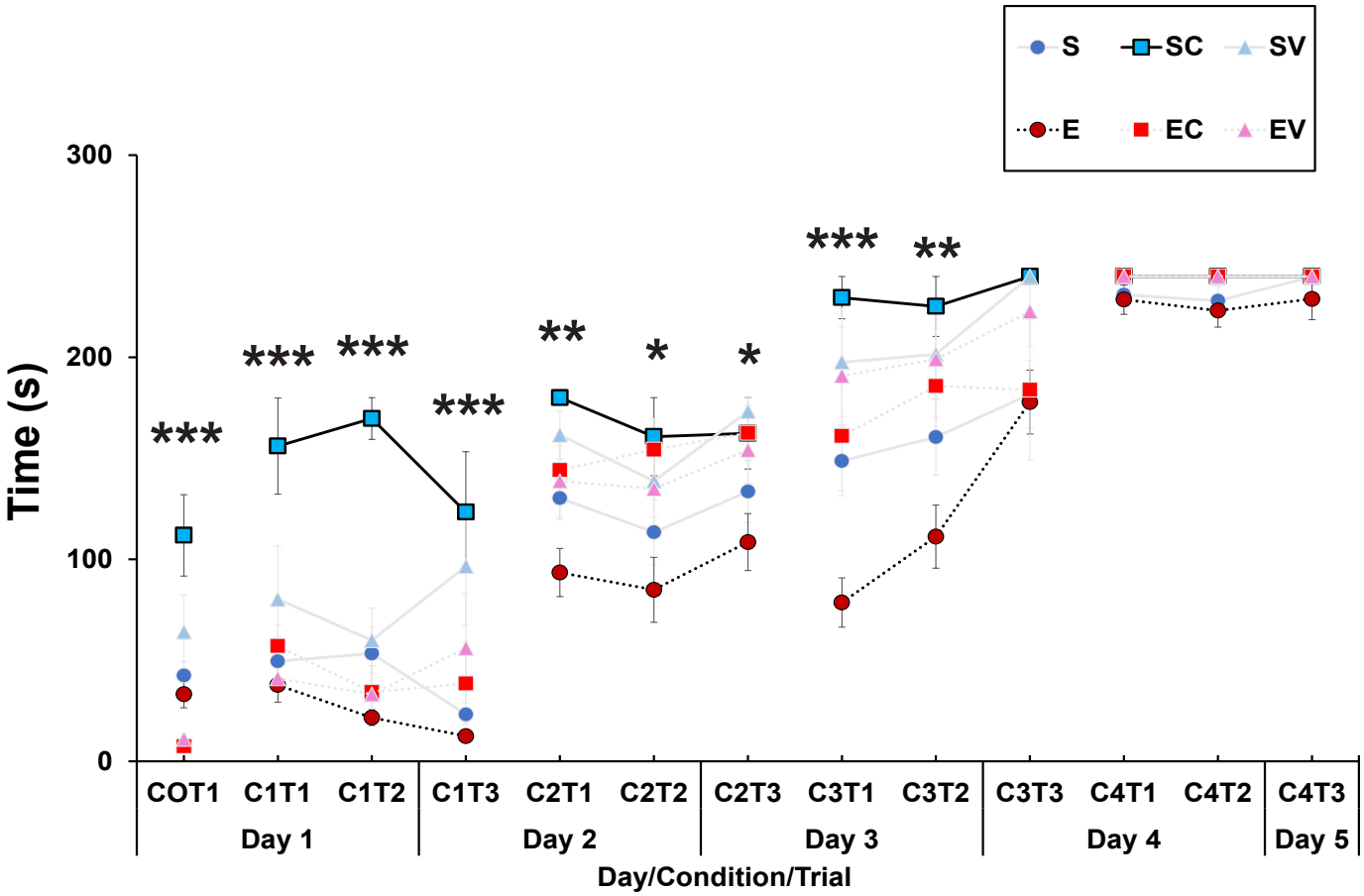
Striatal injection of ChABC impacted both performance and behavioural patterns of animals within the Puzzle-Box apparatus when compared to mice receiving vehicle injections. Further, the changes induced by the treatment seemed to vary depending on the housing history of the animal. In order to determine the degree to which vehicle and ChABC injections affected puzzle box performance, I conducted an exploratory analysis comparing latency and area measurements from all treatment cohorts with recorded values of untreated standard and enriched mice.

I found that animal group had a significant impact upon the time taken to solve obstruction conditions within the Puzzle-Box behavioural test (repeated measures ANOVA, group as between-subjects factor, $F(5, 74)=15.288$, $P<0.001$) (Fig. 4.9). Surprisingly, while posthoc analysis revealed that enriched non-surgery mice entered the goal-box zone faster than all other groups of animals (multiple comparisons posthoc analysis: E vs EC $P=0.001$, E vs EV $P<0.001$, E vs S $P=0.027$, E vs SC $P<0.001$, E vs SV $P<0.001$), both vehicle and ChABC treated E cohorts exhibited latencies that were comparable to untreated S animals. SV and SC groups also exhibited increased latencies compared to untreated S animals: SC mice took longer to enter the goal-box than all other groups except SV (multiple comparisons posthoc analysis: SC vs E $P<0.001$, SC vs EC $P=0.007$, SC vs EV $P=0.007$, SC vs S $P<0.001$, SC vs SV $P=0.544$); and SV mice took longer to enter the goal-box than S animals (multiple comparisons posthoc analysis: SV vs S $P=0.037$).

Trial by trial analysis revealed that animal group significantly impacted performance as measured by latency to goal box zone in nearly all individual trials except the most difficult: specifically, upon first exposure to the Puzzle-Box (univariate ANOVA, group as between-subjects factor, C0T1, $F(5, 74)=15.714$, $P<0.001$) due to SC mice taking significantly longer to enter the goal-box than all other groups (multiple comparisons posthoc analysis C0T1: SC vs E $P<0.001$, SC vs EC $P<0.001$, SC vs EV $P<0.001$, SC vs S $P<0.001$, SC vs SV $P=0.022$); during all trials of the open channel obstruction

(univariate ANOVA, group as between-subjects factor: C1T1, $F(5, 74)=9.359$, $P<0.001$; C1T2, $F(5, 74)=14.743$, $P<0.001$; C1T3, $F(5, 74)=7.684$, $P<0.001$), again due to SC mice taking longer than all other groups to enter the goal-box during the first two trials, and nearly all groups during the last trial, of this obstruction (multiple comparisons posthoc analysis: C1T1: SC vs E $P<0.001$, SC vs EC $P<0.001$, SC vs EV $P<0.001$, SC vs S $P<0.001$, SC vs SV $P=0.008$; C1T2: SC vs E $P<0.001$, SC vs EC $P<0.001$, SC vs EV $P<0.001$, SC vs S $P<0.001$, SC vs SV $P=0.001$; C1T3: SC vs E $P<0.001$, SC vs EC $P<0.001$, SC vs EV $P=0.115$, SC vs S $P<0.001$, SC vs SV $P=0.938$).

There continued to be a significant effect of animal group throughout the more difficult filled channel obstruction (univariate ANOVA, group as between-subjects factor: C2T1, $F(5, 74)=4.491$, $P=0.001$; C2T2, $F(5, 74)=2.784$, $P=0.023$; C2T3, $F(5, 74)=2.395$, $P=0.045$) due to enriched untreated animals entering the goal-box sooner than most groups during C2T1, and faster than SC mice during C2T2 (multiple comparisons posthoc analysis: C2T1: E vs EC $P=0.023$, E vs SC $P=0.006$, E vs SV $P=0.013$; C2T2: E vs SC $P=0.032$); and in the first two trials of the more difficult tissue plug obstruction (univariate ANOVA, group as between-subjects factor: C3T1, $F(5, 74)=10.750$, $P<0.001$; C3T2, $F(5, 74)=4.959$, $P=0.001$), which posthoc analysis revealed to be due to enriched untreated animals entering the goal-box sooner than all other groups of animals in C3T1, and nearly all groups in C3T2 (multiple comparisons posthoc analysis: C3T1: E vs EC $P=0.005$, E vs EV $P<0.001$, E vs S $P=0.010$, E vs SC $P<0.001$, E vs SV $P<0.001$; C3T2: E vs EC $P=0.032$, E vs EV $P=0.015$, E vs SC $P=0.002$, E vs SV $P=0.031$) and SC animals taking longer to enter the goal-box than untreated S animals in C3T1 (multiple comparisons posthoc analysis, C3T1: S vs SC $P=0.028$) (Fig. 4.9).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C4T3
Day 1
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Day 4
Day 5
Day/Condition/Trial

Figure 4.9: ChABC treatment impacts the performance of standard mice and enriched animals differently.

Graph plotting the average time taken (s) for all four paws of an animal to enter the goal-box zone of the Puzzle-Box, upon which time a behavioural task was terminated, for mice raised in enriched (E) and standard (S) housing receiving vehicle (EV, SV) or chondroitinase (EC, SC) striatal injection. Treatment group significantly impacted the latency to goal-box (repeated measures ANOVA, $F=15.288$, $P<0.001$), due to enriched mice taking less time to solve obstruction puzzles than all other groups, and standard mice receiving ChABC treatment taking longer to solve obstruction puzzles than all other groups except SV (see text). The effect of animal group was particularly evident at nearly all individual trials: C0T1 (univariate ANOVA, $F=15.714$, $P<0.001$); C1T1 (univariate ANOVA, $F=9.359$, $P<0.001$); C1T2 (univariate ANOVA, $F=14.743$, $P<0.001$); C1T3 (univariate ANOVA, $F=7.684$, $P<0.001$); C2T1 (univariate ANOVA, $F=4.491$, $P=0.001$); C2T2 (univariate ANOVA, $F=2.784$, $P=0.023$); C2T3 (univariate ANOVA, $F=2.395$, $P=0.045$); C3T1 (univariate ANOVA, $F=10.750$, $P<0.001$); C3T2 (univariate ANOVA, $F(5, 74)=4.959$, $P=0.001$). *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$. E n=21, EV n=13, EC n=13, S n=18, SV n=7, SC n=8. Error bars=SEM.

Both vehicle and ChABC injection were also found to impact the patterns of movement within the Puzzle-Box apparatus, when compared to untreated animals. I found that animal group had a significant effect upon the proportion of time spent within the “wall” (repeated measures ANOVA, group as between-subjects factor, $F(5, 74)=10.240$, $P<0.001$) (Fig. 4.10A), “centre” (repeated measures ANOVA, group as between-subjects factor, $F(5, 74)=2.722$, $P=0.026$) (Fig. 4.10B) and “obstruction” (repeated measures ANOVA, group as between-subjects factor, $F(5, 74)=9.904$, $P<0.001$) (Fig. 4.10C) areas of the open-field zone within the Puzzle-Box.

Posthoc analysis revealed behavioural patterns consistent with the results of area comparisons described above (see section 3.1 and 3.2). Specifically, enriched untreated animals spending a lesser proportion of time within the “wall” than all other groups, including EV and EC animals (multiple comparisons posthoc analysis: E vs EC $P=0.002$, E vs EV $P<0.001$, E vs S $P=0.012$, E vs SC $P<0.001$, E vs SV $P=0.005$). E mice also spent a greater proportion of time engaged with the “obstruction” than all other groups of animals (multiple comparisons posthoc analysis: E vs EC $P<0.001$, E vs EV $P<0.001$, E vs S $P<0.001$, E vs SC $P<0.001$, E vs SV $P=0.001$). SC mice spent a greater proportion of time within the “wall” area than did untreated standard animals (multiple comparisons posthoc analysis: S vs SC $P=0.033$). No differences in performance were detected for the “centre” area, except for untreated standard animals exhibiting a greater proportion of time spent in this region compared to EV mice multiple comparisons posthoc analysis: S vs EV $P=0.015$).

The effect of animal group upon the proportion of time spent within the “wall” section of the open-field in the Puzzle-Box was particularly evident at all individual trials where an obstruction condition was placed within the entrance to the goal-box: throughout the empty channel obstruction (univariate ANOVA, group as between-subjects factor: C1T1, $F(5, 74)=4.470$, $P=0.001$; C1T2, $F(5, 74)=5.704$, $P<0.001$; C1T3, $F(5, 74)=2.959$, $P=0.017$) due to enriched groups spending a lesser proportion of time within the “wall” than standard groups (multiple comparisons posthoc analysis: C1T1: E vs S $P=0.007$, E vs SC $P=0.032$; C1T2: E vs S $P=0.003$, E vs SC $P=0.001$, EC vs SC $P=0.012$; C1T3:

E vs SC P=0.020); for the entirety of the more difficult filled channel obstruction (univariate ANOVA, group as between-subjects factor: C2T1, $F(5, 74)=2.962$, $P=0.017$; C2T2, $F(5, 74)=2.655$, $P=0.029$; C2T3, $F(5, 74)=5.930$, $P<0.001$) due to enriched untreated animals spending a lesser proportion of time and SC mice spending a greater proportion of time within the "wall" region of the open-field (multiple comparisons posthoc analysis: C2T1: E vs EC P=0.019; C2T3: E vs EV P=0.013, E vs SC P=0.002, SC vs EC P=0.036, SC vs S P=0.003). There were no significant posthoc effects of animal group upon C2T2.

The significant effect of animal group was maintained throughout the more difficult tissue plug obstruction (univariate ANOVA, group as between-subjects factor: C3T1, $F(5, 74)=3.473$, $P=0.007$; C3T2, $F(5, 74)=5.564$, $P<0.001$; C3T3, $F(5, 74)=3.458$, $P=0.007$), again due to enriched untreated animals spending a lesser proportion of time and SC mice spending a greater proportion of time within the "wall" region of the open-field (multiple comparisons posthoc analysis: C3T1: E vs SC P=0.044; C3T2: E vs EC P=0.017, E vs EV P=0.005, E vs SC P=0.004, E vs SV P=0.049; C3T3: E vs SC P=0.007); and finally, during the most difficult foam plug obstruction (univariate ANOVA, group as between-subjects factor: C4T1, $F(5, 74)=2.645$, $P=0.030$; C4T2 $F(5, 74)=6.281$, $P<0.001$; C4T3, $F(5, 74)=2.584$, $P=0.033$) due to enriched untreated mice spending a lesser proportion of time within the "wall" (multiple comparisons posthoc analysis: C4T1: E vs EV P=0.015; C4T2: E vs EC P<0.001, E vs EV P<0.001, E vs S P=0.019; C4T3: E vs EV P=0.012) (Fig. 4.10A).

There were also significant effects of animal group upon the proportion of time spent within the "centre" during individual trials: specifically, during the first and third trials of the open and filled channel obstructions (univariate ANOVA, group as between-subjects factor: C1T1, $F(5, 74)=3.660$, $P=0.005$; C1T3, $F(5, 74)=2.716$, $P=0.026$; C2T1, $F(5, 74)=6.440$, $P<0.001$; C2T3, $F(5, 74)=2.652$, $P=0.029$) due to ChABC treated animals spending a greater proportion of time within the "centre" than untreated animals during the open channel obstruction and the first trial of the filled channel obstruction (multiple comparisons posthoc analysis: C1T1: E vs EC P=0.009, E vs SC P=0.030; C1T3: S

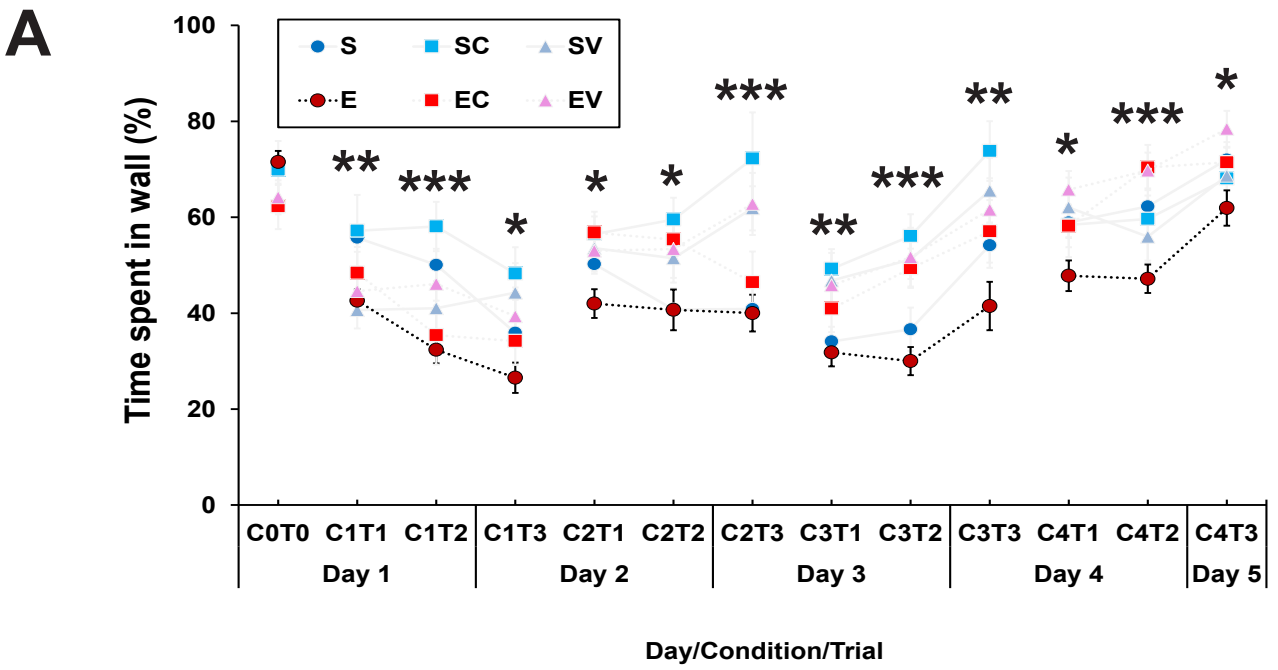
vs EC P=0.011; C2T1: E vs EC P=0.001, E vs EV P=0.003, E vs SC P=0.002, E vs SV P=0.013) and standard untreated animals spending a greater proportion of time within the “centre” than enriched untreated animals during the third trial of the filled channel obstruction (multiple comparisons posthoc analysis, C2T3: E vs S P=0.017).

The significant effect of animal group was maintained in the second trial of the more difficult foam plug obstruction (univariate ANOVA, group as between-subjects factor, C3T2, $F(5, 74)=3.611$, $P=0.006$) due to standard untreated mice spending a greater proportion of time within the “centre” (multiple comparisons posthoc analysis, C3T2: S vs EV P=0.014, S vs SC P=0.030) and throughout the most difficult foam plug obstruction (univariate ANOVA, group as between-subjects factor: C4T1, $F(5, 74)=2.491$, $P=0.039$; C4T2, $F(5, 74)=7.657$, $P<0.001$; C4T3, $F(5, 74)=2.675$, $P=0.028$) due to enriched untreated animals spending a greater proportion of time within the “centre” region of the open-field in the second trial, with no significant posthoc differences observed in the first and third trials (multiple comparisons posthoc analysis, C4T2: E vs EC P=0.005, E vs EV P<0.001, E vs SC P<0.001) (Fig. 4.10B).

The effect of animal group upon the proportion of time spent within “obstruction” was also particularly evident at certain trials within the task: specifically, upon first exposure to the Puzzle-Box (univariate ANOVA, group as between-subjects factor, C0T1, $F(5, 74)=3.263$, $P=0.010$) due to EV mice spending a proportion of time within the “obstruction” between that of SC and standard untreated animals (multiple comparisons posthoc analysis, C0T1: EV vs S P=0.019, EV vs SC P=0.018); throughout the open channel obstruction (univariate ANOVA, group as between-subjects factor: C1T1, $F(5, 74)=7.037$, $P<0.001$; C1T2, $F(5, 74)=7.193$, $P<0.001$; C1T3, $F(5, 74)=3.719$, $P=0.005$) due to enriched groups spending a greater proportion of time within this region than standard mice (multiple comparisons posthoc analysis: C1T1: E vs S P<0.001, E vs SC P<0.001, EV vs SC P=0.023; C1T2: E vs S P<0.001, E vs SC P<0.001, EV vs SC P=0.009; C1T3: E vs SC P=0.005).

The significant effect of animal group was maintained during the first and third trials of the more difficult filled channel and tissue plug obstructions (univariate ANOVA, group as between-subjects factor: C2T1, $F(5, 74)=7.100$, $P<0.001$; C2T3, $F(5, 74)=6.054$, $P<0.001$; C3T1, $F(5, 74)=2.715$, $P=0.026$; C3T3, $F(5, 74)=3.014$, $P=0.016$), due to enriched untreated animals spending a greater proportion of time interacting with the "obstruction" (multiple comparisons posthoc analysis: C2T1: E vs EC $P<0.001$, E vs EV $P<0.001$, E vs S $P=0.045$, E vs SC $P=0.001$, E vs SV $P=0.014$; C2T3: E vs EV $P=0.001$, E vs S $P=0.001$, E vs SV $P=0.030$; C3T1: E vs SC $P=0.044$; C3T3: E vs SC $P=0.033$). Finally, animal group had a significant effect on the first trial of the most difficult foam plug obstruction (univariate ANOVA, group as between-subjects factor, C4T1, $F(5, 74)=3.168$, $P=0.012$) due to enriched non-surgery animals spending a greater proportion of time within "obstruction" than standard untreated mice (multiple comparisons posthoc analysis, C4T1: E vs S $P=0.025$) (Fig. 4.10C).

These results suggest that enriched non-surgery mice display the greatest level of engagement with a novel obstruction puzzle, whilst ChABC treatment appears to increase thigmotaxis in standard mice relative to non-surgery counterparts.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Time spent in wall (%)
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Day 1
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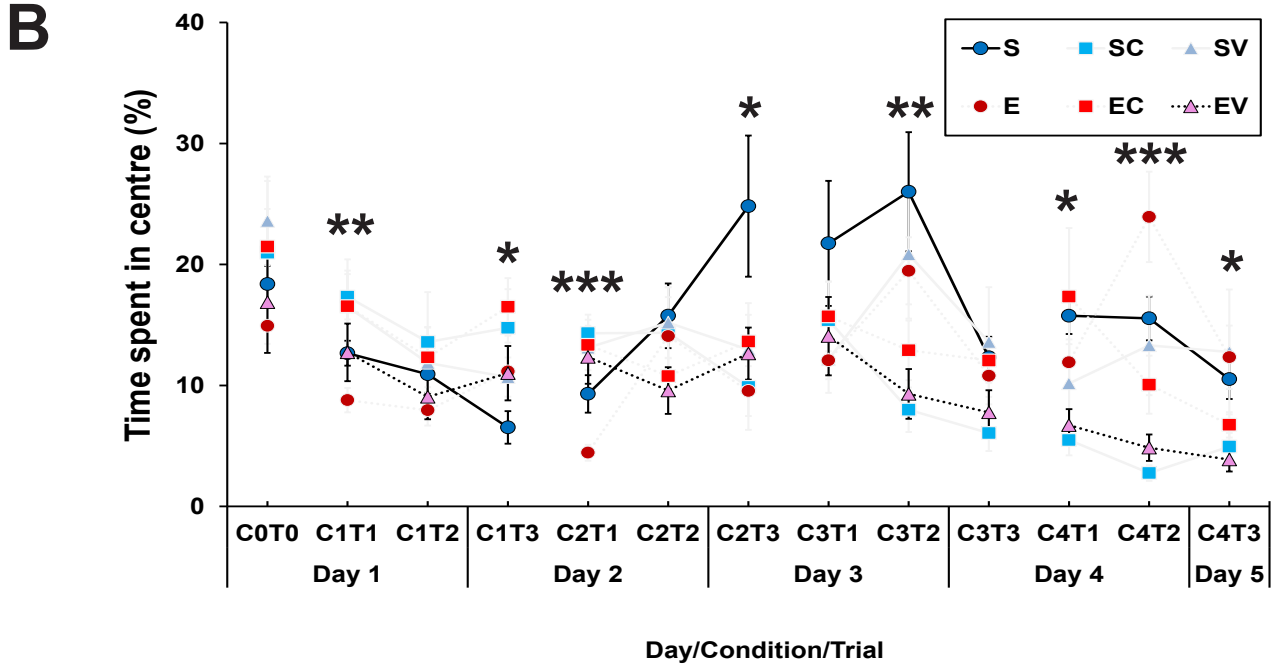
Day 5
Day/Condition/Trial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Day 3
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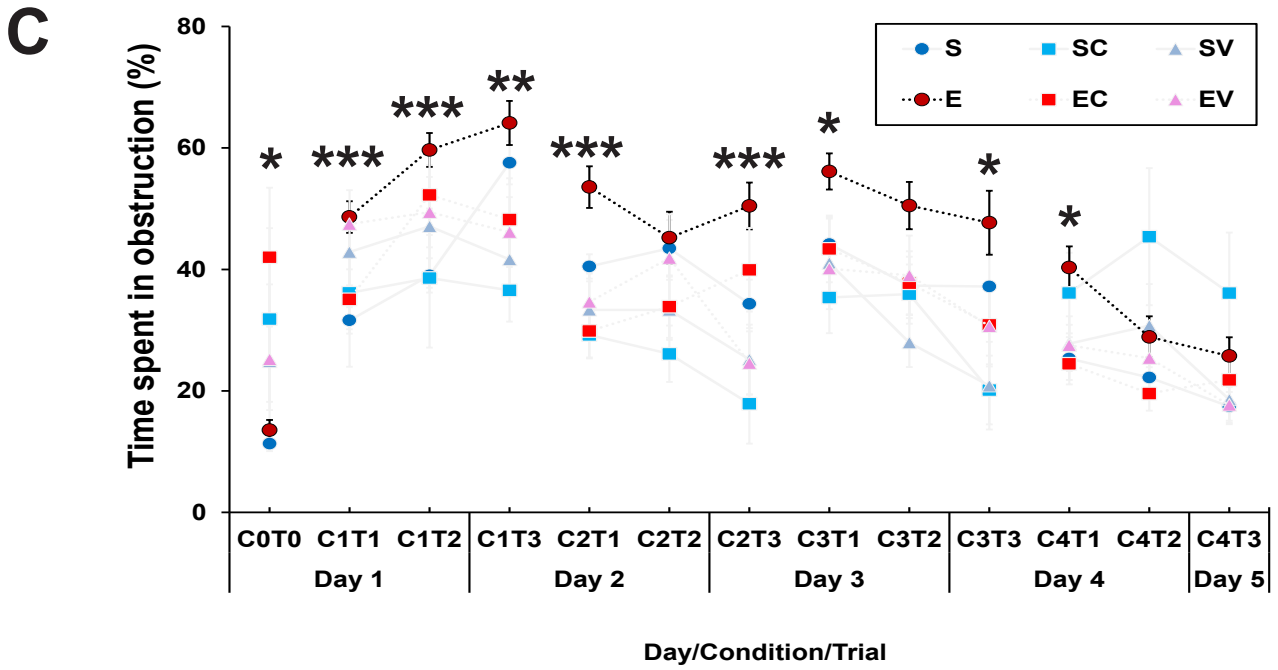
Day 5
Day/Condition/Trial


C
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Time spent in obstruction (%)
80
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C0T0 C1T1 C1T2 C1T3 C2T1 C2T2 C2T3 C3T1 C3T2 C3T3 C4T1 C4T2 C4T3
Day 1
Day 2
Day 3
Day 4
Day 5
Day/Condition/Trial

Figure 4.10: Both vehicle and ChABC treatment impact patterns of movement within the Puzzle-Box apparatus.

(A – C) Graphs plotting the average percentage of total time spent within the Puzzle-Box where an animal was within specified areas in the open-field start zone for mice raised from birth in enriched and standard housing receiving no treatment (E, S), vehicle (EV, SV) or chondroitinase (EC, SC) striatal injection. (A) Animal group significantly impacted the proportion of time mice spent within the "wall" section of the open-field (repeated measures ANOVA, $F=10.240$, $P<0.001$), due to enriched non-surgery mice spending less time within this region than all other groups, and SC mice spending more time within this region than standard non-surgery mice (see text). The effect of animal group was notable at all individual trials except C0T1 (see text) (B) Animal group significantly impacted the proportion of time spent within the "centre" zone of the open-field (repeated measures ANOVA, $F=2.722$, $P=0.026$), due to standard non-surgery animals spending a greater proportion of time within this region than EV mice (see text). This effect of animal group was particularly evident during individual trials: C1T1 (univariate ANOVA, $F=3.660$, $P=0.005$); C1T3 (univariate ANOVA, $F=2.716$, $P=0.026$); C2T1 (univariate ANOVA, $F=6.440$, $P<0.001$); C2T3 (univariate ANOVA, $F=2.652$, $P=0.029$); C3T2 (univariate ANOVA, $F=3.611$, $P=0.006$); C4T1 (univariate ANOVA, $F=2.491$, $P=0.039$); C4T2 (univariate ANOVA, $F=7.657$, $P<0.001$); and C4T3 (univariate ANOVA, $F=2.675$, $P=0.028$). (C) Animal group significantly impacted the proportion of time spent within the "obstruction" area of the open-field zone (repeated measures ANOVA, $F=9.904$, $P<0.001$), due to enriched non-surgery animals spending a greater proportion of time engaged with the obstruction puzzles than all other groups (see text). Again, the effect of animal group was particularly notable during individual trials: C1T1 (univariate ANOVA, $F=7.037$, $P<0.001$); C1T2 (univariate ANOVA, $F=7.193$, $P<0.001$); C1T3 (univariate ANOVA, $F=3.719$, $P=0.005$); C2T1 (univariate ANOVA, $F=7.100$, $P<0.001$; C2T3 (univariate ANOVA, $F=6.054$, $P<0.001$); C3T1 (univariate ANOVA, $F=2.715$, $P=0.026$); C3T3 (univariate ANOVA, $F=3.014$, $P=0.016$); and C4T1 (univariate ANOVA, $F=3.168$, $P=0.012$). ***: $P<0.001$, **: $P<0.01$, *: $P<0.05$. E n=21, EV n=13, EC n=13, S n=18, SV n=7, SC n=8. Error bars=SEM.

Similar trends were observed when the distance traversed within these regions were compared across all cohorts. Quantitative analysis revealed that animal group had a significant effect on the distance travelled in the “wall” (repeated measures ANOVA, group as between-subjects factor, $F(5, 74)=13.010$, $P<0.001$) (Fig. 4.11A), “centre” (repeated measures ANOVA, group as between-subjects factor, $F(5, 74)=10.275$, $P<0.001$) (Fig. 4.11B) and “obstruction” (repeated measures ANOVA, group as between-subjects factor, $F(5, 74)=7.341$, $P<0.001$) (Fig. 4.11C) areas of the open-field zone within the Puzzle-Box.

Posthoc analysis revealed that enriched non-surgery animals travelled less distance in the “wall” (multiple comparisons posthoc analysis: E vs EC $P=0.003$, E vs EV $P<0.001$, E vs S $P<0.001$, E vs SC $P<0.001$, E vs SV $P<0.001$) and “centre” (multiple comparisons posthoc analysis: E vs EC $P<0.001$, E vs EV $P=0.042$, E vs S $P=0.001$, E vs SC $P=0.001$, E vs SV $P<0.001$) areas than all other groups of animals. Standard housed non-surgery and EV animals also travelled less distance within the “centre” than did SV mice (multiple comparisons posthoc analysis: SV vs S $P=0.040$, SV vs EV $P=0.010$). The posthoc differences observed in the “obstruction” area were more limited: standard non-surgery animals covered a greater distance than SC, EC and EV mice (multiple comparisons posthoc analysis: S vs SC $P=0.002$, S vs EC $P<0.001$, S vs EV $P<0.001$).

The effect of animal group upon distance travelled within the various zones of the open-field section of the Puzzle-Box was particularly notable in nearly all individual trials during the task. Animal group significantly affected the distance traversed within the “wall” for all trials except the third trial of the filled channel obstruction (C2T3) and the second trial of the most difficult foam plug obstruction (C4T2). Specifically, significant differences were observed upon first exposure to the Puzzle-Box (univariate ANOVA, group as between-subjects factor: C0T1, $F(5, 74)=8.371$, $P<0.001$) due to SC animals covering a greater distance than all other groups except SV mice (multiple comparisons posthoc analysis, C0T1: SC vs E $P<0.001$, SC vs EC $P<0.001$, SC vs EV $P<0.001$, SC vs S $P=0.034$, SC vs SV $P=0.729$); during the open channel obstruction (univariate ANOVA, group as between-subjects

factor: C1T1, F(5, 74)=6.339, P<0.001; C1T2, F(5, 74)=10.525, P<0.001; C1T3, F(5, 74)=10.834, P<0.001) due to enriched groups covering less distance in the first two trials than standard animals and SC mice covering a greater distance in the third trial than all other groups except SV (multiple comparisons posthoc analysis: C1T1: E vs S P=0.008, E vs SC P<0.001; C1T2: E vs S P<0.001, E vs SC P<0.001, EC vs S P=0.001, EC vs SC P<0.001, EV vs SC P=0.029; C1T3: SC vs E P<0.001, SC vs EC P<0.001, SC vs EV P<0.001, SC vs S P<0.001, SC vs SV P=0.865).

The effect of animal group was maintained in the first and second trials of the more difficult filled channel obstruction (univariate ANOVA, group as between-subjects factor: C2T1, F(5, 74)=8.929, P<0.001; C2T2, F(5, 74)=5.487, P<0.001) due to enriched untreated animals covering less distance than almost all other groups (multiple comparisons posthoc analysis: C2T1: E vs EC P=0.006, E vs S P=0.016, E vs SC P<0.001, E vs SV P=0.006; C2T2: E vs EC P=0.016, E vs EV P=0.032, E vs SC P=0.001, E vs SV P=0.005); throughout the more difficult tissue plug obstruction(univariate ANOVA, group as between-subjects factor: C3T1, F(5, 74)=10.203, P<0.001; C3T2, F(5, 74)=5.404, P<0.001; C3T3, F(5, 74)=7.114, P<0.001), again due to enriched groups covering less distance than standard animals (multiple comparisons posthoc analysis: C3T1: E vs S P=0.019, E vs SC P<0.001, E vs SV P<0.001, EC vs SC P=0.010, EC vs SV P=0.036; C3T2: E vs SC P=0.015, E vs SV P<0.001; C3T3: E vs S P<0.001, E vs SV P<0.001, EC vs SV P=0.009, EV vs SV P=0.046). Finally, there was a significant effect of animal group during the first and third trials of the most difficult foam plug obstruction C4T1, F(5, 74)=4.353, P=0.002; C4T3, F(5, 74)=5.504, P<0.001) due to enriched untreated mice covering less distance in the "wall" than standard untreated animals (multiple comparisons posthoc analysis: C4T1: E vs S P=0.002; C4T3: E vs S P=0.003, E vs SV P=0.001) (Fig. 4.11A).

A similar effect of animal group was observed when performing a trial by trial analysis upon the distance travelled within the "centre", with all trials except the third trial of the filled channel obstruction (C2T3) and the second trial of the tissue plug obstruction (C3T2) impacted. Specifically, upon first exposure to the Puzzle-Box (univariate ANOVA, group as between-subjects factor: C0T1,

F(5, 74)=7.672, P<0.001) due to SC mice covering a greater distance than nearly all other animal groups (multiple comparisons posthoc analysis, C0T1: SC vs E P<0.001, SC vs EC P=0.001, SC vs EV P=0.001, SC vs S P=0.020, SC vs SV P=0.978); throughout the first open channel obstruction (univariate ANOVA, group as between-subjects factor: C1T1, F(5, 74)=9.615, P<0.001; C1T2, F(5, 74)=6.956, P<0.001; C1T3, F(5, 74)=18.949, P<0.001) due to enriched untreated mice covering less distance and SC animals covering a greater distance than most other groups (multiple comparisons posthoc analysis: C1T1: E vs EC P=0.003, E vs SC P<0.001, E vs SV P<0.001, SC vs EV P=0.005, SC vs S P<0.001; C1T2: SC vs E P<0.001, SC vs EC P=0.003, SC vs EV P=0.015, SC vs S P=0.007; C1T3: E vs EC P=0.015, E vs EV P=0.003, E vs SC P<0.001, E vs SV P<0.001, SC vs EC P<0.001, SC vs EV P=0.001, SC vs S P<0.001).

The significant effect of animal group was maintained in the first and second trials of the more difficult filled channel obstruction (univariate ANOVA, group as between-subjects factor: C2T1, F(5, 74)=11.707, P<0.001; C2T2, F(5, 74)=2.478, P=0.039), due to enriched animals covering less distance and SC animals travelling further within the “centre” (multiple comparisons posthoc analysis: C2T1: E vs EC P<0.001, E vs EV P=0.012, E vs SC P<0.001, E vs SV P<0.001, SC vs EV P=0.048, SC vs S P=0.001, no significant posthoc effects for C2T2); and the first and third trials of the more difficult tissue plug obstruction (univariate ANOVA, group as between-subjects factor: C3T1, F(5, 74)=4.456, P=0.001; C3T3, F(5, 74)=2.673, P=0.028) again due to enriched untreated animals covering less distance within the “centre” of the open-field (multiple comparisons posthoc analysis: C3T1: E vs EC P=0.024, E vs EV P=0.041, E vs SC P=0.015, E vs SV P=0.010; C3T3: E vs SV P=0.013). Finally, the significant effect of animal group was maintained throughout the most difficult foam plug obstruction (univariate ANOVA, group as between-subjects factor: C4T1, F(5, 74)=5.229, P=0.002; C4T2, F(5, 74)=3.106, P=0.013; C4T3, F(5, 74)=3.307, P=0.014) due to standard untreated animals covering a greater distance in the first, SC mice covering less distance in the second, and SV mice covering more distance in the third, trials (multiple comparisons posthoc analysis: C4T1: S vs E P=0.032, S vs SC

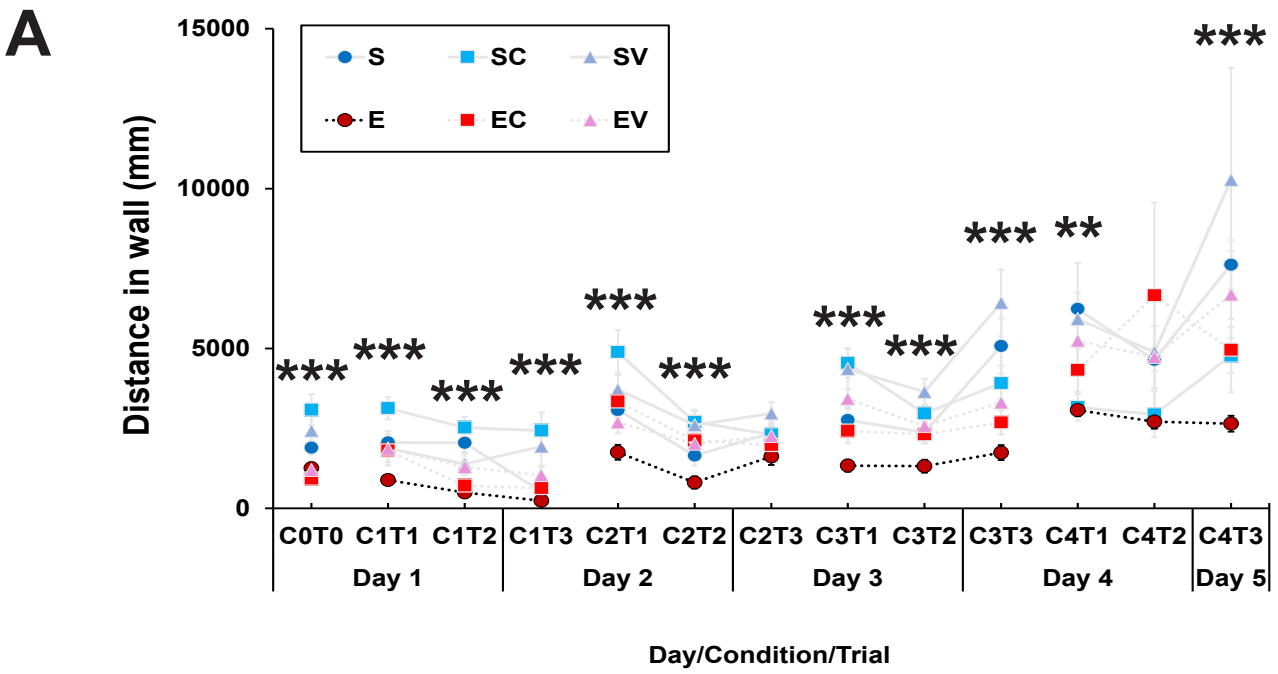
P<0.001; C4T2: SC vs E P=0.034, SC vs S P=0.030; C4T3: SV vs E P=0.024, SV vs EV P=0.034, SV vs SC P=0.046) (Fig. 4.11B).

The “obstruction” region of the open-field in the Puzzle-Box was likewise affected, with distance travelled during all individual trials impacted by animal group, with the exception of the third trial of the tissue plug obstruction (C3T3). Specifically, upon first exposure to the Puzzle-Box (univariate ANOVA, group as between-subjects factor: C0T1, F(5, 74)=11.696, P<0.001) due to enriched untreated animals covering less distance than treated groups (multiple comparisons posthoc analysis, C0T1: E vs EC P=0.037, E vs EV P<0.001, E vs SC P<0.001, E vs SV P<0.001); throughout the open channel obstruction (univariate ANOVA, group as between-subjects factor: C1T1, F(5, 74)=13.227, P<0.001; C1T2, F(5, 74)=5.673, P<0.001; C1T3, F(5, 74)=2.834, P=0.021) due to untreated animals covering less distance than treated mice (multiple comparisons posthoc analysis: C1T1: E vs EV P<0.001, E vs SC P=0.010, E vs SV P<0.001, S vs EV P<0.001, S vs SC P=0.010, S vs SV P<0.001; C1T2: E vs EV P=0.003, E vs SV P=0.004, S vs EV P=0.025, S vs SV P=0.018; C1T3: E vs SC P=0.015, EV vs SC P=0.029).

The significant effect of animal group was maintained during the more difficult filled channel obstruction (univariate ANOVA, group as between-subjects factor: C2T1, F(5, 74)=9.959, P<0.001; C2T2, F(5, 74)=3.254, P=0.010; C2T3, F(5, 74)=7.641, P<0.001) due to untreated animals travelling a greater distance within “obstruction” during these trials (multiple comparisons posthoc analysis: C2T1: E vs EV P=0.002, S vs EC P<0.001, S vs EV P<0.001, S vs SC P=0.027, S vs SV P=0.014; C2T2: S vs EC P=0.039, S vs EV P=0.008; C2T3: E vs EC P=0.002, E vs EV P=0.001, E vs SC P=0.016, S vs EC P=0.002, S vs EV P=0.001, S vs SC P=0.016); in the first and second trials of the more difficult tissue plug obstruction (univariate ANOVA, group as between-subjects factor: C3T1, F(5, 74)=9.070, P<0.001; C3T2, F(5, 74)=7.515, P<0.001) due to standard untreated animals traversing a greater distance (multiple comparisons posthoc analysis: C3T1: S vs E P=0.015, S vs EC P<0.001, S vs EV P<0.001, S vs SC P=0.001, S vs SV P=0.002; C3T2: S vs EC P<0.001, S vs EV P<0.001, S vs SC P=0.016, S

vs SV P=0.005). Finally, animal group had a significant effect during the entirety of the most difficult foam plug obstruction (univariate ANOVA, group as between-subjects factor: C4T1, $F(5, 74)=8.401$, $P<0.001$; C4T2, $F(5, 74)=9.759$, $P<0.001$; C4T3, $F(5, 74)=5.331$, $P<0.001$), again due to untreated animals covering a greater distance within the "obstruction" zone of the open-field than treated mice (multiple comparisons posthoc analysis: C4T1: E vs EC $P<0.001$, E vs EV $P=0.001$, E vs SC $P=0.018$, E vs SV $P=0.021$, S vs EC $P=0.001$, S vs EV $P=0.004$, S vs SC $P=0.036$, S vs SV $P=0.040$; C4T2: E vs EC $P<0.001$, E vs EV $P<0.001$, E vs SC $P<0.001$, E vs SV $P=0.014$, S vs EC $P=0.002$, S vs EV $P=0.012$, S vs SC $P=0.007$; C4T3: E vs EC $P=0.027$, S vs EC $P=0.002$, S vs EV $P=0.012$, S vs SC $P=0.032$) (Fig. 4.11C).

These analyses reveal that both vehicle and ChABC treatments can affect performance, but that the degree to which they influence performance depends on the housing history of the animals. While treatment groups generally exhibited increased latencies to complete the puzzle box task compared to untreated cohorts, remarkably for E mice, the change in performance levels was limited, with both EV and EC groups exhibiting latencies that were indistinguishable from that of untreated S animals. This is in contrast to S mice, where SC animals exhibited generally longer latencies than both SV and untreated cohorts. Trial by trial and area analyses also revealed that the effect of treatment extended across all testing conditions for enriched mice, while for standard animals the changes due to vehicle and ChABC injections were more dramatic earlier in the testing period.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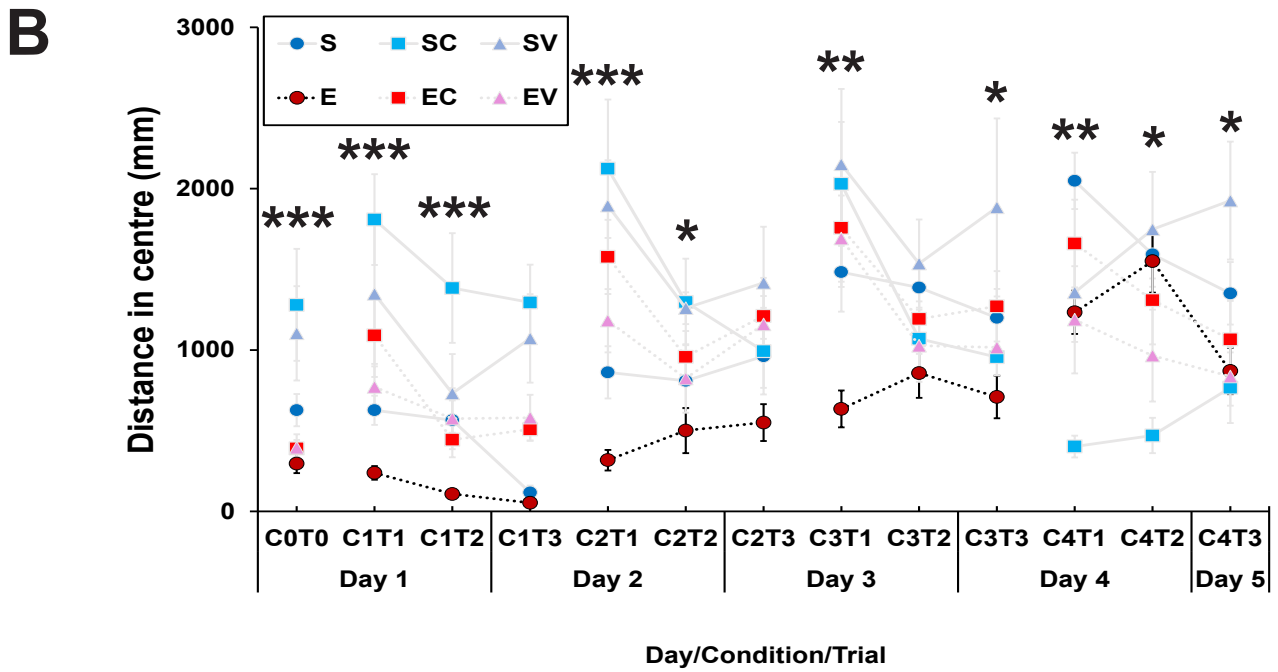
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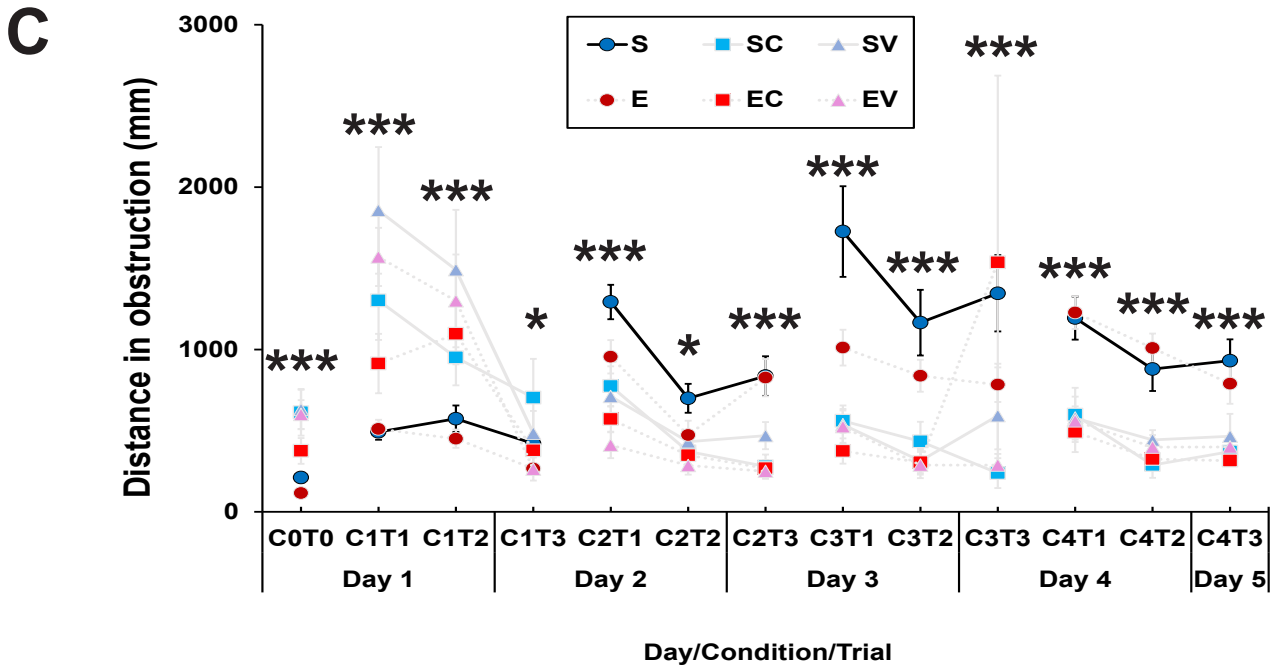
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Day/Condition/Trial

Figure 4.11: Both vehicle and ChABC treatment increase locomotor activity of enriched mice.

(A – C) Graphs plotting the average distance travelled within specified areas in the open-field start zone for mice raised from birth in enriched and standard housing receiving no treatment (E, S), vehicle (EV, SV) or chondroitinase (EC, SC) striatal injection. (A) Animal group significantly impacted the distance travelled within the "wall" section of the open-field start zone (repeated measures ANOVA, $F=13.010$, $P<0.001$), due to enriched non-surgery mice covering less distance within this region than all other animal groups (see text). The effect of animal group was notable in all individual trials except C2T3 and C4T2 (see text). (B) Animal group also significantly impacted the distance traversed within the "centre" region of the open-field (repeated measures ANOVA, $F=10.275$, $P<0.001$), again due to enriched non-surgery mice travelling less distance within this area (see text). This effect of animal group was notable in all trials except C2T3 and C3T2 (see text for details). (C) Animal group significantly affected the distance covered within the "obstruction" zone of the open-field (repeated measures ANOVA, $F=7.341$, $P<0.001$), due to standard non-surgery animals covering a greater distance than SC, EV and EC mice (see text). Again, the effect of animal group was notable in nearly all individual trials, with the exception of C3T3 (see text). *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$. E n=21, EV n=13, EC n=13, S n=18, SV n=7, SC n=8. Error bars=SEM.

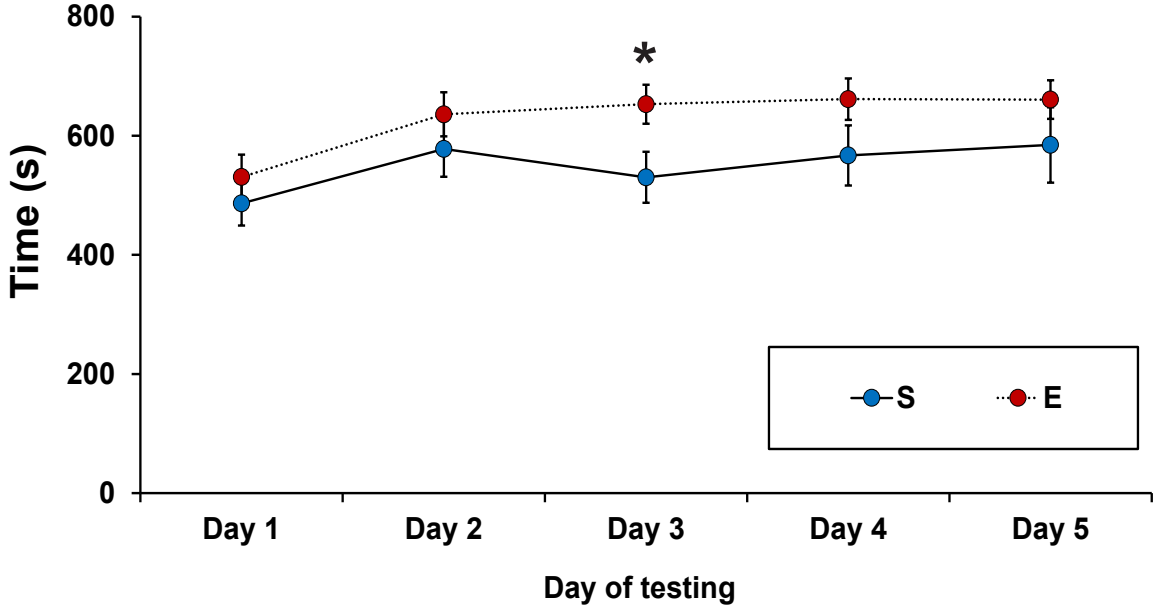
3.4 Environmental enrichment has a minor effect on the acquisition of a sensorimotor coordination task

Previous work from our laboratory has demonstrated that early EE improves the sensorimotor coordination of young animals, as measured by a forced-swim task [22]. Recent work from another laboratory has demonstrated an improvement in performance due to EE during a one day assessment using the rotarod behavioural task [62]. This flexible apparatus has also been used recently to assess the ability of subjects to acquire motor skills by monitoring changes in their performance over multiple trials across several days [27, 63]. The impact of enrichment on subjects using this protocol is not known. Accordingly, I compared the performance of adult animals raised in enriched (E, n=13) and standard (S, n=11) environments on this combined sensorimotor coordination and motor skill acquisition version of the task.

Animals were tested at 12 – 14 weeks of age upon a rotarod apparatus for five consecutive days. I found that housing condition had no effect on overall performance within this task (repeated measures ANOVA, housing condition as between-subjects factor, $F(1, 22)=2.364$, $P=0.138$) (Fig. 4.12A). Performance analysed by day of testing, however, was significantly affected in animals raised within an enriched environment (repeated measures ANOVA, day as within-subjects factor, $F(4, 48)=11.629$, $P=0.001$) but not in animals raised within a standard environment (repeated measures ANOVA, day as within-subjects factor, $F(4, 40)=2.006$, $P=0.198$), although no interaction between housing condition and day of testing was detected (repeated measures ANOVA, housing condition*day, $F(4, 48)=0.947$, $P=0.441$) (Fig. 4.12A). Exploratory day by day analysis revealed that enriched animals performed significantly better than standard mice on a single day of testing (day 3) (univariate ANOVA, housing condition as between-subjects factor $F(1, 22)=5.372$, $P=0.030$) (Fig. 4.12A).

These findings suggest that, relative to goal seeking behaviour, enrichment has a relatively minor influence on the acquisition of a specific motor skill.

A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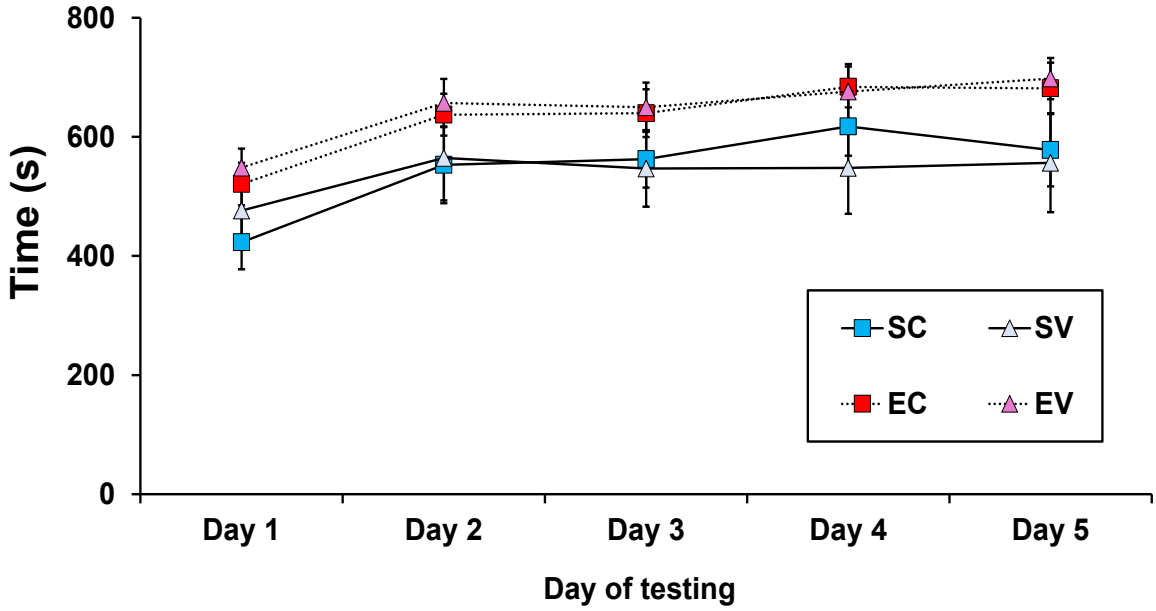
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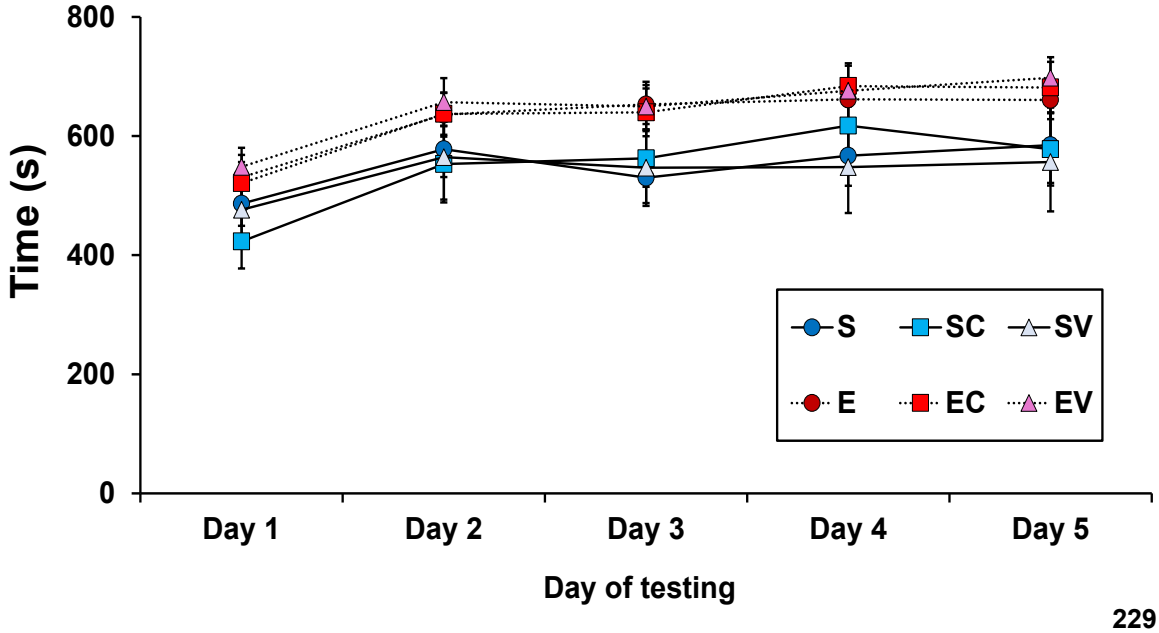
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Figure 4.12: Enrichment-induced improvement in motor learning is prevented by ChABC treatment

(A – C) Average of the sum of the total time spent per day of testing (seconds) upon the rotor during the rotarod behavioural task for mice raised from birth in enriched and standard housing receiving no treatment (E, S), vehicle (EV, SV) or chondroitinase (EC, SC) striatal injection. (A) There was no significant effect of housing condition upon overall performance of non-surgery animals (repeated measures ANOVA, $F=2.364$, $P=0.138$). However, adult animals raised within an enriched environment (E) showed a significant effect of day of testing upon performance (repeated measures ANOVA, $F=11.629$, $P=0.001$), whilst standard housed animals (S) did not (repeated measures ANOVA, $F=2.006$, $P=0.198$). This was particularly so on day 3, when E animals performed significantly better than S mice (univariate ANOVA, $F=5.372$, $P=0.030$). (B) Similarly, there was no significant effect of treatment group (repeated measures ANOVA, $F=1.893$, $P=0.148$) on performance. However, adult animals raised within an enriched environment receiving vehicle injection into the striatum (EV) showed a significant effect of day of testing upon performance (repeated measures ANOVA, $F=5.334$, $P=0.018$), whilst adult animals raised within a standard environment receiving vehicle injection into the striatum (SV) (repeated measures ANOVA, $F=2.665$, $P=0.223$), enriched animals receiving ChABC injection (EC) (repeated measures ANOVA, $F=3.034$, $P=0.077$) and standard (SC) animals receiving ChABC injection (repeated measures ANOVA, $F=2.529$, $P=0.134$) did not. (C) There was no significant effect of surgical treatment on performance when comparing non-treated animals with those receiving vehicle or ChABC treatment (repeated measures ANOVA, $F=1.635$, $P=0.165$). E n=13, EV n=13, EC n=13, S n=11, SV n=7, SC n=8. *: $P<0.05$. Error bars=SEM.

3.5 ChABC treatment has minimal influence on rotarod performance

While the current study has revealed that the digestion of striatal PNNs can have an effect on a task designed to assess goal seeking behaviour (see above), whether the treatment will influence the ability of mice to acquire a motor skill, another behaviour thought to be regulated by striatal function [63], has yet to be determined. Accordingly, ChABC and vehicle treated animals raised in S and E conditions that were tested within the Puzzle-Box (Sv, SC, EV and EC) were further assessed on the skill acquisition version of the rotarod. I found that treatment group (repeated measures ANOVA, group as between-subjects factor, $F(3, 37)=1.893$, $P=0.148$) did not affect performance on the rotarod, as measured by the sum of total time spent on the rotor each day (Fig. 4.12B).

Similar to untreated animals, vehicle treated enriched mice exhibited an improvement in performance across days of testing (repeated measures ANOVA, day as within-subjects factor, $F(4, 48)=5.334$, $P=0.018$) (Fig. 4.12B). No significant effect of day upon performance was observed for standard vehicle (repeated measures ANOVA, day as within-subjects factor, $F(4, 24)=2.665$, $P=0.223$) (Fig. 4.12B) or either ChABC cohort (repeated measures ANOVA, day as within-subjects factor: EC, $F(4, 48)=3.034$, $P=0.077$; SC, $F(4, 28)=2.529$, $P=0.134$) (Fig. 4.12B). Furtjer, there was no significant interaction of treatment group with day of testing (repeated measures ANOVA, treatment group*day, $F(4, 24)=0.597$, $P=0.621$).

Finally, performance of vehicle and ChABC treated animals was compared to that of untreated cohorts to determine whether the effects of treatment varied depending on housing history. There were no significant effects of surgical treatment upon overall performance on the rotarod (repeated measures ANOVA, group as between-subjects factor, $F(5, 74)=1.635$, $P=0.165$) and no significant interaction of animal group with day of testing (repeated measures ANOVA, animal group*day, $F*(5, 74)=0.547$, $P=0.740$) (Fig. 4.12C).

These results suggest that although the striatum is involved in motor learning neither enrichment nor enzymatic digestion of striatal PNNs dramatically affected motor skill acquisition for this rotarod based test.

4. Discussion

This study confirms that EE is capable of dramatically affecting the performance of adult animals during a behavioural task testing goal seeking behaviour. Animals raised within enriched housing solved obstruction puzzles within the Puzzle-Box significantly faster than did standard housed animals, due at least in part to differing movement patterns within this testing apparatus. At the same time, EE has only a minor effect on the motor skill acquisition of a rotarod task. In contrast, striatal PNN dissolution compromised the performance of animals within the Puzzle-Box and also impaired motor skill acquisition. Finally, EE reduced striatal ChABC-induced behavioural deficits within the Puzzle-Box, but had little impact upon impairment observed in rotarod motor skill acquisition. Together, these results have important implications for the role of environmental influences upon the striatal function of mice, and suggest that plasticity effects induced by ChABC treatment manifested at potentially different levels of striatal processing for enriched and standard mice.

4.1 Environmental enrichment improves performance in the Puzzle-Box behavioural task

In the current study, enriched animals demonstrated lower latencies to solve obstruction puzzles within the Puzzle-Box behavioural task, particularly during the first trial of an obstruction condition. This first trial is meant to assess the native problem-solving ability of animal subjects [51-53], suggesting that EE, whilst having some effect on the ability of animals to retain and recall solutions to obstruction challenges, has a greater impact upon native problem-solving ability of an animal.

Enriched animals were found to spend less time within the testing arena, and a greater proportion of time interacting with obstruction puzzles, suggesting that EE increases engagement with these behavioural challenges when animals are placed into the Puzzle-Box arena. Very few animals were capable of solving the fourth obstruction puzzle in the allotted time, suggesting that this obstruction condition may have been too difficult, to the extent that even enriched animals struggled to remove the foam plug. Despite there being no effect of EE on performance, enriched animals still displayed a differing behavioural pattern within the Puzzle-Box when approaching the fourth obstruction puzzle, suggesting that strategies for solving obstruction conditions were maintained even when they were too difficult to actually remove.

Why do enriched untreated mice show greater engagement with obstruction obstacles? One possibility is that standard and enriched cohorts exhibited different levels of anxiety towards open environments. One metric often used in standard open field tests to assess stress levels is to measure the degree to which subjects exhibit thigmotaxis, or the propensity to avoid the central portion of the environment, maintaining close proximity to the boundaries or walls [64, 65]. While standard mice did spend a greater amount of time and traversed a greater distance along the walls of the Puzzle-Box open-field, they also exhibited increased traversal within the centre of the arena. Indeed, the fact that enriched mice spent less time in the centre, instead seeking the obstruction area, which also happens to be proximal to a wall, suggests that they may be even more anxious to remove themselves from the stressful open environment than standard untreated animals. This interpretation is consistent with previous findings showing that environmentally enriched mice exhibit improved performance on open water tasks such as the Morris Water Maze [22]. That there was little difference in the behaviour of standard and enriched untreated animals in the initial unobstructed, habituation trial of the task, however, suggests that there may be little base-line differences between the two groups with respect to open-field anxiety. Without directly comparing post-test anxiety levels via measurement of cortisol levels, differences in stress response to the task

is not explicitly determinable. Nevertheless, my evidence suggests that anxiety in itself is not likely to be the sole mechanism underlying the differences observed in S and E performance.

Could the enriched animals be more prone to novelty seeking? The propensity to explore novel environments or objects has been shown to be associated with, yet differentiable from, anxiety [66]. Recent work has indicated that exposure to stimulating environments can increase sensitivity to novelty in rats, albeit in a different context [67]. Whether this would in turn translate into increased novel object exploration is unclear. Nevertheless, the finding that enriched animals spend a significantly greater amount of time proximal to the goal-box entry in nearly all presentations of new obstacles, relative to standard housed cohorts, suggests that this or a related attribute may contribute to the behavioural disparity observed here.

Another possible explanation for the improved performance differences between standard and enriched untreated cohorts is a generalised improvement in cognitive ability: enriched mice may be more willing to engage with obstruction puzzles as they have figured out that removing obstructions grants access to the covered goal-box. This is supported by the reduced locomotion displayed by enriched animals, suggesting that these mice hone in and remain focused on obstruction puzzles in order to escape the open-field start zone, spending less time roaming the arena. An enrichment-induced increase in motivational drive would also explain the greater amount of time enriched animals spent engaged with obstruction puzzles. In order to better understand the improvement that EE induces in cognitive-based tasks, further studies into the exact neural and behavioural mechanisms underlying this phenomenon are required.

4.2 Environmental enrichment reduces ChABC-induced deficits in Puzzle-Box performance

Damage to the striatum has been shown to interfere with a wide range of behaviours, including sensorimotor coordination [26, 68], volitional drive, motivation, and cognitive processes such as rule

learning and task acquisition [28-31]. Solving the Puzzle-Box behavioural task may be considered to contain elements of all of these behaviours: sensorimotor coordination is required to successfully remove obstruction puzzles; volitional drive to engage with obstructions; motivation to drive animals to seek the sheltered goal box; and cognitive processes to learn how to remove obstruction puzzles.

The incentive to solve the Puzzle-Box is considered to be mixed itself – an animal's desire to seek out a small, dark enclosed space, and objects to explore and hide in within such a sheltered area are thought to be the main drivers of behaviours in this testing arena [51-53]. Previous studies have demonstrated that lesions within the hippocampus lead to generalised deficits in behavioural performance and cognitive ability within the Puzzle-Box testing apparatus [51]. In contrast, lesions to the medial prefrontal cortex have been shown to result in a specific deficit in problem solving ability within the Puzzle-Box behavioural task [69]. Given the mixed motivation for solving this task, and the varied functions undertaken by the striatum, it is not unexpected that this area of the brain might mediate behaviours within the Puzzle-Box task.

The effect of striatal ChABC treatment on performance as measured by latency to goal-box was particularly pronounced during the first day of testing for standard mice, with a smaller and more dispersed effect upon the behaviour of enriched animals. Curiously, enriched animals receiving vehicle or ChABC demonstrated a similar level of performance. Even more remarkably, with the exception of the initial open entry condition, both cohorts exhibited performance latencies similar to vehicle treated standard housed animals. The mechanism underlying the observed changes due to striatal PNN digestion is unclear. Area analyses revealed that ChABC treated mice exhibited an increase in time spent and distance travelled within both the wall and centre regions of the open-field. These changes appeared to be slightly more prominent early in testing, although some of the effects persisted across most conditions. High novelty-induced locomotor activity is reminiscent of behaviour exhibited by rats that have a tendency to acquire cocaine self-administration [70], which in turn has been shown to depend upon dopaminergic input to the dorsal striatum [71]. The

digestion of striatal PNNs may possibly disrupt transmission of the reward signal to one of the key recipients of dopaminergic input within the nucleus, parvalbumin-expressing (PV+) cells [58]. Although further work is required to assess this possibility, the observed behavioural changes are not inconsistent with striatal ChABC treatment contributing to a shift – at least temporarily – in the overall efficiency of the striatal dopamine signal, leading to an increase in sensory seeking and this hyperactive open-field behaviours.

In light of this, previous studies have demonstrated the ability of EE from birth to blunt the rewarding effects of cocaine, as well as reduce cocaine induced locomotor activity [72, 73]. The current results are consistent with these findings: ChABC treatment did affect Puzzle-Box performance in enriched mice, but not to the same degree observed in standard cohorts. While further studies would be required to elucidate the exact mechanisms by which EE reduces the full behavioural effects of striatal PNN dissolution, the evidence presented here suggests that enrichment may be limiting, and thus protecting against, ChABC-induced changes.

Standard housed animals with ChABC injection were more affected than standard animals receiving vehicle in the early stages of testing, and overall more affected than enriched mice receiving ChABC. It would appear that striatal PNN dissolution has the greatest impact on performance of standard housed animals when first encountering testing arenas, and that EE reduces these effects.

4.3 Differential effects of ChABC treatment on enriched and standard housed mice suggest multiple levels of malleable striatal networks

Arguably the most surprising finding from this study is the observation that not only did enrichment limit the effect of PNN digestion, but both vehicle and ChABC treatments led to a Puzzle-Box behavioural profile in enriched mice that was nearly indistinguishable from standard untreated and vehicle treated animals. Both vehicle treatment and ChABC injections were found to reduce PNN

density within the striatum, with a greater effect from ChABC. The reduction in PNNs resulting from vehicle treatment was unexpected, but may explain the behavioural differences observed between vehicle treated and untreated animals. The changes in performance of treated relative to untreated enriched mice may at least in part be due to damage done to the striatum as a result of the injection procedures used to deliver the treatment. Previous studies have shown that lesions to dorsal striatum induce rule-based learning impairments [28] and can impact cocaine and morphine self-administration, leading to an overall decrease in reward-seeking behaviour [74]. In rats, however, a decreased drive to self-administer cocaine is usually correlated with reduced activity in novel environments [70], opposite to the effect described here of ChABC treatment on enriched animals. Moreover, this model does not provide any straightforward explanations as to how potentially minor, yet indiscriminate, damage to dorsal striatum would "pin" its behavioural effects to levels comparable to untreated standard housed cohorts.

Similarly, while enrichment does limit the effects of ChABC treatment on Puzzle-Box performance, it does so only to the level of vehicle treated enriched animals. As no differences were observed in the degree of WFA staining due to enzymatic treatment between SC and EC cohorts (Fig. 5), the effect of ChABC treatment must be downstream of PNN digestion.

This gives rise to the intriguing possibility that the striatum has potentially two levels of circuitry involved in the regulation of goal-seeking behaviour: a "hard", relatively fixed version that serves to regulate base-line levels of striatal function observed in standard animals; and a second "soft", pliable version that underlies the improves performance seen in enriched mice. These two levels can be established during the putative PV+ dependent striatal critical period, manifested in potentially increased afferent drive and corresponding increases in PV expression (see Chapter 2), as well as morphological changes observed at the level of individual neurons within the striatum [22]. Removal of PNNs or incidental damage to the nucleus in enriched animals may lead to a selective culling of these soft networks, leaving the hard circuits intact, whilst ChABC treatment in standard mice,

without the buffer of these extra connections, may yield a more dramatic change in behaviour. The presence of circuit level function that is relatively impermeable to manipulation is well established [44]. Although not analogous, such properties provide precedence for the model proposed here. Whether this, or comparable mechanisms underlie the disparity in treatment effects observed in standard and enriched cohorts is a topic for future study.

It is not entirely clear what effects PNN digestion has on the neurons these extracellular matrix structures associate with, nor why their removal is capable of inducing such large behavioural effects. PNNs are known to associate with fast-spiking inhibitory interneurons, providing a highly anionic environment to buffer against the flow of cations around this neural population [38-41], and are also thought to consolidate synaptic connections [35-37], “locking” circuitry into place. The removal of PNNS may expose the cells they associate with to calcium excitotoxicity, resulting in the loss of particular neural populations. This would be particularly injurious within striatal circuitry, where PV+ inhibitory interneurons associated with PNNs [41] play a large role in feedforward inhibition and modulating the activity of medium spiny neurons, the output population of the striatum [58, 60, 61, 75, 76]. Previous work, however, has demonstrated that the removal of PNNs does not impact the immunoreactivity nor density of PV+ inhibitory interneurons within the cortical regions [77], suggesting that behavioural consequences of PNN digestion are mediated by a mechanism other than neuronal loss. It is known from experiments using hippocampus-derived cell cultures that PNN digestion results in increased excitability of the inhibitory interneurons these structures associate with, whilst having little effect on GABAergic synaptic contacts of neurons that were previously enveloped by PNNs [78]. PNN removal may result in altered synaptic connectivity and activity such that normal circuitry function is disrupted, as mentioned above regarding the possible effects of ChABC treatment on dopaminergic transmission within the striatum. Further analyses assessing how PNN removal may impact the associated population of PV+ inhibitory interneurons within the striatum may shed light on why striatal ChABC treatment has such dramatic behavioural effects.

4.4 Neither EE nor striatal PNN dissolution dramatically affected motor skill acquisition

Unlike goal-seeking behaviour assessed in the Puzzle-Box, both enrichment and ChABC treatment had little effect on another striatally mediated behaviour, the acquisition of a motor skill. While some effects were observed, none of them were particularly dramatic. This was surprising, given that previous work from our laboratory has demonstrated that enrichment from birth accelerated the emergence of striatally mediated behaviours in developing mice [22], ChABC digestion of striatal PNNs led to changes in hind limb ambulation suggestive of a regression to a more juvenile gait [41], and a mouse model with a known projection defect in the thalamostriatal pathway exhibited a significant delay in acquiring the same rotarod based motor skill task used here [63].

A number of factors may have contributed to this relative lack of change. One possibility is that the rotarod task may not have been sensitive enough to pick up subtle changes attributable to the acquisition of a motor skill [41]. A more discerning test may be needed to determine whether there is any interaction between EE and striatal ChABC infusion with relation to striatally mediated sensorimotor behaviours.

Alternately, the timing of the testing itself may have compromised an ability to adequately examine the influence of both enrichment and ChABC treatment on this particular motor skill task. All rotarod assessments occurred after subjects were tested within the Puzzle-Box. Accordingly, while PNNs remained depleted even after completion of all behavioural testing (see Fig. 5), it is possible that any plasticity in striatal circuitry induced by ChABC treatment may have waned over the duration of testing. Indeed, in other brain areas the maturation of inhibitory networks, and the resulting termination of the developmental critical period, precedes PNN formation [36]. Thus, while the influence of enrichment remained relatively intact, as evidenced by the differences in S and E cohorts in latency changes across the period of testing, the effect of the enzymatic treatment may have been curtailed. The finding that PNN digestion appeared to have the greatest influence on the

first day of the Puzzle-Box protocol is also consistent with this possibility. Further experiments initiating testing closer to the onset of treatment will be required to more adequately assess the roles of enrichment and PNNs on motor learning.

4.5 Conclusions.

The behavioural analyses used within this study have granted insight into the whole animal effects of EE and ChABC-induced PNN dissolution within the striatum, along with the ways in which these two treatments interact with one another. These results provide evidence that EE from birth can induce measurable changes in striatally dependent behaviours, potentially increasing motivational drive and novelty seeking, along with improving problem-solving and task acquisition capabilities. Some of these aspects are shown to be affected by PNN dissolution within the striatum, with a lesser impact observed on the same behaviours of enriched animals. Determining the exact mechanisms underlying how enrichment limits the effects of this intervention may provide a deeper understanding of how plasticity is regulated in these vital neural networks.

5. References

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Chapter 5:

Assessing the Impact of Environmental Enrichment upon Decision-Making Behaviours within the IntelliCage System

Abstract

It is known that environmental enrichment can influence several cognitive behaviours associated with striatal function. The effect of enrichment on more complex striatally mediated behaviours, however, has not yet been investigated. Accordingly, a novel rodent version of the Iowa Gambling Task (RGT), developed using the IntelliCage behavioural testing system, was used to determine the impact of both life-long and adulthood environmental enrichment upon decision-making behaviours. Animals raised from birth in an enriched environment and animals brought into the testing facility as young adults demonstrated more exploratory behaviour upon first exposure to the IntelliCage apparatus. All animals were able to successfully discriminate between low and high risk decisions within my version of the RGT, regardless of housing condition. Enrichment, however, did not impact decision-making within the RGT compared to standard housed cohorts. These results provide evidence that mice are capable of performing complex behavioural tasks previously only conducted using rats as subjects; that the IntelliCage testing arena is able to be used to develop behavioural tasks similar to those conducted using human subjects; and that environmental enrichment has no impact on reward-based decision-making within the paradigm presented here.

Keywords: Environmental enrichment; Behaviour; Rodent/Iowa Gambling Task; IntelliCage

1. Introduction

The basal ganglia are involved in mediating a wide variety of behaviours, ranging from voluntary movement, sensorimotor coordination, skeletomotor, oculomotor, cognitive and emotional functions [1-3]. Of particular interest is the role of the striatum in decision-making behaviours.

Previous work has revealed that the striatum is involved in moderating important features of the decision-making process, due to the ability of this brain region to integrate sensory, motor, emotional and cognitive function [2, 4]. Striatal circuitry is thought to be involved in selection and initiation of actions based upon potential reward value [1], with the dorsal striatum particularly involved in this process.

Environmental manipulations, particularly environmental enrichment (EE) can affect the development and function of neural circuits, and striatal pathways are no exception. The striatum is known to be sensitive to EE in several ways. Early enrichment can accelerate the formation of striatal perineuronal nets (PNNs), an extracellular matrix structure crucial for the maturation of circuits within this nucleus ([5]; see also Results Chapter 1, above). Exposure to EE during adulthood increases dendritic branching and spine density of striatal medium spiny neurons [6, 7]. Along with anatomical effects, EE has been shown to affect the levels of neurotrophic growth factors [8-10], up-regulate metabolic activity [11], and influence the expression of genes within the striatum involved in cell proliferation, differentiation, structure and metabolism, and signal transduction, transcription and translation [12]. As demonstrated within results chapter one of this thesis, EE is capable of accelerating the morphological maturation of a population of inhibitory interneurons within the striatum. Given the anatomical and molecular changes induced within the striatum by EE, enrichment would likely affect behaviours that are dependent on striatal function. Previous studies have revealed improved cognitive performance resulting from EE [5, 13] (see also Results Chapter 3, above), but as yet there has not been a thorough investigation of the effects of EE upon decision-making behaviours.

One behavioural test used to assess decision-making behaviours is the Iowa Gambling Task (IGT). A task traditionally used in human psychological studies, the IGT tests the ability of a subject to postpone an immediate reward for a greater outcome in the longer term [14]. In the human version of this task subjects are asked to choose between four decks of cards: two of which give large wins and larger losses, and are thus overall disadvantageous; and two of which give small wins and smaller losses, and are thus overall advantageous [14, 15]. Healthy subjects will generally favour the advantageous decks as testing progresses, whilst subjects with an impaired decision-making ability will generally favour the decks with large wins and larger losses [16]. The IGT has previously been adapted for use on rodent subjects, with the supply and withdrawal of food used as reward and loss factors [17]. One such study using rats revealed that greater levels of early life maternal care can lead to more advantageous decision-making behaviours as adults in a version of the RGT [18]. Whether environmental factors influence the RGT choice behaviours of mice, however, has yet to be determined.

The IntelliCage system is an automated behavioural testing apparatus in which animals are housed during the duration of a task, and the behaviour of each individual within the cage continually monitored. The system requires minimal handling of mice, eliminating a source of distress for animals [19-21]. The IntelliCage has proven to be robust to strain and laboratory differences [55], and the system's flexible design provides an ideal foundation for developing and/or adapting complex cognitive tasks to rodents. Thus far, no version of the RGT has been implemented for mice, using the IntelliCage or otherwise.

Given the role striatal circuits play in regulating reward seeking and decision-making behaviours, and the large impact EE has upon this nucleus, it may be expected that performance within a RGT is affected by enrichment. This study proposes to use the IntelliCage system as a testing arena for a novel gambling task investigating the impact of both lifelong and adulthood EE on reward/loss based decision-making behaviours.

2. Materials & Methods

2.1 Ethics Statement

All procedures were approved by the Animal Ethics Committee of the University of Sydney and conformed to National Health and Medical Research Council of Australia guidelines under the ethics numbers K22/09-09/3/5128 and K22/11-12/3/5838. Experiments were performed using C57/BL6J mice reared and/or housed within the University of Sydney Animal Housing Facility. All mice were kept in individually ventilated cages at 21^{o}C ambient temperature with *ad libitum* access to both water and food when not undergoing behavioural testing.

2.2 Housing of animals in standard and enriched environments

On arrival, half of the animals were randomly assigned to standard housing (30cm x 15cm x 13cm cage), and the other half to enriched housing (46cm x 40cm x 40cm cage), adapted from the protocol used in Simonetti et al, 2009 [5]. Standard environments contained a single red mouse igloo and extra material for bedding. Enriched housing contained red mouse igloos, a running wheel, marbles, tunnels, extra housing and material for bedding, Velcro, scented plush balls (vanilla, strawberry and cinnamon), a rubber ball and two high-contrast visual stimuli. These items were moved around the cage every two to three days and refreshed once a month to ensure novelty.

Two pregnant dams were housed within each enriched cage, and one pregnant dam within each standard cage. Young animals were weaned at 21 days postnatal (P21) and placed into male-female segregated housing conditions the same as that which they were born into. On arrival, 8 – 10 week old C57/Bl6J mice were housed 5 to a cage within standard or enriched cages for six weeks prior to behavioural testing. Behavioural testing was carried out once animals reached 14 – 16 weeks of age. A total of four groups were tested: animals raised from birth in EE (E); animals raised from birth in a

standard environment (S) animals housed in EE upon arrival as young adults (SE); and animals housed in a standard environment upon arrival as young adults (SS).

2.3 Microchipping

A week prior to behavioural testing, animals were anaesthetised with 2-4% isoflurane in oxygen, and a radio-frequency-identification (RFID) chip (Planet-ID, 12mm, 0.09g) was injected into the adipose tissue at the nape of the neck. Animals were returned to their home cages following this procedure to allow for recovery.

2.4 Behavioural testing – the IntelliCage System

The IntelliCage system consists of a large (20.5cm x 40cm x 50cm) transparent cage, outfitted with four identical operant conditioning corners (Fig. 5.1A – B). Each corner contains two water sippers from which animals may drink, and is accessible to only one animal at a time (Fig. 5.1B). There is a microchip reader placed within the access point of each corner, to monitor the movements of individual animals within the cage. Access to the water sippers is controlled by means of a small door on the front of each sipper, which may be opened by animals performing various behaviours (e.g. nose-pokes).

Animals were placed into the IntelliCage system to undergo a modified version of the rodent gambling task (RGT) [17], using water access as a reward. Subjects were exposed to three training stages leading up to the RGT: free adaptation; nose-poke adaptation; and drinking session adaptation (Table 5.1). Animals were continuously monitored. Mice exhibiting adverse signs of water limitation, or who had not drunk for a period of 24 hours or more were removed from the testing arena. Unless stated otherwise, the following number of animals were tested at each stage of the

task: E n=16 (8 male + 8 female); S n=13 (7 male + 6 female); SE n=20 (10 male + 8 female); and SS n=20 (10 male + 10 female).

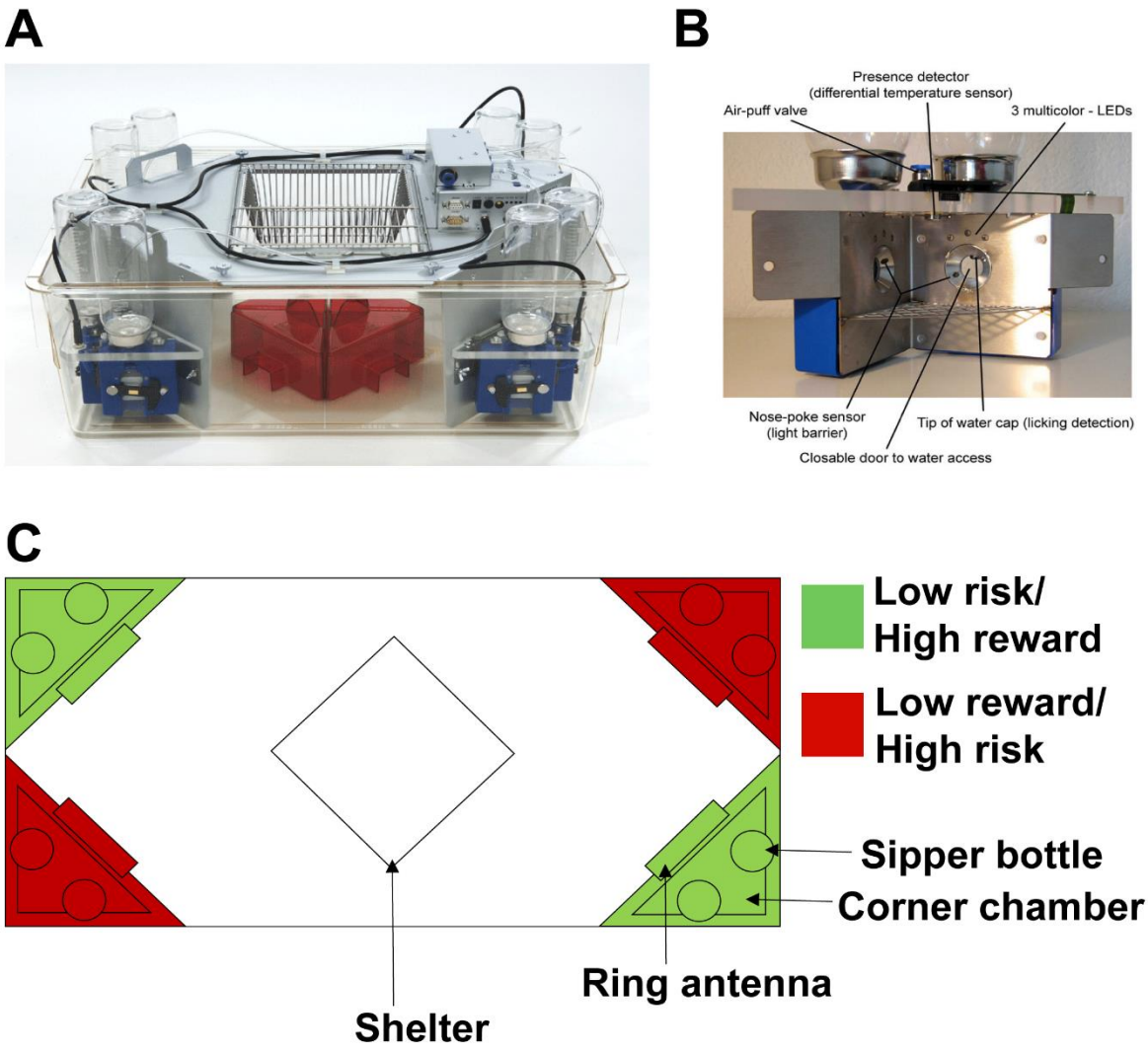


Figure 5.1: The IntelliCage testing system.

(A) The IntelliCage testing system. A large (20.5 x 40 x 50 cm) transparent cage, with triangular conditioning corners (15cm x 15cm x 20cm) that may be accessed via a ring antenna (30mm diameter at smallest point), allowing only one animal to pass into the chamber at a time. (B) A conditioning corner within the IntelliCage system, consisting of a mesh floor and two walls with 13mm wide holes allowing access to water sippers. A detector determines the presence of an animal within the conditioning corner; LEDs may be used to provide stimulus cues, and the air-puff valve to provide negative reinforcement. Doors may be closed across the water sipper holes, and access gained by nose-poking to break a laser beam across the doorway. (C) Schematic of the rodent gambling task (RGT) run within the IntelliCage system. Animals were individually assigned two diagonally opposed low risk/high reward (overall advantageous) corners, and two diagonally opposed high risk/low reward (overall disadvantageous) corners. Water access was restricted to three, one hour periods a day during which animals were required to nose-poke in order to open the doorways.

Table 5.1: Schematic of the IntelliCage testing phases during the Rodent Gambling Task

Testing phase	Free Adaptation	Nose-poke Adaptation	Drinking Session Adaptation	Rodent Gambling Task
Door state	Open	Closed	Closed	Closed
Nose-poke to open door	N/A	Yes	Yes	Yes
Limited access time	No	No	Yes: 8pm – 9pm 3am – 4am 11am – 12pm	Yes: 8pm – 9pm 3am – 4am 11am – 12pm
Time doors are open	N/A	1.5 seconds	1.5 seconds	"Low risk" choice: 1.5 seconds "High risk" choice: 3 seconds
Time out following drinking	No	No	No	"Low risk" choice: 30 seconds "High risk" choice: 240 seconds

2.4.1 Free Adaptation (FA)

Animals from the same home cage were placed into a single IntelliCage (Fig. 5.1) that had been cleaned with 70% ethanol, filled with fresh bedding, and supplied with autoclaved feed and a clean red mouse igloo. Two to four cages were run on the same program simultaneously, with all animals following the same protocol. During free adaptation (FA), both food and water were available *ad libitum* – the doors restricting access to the water sippers were open the entire time. This phase of testing lasted 3 days (Fig. 5.2), and the number of visits to corners during initial exposure and throughout FA recorded to assess exploratory and adaptation behaviours [20] [E n=16 (8 male + 8 female), S n=13 (7 male + 6 female), SE n=20 (10 male + 8 female), SS n=20 (10 male + 10 female)].

2.4.2 Nose-poke Adaptation (NPA)

Following FA, the doors on the water sippers were closed. Animals were able to access the sippers by breaking a laser beam across the door with their nose, causing the door to open for 1.5 seconds. Animals then had to leave the conditioning corner before they could nose-poke once again and access more water. From NPA onwards, the incidence of nose-poking during visits may be considered as a deliberate behaviour, as animals attempt to open the water sipper access doors. Both water and food were available *ad libitum* during this time. This phase of testing lasted 4 days (Fig. 5.2), and the number of visits to corners and the proportion of nose-pokes and visits made both with and without drinking were recorded [E n=16 (8 male + 8 female), S n=13 (7 male + 6 female), SE n=20 (10 male + 8 female), SS n=20 (10 male + 10 female)].

2.4.3 Drinking Session Adaptation (DSA)

Following NPA, animals underwent temporal training where access to the water sippers was restricted to three, one hour long periods per every 24 hours (8pm – 9pm, 3am – 4am, 11am – 12pm). During these times, animals were able to access the sippers by breaking a laser beam across the door with their nose, causing the door to open for 1.5 seconds. Similar to NPA, animals had to leave the conditioning corner before they could again access the water sippers. This phase of testing lasted five days (Fig. 5.2), with a minimum of 15 drinking sessions experienced by animals. The number of visits to corners during each drink session, the incidence of nose-poking and the proportion of visits with nose-pokes and visits were recorded for analysis [E n=14 (7 male + 7 female), S n=13 (7 male + 6 female), SE n=15 (10 male + 5 female), SS n=15 (10 male + 5 female)].

2.4.4 Rodent Gambling Task (RGT)

Following DSA, animals underwent a Rodent Gambling Task protocol (RGT) within the IntelliCage. During this time, access to water sippers was maintained on the same schedule as that during DSA, and access to the sippers was gained by means of breaking a proximity detector beam. Each individual animal was assigned two diagonally-opposite corners within the IntelliCage classed as either low risk/high reward (overall advantageous), or high risk/low reward (overall disadvantageous) for that mouse (Fig. 5.1C). Animals within a cage were allocated evenly between each combination of corners such that the high risk/low reward corners for one half of a cohort within an IntelliCage were the low risk/high reward corners for the other half. This was done to prevent any "follow the leader" effects upon the behaviour of animals housed within the same IntelliCage [22].

Low risk/high reward corners allowed an animal to access the water sippers for 1.5 seconds, followed by a "timeout" of 60 seconds, during which animals were not able to access any water sippers. High risk/low reward corners allowed an animal to drink for 3 seconds, followed by a timeout of 240 seconds during which animals were not able to access any water sippers. Overall, animals received 50% more drinking time within an hour-long drinking session by consistently choosing to drink from low risk/high reward corners (see Table 1 above). Note was made of the number of visits to corners, the incidence of nose-poking and the proportion of visits with nose-pokes occurring during each drinking session, and analyzed according to the risk/reward classification of conditioning corners. E n=14 (7 male + 7 female), S n=13 (7 male + 6 female), SE n=15 (10 male + 5 female), SS n=15 (10 male + 5 female).

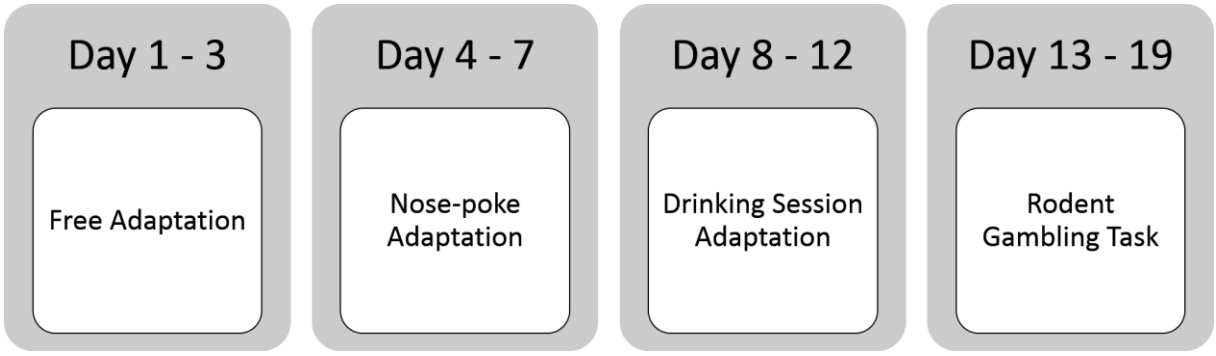


Figure 5.2: Timeline of the RGT.

Animals within the IntelliCage underwent a series of training steps prior to undertaking the RGT. Free adaptation lasted for three days; nose-poke adaptation for four days; and drinking session adaptation for five days, before the RGT was run for seven days.

2.4.5 Analysis

Patterns of visitation to conditioning corners during the first hour within the IntelliCage are thought to be indicative of the exploratory behaviour of an animal [20], whilst the overall number of visits made throughout testing are thought to reflect adaptation and ongoing activity levels [20].Visitation to conditioning corners upon initial exposure to the IntelliCage was analysed using a univariate ANOVA with housing condition as between-subjects factor. The behaviour of animals was analysed by day within each testing phase, using repeated measures ANOVA with housing condition as between-subjects factor and multiple comparisons posthoc analysis to determine any differences between individual groups. In all analyses, comparisons were made across all four housing groups and population was regarded as the number of animals within each housing condition group, as described above in *Materials and Methods* and in figure legends.

3. Results

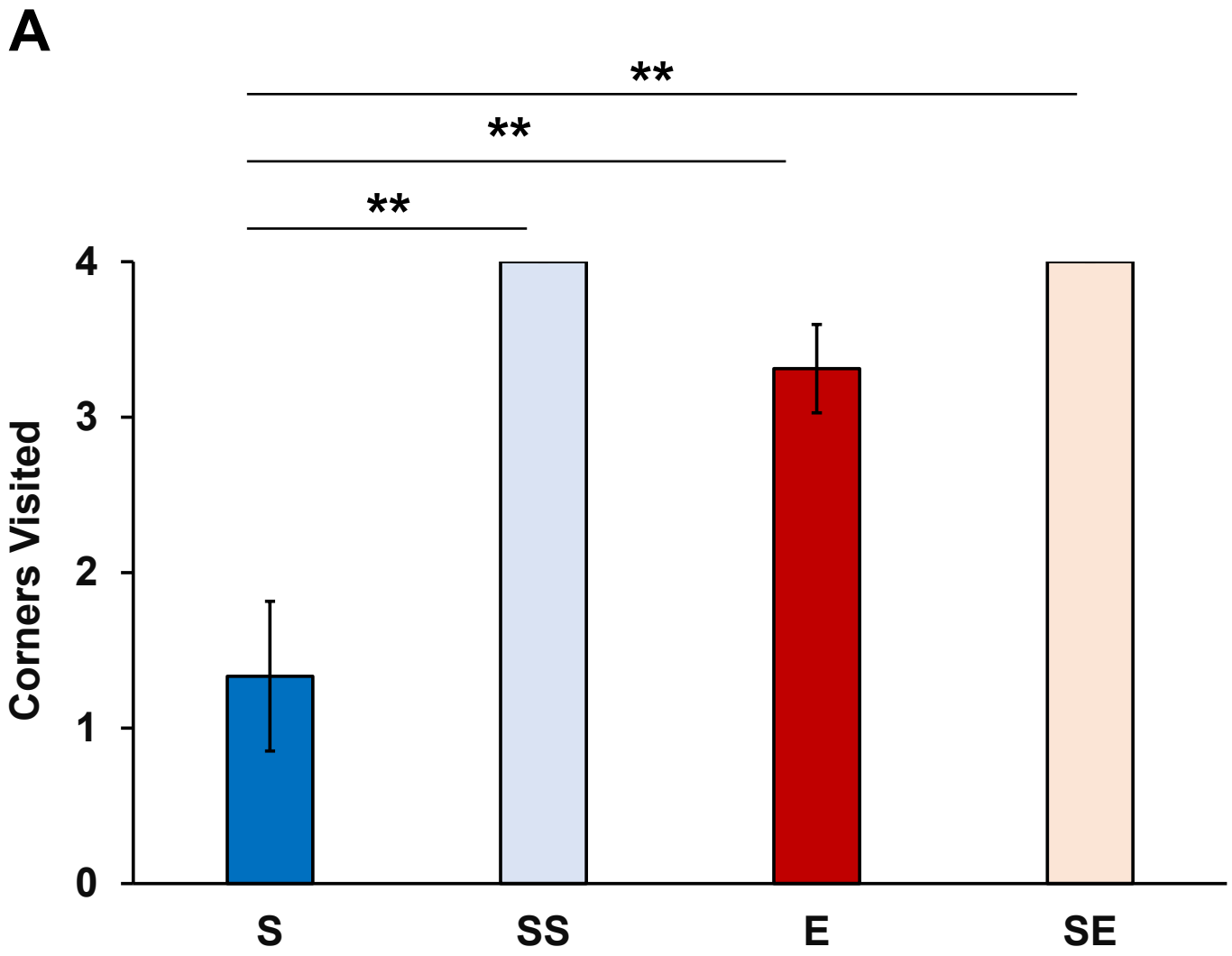
3.1 Environmental enrichment increases exploratory behaviour upon initial exposure to the IntelliCage

Patterns of visitation to conditioning corners during the first hour within the IntelliCage are thought to be indicative of the exploratory behaviour of an animal, whilst the overall number of visits made during free adaptation are thought to reflect adaptation to novelty [20]. I found that housing condition impacted both exploratory behaviours upon first exposure to the IntelliCage system, and the overall number of visits made to conditioning corners during free adaptation (Fig. 5.3).

Quantitative analysis of the number of conditioning corners visited during the first hour within the IntelliCage system revealed that housing condition significantly affected whether animals visited all four corners during this time (univariate ANOVA, housing condition as between-subjects factor, $F(3, 65)=27.976$, $P<0.001$) (Fig. 5.3A). Posthoc analysis revealed this effect to be due to S animals not visiting all four conditioning corners in the first hour within the IntelliCage (multiple comparisons posthoc analysis: E vs S, $P<0.001$; SE vs S, $P<0.001$; SS vs S, $P<0.001$). All SS and SE animals visited all four conditioning corners during the first hour within the IntelliCage system (Fig. 5.3A).

Housing condition also significantly impacted the overall total number of visits made to conditioning corners during FA (repeated measures ANOVA, housing condition as between-subjects factor, $F(3, 65)=11.614$, $P<0.001$). Posthoc analysis revealed this effect to be due to both S and SS animals making a greater number of visits to conditioning corners than E and SE cohorts (multiple comparisons posthoc analysis: E vs S, $P<0.001$; E vs SS, $P=0.032$; SE vs S, $P<0.001$, SE vs SS, $P=0.039$) (Fig. 5.3B).

These results suggest that the environmental condition of home cages can influence exploratory and adaptation behaviours within the IntelliCage during the FA period of testing.


A
**
**
**
Corners Visited
4
3
2
1
0
S
SS
E
SE

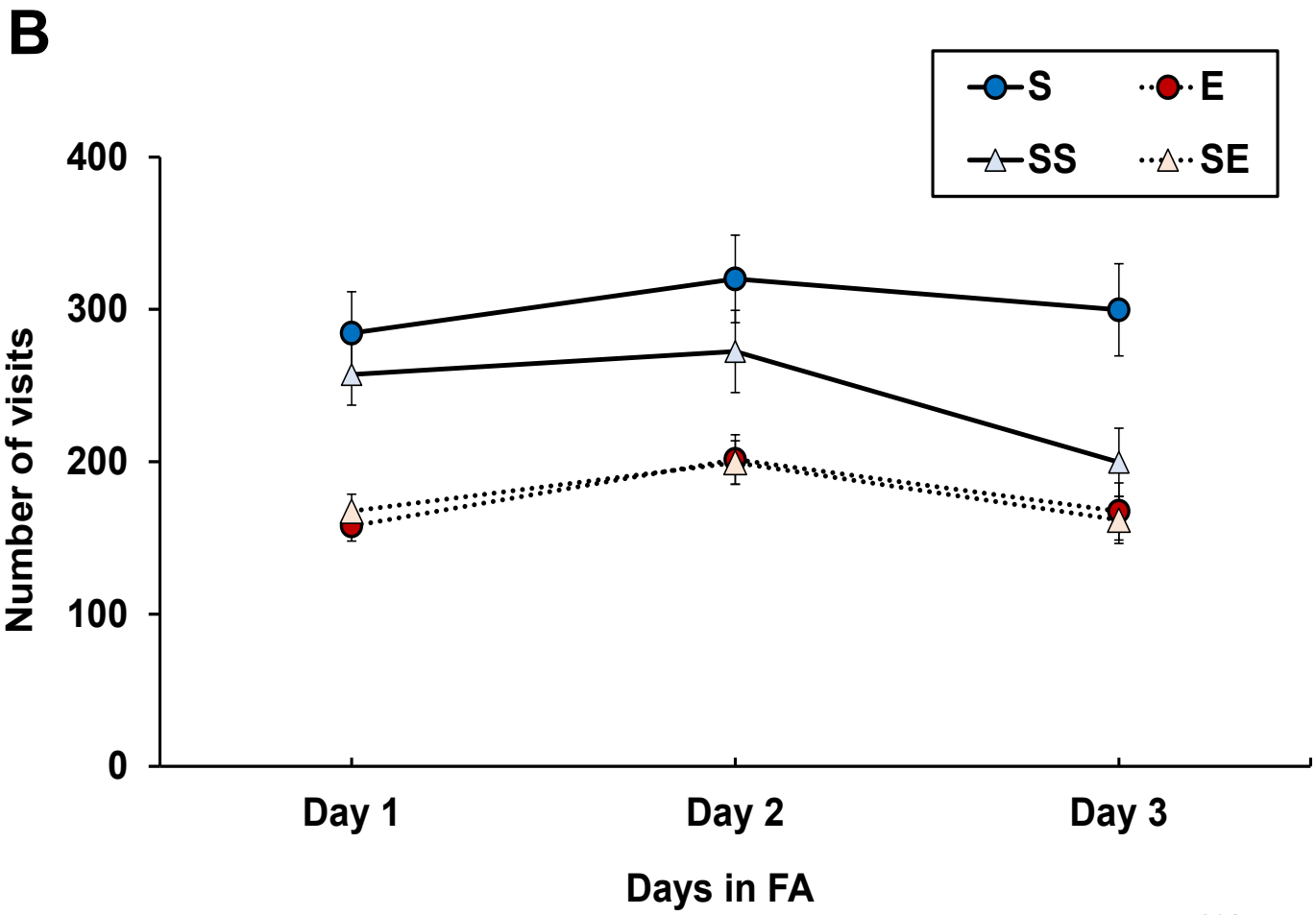

B
S
E
SS
SE
Number of visits
400
300
200
100
0
Day 1
Day 2
Day 3
Days in FA

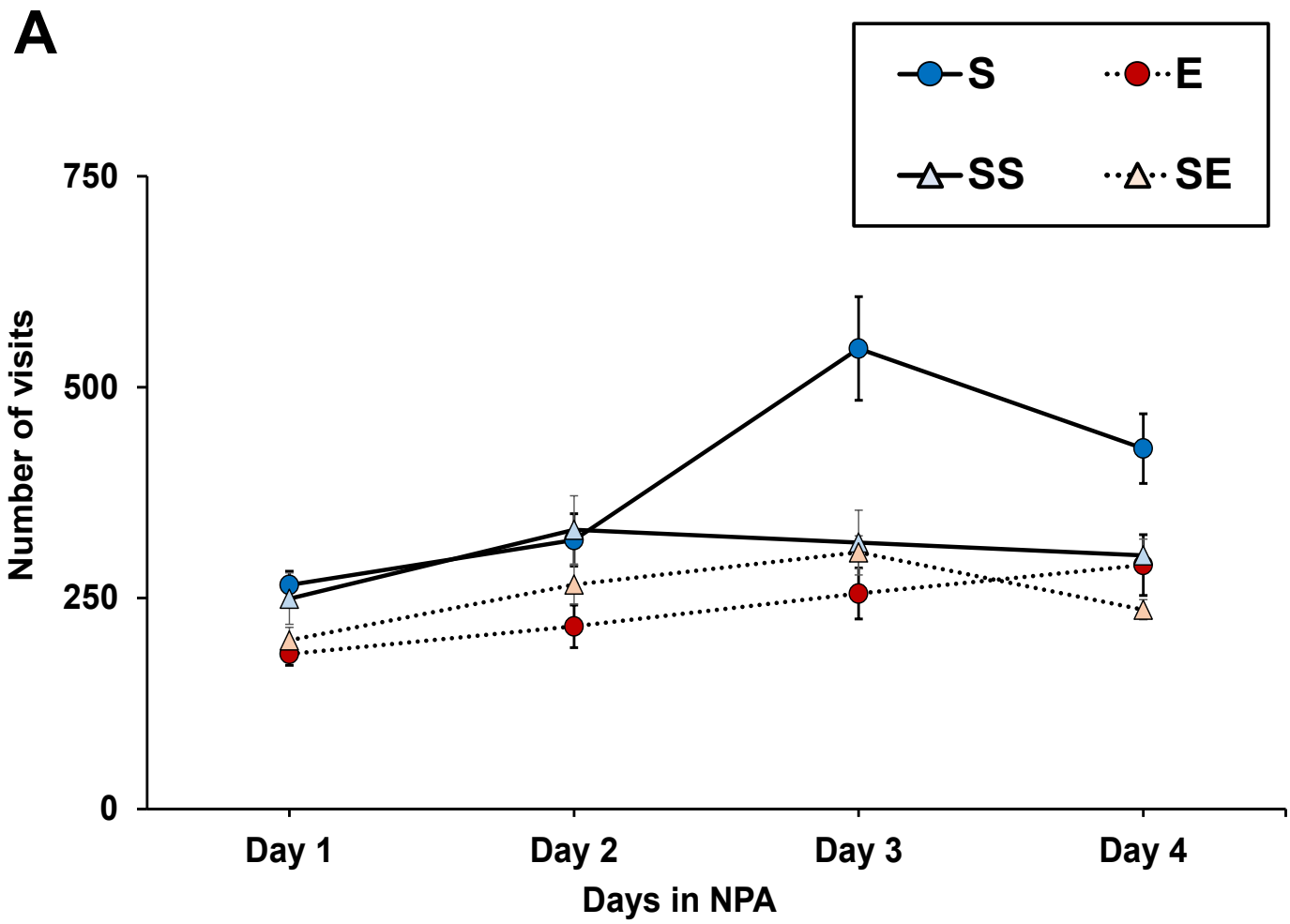
Figure 5.3: Housing condition impacts visitation behaviour within the IntelliCage during FA

(A) Average number of corners visited in the first hour immediately after introduction to the IntelliCage (cumulative, maximum possible 4) for animals raised from birth in an enriched (E) and standard environment (S), and animals housed for 6 weeks within an enriched (SE) and standard (SS) environment upon arrival at the University of Sydney animal house at 8 – 10 weeks of age. During the first hour within the IntelliCage, S animals visited significantly fewer of the conditioning corners than did other animals (univariate ANOVA, $F=27.976$, $P<0.001$). (B) Average number of visits made per day during FA. Housing condition significantly impacted the incidence of visits to conditioning corners (repeated measures ANOVA, $F=11.614$, $P<0.001$) due to differences between standard and enriched cohorts (see text). **: $P<0.01$. E n=16, S n=13, SE n=20, SS n=20. Error bars=Standard Error of the Mean (SEM).

3.2 Standard raised animals demonstrate increased exploratory behaviours during the NPA phase of testing

Similar to the effect observed during the free adaptation testing phase, housing condition also impacted the number of visits made to conditioning corners during the nose-poke adaptation (NPA) phase of testing (Fig. 5.4). Quantitative analysis revealed a significant effect of housing condition upon visits (repeated measures ANOVA, housing condition as between-subjects factor, $F(3, 65)=8.392$, $P<0.001$) (Fig. 5.4A), brought about by S animals making a greater number of visits to conditioning corners than other housing conditions (multiple comparisons posthoc analysis: S vs E, $P<0.001$; S vs SE, $P<0.001$, S vs SS, $P=0.033$).

Previously, during FA, nose-pokes made by animals within a conditioning corner elicited no effect and thus could not be considered as a deliberate behaviour. During NPA and all further testing phases nose-poking within a conditioning corner enabled access to water sippers. Thus, from NPA onwards, the incidence of nose-poking during visits may be considered as an important behaviour, as animals attempt to open the water sipper access doors. Housing condition significantly impacted the incidence of nose-poking within conditioning corners during NPA (repeated measures ANOVA, housing condition as between-subjects factor, $F(3, 65)=6.387$, $P=0.001$) (Fig. 5.4B). Posthoc analysis revealed this effect to be due to S animals having more incidences of nose-poking than all other housing conditions (multiple comparisons posthoc analysis: S vs E, $P=0.001$; S vs SE, $P=0.002$, S vs SS, $P=0.041$). These results suggest that, once past initial exposure, S animals continue to display greater exploratory behaviours within the IntelliCage system than do animals from all other housing conditions.


A
S
E
SS
SE
750
500
250
0
Number of visits
Day 1
Day 2
Day 3
Day 4
Days in NPA

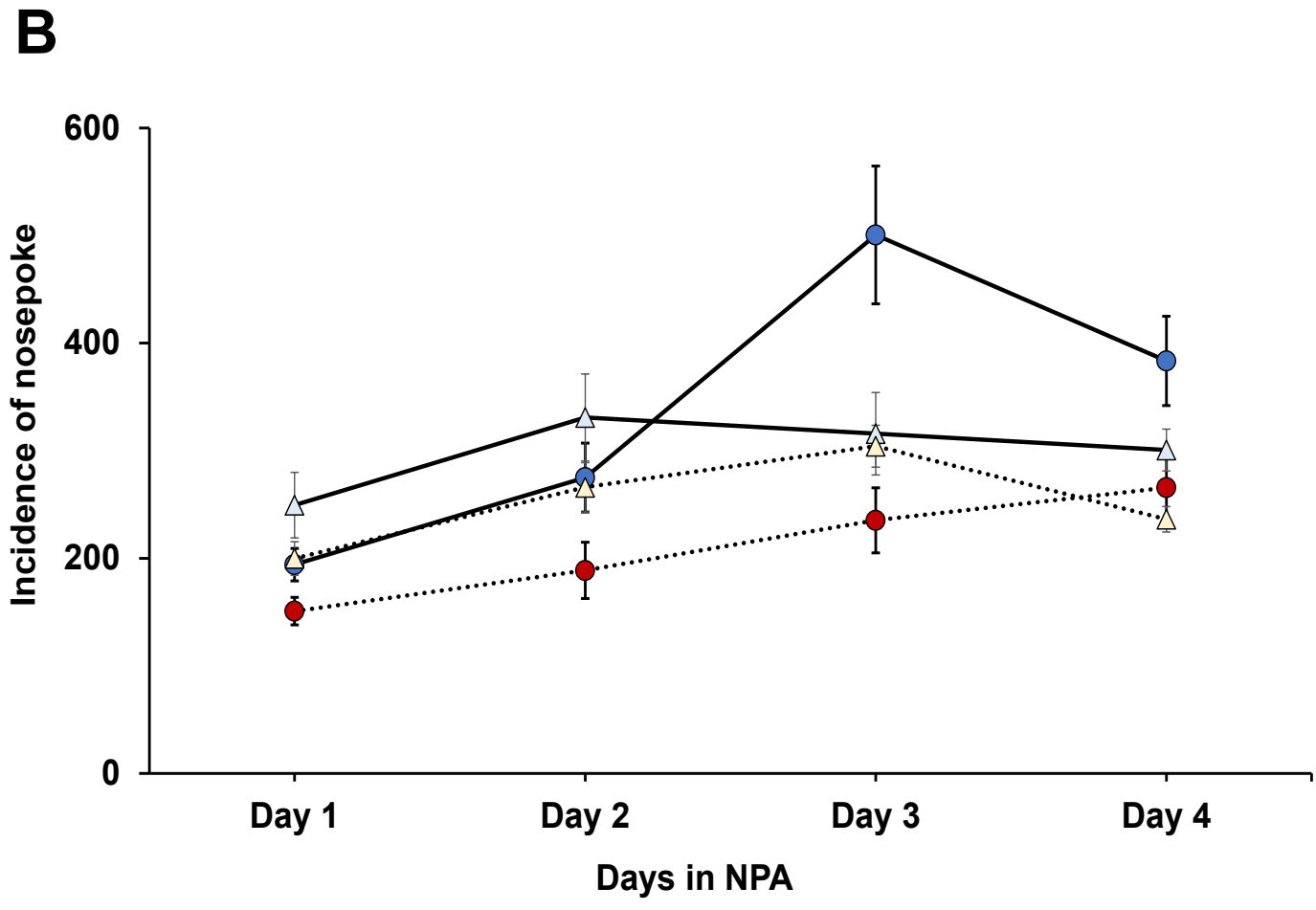

B
600
400
200
0
Incidence of nosepoke
Day 1
Day 2
Day 3
Day 4
Days in NPA

Figure 5.4: S animals show a greater number of visits and nose-pokes during NPA phase of testing

(A) Graph displaying the average number of visits made to conditioning corners every 12 hours throughout NPA by animals raised in an enriched (E) and standard (S) environment, and animals housed for 6 weeks within an enriched (SE) and standard (SS) environment upon arrival at the University of Sydney animal house at 8 – 10 weeks of age. S animals made significantly more visits throughout NPA than did all other housing conditions (repeated measures ANOVA, $F=8.392$, $P<0.001$). (B) A similar graph, displaying the average occurrence of a nose-poke within conditioning corners during visits throughout NPA. S animals made significantly more nose-pokes throughout NPA than did all other housing conditions (repeated measures ANOVA, $F=6.387$, $P=0.001$). E n=16, S n=13, SE n=20, SS n=20. Error bars=SEM.

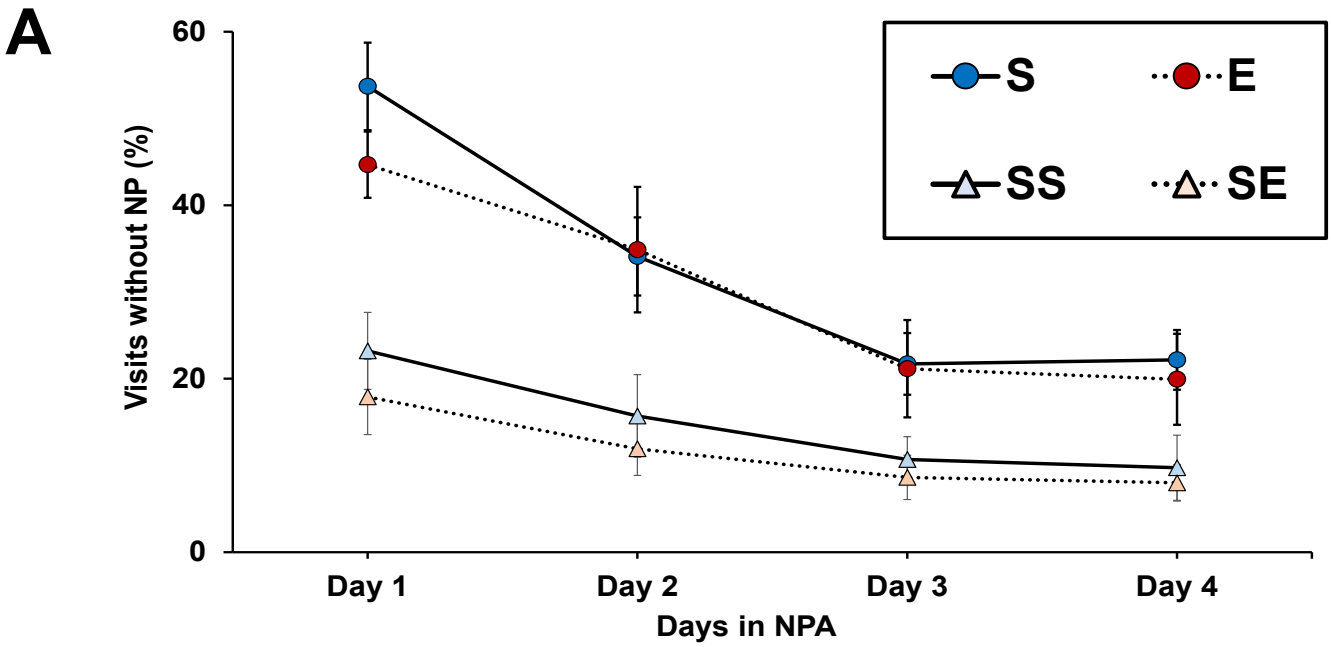
The performance of all animals improved as the NPA testing phase progressed (Fig. 5.5). Quantitative analysis revealed that the incidence of animals making visits to conditioning corners without nose-poking was decreased as testing progressed (repeated measures ANOVA, day of testing as within-subjects factor, $F(3, 195)=32.465$, $P<0.001$), with a significant difference between housing groups (repeated measures ANOVA, housing condition as between-subjects factor, $F(3, 65)=20.210$, $P<0.001$) due to S and E animals having a higher proportion of visits without nose-pokes when compared to SS and SE cohorts (multiple comparisons posthoc analysis: E vs SE, $P<0.001$; E vs SS, $P<0.001$; S vs SE, $P<0.001$; S vs SS, $P<0.001$) (Fig. 5.5A). There was a significant interaction between housing condition and day of testing upon this metric (repeated measures ANOVA, housing condition*day of testing, $F(9, 195)=2.233$, $P=0.022$).

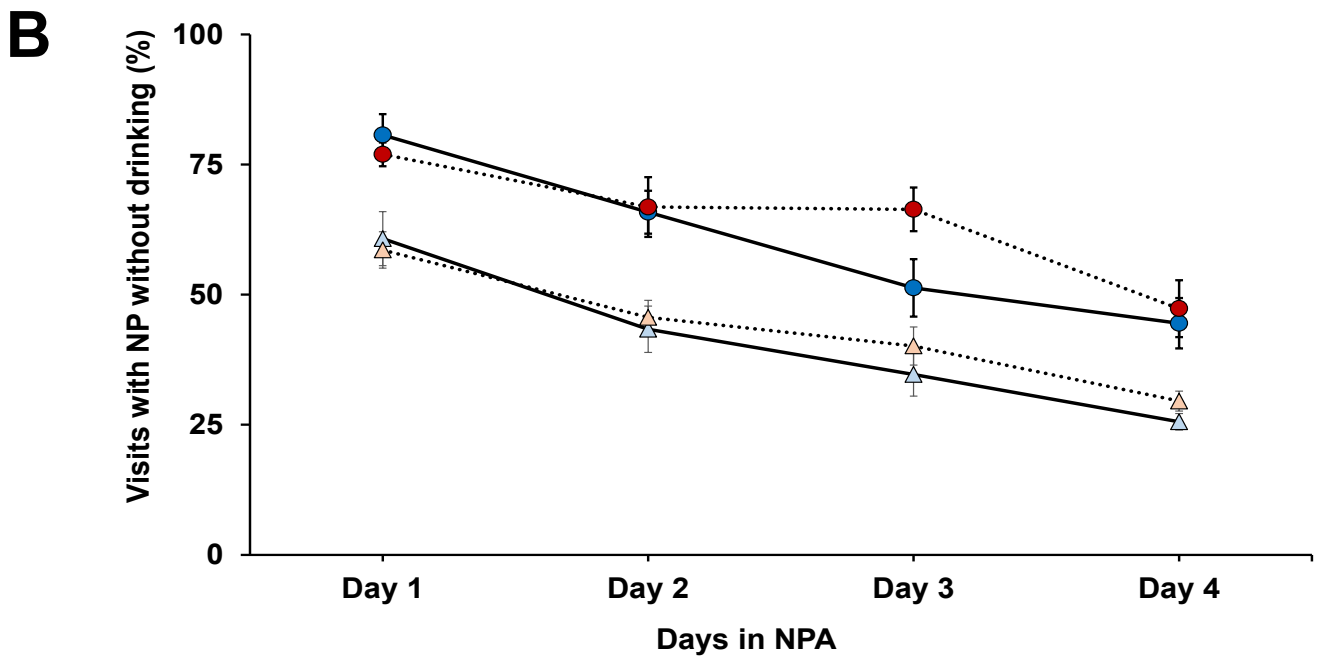
Similarly, the proportion of visits with nose-pokes where drinking did not occur also decreased as testing progressed (repeated measures ANOVA, day of testing as within-subjects factor, $F(3, 195)=56.916$, $P<0.001$); again, there was a significant effect of housing condition upon this metric (repeated measures ANOVA, housing condition as between-subjects factor, $F(3, 65)=19.334$, $P<0.001$), again due to E and S animals having a higher proportion of visits with nose-pokes without drinking compared to SS and SE cohorts (multiple comparisons posthoc analysis: E vs SE, $P<0.001$; E vs SS, $P<0.001$; S vs SE, $P<0.001$; S vs SS, $P<0.001$) (Fig. 5.5B). There was no significant interaction between housing condition and day of testing upon this metric.

A similar improvement in performance was observed in the proportion of visits to conditioning corners with nose-pokes where drinking occurred (repeated measures ANOVA, day of testing as within-subjects factor, $F(3, 195)=55.675$, $P<0.001$). Once more, there was a significant effect of housing condition upon this metric (repeated measures ANOVA, housing condition as between-subjects factor, $F(3, 65)=19.471$, $P<0.001$), again due to E and S animals differing in performance to SS and SE cohorts (multiple comparisons posthoc analysis: E vs SE, $P<0.001$; E vs SS, $P<0.001$; S vs SE,

P<0.001; S vs SS, P<0.001) (Fig. 5.5C). There was no significant interaction between housing condition and day of testing upon this metric.

These results suggest that home cage environment can continue to influence behaviours within the IntelliCage apparatus beyond the FA phase of testing.


A
60
40
20
0
Visits without NP (%)
S
E
SS
SE
Day 1
Day 2
Day 3
Day 4
Days in NPA


B
100
75
50
25
0
Visits with NP without drinking (%)
Day 1
Day 2
Day 3
Day 4
Days in NPA

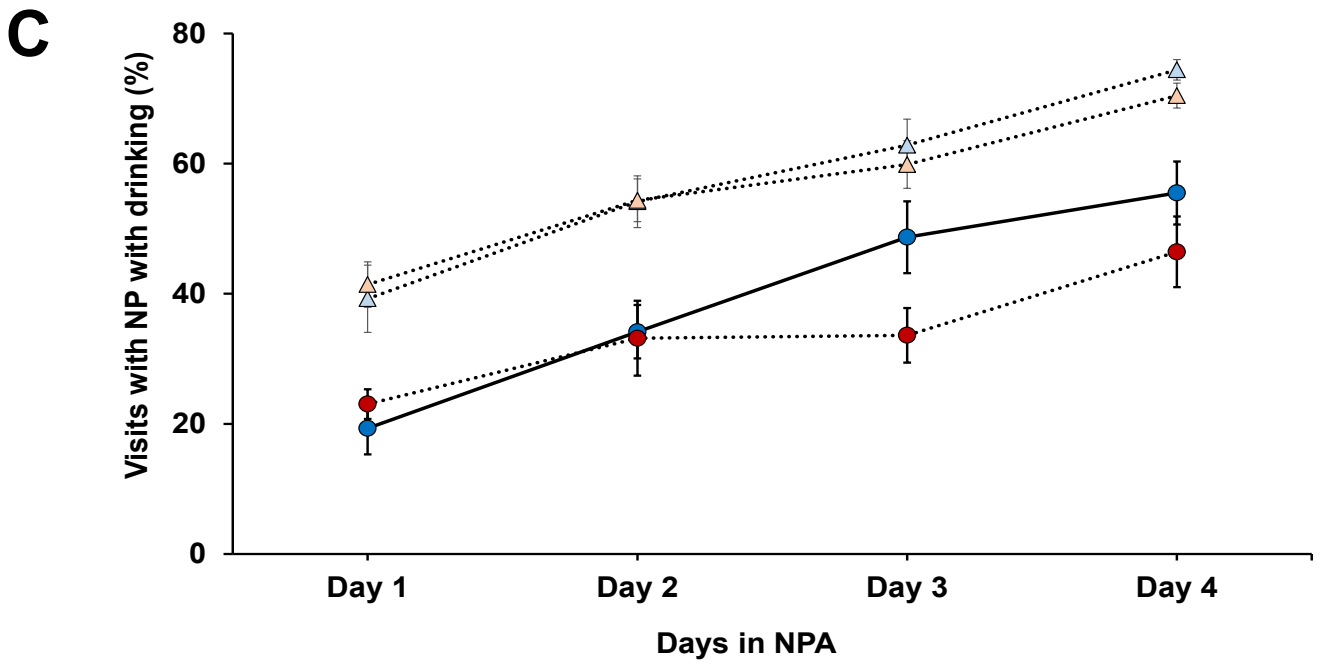

C
80
60
40
20
0
Visits with NP with drinking (%)
Day 1
Day 2
Day 3
Day 4
Days in NPA

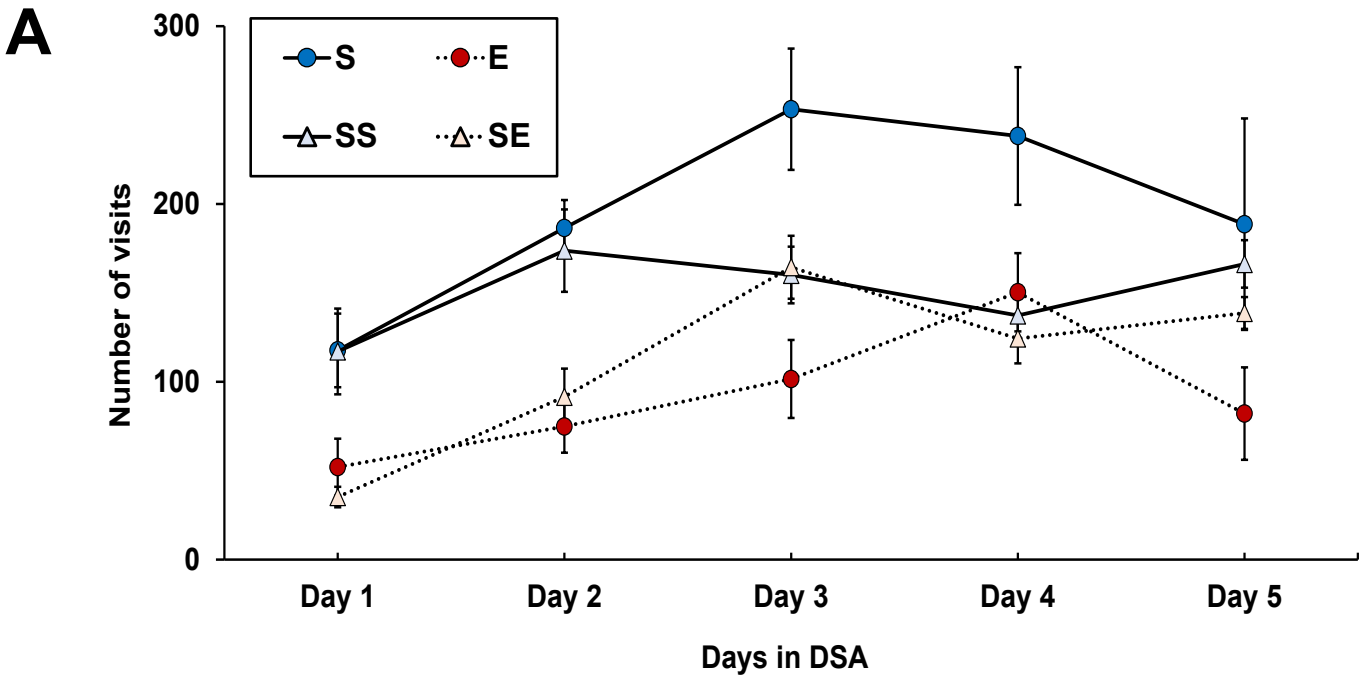
Figure 5.5: The performance of all animals improved as NPA progressed

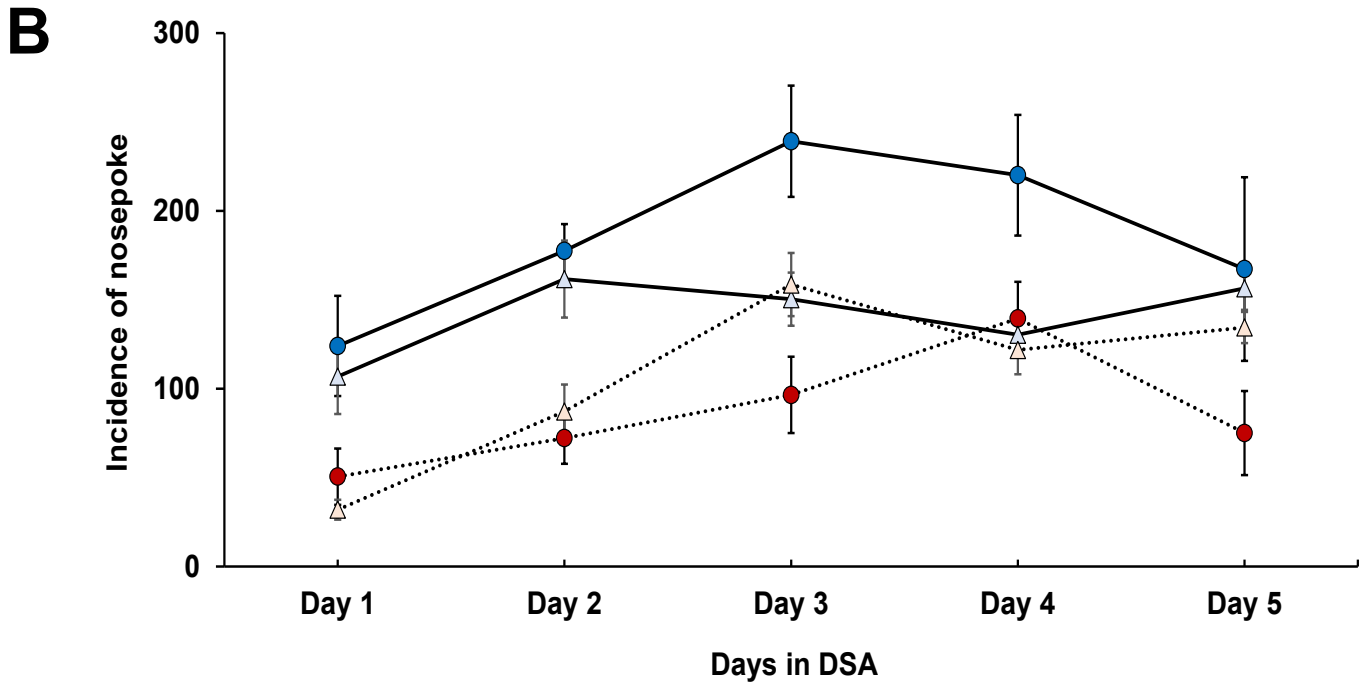
(A) Graph displaying the average proportion of visits to conditioning corners without nose-pokes made by animals raised in an enriched (E) and standard (S) environment, and animals housed for 6 weeks within an enriched (SE) and standard (SS) environment upon arrival at the University of Sydney animal house at 8 – 10 weeks of age during NPA. There was a significant reduction in the proportion of visits without nose-pokes as time within NPA progressed (repeated measures ANOVA, $F=32.465$, $P<0.001$). (B) A similar graph, but for the average proportion of visits to conditioning corners with nose-pokes where no drinking occurred. Again, as time within NPA progressed, there was a significant reduction in the proportion of visits with nose-pokes where no drinking occurred (repeated measures ANOVA, $F=56.916$, $P<0.001$). (C) A similar graph, but for the average proportion of visits to conditioning corners with nose-pokes where drinking did occur. As time within NPA progressed, there was a significant increase in the proportion of visits with nose-pokes where drinking did occur (repeated measures ANOVA, $F=55.675$, $P<0.001$). E n=16, S n=13, SE n=20, SS n=20. Error bars=SEM.

3.3 Standard raised animals demonstrate a greater number of visits and incidence of nose-pokes during Drinking Session Adaptation

Following NPA, animals were subjected to a drinking session adaptation (DSA) testing phase. During this time access to water was restricted to three one-hour long periods every 24 hours (8pm – 9pm; 3am – 4am; 11am – 12pm), and animals were required to nose-poke in order to access the water sippers. Data was analysed by day of testing using measurements taken during drinking sessions. Housing condition significantly affected the number of visits made to conditioning corners during DSA (repeated measures ANOVA, housing condition as between-subjects factor, $F(3, 53)=12.340$, $P<0.001$) (Fig. 5.6A). Posthoc analysis revealed this effect to be due to S and SS animals making a greater number of visits to conditioning corners than enriched groups (multiple comparisons posthoc analysis, $F(3, 53)= 12.340$: S vs E, $P<0.001$; S vs SE, $P<0.001$: SS vs E, $P=0.010$).

The incidence of nose-pokes within conditioning corners during DSA was also impacted by housing condition (repeated measures ANOVA, housing condition as between-subjects factor, $F(3, 53)=10.470$, $P<0.001$) (Fig. 5.6B), and came about due to a greater incidence of nose-pokes being made by standard animals (multiple comparisons posthoc analysis: S vs E, $P<0.001$; S vs SE, $P=0.001$; SS vs E, $P=0.012$). Housing condition also had an impact upon the proportion of visits to conditioning corners with nose-pokes occurring (repeated measures ANOVA, housing condition as between-subjects factor, $F(3, 53)=3.307$, $P=0.027$), coming about due to a lesser proportion of visits with nose-pokes made by S animals relative to SE animals (multiple comparisons posthoc analysis: S vs SE, P0.015) (Fig. 5.6C). These results suggest that, despite a higher incidence of visits and nose-pokes within conditioning corners, S animals do not nose-poke any more efficiently than animals from other housing conditions.


A
300
200
100
0
Number of visits
S
E
SS
SE
Day 1
Day 2
Day 3
Day 4
Day 5
Days in DSA


B
300
200
100
0
Incidence of nosepoke
Day 1
Day 2
Day 3
Day 4
Day 5
Days in DSA

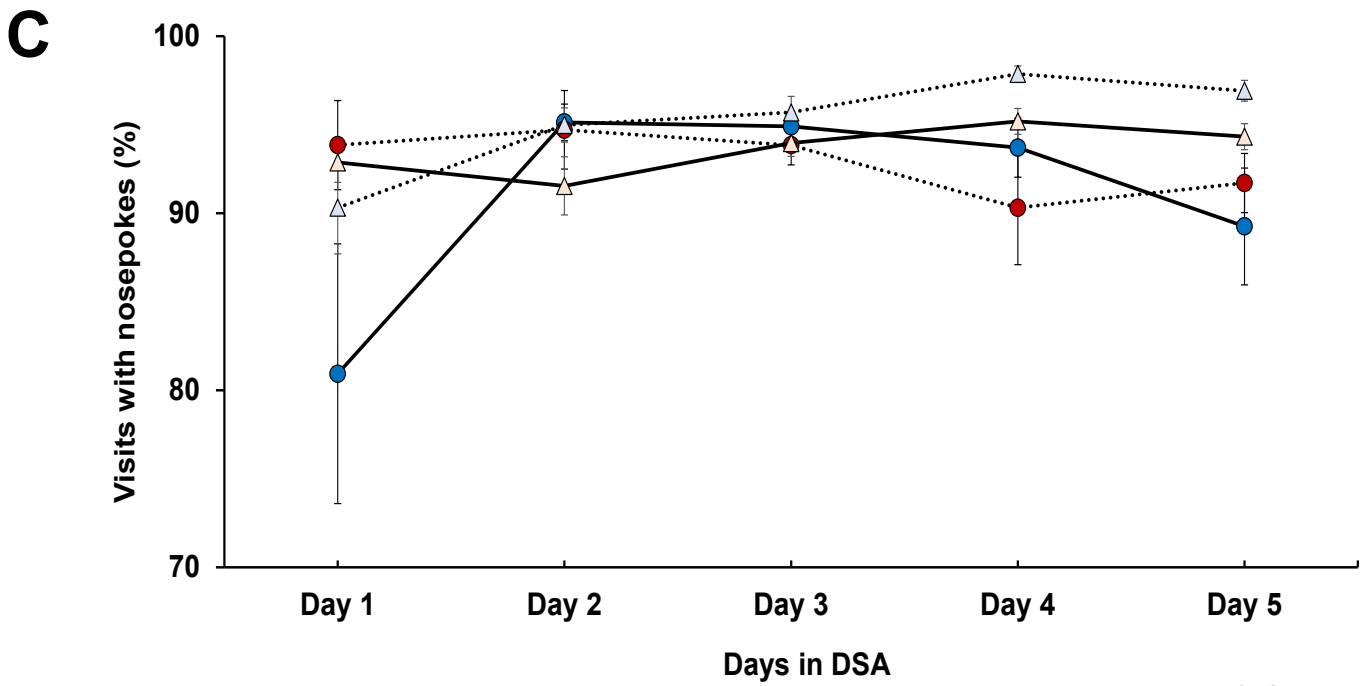
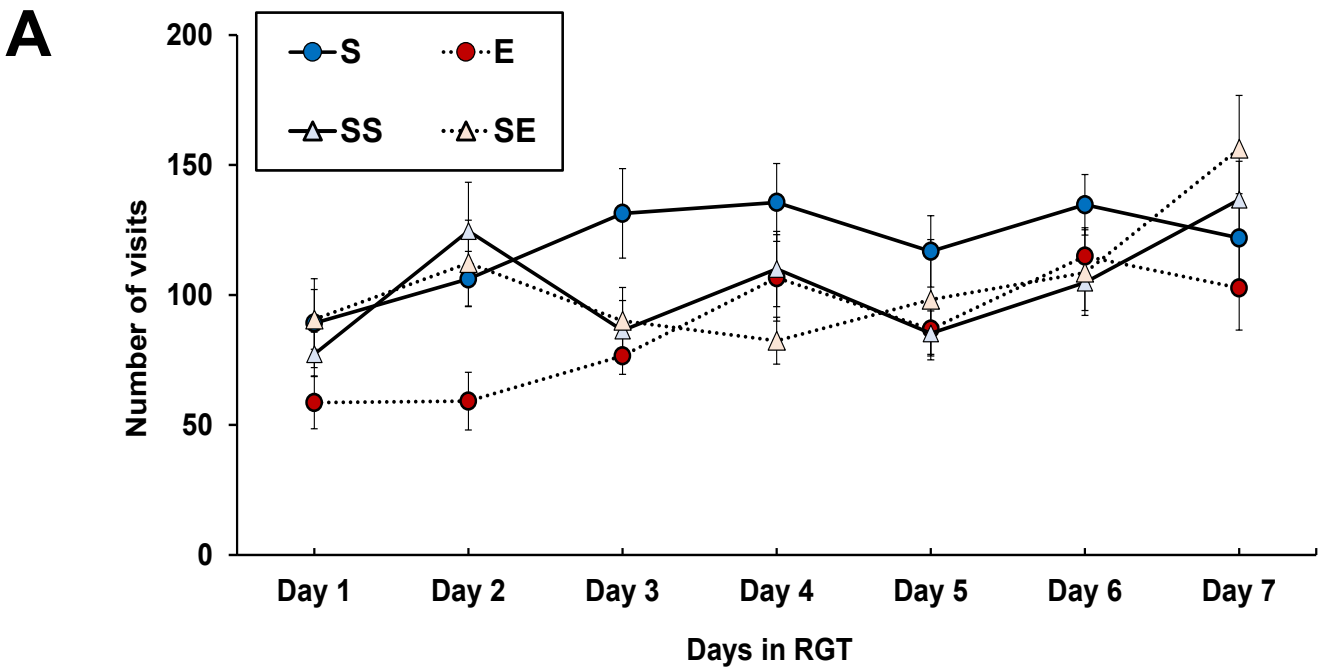

C
100
90
80
70
Visits with nosepokes (%)
Day 1
Day 2
Day 3
Day 4
Day 5
Days in DSA

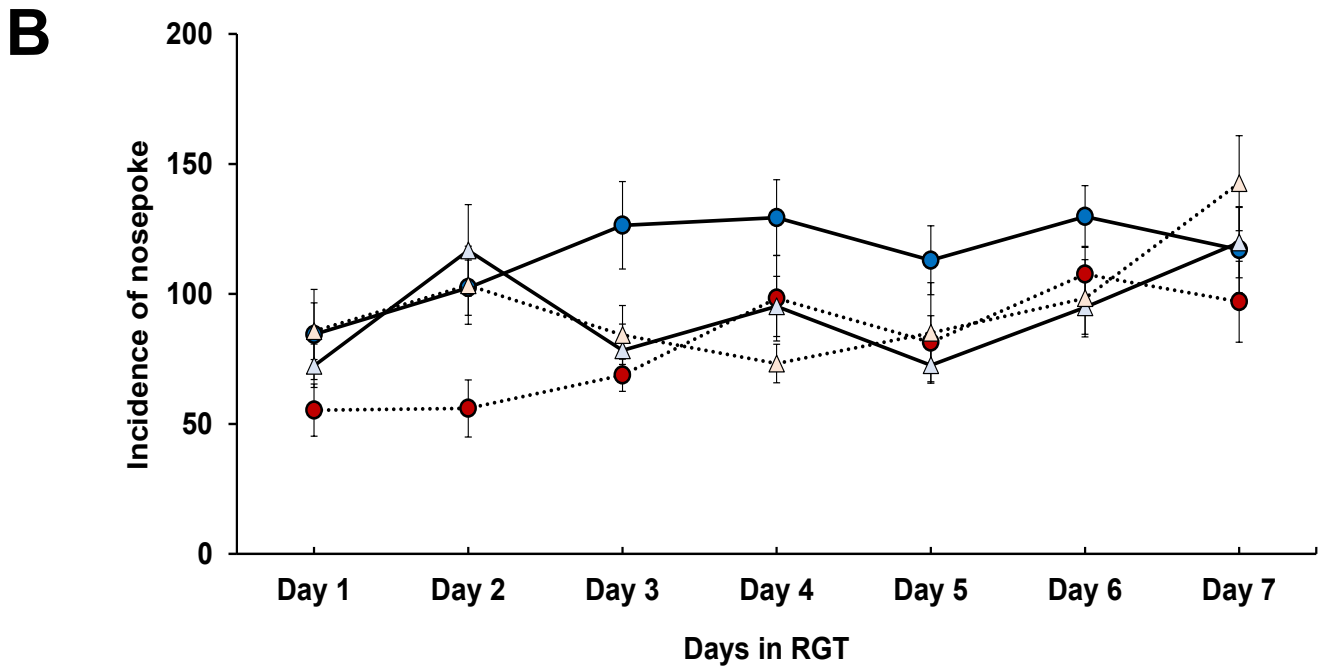
Figure 5.6: S animals show increased incidence of visits and nose-pokes during the DSA testing phase

(A) A graph displaying the average number of visits made to conditioning corners per day during DSA by animals raised in an enriched (E) and standard (S) environment, and animals housed for 6 weeks within an enriched (SE) and standard (SS) environment upon arrival at the University of Sydney animal house at 8 – 10 weeks of age. S animals made significantly more visits to conditioning corners than all other housing conditions (repeated measures ANOVA, $F=12.340$, $P<0.001$). (B) A similar graph, but for the average incidence of nose-poke during visits to conditioning corners. S animals had significantly more incidences of nose-poking within conditioning corners than did all other animals (repeated measures ANOVA, $F=10.470$, $P<0.001$). (C) A similar graph, but for the average proportion of visits to conditioning corners where nose-pokes occur. S and SE animals had significantly different proportions of visits with nose-pokes (repeated measures ANOVA, $F=3.307$, $P=0.015$). E n=14, S n=13, SE n=15, SS n=15. Error bars=SEM.

3.4 Lifelong environmental enrichment decreases the incidence of visits and nose-pokes within conditioning corners during the RGT

Upon completion of the DSA, formal training on the RGT was initiated. Similar to previous testing phases, the number of visits and nose-pokes made within conditioning corners were taken as a measure of task acquisition [19, 20, 23]. Data was analysed by day of testing using measurements taken during drinking sessions. In contrast to the effect observed in previous testing phases, housing condition did not significantly impact the number of visits made to conditioning corners during the RGT (repeated measures ANOVA, housing condition as between-subjects factor, $F(3, 53)=1.798$, $P=0.159$) (Fig. 5.7A). Similarly, there was no significant impact of home cage environment upon the incidence of nose-poke occurring within conditioning corners during the RGT (repeated measures ANOVA, $F(3, 53)=2.409$, $P=0.077$) (Fig. 5.7B). However, exploratory posthoc analysis revealed that E animals had significantly fewer incidences of nose-poking within conditioning corners than did S animals (multiple comparisons posthoc analysis: E vs S, $P=0.049$). Housing condition also had no significant effect upon the proportion of visits to conditioning corners with nose-pokes during RGT (repeated measures ANOVA, housing condition as between-subjects factor, $F(3, 53)=2.061$, $P=0.116$) (Fig. 5.7C). These results suggest that lifelong EE slightly reduces the incidence of nose-poking within conditioning corners, but not so much that it impacts upon the proportion of visits where nose-pokes occur.


A
S
E
SS
SE
Number of visits
200
150
100
50
0
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Days in RGT


B
Incidence of nosepoke
200
150
100
50
0
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Days in RGT

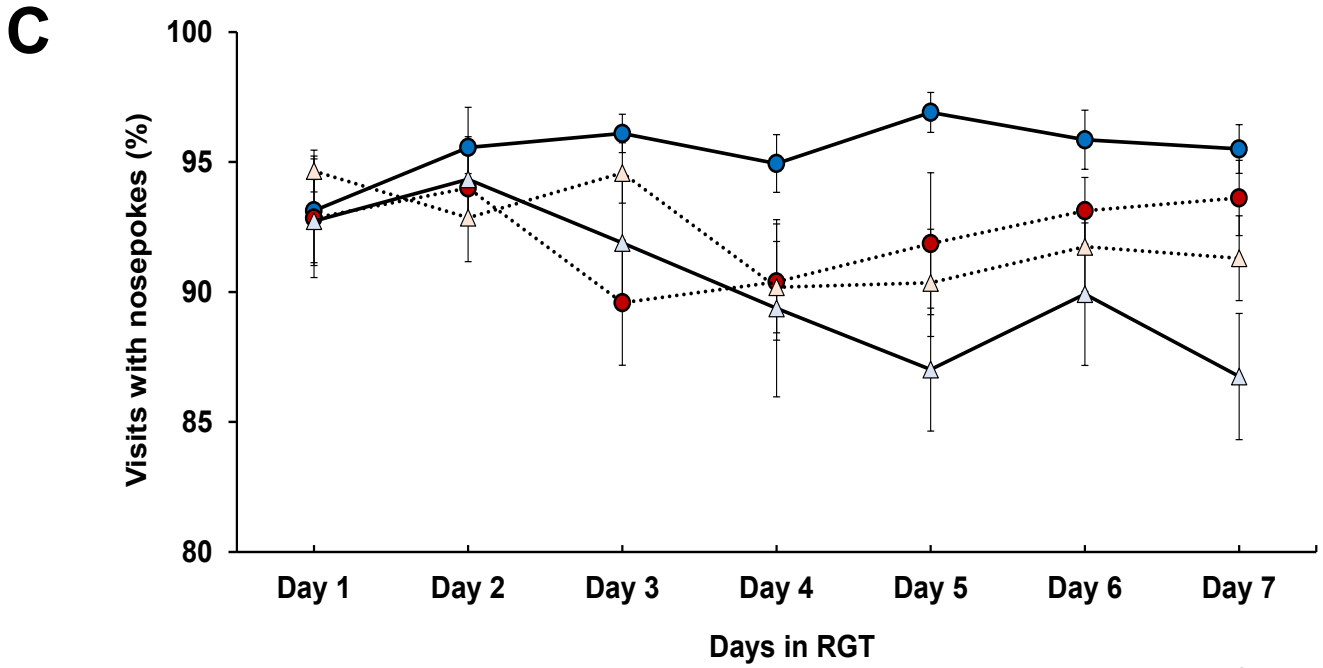

C
Visits with nosepokes (%)
100
95
90
85
80
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Days in RGT

Figure 5.7: E animals show slightly reduced incidence of nose-pokes during the RGT testing phase

(A) A graph displaying the average number of visits made to conditioning corners per day by animals raised in an enriched (E) and standard (S) environment, and animals housed for 6 weeks within an enriched (SE) and standard (SS) environment upon arrival at the University of Sydney animal house at 8 – 10 weeks of age during RGT. There was no overall significant effect of housing condition upon this metric. (B) A similar graph, but for the average incidence of nose-pokes during visits to conditioning corners. There was no overall significant effect of housing condition upon this metric. E animals had fewer incidences of nose-poke than did S animals (repeated measures ANOVA, F=2.409: E vs S, P=0.049). (C) A similar graph, but for the average proportion of visits to conditioning corners where nose-pokes occur. There was no overall significant effect of housing condition upon this measurement. E n=14, S n=13, SE n=15, SS n=15. Error bars=SEM.

3.5 Housing condition has little impact upon the proportion of visits and nose-pokes to different types of conditioning corners

In order to assess decision-making within the IntelliCage during the RGT, the proportion of visits made to, and incidence of nose-pokes within, conditioning corners of varying types were assessed. Within a given drinking session, animals that only visited low risk/high reward (overall advantageous) conditioning corners would receive twice as much drinking time than those only visiting high risk/low reward (overall disadvantageous) conditioning corners.

Home cage environment had little impact upon the choice of which corners to visit or nose-poke in (Fig. 5.8, 5.9). The proportion of visits to low risk corners (repeated measures ANOVA, housing condition as between-subjects factor, $F(3, 53)=0.648$, $P=0.588$) (Fig. 5.8A), the proportion of nose-pokes occurring in low risk corners (repeated measures ANOVA, housing condition as between-subjects factor, $F(3, 53)=0.890$, $P=0.452$) (Fig. 5.8B), and the proportion of visits to low risk corners with nose-pokes (repeated measures ANOVA, housing condition as between-subjects factor, $F(3, 53)=1.309$, $P=0.281$) (Fig. 5.8C) were not significantly affected by housing condition, and there were no significant posthoc effects of housing condition.

Similarly, the proportion of visits to high risk corners (repeated measures ANOVA, housing condition as between-subjects factor, $F(3, 53)=0.808$, $P=0.495$) (Fig. 5.9A), the proportion of nose-pokes occurring in high risk corners (repeated measures ANOVA, housing condition as between-subjects factor, $F(3, 53)=0.890$, $P=0.452$) (Fig. 5.9B), and the proportion of visits to high risk corners with nose-pokes (repeated measures ANOVA, housing condition as between-subjects factor, $F(3, 53)=2.144$, $P=0.106$) (Fig. 5.9C) were not significantly affected by housing condition, and there were no significant posthoc effects of housing condition.

Given the lack of effect from housing condition, measurements were pooled and analysed according to corner type to assess whether animals were able to perform the RGT. Quantitative analysis revealed that the type of conditioning corner did impact upon the proportion of visits received (repeated measures ANOVA, corner type as between-subjects factor, $F(1, 105)=44.568$, $P<0.001$) (Fig. 5.8A, 5.9A), and the proportion of nose-pokes occurring within different corner types (repeated measures ANOVA, corner type as between-subjects factor, $F(1, 105)=49.178$, $P<0.001$) (Fig. 5.8B, 5.9B), with a greater proportion of visits and nose-pokes occurring in low risk corners. There was no effect of corner type upon the proportion of visits to both low and high risk corners with nose-poke occurring (repeated measures ANOVA, corner type as between-subjects factor, $F(1, 105)=1.420$, $P=0.931$) (Fig. 5.8C, 5.9C). These results suggest that all animals are able to differentiate between low risk and high risk conditioning corners, but that exposure to EE does not appear to influence this ability.

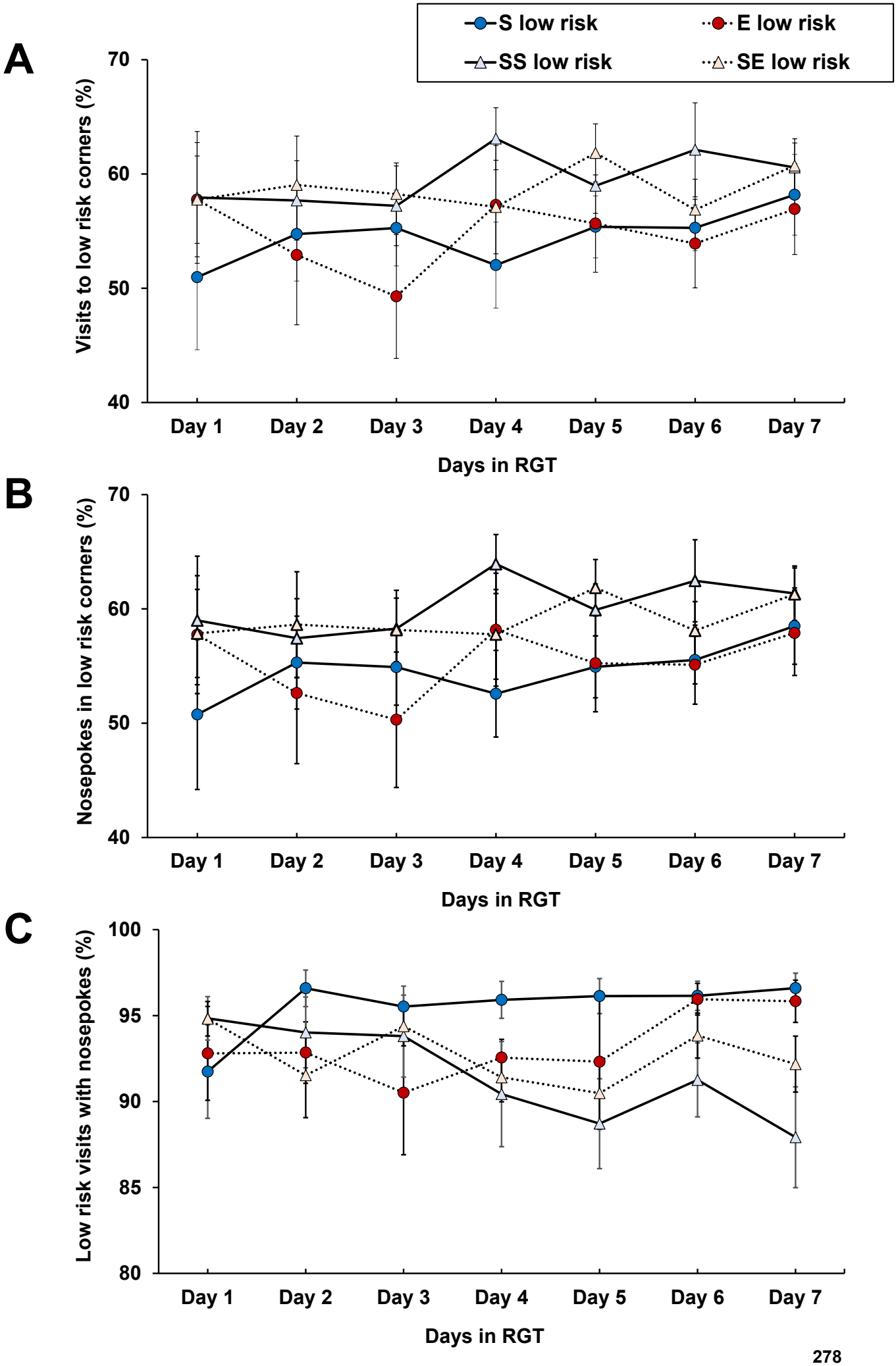

A
S low risk
E low risk
SS low risk
SE low risk
Visits to low risk corners (%)
70
60
50
40
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Days in RGT
B
Nosepokes in low risk corners (%)
70
60
50
40
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Days in RGT
C
Low risk visits with nosepokes (%)
100
95
90
85
80
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Days in RGT

Figure 5.8: Corner type has a greater impact upon visits and nose-pokes than does housing condition

(A) A graph displaying the average proportion of visits made to low risk/high reward (low risk) corners by animals raised in an enriched (E) and standard (S) environment, and animals housed for 6 weeks within an enriched (SE) and standard (SS) environment upon arrival at the University of Sydney animal house at 8 – 10 weeks of age during RGT. There was no significant effect of housing condition upon this measure (repeated measures ANOVA, $F=0.648$, $P=0.588$); however, a significantly greater proportion of visits were made to low risk conditioning corners (repeated measures ANOVA, $F=44.568$, $P<0.001$). (B) A similar graph, but for the average proportion of nose-pokes made in low risk corners. There was no significant effect of housing condition upon this measure (repeated measures ANOVA, $F=0.890$, $P=0.452$); however, a significantly greater proportion of nose-pokes were made within low risk conditioning corners (repeated measures ANOVA, $F=49.178$, $P<0.001$). (C) A similar graph, but for the average proportion of visits with nose-pokes made in low risk corners. There was no significant effect of either housing condition (repeated measures ANOVA, $F=1.309$, $P=0.281$) or conditioning corner type upon this measurement (repeated measures ANOVA, $F=1.420$, $P=0.931$). E n=14, S n=13, SE n=15, SS n=15. Error bars=SEM.

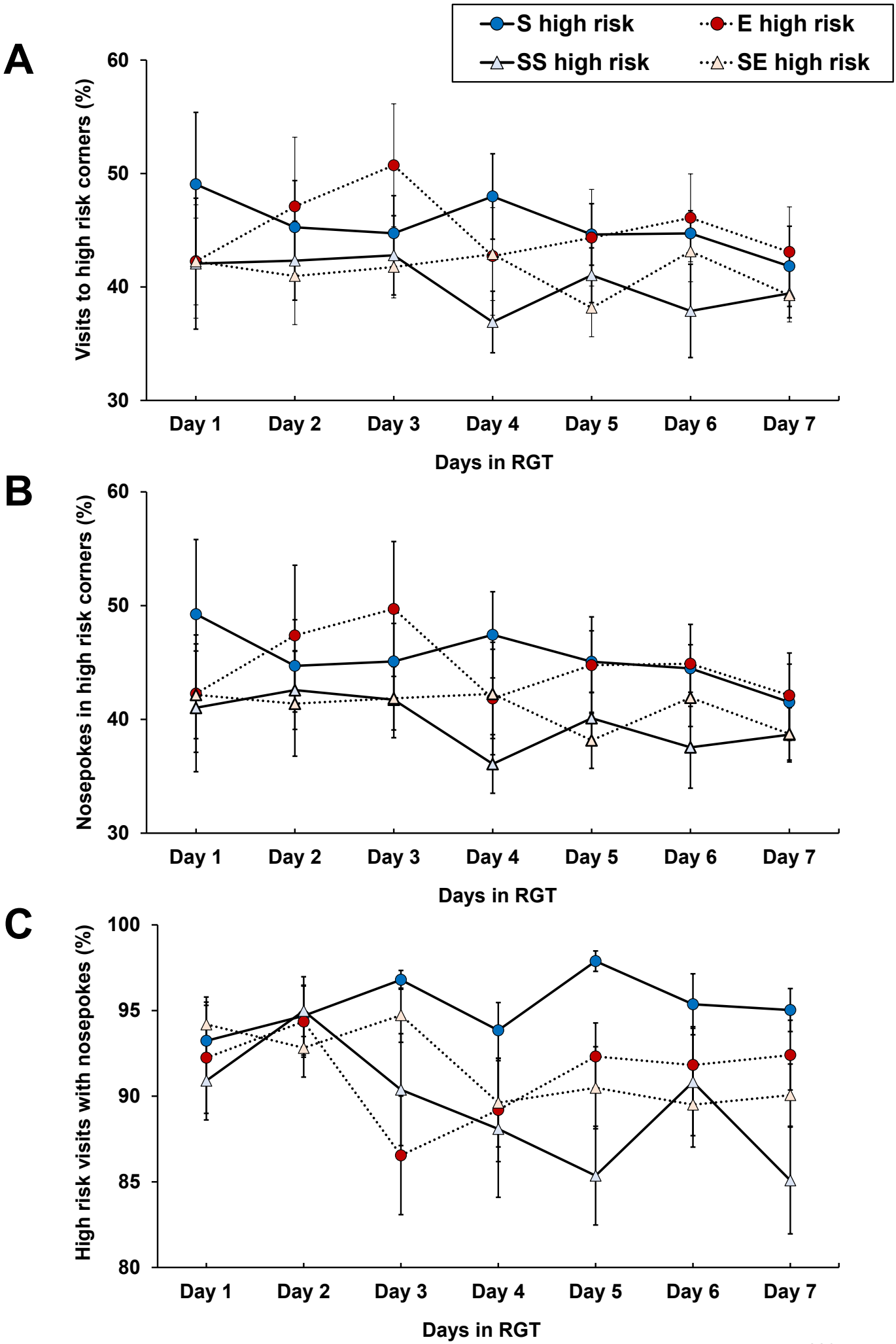

A
S high risk
E high risk
SS high risk
SE high risk
Visits to high risk corners (%)
60
50
40
30
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Days in RGT
B
Nosepokes in high risk corners (%)
60
50
40
30
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Days in RGT
C
High risk visits with nosepokes (%)
100
95
90
85
80
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Days in RGT

Figure 5.9: Corner type has a greater impact upon visits and nose-pokes than does housing condition

(A) A graph displaying the average proportion of visits made to high risk/low reward (high risk) corners by animals raised in an enriched (E) and standard (S) environment, and animals housed for 6 weeks within an enriched (SE) and standard (SS) environment upon arrival at the University of Sydney animal house at 8 – 10 weeks of age during RGT. There was no significant effect of housing condition upon this measure (repeated measures ANOVA, $F=0.808$, $P=0.495$); however, a significantly smaller proportion of visits were made to high risk conditioning corners (repeated measures ANOVA, $F=44.568$, $P<0.001$). (B) A similar graph, but for the average proportion of nose-pokes made in high risk corners. There was no significant effect of housing condition upon this measure (repeated measures ANOVA, $F=0.890$, $P=0.452$); however, a significantly smaller proportion of nose-pokes were made within high risk conditioning corners (repeated measures ANOVA, $F=49.178$, $P<0.001$). (C) A similar graph, but for the average proportion of visits with nose-pokes made in high risk corners. There was no significant effect of either housing condition (repeated measures ANOVA, $F=2.144$, $P=0.106$) or conditioning corner type upon this measurement (repeated measures ANOVA, $F=1.420$, $P=0.931$). E n=14, S n=13, SE n=15, SS n=15. Error bars=SEM.

4. Discussion

This study investigated the impact of EE from birth or adulthood upon reward-based decision-making behaviours of animals within the IntelliCage, an automated behavioural testing apparatus. Exposure to EE was found to alter exploratory and adaptation behaviours upon first exposure to a novel testing environment, despite having little impact on the decision-making process of animals during a rodent version of the IGT (RGT). The development of an automated version of the RGT provides a launch pad for the systematic assessment of decision-making behavior in laboratory mice, and may prove to be a valuable tool when assessing animal models of human neurological disorders.

4.1 Environmental enrichment influences exploratory behaviours within the IntelliCage

Consistent with previous work, animals that had experienced any EE– both those raised from birth (E) and those briefly housed as adults within an enriched environment (SE) – made fewer overall visits to conditioning corners within the IntelliCage system [23]. Despite this, E animals demonstrated a higher level of exploratory behaviour during the first hour within the IntelliCage than did standard raised cohorts. It would appear that EE leads to a decrease in the level of exploratory behaviour within the IntelliCage after initial exposure, and that this trend is maintained throughout the rest of the time spent within the system. This may come about due to a "novelty saturation" effect – animals that have been exposed to EE may not experience the same drive to continually explore a new environment that their standard counterparts do. It may be that enriched animals are less anxious and more exploratory when first placed into the IntelliCage system, but that the novelty of a new housing situation wears off more quickly than it does for standard housed counterparts.

Previous work from our laboratory has demonstrated that EE in young animals leads to an increase in exploratory behaviours when mice are placed into a novel environment [5]. Whether this effect is

maintained past the initial exposure time of two minutes was not determined [5]. Unpublished observations made by a colleague within our laboratory suggest that enriched animals show reduced levels of activity within another automated behavioural testing paradigm (the operant conditioning box). Further studies specifically investigating the effect of EE upon the activity levels of animals within an automated testing situation would be of interest.

4.2 Environmental enrichment does not impact choice of corner during the RGT

During the RGT phase of testing animals were assigned two diagonally opposite low risk/high reward (overall advantageous) conditioning corners, and two diagonally opposite high risk/low reward (overall disadvantageous) conditioning corners within the IntelliCage system. The traditional IGT used during human psychological assessment employs decks of cards as the "sites" of choice, and uses the gain and loss of money as reward and loss [14-16]. Previous work has confirmed the ability of rats to undertake a version of the RGT using food supply and restriction as reward and loss [17, 24], and has revealed that, similar to humans, a majority of animals consistently choose the overall advantageous options, with higher overall reward gained [17].

When interpreting the data gained from within the IntelliCage system, there are several measurements that may be taken as indicators of performance; this study looked at the incidence of visits to, and nose-pokes within, conditioning corners of both types. The occurrence of visits to conditioning corners, and nose-pokes made during those visits, may be interpreted as indicators of intent – that is, whether animals are able to differentiate between corner type on a macro scale such that they do not make visits to overall disadvantageous conditioning corners. By the metrics of number of visits and nose-pokes, all animals were able to successfully differentiate between overall advantageous and disadvantageous corners, regardless of housing condition.

Interestingly, exposure to EE did not improve drinking choices above the level observed in standard cohorts. It is known that inability to discriminate between low and high risk choices within the human IGT indicates either impaired inhibition during decision making, or a reduced ability to learn through reward/loss paradigms [15, 25-30]. It is also known that a certain percentage of subjects (20 – 25%) demonstrated poor decision making within the RGT previously run by another group, thought to result from a hypersensitivity to reward [17]. These animals also showed higher levels of risk taking behaviour in anxiogenic behavioural analyses, suggesting that there is a subset of the healthy rodent population that consistently displays poor or risky decision-making [17]. It is possible that EE leads to an increase in the number of poor decision-makers within a population by decreasing anxiety [31, 32], resulting in higher levels of risk taking behaviour. Previous work has demonstrated that standard housed animals are more sensitive to rewards during behavioural testing than enriched animals [33]. It is possible that EE impairs the ability of animals to learn from a reward/loss paradigm by providing constant stimulation, making them less sensitive to a reward that may not be adequately stimulating. If either of these were the case, however, then enriched animals would likely have performed more poorly than standard counterparts, which did not occur. The IntelliCage system itself may be considered as an enriching environment [34]. Given the length of time animals spent in training phases leading up to the RGT testing phase, it is possible that the novelty effect of IntelliCage housing is enough to provide a "boost" to standard mice such that they display the same decision-making ability as enriched counterparts. Further experiments to determine the proportion of poor decision-makers/high risk takers within variously housed populations would be of interest to determine how far housing condition is able to impact upon choice behaviours.

The negative findings with regards to any effect of EE suggest that merely measuring the number of visits and nose-pokes may not be enough to give an accurate reflection of animal behaviours within the IntelliCage system. Nevertheless, the current results demonstrate the feasibility of conducting a complex behavioural task on mice that is analogous to a human psychological test, using an automated behavioural testing apparatus.

4.3 Behaviour of enriched animals within an automated testing environment and the potential for novelty saturation

Automated behavioural testing apparatuses, such as the IntelliCage, are becoming more frequently used by laboratories. These systems are capable of generating consistent results between laboratories by eliminating the effect of variables such as animal handling, exposure to new testing environments, and stress resulting from single housing during intra-cage behavioural testing [20, 23]. Given that I was investigating the impact of EE upon decision-making behaviour during the RGT within the IntelliCage, a reduction in animal handling may be considered advantageous. Handling of young mice has been shown to have similar effects to that of EE, accelerating maturation of the visual system [35, 36].

The benefits gained from lack of experimenter-induced variables and animal handling effects may be offset by the enriching effects of the IntelliCage itself [34] The IntelliCage in particular is a high-throughput behavioural testing arena – up to 16 animals can be placed into an individual cage at one time – allowing for rapid behavioural analyses. Behavioural analyses conducted within the IntelliCage eliminate the impact of variables such as experimenter traits, testing order of animals, and handling effects. Recent findings have illustrated the effect of experimenter gender upon animal behaviours, with male mice displaying higher levels of stress and a stress-induced analgesic effect in response to the presence of male, but not female, researchers [21]. Fear-conditioned learning [37] and pain thresholds [38] of experimental subjects are both known to be influenced by the order in which animals undergo behavioural testing. It is possible that animals influenced one another in their decision-making during the RGT within the IntelliCage – hence enriched and standard cohorts were housed in separate IntelliCage systems, and the allocation of overall advantageous and disadvantageous conditioning corners was reversed for half the mice within a given cage such that an overall advantageous corner was also an overall disadvantageous corner for half of the testing

population. Further studies are needed to determine the potential impact of this testing environment upon base level animal behaviours.

Enriched animals demonstrated less exploratory activity within the IntelliCage than did standard counterparts once past initial exposure to the testing arena. Similar to the rise in the use of automated testing environments, more research is beginning to investigate the effect of EE upon animal behaviours. An enriched environment provides animals with a more naturalistic home cage, and greater levels of stimulation and activity more closely approximating what would be encountered in a natural environment [39, 40]. Previous studies investigating the behavioural effects of EE have demonstrated that enriched animals are less likely to develop addiction behaviours when exposed to addictive substances [8, 41-43], consume less sucrose than standard housed animals when given the opportunity to do so [44], and are less sensitive to reward than standard housed animals [33]. It is possible that animals housed within standard environments are more likely to self-administer and seek out drugs of addiction in response to their relatively deprived home cages, and that enriched animals provide a more realistic model of behaviours. It is also possible that EE induces a form of anhedonia, where the usual rewards employed during behavioural testing are not sufficient to provoke the interest of enriched animals [33, 43].

Similar to previous studies, my work demonstrates that enriched animals display an accelerated habituation to novelty when placed within a behavioural testing arena [45]. This is potentially problematic, particularly when a behavioural task is dependent upon continued levels of activity, such as lever pressing within operant conditioning chambers, and visits to conditioning corners within the IntelliCage. Unpublished observations from our laboratory suggest that enriched animals display reduced responsiveness when placed within an operant conditioning chamber using food as a reward. Behavioural arenas testing reward-based behaviours may not provide enriched animals with an adequate level of stimulation compared to their home cage, resulting in a lack of motivation to perform behavioural tasks. Given the rise of both automated behavioural testing, and the number

of studies investigating the effects of EE, it is advantageous to be wary of unusual behaviours resulting from enrichment.

4.4 The possible role of the striatum in decision-making behaviours within our RGT testing paradigm

A previous study has demonstrated a reduction in the level of striatal damage induced by a model of traumatic brain injury, following a period of housing within the IntelliCage system, [34], suggesting that the striatum is likely involved in behaviours occurring within this apparatus. Previous research has also indicated that the striatum is highly impacted by EE, resulting in an increase in dendritic branching and spine density [6, 7], an increase in striatal levels of BDNF and NGF [8-10], and an up-regulation of striatal metabolic activity and expression of genes involved in various cellular processes [11, 12]. It is difficult to target enrichment towards a particular area of the brain; this is especially true of brain regions involved in varied functions, as is the striatum [1-3]. Given the important role of this nucleus in reward behaviours and decision-making [1, 46-48], it is likely that the striatum was involved in decision-making behaviours during the RGT testing phase within the IntelliCage system.

Despite the many noted effects of EE upon the striatum, enrichment did not appear to impact decision-making during the RGT conducted in this study. Many areas of the brain may be involved in learning the processes necessary to undertake the RGT, and in decision-making during the task itself. The prefrontal cortex, which has been implicated in mediating the behaviour of human subjects during the IGT [14, 16], would likely be involved in decision-making during the RGT. It is also probable that the hippocampus was involved in remembering the processes necessary to access water during the RGT. Further studies perhaps making use of lesions within the brain or utilising opto- or pharmaco-genetic inactivation throughout the duration of IntelliCage testing may shed some light upon the exact role of the striatum during behavioural tasks within this apparatus.

4.5 Conclusions

In conclusion, this study successfully determined that it is possible to design and implement a rodent version of the Iowa Gambling Task within an automated behavioural testing system, using mice as subjects and water access and restriction as the reward and loss components of this task. Further, these findings provide evidence for the influence of both lifelong and brief adulthood environmental enrichment upon behaviours within the IntelliCage, ranging from basic metrics during free adaptation through to the effect of enrichment upon a complex reward-based decision-making task. Further experiments as suggested throughout the discussion section would aid in shedding light upon the exact mechanisms behind the observations made within this study.

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Chapter 6: Discussion

"The most exciting phrase to hear in science, the one that heralds the most discoveries, is not 'Eureka (I found it!)' but 'That's funny…'"

- Isaac Asimov

This thesis successfully established the impact of environmental enrichment (EE) upon various juvenile and adult striatally-mediated behaviours, and investigated the effect of EE upon the murine striatum during a putative early life critical period and in adulthood.

1. Environmental enrichment increases the number of parvalbumin-expressing neurons within the striatum of the adult mouse

This thesis successfully determined the effects of lifelong EE upon the striatal population of Parvalbumin-expressing (PV+) inhibitory interneurons, by assessing the correlation of these neurons with PNNs within the striatum of adult animals. PNNs are extracellular matrix structures associated with PV+ inhibitory interneurons in several areas of the brain, including the striatum [1-5]. Within other regions of the brain, the formation of mature PNNs is associated with the end of the critical period and the maturation of PV+ neurons [6, 7]. Both the presence of mature PNNs [8] and expression levels of the calcium binding protein PV are able to be influenced by EE [9]. PV expression is regulated by cellular activity levels, as this calcium binding protein is thought to protect against excitotoxicity induced by fast-spiking action potentials [10, 11]. I found that lifelong EE increases the number of PV+ inhibitory interneurons, but has no effect upon the number of PNNs, present within the striatum of adult animals. This result provides evidence of an ongoing impact of EE beyond the critical period, and provides insight into how environment may be capable of influencing mature neural circuitry.

1.1 Future Directions

Work from other laboratories has demonstrated the ability of EE to regulate PV expression within inhibitory interneurons of the hippocampus [9]. It is not fully clear whether the increase in PV+ inhibitory interneurons I observed in the striatum of enriched adult animals is due to an activity-

dependent up-regulation of PV in otherwise quiescent neurons, or whether EE leads to an increase in the total number of striatal PV+ inhibitory interneurons. That there is no effect of EE upon PNN density within the striatum suggests that the increase in numbers of PV+ inhibitory interneurons is most likely due to an up-regulation of the PV calcium-binding protein in usually inactive neurons. This may result from increased activity levels within striatal circuitry in response to a greater level of stimulation from the environment.

PV is expressed in electrophysiologically active neurons to buffer ionic movement during fast-spiking action potentials [10, 11]. Electrophysiological studies investigating the impact of EE upon cellular activity levels within striatal PV+ inhibitory interneurons may shed further light upon the exact mechanism underlying a potential increase in striatal PV expression in response to EE. Corticostriatal projections are the main source of afferent input to striatal PV+ inhibitory interneurons, and the activation they provide is thought to influence PV expression levels [12-14]. EE has been shown in several regions of the cortex to increase dendritic branching [15-18] and synaptic density [19]. It would be of interest to determine whether lifelong enrichment influences the number or innervation pattern of corticostriatal afferents synapsing within the striatum, potentially resulting in a greater number of electrophysiologically active PV+ inhibitory interneurons.

2. Environmental enrichment accelerates the maturation of parvalbumin-expressing neurons within the striatum of the mouse

2.1 Juvenile anatomical and protein findings

This thesis successfully characterised PV+ inhibitory interneurons and Brain-Derived Neurotrophic Factor (BDNF) protein levels within the murine striatum during a putative early life striatal critical period, by determining the effect of EE upon these measures. In primary sensory cortex, both PV+

inhibitory interneurons and BDNF protein have been shown to play a vital role in determining timing of the critical period within neural systems [6, 20].

The presence of a late postnatal motor critical period within the striatum beginning at P30, sensitive to protein levels of Neurotrophic Growth Factor (NGF), and mediated by the cholinergic and dopaminergic systems within this area of the brain, has been well established [21-24]. Other striatally-mediated behaviours, such as Ultrasonic Vocalisations (USVs) and coordinated sensorimotor behaviours, are known to develop earlier on in the postnatal period [25-29]. These behaviours emerge around the same time that PV+ inhibitory interneurons within the striatum begin to mature [13, 30], suggesting the presence of an early life critical period within the striatum, mediated by the maturation of PV+ inhibitory interneurons and likely sensitive to protein levels of BDNF. I found that animals raised within enriched housing demonstrate an accelerated maturation of PV+ inhibitory interneurons and elevated BDNF protein levels within the striatum of young animals, providing further support for the presence of an early life striatal critical period, and evidence for the important impact that an animals' environment can have upon neural development.

2.1.1 Future Directions

The immunohistochemically detectable presence of PV+ inhibitory interneurons within the striatum has been shown to depend upon adequate levels of BDNF protein, transported into the striatum via corticostriatal afferent projections [31]. Striatal PV+ inhibitory interneurons begin to mature around the same time that corticostriatal afferents form synapses within the striatum, and are known to depend upon the electrophysiological activity provided by these connections for full maturation [13, 32]. It is likely that the BDNF protein provided by corticostriatal afferents also contributes to the maturation of striatal PV+ inhibitory interneurons.

This thesis demonstrates that EE leads to an accelerated maturation of PV+ inhibitory interneurons and elevated BDNF protein levels within the juvenile striatum. It would be of interest to determine the impact of EE upon the development of corticostriatal afferents during the early postnatal period. Further studies utilising neural tracers or markers for corticostriatal projections in conjunction with staining for PV+ inhibitory interneurons might provide a more thorough explanation for the mechanisms underlying the accelerated maturation of this neural population within enriched animals. Electrophysiological studies assessing activity levels of PV+ inhibitory interneurons within the striatum of juvenile animals may also provide further insight into the impact of EE upon both these neurons and the corticostriatal afferent projections they receive.

Along with modulating the levels of PV present within striatal interneurons [13], the activity of corticostriatal afferent projections is also known to moderate the permeability of gap junction networks within the striatum [33]. Gap junctions are cell-to-cell channels that enable the transfer of ions between coupled cells [34], enabling electrotonic coupling between neurons and coordinated action potential firing within neural circuitry [35-39]. PV+ inhibitory interneurons within the striatum have been shown to form a syncytium, regulating action potentials through the use of gap junction connectivity [39]. Gap junctions are composed of transmembrane proteins known as connexins [34], of which there are several known types whose expression profiles are modulated throughout development [40, 41]. It is known that striatal PV+ inhibitory interneurons express connexin36 [42, 43], the expression of which peaks within the striatum at postnatal day 16, followed by a decline [42]. It would be of interest to determine whether EE accelerates this pattern of maturation within the striatum, and to what extent it may influence electrotonic coupling between maturing striatal PV+ inhibitory interneurons.

BDNF is not the only growth factor present within the striatum, nor the only growth factor capable of being impacted by EE. NGF is also present within the striatum, and plays a role in determining the timing of a late postnatal motor critical period [21-24]. Exposure to EE has been shown to increase

levels of NGF within the adult rat striatum [44], and slightly decrease levels of this growth factor within the adult murine striatum [45]. Protein levels of Insulin-like Growth Factor-1 (IGF-1) are able to be impacted by EE in the visual system [46, 47], although whether this growth factor is present within the striatum has not yet been determined. Given that homozygous IGF-1 knockout animals display a reduction in the number of immunohistochemically visible PV+ neurons within the striatum [48] and that application of exogenous IGF-1 protein has been shown to ameliorate the symptoms of MeCP2 Rett Syndrome mice [49], it is highly likely that this growth factor also plays an important role in striatal development. Future studies assessing the effect of EE upon these other growth factors within the striatum of juvenile animals would provide a more thorough view of how enrichment is capable of impacting this nucleus, particularly with regards to NGF, given the role this neurotrophic factor has been shown to play in striatal maturation [21-24].

2.2 Juvenile Behavioural Findings

This thesis successfully assessed the effect of EE upon juvenile striatally-mediated behaviours. The striatum is involved in the production of USVs and coordinated sensorimotor behaviours, both of which emerge during the first three weeks of life [25-29]. The USV calls profiled in this thesis are those produced in response to maternal separation, known to be uttered from as young as 3 days postnatal and to follow a set pattern of development, with occurrence decreasing as pups age and become independently mobile [50-54]. I found that EE impacted the call profile and duration of USVs produced by pups in response to maternal separation, but had no effect upon the incidence of USV production as pups aged. The current results are in line with evidence provided by other work of the ability of animals' environment and upbringing to influence the production of USVs [54-56], and also support the worth of the maternal separation paradigm as a successful means for eliciting the production of USVs in young animals, providing evidence for the manner in which postnatal environment and age interact with the effects of short term isolation. A consistently successful

means of eliciting USVs is highly beneficial, as these calls are a potentially useful tool for assessing the ontogeny of behaviour and the maturation of the neural circuitry involved in their production.

Coordinated sensorimotor behaviours were assessed by use of the rotarod behavioural apparatus, a test of speed, endurance and coordination. Previous work from our laboratory has shown that EE results in accelerated maturation of coordinated sensorimotor behaviours of very young (postnatal day 10) animals [57]. In order to determine whether EE has an ongoing impact upon development of coordinated sensorimotor behaviours, I assessed animals at an older age (postnatal days 21 to 26) prior to the onset of an already-defined striatal motor critical period [21-24], and towards the end of a putative early life striatal critical period [57]. I found that enrichment had no impact upon coordinated sensorimotor behaviours of juvenile animals as measured by performance on the rotarod behavioural task. These results suggest that the impact of EE upon particular behaviours or neural systems may only be observable during certain periods of behavioural ontogeny, and that this should be kept in mind when investigating the impact of environment upon brain development.

2.2.1 Future Directions

Raising pups within enriched housing has been shown to impact upon maternal behaviours, resulting in less time spent upon the nest overall, with a concomitant increase in the level of grooming and attention received by pups [58, 59]. Maternal behaviours have been shown to influence the production of USVs [60, 61], suggesting the differential USV call profiles observed within this study were likely due to the impact of enriched housing upon maternal behaviours. Further investigation is required to determine the exact interaction between EE, maternal behaviours and USV call production. One possibility for future studies would be assessment of the maternal response to USV recordings made of pups, to determine whether enriched housing leads to differences in maternal responsiveness. It would also be of interest to examine the effect of EE upon USV production by pups in response to other social stimuli, such as an intruder male or strange female. The recording

apparatus used was capable of only recording a 20 kHz range of sound; future studies assessing the impact of EE upon a wider range of calls would shed further light on the maturation of systems involved in USV production [25].

The effect of EE upon developing coordinated sensorimotor behaviours may be further assessed by the use of a gait analysis apparatus, balance beam apparatus, or forced swimming tests similar to those used in a previous study from our laboratory [57]. The tendency of young animals to jump off the rotarod behavioural apparatus before the completion of a trial means that any future experiments investigating the sensorimotor capabilities of animals at this age (postnatal day 21 to 26) should take this "non-compliance" behaviour into account.

3. Environmental enrichment improves problem-solving and goal seeking and ameliorates the effects of striatal PNN dissolution upon these behaviours

This thesis successfully developed a consistent and relatively simple behavioural test by which to determine the efficacy of EE upon animal behaviours, and assessed the impact of EE upon goal-orientated learning and sensorimotor coordination behaviours. The role of inhibitory striatal circuitry within these behaviours was ascertained by the use of Chrondroitinase ABC (ChABC), and the manner in which EE interacted with Perineuronal Net (PNN) dissolutions during behavioural testing was assessed.

Exposure to EE is known to improve task acquisition in a goal-orientated learning behavioural paradigm [57, 62], and to also improve coordinated sensorimotor behaviours [63]. I found that animals raised in enriched housing demonstrated faster problem solving within the Puzzle Box during a test of goal-orientated learning, but only slightly improved acquisition of a rotarod based motor skill. These results demonstrate that exposure to EE can impact upon a variety of behaviours, and that the Puzzle Box goal-orientated learning task used in our studies is a consistent and useful

test for the effects of enrichment. I also found that animals raised within enriched housing approach problems within the Puzzle Box in a different manner to those raised within a standard laboratory environment, displaying more interaction with novel obstruction puzzles and less locomotor activity. These results demonstrate that performing a thorough analysis of movement patterns during behavioural tasks can provide greater insight into neural functioning.

ChABC is a bacterial enzyme that digests PNNs into their constituent molecules, dissolving a component of the extracellular matrix [5, 64]. It is thought that PNN dissolution increases the level of neuroplasticity within a circuit, allowing for the formation of new connections between neurons [64, 65]. Previous work from our laboratory assessing the removal of PNNs within the striatum suggests that these extracellular matrix structures play a role in regulating behavioural functions mediated by this nucleus [5]. Permanent lesions to the striatum have been shown to interfere with performance of both cognitive and sensorimotor behavioural tasks [66-69], although exposure to EE is capable of reducing the severity of behavioural deficits induced by these lesions [68]. I found that injecting ChABC into the striatum successfully reduces the number of mature PNNs within this brain area, with no significant difference between the level of PNN digestion observed in both enriched and standard housed adult animals.

ChABC treatment affected animal behaviour within the Puzzle Box task, but had little effect on motor task acquisition on the rotarod. These results demonstrate that PNN dissolution within the striatum is capable of modulating specific cognitive processes, providing further evidence for the important role of this nucleus – and by extension the basal ganglia – in a variety of behaviours. I also found that animals raised within enriched housing receiving ChABC did not demonstrate the same level of changes in goal-orientated learning behaviours as standard housed animals receiving ChABC. Taken together with the fact that enrichment improved behavioural performance, these results suggest that striatal PNN digestion has an opposite effect to that of EE on behaviours both within the Puzzle-Box and upon the rotarod. Despite both enrichment [8, 70, 71] and ChABC treatment [7, 64,

72] increasing plasticity within the CNS, the current results suggest that these two treatments can act on different, and potentially distinguishable, levels of striatal circuits.

3.1 Future Directions

The striatum is involved in cognitive processes such as decision-making [73, 74], choice of action [74], goal-orientated learning [75], rule-based learning [69], and task acquisition [67]; as well as sensorimotor behaviours, including motor coordination [68, 76], balance [68, 77], motor skill acquisition [78, 79], and synchronisation of sensory and motor input [68, 76, 77]. I established that striatal ChABC injection affects goal-orientated learning and problem-solving behaviours, and prevented enrichment-induced improvement in motor task acquisition on the rotarod. It would be of interest to determine whether PNN dissolution affects other striatally-mediated behaviours, such as instrumental learning and decision-making.

The Puzzle-Box provides a simple and efficient means of assessing the efficacy of enrichment protocols. Accordingly, future experiments should endeavour to determine whether different forms of enrichment exhibit similar performance benefits on this goal-orientated learning task. For example, both full scale EE [45, 80] as well as voluntary exercise [81-83] in isolation can have similar benefits on delaying the onset of symptoms in Huntington's disease mouse models. It would be of interest to determine how these two methods of enrichment affect specific behaviours exhibited in the solving of obstruction challenges within the Puzzle-Box task.

Further studies determining how EE ameliorates the ChABC-induced changes in behaviour observed in this study would also be of import. Given that there was no detectable difference in the level of PNN dissolution between enriched and standard housed animals, there may other regions of the brain compensating for the loss of these structures within the striatum, or there may be a protective

effect of EE within the striatum preventing the modification of function associated with the removal of PNNs.

Determining the extent of EE needed to limit ChABC-induced behavioural changes would also be of interest – whether EE confined to the early postnatal period, adulthood, or immediately following surgical intervention might have a similar protective effect as lifelong EE has not been established. Further studies into the potential protective effects of EE in this context may provide insights into recovery mechanisms, and contribute to the development of rehabilitative therapies.

As noted above, a thorough anatomical and physiological assessment of afferent input to the striatum resulting from both enrichment and striatal PNN digestion should be prioritised.

Surprisingly, motor skill acquisition, another behaviour dependent on striatal function [78, 79], exhibited little change due to both enrichment and ChABC treatment. One possible reason may be the time that had elapsed between initial PNN removal and the onset of rotarod testing. Accordingly, the same cohort of animals should be assessed on this motor skill acquisition task, soon after pharmacological treatment.

4. Assessing the impact of environmental enrichment upon decision-making behaviours within the IntelliCage system

Finally, this thesis successfully determined the effects of EE upon reward-based decision-making within a novel testing environment. One behavioural test that assesses the decision-making process by using rewards based on a set of rules is the Iowa Gambling Task (IGT), used to characterise decision-making behaviours by humans during psychological evaluations [84, 85]. The IGT determines the ability of an individual to balance potential future rewards against potential future losses. The striatum is known to be involved in rule-based learning [69], decision-making [73, 74] and choice of action behaviours [74], and thus is likely involved in mediating behaviour during this task.

Previous studies from other laboratories have developed rodent versions of the IGT – Rodent Gambling Task (RGT) – using food supply and withdrawal as rewards and losses, and rats as experimental subjects [86-88].

I developed a mouse version of the RGT conducted within the self-contained behavioural testing IntelliCage arena, using water supply and withdrawal as rewards and losses. I found that all animals, regardless of housing condition, were able to discriminate between overall disadvantageous and overall advantageous choices within the IntelliCage, consistently preferring overall advantageous choices. These results demonstrate that mice are capable of performing complex behavioural tasks such as the RGT, providing another option for behavioural testing when assessing decision-making behaviours. A mouse version of the RGT is potentially useful for analysis of murine models of human diseases such as Schizophrenia, which has been shown to impact performance of human subjects in the IGT [89-91]. These results also demonstrate that a self-contained testing arena such as the IntelliCage is able to be adapted to perform more complex behavioural tasks than initially intended.

There was no discernible difference in the performance of animals raised in or exposed to EE as adults and standard housed cohorts during the RGT. Animals raised from birth in enriched housing showed greater levels of exploratory behaviour upon first encountering the IntelliCage when compared to standard raised mice. This effect soon wore off, with both animals raised from birth in enrichment and housed briefly in enrichment as adults displaying lower levels of exploratory behaviours during the remainder of their time within the IntelliCage than either group of standard housed animals. These results are consistent with previous studies demonstrating that animals exposed to EE habituate faster to novel situations than those housed within standard cages [92]. This effect of EE is worth keeping in mind when designing behavioural tasks, particularly those dependent upon animals escaping an aversive arena or maintaining high levels of activity, such as the Morris Water Maze or IntelliCage, respectively. It may also be that enriched animals require greater reward or stimulation during behavioural tasks than those housed in standard laboratory

cages, due to experiencing a higher level of stimulation within home cages. Given the growing body of research investigating the effects of environmental manipulations, determining the effect of EE upon baseline behaviours such as habituation to testing arenas and sensitivity to reward is of importance.

4.1 Future Directions

The IntelliCage provides a valuable option for behavioural testing, reducing experimenter contact and handling that can cause stress during more conventional behavioural tasks, and reflecting a more "naturalistic" behavioural pattern due to co-housing of animals during behavioural testing [93-95].

While the current study confirmed the feasibility of developing an RGT using the IntelliCage platform, the prolonged occupancy of subjects within the home/testing arena, and the corresponding enrichment experienced by all animals within this system may have inadvertently reduced any potential differences in performance of cohorts raised in enriched and standard housing conditions. Further, the particular combination of reward/penalty used in this version of the task may not have been optimal to drive the desired choice behaviour. Future studies should examine ways of improving the task to elicit a stronger response from test subjects.

It would be worth determining whether there are other human-based behavioural tests that may translate to the IntelliCage, particularly for the assessment of animal models of human diseases. The rodent version of the IGT developed during this study is potentially of great use to researchers investigating animal models of human conditions such as Schizophrenia, addiction, anxiety, and obesity, all of which have been shown to impair decision-making behaviours of subjects during the human IGT [89, 91, 96-100]. Determining the effect of disease models upon decision-making behaviours during the RGT would provide a more detailed picture of behavioural impairments in

these animal models, and may provide confirmation that animal models match the cognitive and behavioural profile of human diseases.

The effect of EE upon exploratory behaviour and habituation within the IntelliCage was unexpected and of great interest. Unpublished results from another student in our laboratory suggest that exposure to EE has a similar impact upon behaviour within an operant conditioning chamber using food as a reward. Given the increasing prevalence of studies investigating EE, and the possibility of all laboratory animal housing containing enriching elements in the future, it would be of interest to determine the exact effects of EE upon reward sensitivity and habituation behaviours within automated behavioural testing paradigms. It may be that animals exposed to EE require higher levels of reward and stimulation within a testing arena than those housed in standard laboratory conditions, perhaps requiring a new "baseline" for behavioural testing.

5. Final Conclusions

The basal ganglia is integral to the healthy and whole functioning of an organism, mediating interactions with and responses to an organism's surroundings. The effects of environmental enrichment upon the striatum and animal behaviours documented within this thesis provide evidence for the significant impact that an organism's surroundings may exert upon this important part of the brain. Determining the manner in which environmental enrichment influences specific cognitive neural networks may assist in the development of early education and intervention programs targeted at young children, or environmentally-based therapies for individuals suffering from neurological disease or injury.

6. References

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