A Novel Role for Adult Neurogenesis in a Two-Armed Bandit Reversal Learning Task

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CA2:	cornu ammonis 2
CA3:	cornu ammonis 3
CNO:	clozapine N-oxide
D1:	dopamine 1 (receptor)
D2:	dopamine 2 (receptor)
DCX:	doublecortin
DREADD:	designer receptor exclusively activated by designer drugs
GFAP:	glial fibrillary acidic protein
HSV-TK:	herpes simplex virus thymidine kinase
MDD:	major depressive disorder
PBS:	phosphate-buffered saline
PCR:	polymerase chain reaction
PFC:	prefrontal cortex
PRL:	probabilistic reversal learning
RR2:	random ratio (average: 2)
SGZ:	subgranular zone
TK:	used to refer to transgenic rats expressing the HSV-TK gene
WT:	wild-type

Chapter 1:
Background

1.1 Introduction

The mammalian hippocampus is a multifunctional brain structure that plays an essential role in spatial navigation, stress response, and regulation of episodic memory (Corcoran & Maren, 2001; Herman et al., 1998; Olton et al., 1978). Within the hippocampus, the dentate gyrus is unique in its ability to continuously add new neurons, even well into adulthood (Altman & Das, 1965a). Although adult neurogenesis has been observed in other brain areas – most notably in the subventricular zone of the lateral ventricles (Pencea et al., 2001) – nowhere has it been more studied than in the dentate gyrus (Kempermann, 2011, p. 185). Diminished levels of hippocampal adult neurogenesis have been associated with altered stress response (Snyder et al., 2011), impaired pattern separation (Clelland et al., 2009), and reduced distractibility (Weeden et al., 2019). However, the ways in which newly-born neurons directly influence behavior remain poorly understood.

This dissertation explores a novel role for adult neurogenesis in probabilistic decision-making through the use of a two-armed bandit reversal learning task. Additionally, this work provides evidence that neurogenesis ablation can confer a distinct behavioral advantage in certain contexts – a marked departure from most prior literature.

1.2 Adult Neurogenesis

1.2.1 History

In 1963, Joseph Altman published the first image of an adult-born neuron: a granule cell in the hippocampus of a mature rat labelled with tritiated thymidine (Altman, 1963). By injecting the radioactive nucleoside into rodent and feline brains, Altman labelled dividing cells, quickly identifying the dentate gyrus as a region with large populations of newly-born cells. In a later

paper, Altman demonstrated the permanence of the new granule cells, finding evidence of the marker up to eight months after injection (Altman & Das, 1965a). Unfortunately, these findings were met with skepticism, and the field of adult neurogenesis was largely abandoned.

Decades later, Heather Cameron and Elizabeth Gould definitively proved the existence of hippocampal neurogenesis while working in Bruce McEwen's lab. Before, many had argued that the tritiated cells observed by Altman might in fact be glial cells, and could not be conclusively identified as neurons. However, using both radioactive thymidine and neuron-specific enolase as markers, Cameron and colleagues found widespread double-labelling, demonstrating that the majority of the thymidine-labeled cells were, indeed, neurons (Cameron et al., 1993).

Although much neurogenesis research has focused on the rodent dentate gyrus, there is a long history of study in other brain regions and species. After publishing his seminal works, Joseph Altman identified proliferating cells in the mammalian olfactory bulb (Altman & Das, 1965b), a finding replicated by many others (Corotto et al., 1993; Kaplan & Hinds, 1977; Kornack & Rakic, 2001). Adult neurogenesis has also been observed (though not without controversy) in other brain regions, including the striatum (Dayer et al., 2005), amygdala (Bernier et al., 2002), and cortex (Gould, Reeves, et al., 1999). Further, neurogenesis research has not been restricted to mammals: adult-born neurons have now been identified in canaries (Goldman & Nottebohm, 1983), turtles (Pérez-Cañellas et al., 1997), and knifefish (Zupanc & Horschke, 1995), among other species.

Adult neurogenesis in humans remains a contested topic (Sorrells et al., 2021), despite abundant evidence in its favor (Boldrini et al., 2018; Eriksson et al., 1998; Moreno-Jiménez et al., 2019; Spalding et al., 2013). Nevertheless, the study of human adult neurogenesis has significant clinical implications. Many have proposed a link between neurogenesis level and

depressive symptoms (Jacobs et al., 2000; Sahay & Hen, 2007). Neurogenesis ablation renders mice more susceptible to depression-like phenotypes (Mateus-Pinheiro et al., 2013; Snyder et al., 2011), whereas systemically increased neurogenesis reduces their incidence (Hill et al., 2015). Further, neurogenesis levels rise following antidepressant administration (Malberg et al., 2000; Perera et al., 2007). In light of these findings and others, enhancement of neurogenesis has been suggested as a future therapy for depression (Svoboda, 2022). Similarly, a better understanding of adult neurogenesis could provide insight into how the brain recovers from injuries and stroke (Ceanga et al., 2021).

However, adult neurogenesis is relevant to humans even outside of the clinic. Animal studies suggest many critical functions of hippocampal neurogenesis, including the regulation of motivation (Karlsson et al., 2018), stress response (Levone et al., 2015; Snyder et al., 2011), pattern separation (Clelland et al., 2009), and fear learning (Drew et al., 2010; Shors et al., 2001): the mechanisms of which are all incompletely understood in humans.

1.2.2 Origins and Maturation of Adult-Born Hippocampal Neurons

Adult-born hippocampal neurons begin as radial precursor cells in the subgranular zone (SGZ), a thin strip of cells separating the granule cell layer from the hilus. The daughter cells of the radial precursors are highly proliferative, eventually maturing into putative neurons which migrate to the granule cell layer (Kempermann, 2011, pp. 190–202; Kempermann et al., 2015). The new neurons are then incorporated into circuits, sending axons through the mossy fiber tract to CA2 and CA3 (Llorens-Martín et al., 2015), and receiving inputs primarily from within the dentate gyrus – but also from the superior colliculus, amygdala, and thalamus, among other regions (Terreros-Roncal et al., 2019; Vivar et al., 2012).

It takes at least seven weeks for an adult-born neuron to reach full maturity (Jessberger & Kempermann, 2003; Toni et al., 2007), behaving identically to the pre-existing granule cell population (Laplagne et al., 2006). However, new granule cells axons reach CA3 as soon as four days after birth (Hastings & Gould, 1999) and develop glutamatergic synapses and dendritic spines after three to five weeks (Toni et al., 2007). Peak synaptic plasticity of the new neurons occurs during this intermediate period, when the cells exhibit many of the morphological characteristics of mature granule cells, but remain functionally immature (Schmidt-Hieber et al., 2004).

At this stage, extrinsic factors can influence the eventual survival of the new granule cells. For example, environmental enrichment (Kempermann et al., 1997), hippocampal-dependent learning (Gould, Beylin, et al., 1999), exercise (Snyder et al., 2009), and mild caloric restriction (Lee et al., 2002) have each been found to exert survival-promoting effects. Furthermore, differences in survival rate, not proliferation, explain most of the genetic variation in neurogenesis rates between mouse strains (Kempermann et al., 2006). While not as affected by genetics, the early proliferation rate can also be affected by environmental factors: acute stress (Gould et al., 1997; Malberg & Duman, 2003), advanced age (Cameron & McKay, 1999), and chronic sleep deprivation (Mirescu et al., 2006) reduce proliferation in the SGZ, whereas exercise (van Praag et al., 1999) has the opposite effect.

Additionally, sex hormones play an important role in the regulation of adult neurogenesis. Androgen exposure promotes granule cell survival only in male rats (Duarte-Guterman et al., 2019), whereas cell proliferation rates fluctuate with the estrous cycle in females (Tanapat et al., 1999). Moreover, the early post-partum period is associated with decreased

hippocampal cell proliferation (Darnaudéry et al., 2007), a reduction most likely mediated by hormonal changes (Green & Galea, 2008; Leuner et al., 2007).

Overall, an estimated 9000 new neurons are born daily in the rat hippocampus (Cameron & Mckay, 2001). Altering the rate of new cell birth and survival *in vivo* provides one of the most straightforward methods for studying the ethological relevance of adult neurogenesis.

1.2.3 Methods of Neurogenesis Ablation

The least ambiguous way to regulate neurogenesis *in vivo* is to ablate it completely. Early neurogenesis ablation studies relied primarily on irradiation or chemical treatment (Monje et al., 2002; Parent et al., 1999; Shors et al., 2002). Unfortunately, neither method can guarantee specificity. Irradiation studies often employ whole-brain X-rays, potentially eliminating not only dividing neurons, but also any other proliferating cell types nearby. Similarly, cytotoxic drugs (like methylazoxymethanol and chemotherapy drug temozolamide) poison all proliferating cells in the body, potentially damaging digestive (Zedeck & Sternberg, 1974), immune (Sampson et al., 2011), and locomotor function (Suhovskih et al., 2020).

Newer studies have employed optogenetic-based ablation techniques, using retroviruses specifically engineered to target neural precursors. In those studies, the viruses are often directly injected into the dentate gyrus, and the labelled cells are silenced with an implanted optic fiber (Gu et al., 2012; Masachs et al., 2021). While highly specific, this method subjects animals to surgical stress, anesthesia exposure, and neural injury. Chemogenetic techniques are somewhat similar, using designer receptors exclusively activated by designer drugs (DREADDs) to specifically suppress or excite newly-born neurons (Quintanilla et al., 2019; Zhou et al., 2018). Using DREADDs also requires intracranial injection and risks similar side effects to optogenetic

ablation. Additionally, clozapine N-oxide (CNO), the most common DREADD activator, has been shown to induce off-target effects in rats (MacLaren et al., 2016).

This dissertation takes a different approach to neurogenesis ablation, employing a pharmacogenetic method that is both time- and cell type-specific (Snyder et al., 2016). In general, expression of the herpes simplex virus thymidine kinase (HSV-TK) gene disrupts cell division in the presence of nucleoside analogs (Heyman et al., 1989). Our transgenic rat line expresses the HSV-TK gene under the human glial fibrillary acidic protein (GFAP) promoter. Although commonly known as an astrocyte marker, GFAP is also expressed by the radial precursor cells in the SGZ that give rise to new hippocampal neurons. Thus, oral administration of valganciclovir, a nucleoside analog, inhibits SGZ cell proliferation in our transgenic "TK" rats. The rats do not receive drug treatment until reaching eight weeks of age, thus preserving normal juvenile brain development. This time course is particularly important given that most rat granule cells are born during early postnatal days, remaining immature for up to eight weeks.

This pharmacogenetic method completely ablates hippocampal neurogenesis while sparing other dividing cells, and the rats avoid the stress, anesthesia exposure, and inflammation associated with other alternative ablation methods. Furthermore, TK rats are generally healthy and unimpaired cognitively (Snyder et al., 2016), making them ideal subjects for a longitudinal decision-making study.

1.2.4 Behavioral Effects of Neurogenesis Ablation

Animals lacking adult neurogenesis perform normally on many standard learning tests – surprising, given the numerous learning deficits observed in hippocampal lesion studies (Broadbent et al., 2004; Fortin et al., 2002; Isaacson et al., 1966; Kaada et al., 1961). Rats with

ablated neurogenesis learn the location of a submerged platform in a Morris water maze (Groves et al., 2013; Jessberger et al., 2009; Snyder et al., 2005) and the correct path in a dry flex maze (Schoenfeld et al., 2021) just as quickly as intact controls. They also show no impairments in fixed-ratio lever pressing or Pavlovian conditioning (Karlsson et al., 2018). Similarly, learned fear response appears preserved: neurogenesis ablation has no effect on behavior in most cued (Glover et al., 2016) and contextual (Clark et al., 2008) fear conditioning paradigms. Furthermore, animals without neurogenesis perform normally on many tests of anxiety, behaving the same as controls in open-field, novelty-suppressed feeding, and elevated-plus maze assays (Shors et al., 2002; Snyder et al., 2016).

However, behavioral differences emerge when testing animals under uncertain or ambiguous conditions, when actions or cues do not guarantee subsequent outcomes. For example, fear-conditioned TK mice lacking new hippocampal neurons respond more "optimistically" to probabilistic threat cues compared to wild-type littermate controls (Glover et al., 2016). Additionally, rodents with ablated neurogenesis show decreased motivation to work for rewards when the required effort level is unclear (Karlsson et al., 2018) and impaired learning when distinguishing between highly similar cues (Clelland et al., 2009; Tronel et al., 2012). Further, mice without neurogenesis do show deficits in water maze learning when spatial cues are highly complex and therefore, difficult to differentiate (Garthe & Kempermann, 2013).

Together, these findings suggest a role for adult neurogenesis in the cognitive processing of ambiguity, probability, or uncertainty. In humans, altered cognitive bias – which can manifest as impairments in probability processing – is a hallmark of major depressive disorder (MDD) (Elliott et al., 1996; Murphy et al., 2003). As previously mentioned, a connection between neurogenesis level and depression has long been suspected (Jacobs et al., 2000; Sahay & Hen,

2007). Notably, neurogenesis depletion increases vulnerability to depressive phenotypes (Mateus-Pinheiro et al., 2013; Snyder et al., 2011), while elevated neurogenesis protects against them (Hill et al., 2015). Given these findings, it seems possible that reduced neurogenesis levels might underlie the altered judgment bias seen in humans with MDD.

However, not every behavioral effect of neurogenesis loss can be immediately related to ambiguity or uncertainty. For instance, animals without new neurons appear less easily distracted, paying less attention than controls to novel cues (Schoenfeld et al., 2021; Weeden et al., 2019). Further, neurogenesis ablation alters stress response, rendering animals more susceptible to developing anxiety- or depression-like phenotypes following acute stress (Mateus-Pinheiro et al., 2013; Snyder et al., 2011). Still, the connection between adult neurogenesis and the processing of ambiguous feedback is promising – especially given the clinical relevance of impaired judgment bias. This dissertation probes the strength of that connection by testing rats with ablated neurogenesis on a probabilistic two-armed bandit reversal learning task, a paradigm characterized by uncertain outcomes and ambiguous feedback.

1.3 Probabilistic Reversal Learning

1.3.1 Overview

The probabilistic reversal learning (PRL) task is a well-established test of feedback sensitivity used in humans (Swainson et al., 2000), non-human primates (Costa et al., 2015), and rodents (Ineichen et al., 2012; Rychlik et al., 2017). In an appetitive PRL task, correct choices are reinforced by a reward most of the time, at probability p (usually between 60-90%). Similarly, incorrect choices are rarely reinforced, usually at probability $1\text{-}p$. At various points during the test session, the identities of the correct and incorrect choices switch, along with their

corresponding reinforcement probabilities. In contrast to most other reversal tasks, outcomes are probabilistic and feedback is ambiguous. (For instance, a previously lucrative choice failing to produce a reward could indicate either a reversal, or merely the *1-p* chance that the correct choice is non-reinforced.) Individual PRL test sessions can include hundreds of trials, producing high-volume datasets that record responses to wins and losses, both expected and unexpected. Tracking performance across trials and sessions provides insight into subjects' developing decision-making frameworks and how those frameworks differ between individuals and groups.

After nearly a decade of use in humans (Cools et al., 2002; Lawrence et al., 1999), the PRL task was first adapted to for rodent operant use in 2010 (Bari et al., 2010). Traditionally, animal studies have used deterministic reversal learning to test cognitive flexibility. However, deterministic tasks have less relevance to studies in humans (for whom deterministic tasks are too easy) and are less applicable to decision-making in the real world, where the outcomes of choices are seldom guaranteed. While the use of the PRL task – or variations upon it – in both mice and rats has increased in recent years (Amodeo et al., 2014; Chen, Ebitz, et al., 2021; Seib et al., 2020; Spinelli et al., 2013), it remains an underutilized tool for testing feedback sensitivity and judgment bias in rodents.

1.3.2 Neural Correlates of Probabilistic Reversal Learning

Lesion and inactivation studies in both rodents and non-human primates support roles for the basal ganglia (Costa et al., 2016; Ragozzino, 2007) and the prefrontal cortex (PFC; Dalton et al., 2016; Dias et al., 1996) in reversal learning. Specifically, previous literature suggests that the striatum might be responsible for learning new associations (Cools et al., 2002), whereas the PFC might inhibit incorrect responses (Ghahremani et al., 2010). Others have proposed a more

nuanced role for the PFC in attentional control of the basal ganglia during reversal learning (Erdeniz & Atalay, 2010; Mitchell et al., 2008; Schirru et al., 2022).

This corticostriatal loop, linking dopamine signaling to action selection, has been extensively modeled (Frank et al., 2004; Hazy et al., 2007; O'Reilly & Frank, 2006). In most computational models, striatal "Go" cells – mediated by D1 receptors – promote behavior by disinhibiting the thalamus, whereas "No-Go" cells – mediated by D2 receptors – suppress behavior by increasing thalamic inhibition. Both populations of cells strongly innervate the substantia nigra pars compacta, which in turn modulates future striatal firing, along with top-down control from the PFC. This network has been implicated in probabilistic reversal learning, response impulsivity, and both probabilistic and deterministic transitive inference (Cools et al., 2002; Dalley et al., 2011; Frank et al., 2004).

The involvement of the corticostriatal loop in probabilistic reversal learning is largely unsurprising. Success on reversal tasks requires not only the execution of new action-outcome pairs ("Go" responses), but also the suppression of previously-learned actions ("No-Go" responses). However, on probabilistic tasks, widespread feedback ambiguity further complicates decision-making, prompting some to propose the recruitment of an additional, uncertainty-focused network (Soltani & Izquierdo, 2019). In addition to the striatum and PFC, many other brain regions have been implicated in uncertainty signaling, including the hippocampus, basolateral amygdala, and mediodorsal thalamus.

Traditionally excluded from the basal ganglia-cortical action selection loop, the hippocampus could play an important role in a distributed uncertainty network (Soltani & Izquierdo, 2019), especially given its demonstrated involvement in the encoding of choice-outcome predictions and neural correlates of "surprise" (Duncan et al., 2012; Kumaran &

Maguire, 2006; Wikenheiser & Schoenbaum, 2016). The hippocampus also appears to play an important role in reversal learning specifically: in humans, reduced hippocampal volume is associated with poorer understanding of PRL task structure and weaker anticipation of reversals (Vilà-Balló et al., 2017). Further, on a deterministic task, rabbits with hippocampal lesions require four times as many sessions to reach criterion performance following a reversal as unoperated controls (Berger & Orr, 1982). Similarly, the amygdala is implicated in reversal learning despite not appearing in most corticostriatal action selection models. Bilateral amygdalar lesions produce distinct performance impairments in macaques on both stochastic and deterministic reversal learning tasks, decreasing learning rate and choice consistency (Costa et al., 2016).

Successful PRL performance appears to require the recruitment of both the corticostriatal Go/No-Go loop and a larger "uncertainty network." The exact functions of implicated brain regions – especially those associated with the processing of uncertainty, like the amygdala and hippocampus – remain poorly understood. Only future research can provide a better understanding of how action-selection and uncertainty circuits converge to determine behavior.

1.3.3 Clinical Relevance

The PRL task offers a convenient method for quantifying sensitivity to positive and negative feedback in humans, with the large number of trials allowing for the detection of even subtle behavioral differences. Despite its relatively short history, the PRL task has already revealed many findings of clinical importance. Depressed patients switch more often than controls in response to misleading negative feedback in a PRL task (Murphy et al., 2003; Taylor Tavares et al., 2008), consistent with previous research in other paradigms demonstrating a

strong association between negative cognitive bias and depression (Elliott et al., 1997; Robbins et al., 1994; Steffens et al., 2001). Similarly, both children and adults with bipolar disorder also exhibit heightened sensitivity to negative feedback on PRL tasks (Dickstein et al., 2010; Roiser et al., 2009). Somewhat paradoxically, administration of the selective serotonin reuptake inhibitor – and commonly prescribed antidepressant – citalopram has been shown to impair PRL performance in humans (Chamberlain et al., 2006). However, the opposite effect has been observed in rodents given a single, acute dose, or repeated, moderate doses (Bari et al., 2010; Ineichen et al., 2012). Further, possession of a polymorphism on the *SERT* gene, which encodes the serotonin transporter, is associated in humans with increased shift rates following negative feedback (den Ouden et al., 2013). Together, these findings provide convincing evidence of the serotonergic regulation of mood-related changes in feedback sensitivity.

Changes in dopamine signaling are also associated with abnormal PRL task performance. Patients with Parkinson's disease medicated with levodopa (a catecholamine precursor) perform worse on a PRL task than unmedicated patient controls, struggling to adapt to reversals (Cools et al., 2001; Swainson et al., 2000). Early Parkinson's disease generally depletes dopamine only in the dorsal striatum; as a result, levodopa administration might cause dopamine "overdose" in other, relatively unaffected brain regions. This peripheral increase in dopamine has been proposed to underlie observed impairments in reversal learning following levodopa treatment (Cools et al., 2002). Prior work demonstrates other cognitive impairments in Parkinson's patients following levodopa administration, consistent with the theory that peripheral overdose can cause detrimental effects (Gotham et al., 1988). Interestingly, studies in non-human primates show that dopamine depletion also impairs reversal learning (Clarke et al., 2011) and that administration of either haloperidol (a D2 receptor antagonist) or levodopa can improve PRL performance

(Costa et al., 2015). Further human studies suggest that dopamine levels affect sensitivity to positive and negative feedback (Frank et al., 2004), with associated performance effects varying according to specific testing parameters.

Variations on the PRL task have also identified abnormal feedback sensitivity in other neuropsychiatric disorders aside from Parkinson's disease and depression. For example, youth with severe mood dysregulation and anxiety disorder have been found to commit more reversal errors than healthy controls (Dickstein et al., 2010). In contrast, adults with obsessive-compulsive disorder commit similar numbers of reversal errors as controls, while making fewer correct choices overall (Remijnse et al., 2006). Further, people with schizophrenia are more likely than controls to switch their answers, even after receiving positive feedback (Culbreth et al., 2016).

In sum, performance on PRL tasks appears sensitive to the general state of the brain and is influenced by disease, neurotransmitter level, and genetics. Given that impaired feedback sensitivity and cognitive processing are often hallmarks of underlying neuropathology, the PRL task seems poised to play a key role in the future identification of decision-making phenotypes and in the assessment of novel therapeutics.

1.4 Study Rationale

In this background chapter, I have demonstrated that adult hippocampal neurogenesis is implicated in several critical behaviors, especially those involving the cognitive processing of ambiguity or uncertainty. I have also established the PRL task as an effective and well-established method of studying feedback sensitivity. In the remainder of the dissertation, I bridge

these two concepts, studying the effects of neurogenesis ablation on PRL task performance to better understand how neurogenesis affects behavioral approaches to ambiguity.

The PRL task is characterized by uncertain outcomes and ambiguous feedback, providing the perfect tool to study how rats with ablated neurogenesis approach probabilistic decision-making. In contrast to most rodent reversal paradigms, the PRL task uses probabilistic reward outcomes, more closely resembling human studies and real-life decision-making in nature. Moreover, it is often unclear whether or not the behavioral effects of neurogenesis ablation are helpful or harmful. For example, when mice are presented with probabilistic threat cues (Glover et al., 2016), it is difficult to say whether it is "better" to respond optimistically, risking the possibility of shock, or whether it is "better" to freeze more indiscriminately, potentially responding to countless false alarms. Performance on the PRL task offers a much clearer interpretation: for food-restricted animals, earning more rewards seems unequivocally the "better" outcome.

However, this work is not the first to investigate the relationship between adult hippocampal neurogenesis and reversal learning. Previously, our lab found no significant effect of neurogenesis ablation on a standard operant reversal learning task, in which rats and mice were exposed to a single reversal after several days of training (Karlsson et al., 2018). Another group found that rats without adult neurogenesis committed a similar number of errors on a PRL task when compared to wild-type controls, despite heightened sensitivity to negative feedback (Seib et al., 2020). Nevertheless, I hypothesized that more notable neurogenesis effects might emerge on a reversal task with different parameters. To that end, I designed a reversal learning task with probabilistic outcomes and repeated exposure to reversals, both within-session and across days.

In the following chapters, I report and interpret the findings from several experiments employing the reversal protocol. I first describe the effects of neurogenesis ablation on probabilistic reversal learning when the beginning of each session is predictable (Chapter 3) and when it is randomized (Chapter 4). Next, I discuss whether the behavioral effects of neurogenesis persist when reward outcomes are deterministic (Chapter 5). Finally, I explore the roles played by task difficulty and reversal frequency in determining the effects of neurogenesis upon PRL performance (Chapter 6). Over the course of the dissertation, I demonstrate the importance of adult neurogenesis in shaping probabilistic decision-making and in mediating responses to situational uncertainty.

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Chapter 2:
General Methods

All animal procedures were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Research) and were approved by the National Institute of Mental Health Animal Care and Use Committee.

2.1 Animals

A total of 130 male and 134 female rats were used in the experiments included in this dissertation. All rats were bred in-house on a Long Evans background. Transgenic "TK" rats expressed the herpes simplex virus thymidine kinase gene under the control of the glial fibrillary acidic protein (GFAP) promoter. Wild-type (WT) males were mated with heterozygous TK females to generate litters with roughly equal numbers of WT and TK pups, which were weaned at 21-28 days of age and genotyped by PCR (Transnetyx).

Rats were group-housed and kept on a 12h:12h reversed light cycle with lights off at 9:00 am. Experiments were conducted during the first half of the dark phase. All rats were meal-fed from weaning until study completion, receiving 15 g (males) or 10 g (females) of standard laboratory chow per rat per day. Water was provided *ad libitum*. Both WT and TK rats were given 3.8 mg of valganciclovir twice weekly starting at eight weeks of age. Valganciclovir was administered orally, mixed into 0.5 g balls containing a 1:1 blend of peanut butter and powdered food chow. Rats were fed the peanut butter balls by hand to ensure proper dosing.

Each experiment was conducted with a naïve cohort of rats unless otherwise specified. Rats were between 14 and 17.5 weeks of age at the beginning of training and had been on drug treatment for at least seven weeks. Animals were sacrificed within a week of study completion and their brains were collected for histology.

2.2 Apparatus

Reversal training and testing was conducted in modular operant chambers (Med Associates, St. Albans, VT) contained within sound-attenuating cabinets. Each chamber was fitted with two retractable levers, one on each side of the food magazine. Rewarded trials resulted in the delivery a single 20 mg unflavored food pellet (5TUL, TestDiet, Richmond, IN) via an attached pellet dispenser.

Operant chambers were fitted with a removable plastic floor to cover the built-in (unused) shock grid. This floor was cleaned after every session with sanitizing wipes. MED-PC V software controlled the operation of the chambers and recorded time stamps for all lever presses, pellet deliveries, and head entries into the food magazine.

2.3 Training and Reversal Testing

2.3.1 Habituation

Unhandled, naïve rats were habituated to the operant chambers in a single 30-minute session during which 50 pellets were delivered into the magazine at randomly spaced intervals. Habituation was considered successful if fewer than 12 pellets remained in the magazine at the conclusion of the session; otherwise, rats repeated the habituation protocol on the following day.

2.3.2 Autoshaping

Next, rats were manually autoshaped to learn to lever press. One at a time, rats were placed in operant chambers delivering rewards on an FR1 schedule, with only the right lever extended. The lever (and therefore pellet delivery) was controlled remotely with a portable keyboard. At first, the lever was manually depressed whenever rats were merely in the vicinity of

the lever, with the threshold for depression slowly increasing until rats placed their paws on the lever and learned to press themselves. The lever retracted for two seconds following both natural and artificial lever presses. Autoshaping sessions ended when a rat achieved 50 consecutive unaided lever presses.

2.3.3 Lever Training

Following successful autoshaping, rats received two additional days of lever training. On the first day, only the right lever was extended, retracting for ten seconds following a successful lever press. This training concluded when rats pressed the right lever 50 times, unaided, on an FR1 schedule. If any individual rat remained in their operant chamber for 30 minutes without achieving 50 presses, the rat was removed from the chamber and the protocol was repeated the next day. The second lever training was identical to the first, except that the rats pressed the left lever instead of the right, and only the left lever was extended.

2.3.4 Probability Training

Next, rats experienced two days of probability training in which one lever produced a reward following a lever press 80 percent of the time, and the other, 20 percent, for a total of 200 trials. Levers extended at the beginning of each trial, retracting for seven seconds after either a successful lever press or ten seconds of non-response. Rats were pseudo-randomly assigned to two groups: one in which the left lever was the more rewarding lever, and one in which the right lever was the more rewarding. Any experiment-specific deviations to the probability training procedures are noted in the individual Methods section for each chapter.

2.3.5 Reversal Testing

After the two days of probability training, rats were subjected to at least 28 days of reversal testing, experiencing one session per day. Each reversal session included five blocks of 40 trials each. During the first block, lever reward probabilities were identical to those assigned during probability training and these first block probabilities remained the same for each rat across all sessions. At the end of each block, the lever reward probabilities switched. (The lever that had previously produced a reward 80 percent of the time would now do so only 20 percent of the time, and vice versa.)

If a rat failed to respond to the extended levers for ten seconds, the trial timed out and was recorded as an "omission." Following either a successful lever press or an omission, both levers retracted for seven seconds before extending again to mark the beginning of the next trial. Any experiment-specific deviations to the testing protocol are noted in the individual Methods section for each chapter.

2.4 Histology

Within a week of study completion, rats were deeply anesthetized with an isoflurane vaporizer and decapitated using a rodent guillotine. Brains were then surgically removed and drop-fixed in 4% paraformaldehyde for at least 24 hours before transferal to a 20% sucrose solution for cryoprotection. Brains were sectioned coronally at 40 μm on a sliding microtome and sections were stored in a cryoprotectant solution (0.1% sodium azide in PBS) until staining.

To confirm success of the pharmacogenetic ablation method, sections were stained for doublecortin, a marker of immature neurons. Free-floating sections were rinsed in PBS for five minutes before moving to a blocking solution (0.5% Tween-20 and 3% donkey serum in PBS)

for twenty minutes at room temperature. Sections were then incubated with polyclonal rabbit anti-DCX (1:500, Cell Signaling) for at least 24 hours at 4°C.

After primary antibody incubation, sections received three five-minute rinses in PBS. Sections were then incubated with Alexa Fluor 488 or 555 donkey anti-rabbit antibody (1:200) for two hours at room temperature. After another five-minute rinse in PBS, sections were counterstained with Hoeschst 33258 (1:1000) for five minutes. Sections were then rinsed in PBS for a final time, mounted onto slides, and cover-slipped with Prolong Gold liquid mounting medium. Sections were then inspected under a microscope for the presence or absence of DCX+ cells to confirm both genotype and successful drug treatment.

2.5 Statistical Analysis

All behavioral data was converted into spreadsheets using the MED-PC to Excel (MPC2XL) data transfer tool. Data was analyzed and graphed in GraphPad Prism following sorting and organization in Microsoft Excel. Statistical comparisons were made using two- or three-way ANOVA, applying the Geisser-Greenhouse correction where indicated. Post-hoc testing used the Sidak correction to control for multiple comparisons. All graphs display means + SEM.

Chapter 3:

Probabilistic Reversal Learning with Predictable First Blocks

3.1 Introduction

Neurogenesis ablation appears to influence behavior in situations of uncertainty, when actions or cues are either difficult to differentiate (Clelland et al., 2009), or only partially predictive of subsequent outcomes (Glover et al., 2016). To dissect this effect further, I used a probabilistic reversal learning (PRL) task in transgenic "TK" rats with pharmacogenetically ablated neurogenesis (see Chapter 2: Methods). The PRL task, a well-established test of feedback sensitivity, quantifies behavioral responses to positive and negative feedback, both accurate and misleading. In this study, I employed an operant two-armed bandit PRL task in which rats chose between two levers: one that delivered an appetitive food reward 80 percent of the time, and another that delivered a reward only 20 percent of the time. At four evenly spaced intervals, the reward contingencies switched, and the lever that had previously produced a reward on 80 percent of trials now did so only 20 percent of the time, and vice versa. Thus, rats encountered numerous instances of ambiguous feedback. For example, a previously lucrative lever that failed to produce a pellet might indicate that a reversal had occurred – or it might merely represent the 20 percent chance that the "better" lever would deliver no reward.

In total, this dissertation investigates the effects of neurogenesis ablation on five different versions of the PRL task. The present chapter describes the design, results, and implications of the first reversal task. In this version, sessions were divided into five blocks, each separated by a reversal. The correct lever in the first block was not randomized across sessions, thereby eliminating any effect of initial discrimination ability. Given the finding that TK mice respond more "optimistically" to ambiguous threat cues than WT controls (Glover et al., 2016), I hypothesized that TK rats might have more positive interpretations of their own choices, therefore being more likely to persist with a lever even following negative feedback. Such a

tendency would improve performance when negative feedback is misleading, but hinder it following a reversal, when negative feedback is accurate.

This experiment also investigates the behavioral effects of sex on the PRL task. Historically, very few neurogenesis studies have used animals of both sexes (O'Leary et al., 2022), leaving a vast gap in the knowledge of how, if at all, sex and neurogenesis intersect to influence behavior. In the following sections, I discuss the revealed effects of both neurogenesis ablation and sex on performance and strategy, compare the findings to existing literature, and explore the implications for the proposed function of newly-born hippocampal granule cells.

3.2 Abbreviated Methods

The methods employed in each experiment are highly similar, and a full description of the methods conserved across all experiments can be found in Chapter 2: Methods.

3.2.1 Animals

A total of 33 WT (19 male, 14 female) and 35 TK (16 male, 19 female) rats were used to collect data for this experiment. Rats were between 15 and 16.5 weeks of age at the beginning of training and had been on drug treatment for at least seven weeks. All male rats were naïve and unhandled at the beginning of the experiment. Eighteen of the female rats (7 WT, 11 TK) had been previously used for a distraction task involving mild water restriction.

3.2.2 Reversal Task

The experimental protocols used in this chapter follow the general outline presented in Chapter 2: Methods. In this version of the PRL task, lever identities during the first block were

identical to those assigned during the probability training phase and remained the same for each rat across all 28 sessions. (Therefore, the "best" lever at the beginning of each session was the same across all testing days.)

If a rat omitted 25 or more trials in a single session, the data from that rat-session was excluded and replaced with the data from the following session. The same procedure was followed when sessions were compromised by equipment malfunction. I instituted two more general exclusion criteria: (1) if five or more sessions from the same rat were excluded, that rat would be removed completely from the study, and (2) any rats that failed to press their non-trained levers at least 20 times by the tenth session would also be removed. (No rats in the present chapter met either of those criteria.) Out of a total of 1904 rat-sessions, only 11 were excluded for excessive omissions, with an additional three removed due to equipment malfunction. All rats were run on the reversal protocol for at least 28 days; however, to accommodate for removed sessions, only the first 25 successful sessions for each rat are included in the data analysis for this chapter.

As a result of a programming anomaly, most data measures are currently unavailable from the first testing session. For that reason, analyses in this chapter use data from sessions 2-26, with the exception of unadjusted rewards data. Furthermore, 12 rats (8 WT, 4 TK) received longer training on the right lever than described in Chapter 2: Methods. Neither of these issues were repeated in any other chapter.

3.3 Results

Rats without adult neurogenesis earned more rewards than wild-type controls, even at early timepoints.

Perhaps the most straightforward way to assess performance on the PRL task is to count the number of rewards that rats earn while in the operant chambers. Hypothetically, a rat that failed to recognize or adapt to the reversals would perform at chance (100 rewards) by choosing randomly between the two levers. On the other hand, a rat that successfully learned to adapt to the reversals would earn a higher number of rewards as a direct consequence of pressing the "better" lever more often.

Predictably, rats earned more rewards as they became more familiar with the reversal task (effect of session: F (14.40, 921.7) = 49.59, $p < 0.0001$). Surprisingly, TK rats earned more rewards than their wild-type counterparts (*Fig. 3.1 A*; effect of genotype: F (1, 64) = 20.45, $p < 0.0001$), with the effect growing more pronounced with increasing sessions (session-by-genotype interaction: F (24, 1536) = 2.435, $p = 0.0001$). However, the effect of genotype on performance was apparent even during early sessions, and was first significant on the fifth testing day, as revealed by a post-hoc Sidak test (Day 5: $t_{65.61} = 3.869$, $p = 0.0063$). Overall, the number of rewards earned did not differ between sexes. On average, TK rats earned 128.0 (males) and 129.7 (females) rewards during the final week of testing, whereas WTs earned 120.7 (males) and 124.2 rewards (females).

Performance on the reversal task was not determined solely by side bias or random selection.

In this version of the reversal task, the lever that is "better" during the first block remains consistent across all sessions and matches the lever that was more rewarded during each rat's probability training. Accordingly, with increasing PRL experience, rats learned to begin each session with a correct lever press (*Fig. 3.1 C*; effect of session: F (13.69, 876.3) = 5.646). Even so, TK rats were significantly more likely than WTs to make a correct choice on the first trial (F

(1, 64) = 5.470, p = 0.0225). In later sessions, females appeared more likely to make an initial correct choice than males, as indicated by a significant sex-by-session interaction (F (24, 1536) = 1.584, p = 0.0362). However, the overall performance difference between WT and TK rats was not driven solely by behavior during the first block alone. With data from the first block omitted, male and female TK rats still significantly outperformed WT controls, with the effect appearing to increase over time (*Fig. 3.1 B*: effect of genotype: F (1, 64) = 17.39, p < 0.0001, session-by-genotype interaction: F (24, 1536) = 1.749, p = 0.0139).

Moreover, on the final five days of testing, TK rats outperformed WT controls both on odd blocks, during which the originally trained lever was better, and on even blocks, during which the non-trained lever was better (*Fig. 3.1 D*; F (1, 64) = 15.08, p = 0.0002). I also observed a significant effect of "block type," with all rats earning more rewards on odd blocks than on even blocks, suggesting that a bias for the trained lever persisted even on late testing days (F (1, 64) = 118.1, p < 0.0001). For the purposes of this analysis, the first block was not counted as an odd block to control for any differences in session initiation strategy.

While a completely naïve rat might be expected to choose entirely randomly between levers, earning an average of 100 rewards over 200 trials, this expectation would not hold true for a rat with a preexisting lever preference. Therefore, rather than comparing rat performance to "chance," it might be more prudent to compare it to the performance of a hypothetical rat with intractable side bias. Such a rat would be expected to earn 112 rewards per session, assuming that its preferred lever was the one correct during the first block. All groups exceeded this benchmark as early as the second week of testing (Male WT: p = 0.0259; Female WT: p < 0.0001; Male TK: p < 0.0001; Female TK: p < 0.0001).

Because the rats outperformed both benchmarks of "chance," we can conclude that all groups successfully developed a decision-making strategy for the reversal task distinct from either mere random selection or side bias.

Rats without neurogenesis used different decision-making strategies when compared to WT controls.

Next, I analyzed how rats responded to wins and losses on a trial-by-trial basis. In human, primate, and rodent studies, "win-stay" and "lose-switch" ratios are used frequently as measures of performance and feedback sensitivity (Rygula et al., 2018). In this study, the win-stay ratio represents the probability that a rat will choose a lever that delivered a reward on the previous trial. Conversely, the lose-stay ratio represents the probability that a rat will choose a lever that failed to produce a reward on the previous trial.

On this task, a higher win-stay ratio will earn a rat more rewards. Unsurprisingly, animals exhibited higher win-stay ratios as they became more familiar with the task (effect of session: F (5.924, 379.1) = 21.53, $p < 0.0001$). Additionally, TK rats had significantly elevated win-stay ratios compared to WT controls, representing a distinct strategic divergence between rats with and without neurogenesis (effect of genotype: F (1, 64) = 12.01, $p = 0.0010$). Female rats exhibited elevated win-stay ratios compared to males during early sessions, as indicated by a significant session-by-sex interaction (F (24, 1536) = 1.593, $p = 0.0343$). To test whether the genotype difference depended on the veracity of the feedback received, I separately analyzed rats' win-stay ratios following both wins on the "better" lever (when feedback was accurate), and wins on the "worse lever" (when feedback was misleading). Using a three-way ANOVA with feedback veracity and session as within-subject factors and genotype as a between-subjects

factor, I found that TK rats had higher win-stay ratios than WTs regardless of feedback veracity (*Fig. 3.2 A, 3.2 C:* effect of genotype: F (1, 66) = 8.442, p = 0.0050). Furthermore, both genotypes were less likely to stay following a win following misleading feedback (effect of feedback veracity: F (1.00, 66.00) = 676.9, p < 0.0001), though this effect was not apparent on early sessions (session-by-feedback veracity interaction: F (13.56, 895.1) = 2.005, p = 0.0161).

When analyzing the opposite measure, the lose-stay ratio, the overall effect of genotype was no longer significant, although TK rats trended towards being more likely to persist with a lever following a loss (F (1, 64) = 3.584, p = 0.0629). After separating lose-stay behavior based on feedback veracity, I found a significant veracity-by-genotype interaction (F (1, 66) = 18.66, p < 0.0001), with a post-hoc Sidak test revealing that TK rats were more likely than WTs to persist after a loss only when the negative feedback was misleading (*Fig. 3.2 B, 3.2 D*). In other words, WT rats were more likely to abandon the better lever following a probabilistic loss, a poor strategy on a task with relatively rare reversals. Taken together with the win-stay results, these effects demonstrate an increased tendency for TK rats to persist with a recently lucrative lever – regardless of whether or not that lever was rewarded on the previous trial.

While the effect of genotype was not significant when analyzing overall lose-stay ratios, I did find a significant main effect of sex (effect of sex: F (1, 64) = 11.75, p = 0.0011). Female rats were more likely than males to persist with a lever following a loss: the first major sex difference of the study. This difference persisted when the preceding feedback was both accurate (when a loss occurred on the "worse" lever) and misleading (when a loss occurred on the "better" lever) (*Fig. 3.3 D*: effect of sex after accurate feedback: F (1, 64) = 16.78, p = 0.0001; *Fig. 3.3 B:* effect of sex after misleading feedback: F (1, 64) = 6.781, p = 0.0114). No such differences were

observed when analyzing win-stay ratios, where a significant main effect of sex was not revealed after either accurate or misleading feedback (*Fig. 3.3 A, 3.3 C*).

Female rats complete the task faster and omit fewer trials despite committing more perseverative errors.

Female rats completed all 200 trials significantly faster than males (*Fig. 3.4 A*: effect of sex: $F(1,64) = 7.778$, $p = 0.0070$). This difference became more pronounced on the final testing sessions (session-by-sex interaction: $F\ (24, 1536) = 2.531$, $p < 0.0001$). Although all groups adopted a quicker pace as sessions progressed and animals became more familiar with the task (effect of session: $F\ (7.522, 481.4) = 32.56$, $p < 0.0001$), there was no effect of genotype on overall speed. Female rats also committed fewer omissions – trials that timed out due to non-response (*Fig. 4.4 B*: effect of sex: $F\ (1, 64) = 12.02$, $p = 0.0009$), with this difference exaggerated on later testing days, most likely driving the concurrent increase in overall session time (session-by-sex interaction: $F\ (24, 1536) = 1.835$, $p = 0.0082$).

Additionally, female rats made more consecutive incorrect choices following a reversal, or "perseverative errors," than males (*Fig. 3.4 C*: effect of sex: $F\ (1, 64) = 11.48$, $p = 0.0012$). However, the rewards data indicates that the increased perseveration did not detract significantly from overall performance.

3.4 Discussion

In this chapter, WT and TK rats were tested on a probabilistic reversal task in which the correct lever in the first block was static across sessions. Male and female TK rats significantly outperformed WT controls, earning more food rewards each session. Although TK rats were

more likely to choose the correct lever on the first trial, the overall performance difference was not driven solely by inferior decision-making during the first block. (That is, WTs were not merely worse at remembering how the sessions began.) Notably, the genotype effect on performance was apparent even during early testing days, emerging after only five sessions. Given my lab's previous finding that TK rats earn fewer rewards than WTs when working for the same kind of reward pellets on a fixed ratio lever-pressing schedule, it seems unlikely that the performance effect observed on this task is caused by increased motivation alone (Karlsson et al., 2018). Rather, we can attribute the difference to distinct decision-making strategies employed by WT and TK animals.

The effects of sex and neurogenesis loss on probabilistic reward strategy

On a task with relatively infrequent reversals, it is sound strategy to persist with a lucrative lever until compelling evidence indicates that a switch has occurred. The present PRL task features only four reversals over the course of 200 trials. Therefore, a historically successful lever delivering no reward is far more likely to represent the 20 percent chance of lever failure than the small chance of a reversal. Rats that switch levers after isolated losses are most likely abandoning the "better" lever in favor of the "worse" one, thereby missing out on potential rewards. Likewise, there is little incentive for rats to abandon a lever that produced a reward on the preceding trial, as the probability of a reversal having occurred in that instance would be extremely low.

Win-stay ratios, or the tendency to persist with a choice following a win, are commonly taken as indicators of positive feedback sensitivity (Culbreth et al., 2016; Seib et al., 2020; Stolyarova et al., 2014). In the present study, TK rats without neurogenesis exhibited

significantly higher win-stay ratios than WT controls. This tendency, which is strategically advantageous, coincides with measurable performance gains in TK rats and is present following both accurate and misleading positive feedback. Notably, I also found that female rats of both genotypes were less likely to switch following a loss, regardless of feedback veracity, indicating a generally diminished sensitivity to negative feedback compared to males.

Importantly, I also found that sex affects probabilistic reward behavior outside of sensitivity to negative feedback. Female rats omitted significantly fewer trials than males, paralleling previous findings in marmosets (LaClair & Lacreuse, 2016), although studies in rodents have been mixed, with some finding the opposite effect (Aarde et al., 2021; Aguirre et al., 2020; Bryce & Floresco, 2021; Papaleo et al., 2012). In addition, females completed their sessions significantly faster than males. Other groups have observed faster female response times in reversal learning studies in primates (LaClair & Lacreuse, 2016) and rodents (Chen, Knep, et al., 2021). However, it is worth noting that on more complicated reversal tasks, females often have longer response latencies than males; see (Bissonette et al., 2012; Chen, Ebitz, et al., 2021).

Additionally, I found that female rats committed more perseverative errors following reversals, but I remain wary of overinterpreting this effect. It was not wholly uncommon for rats to commit no perseverative errors following a reversal, meaning that they responded correctly in the trial immediately following a reversal by chance alone. Rather than suggesting an impaired ability to reverse, increased perseveration in females could indicate sounder pre-reversal strategy. (Female rats are less likely than males to switch away from the better lever following misleading negative feedback and are therefore less likely to switch to a new lever at the moment of reversal.) As a whole, these findings point to a role for sex in the development of probabilistic reward strategy and behavior, independent from the effects of neurogenesis loss.

Contradictions in the literature

This is not the first study to evaluate the effects of neurogenesis ablation on reversal learning. Previously, my lab found no significant effect of neurogenesis ablation on a standard operant reversal learning task in TK mice and rats (Karlsson et al., 2018). However, the active and inactive levers switched identities only once during the entire experiment, in contrast to the present study, during which lever contingencies switch four times per session. Although lever outcomes in that study were not purely deterministic – rewards from the active lever were delivered on an RR2 schedule – the inactive lever was never reinforced prior to the reversal. Detection of the reversal was therefore relatively easy, and the task was less about sensitivity to ambiguous feedback than the ability to overcome a previously learned behavior.

The finding that TK rats are more likely to persist with the better lever appears to directly contradict previous work showing no significant difference in win-stay behavior between WT and TK rats, and significantly higher lose-switch ratios in TK rats following misleading negative feedback on the better lever (Seib et al., 2020). This apparent contradiction is likely explained by extensive differences between our experimental protocols. In the previous study, reversals did not occur at regularly spaced intervals and instead were dependent upon rats achieving eight successive "correct" lever presses: a high benchmark, given that rats in both groups only persisted with a choice that was rewarded on the preceding trial about 70 percent of the time. This criterion, while common in probabilistic reversal literature, makes for a markedly more difficult task. Additionally, the study was shorter, ending after 12 testing days. In the present study, each rat received at least 28 days of testing, and some of the behavioral effects strengthening during the second half of testing. Many of the observed effects would not have been robust had testing been halted after only 12 sessions. Finally, the lever contingencies in the

first block were not predictable in the previous study, introducing another distinct kind of uncertainty into their task.

Greater implications of neurogenesis ablation

In the present study, rats without new neurons earn significantly more pellets than WT controls. While it may be surprising that the elimination of a distinct neural population could lead to performance gains, this finding is not without precedent (Schwarting & Busse, 2017). Rats with bilateral hippocampal lesions outperform controls when reproducing both simple and complex lever sequences to receive reward (Jackson & Strong, 1969). Rats with similar lesions are superior at learning (and resisting extinction of) avoidance responses (Isaacson et al., 1961). Further, when cats are trained on an alternating Go/No-Go task, those with surgically lesioned hippocampi learn faster to suppress their responses on non-rewarded trials (Brown et al., 1969). The authors interpret this finding as a partial repudiation of the "response perseveration hypothesis," namely, the idea that hippocampal lesions promote the maintenance of a previously learned behavior, even in the absence of reinforcement.

Similarly, I contend that ablation of hippocampal neurogenesis does not simply induce heightened perseverative response. Although one measure of perseveration, the win-stay ratio, was significantly elevated in TK rats, I did not observe a parallel difference in the total number of perseverative errors following reversals. Furthermore, TK rats successfully navigated the lever reversals, even outperforming WT controls on blocks in which the non-trained lever was correct, demonstrating that any increased perseveration in the TKs was not strong enough to interfere with overall task performance. This evidence suggests that TK rats are more likely than controls to follow existing rules, while still retaining their ability to learn new ones. Theoretically, this

tendency should render TK rats less susceptible to distraction or irrelevant cues – but perhaps more likely to miss or undervalue new stimuli.

Therefore, we might conclude that newly-born granule cells mediate the expression of curiosity or the deviation from previously learned rules, in addition to their proposed role in the processing of ambiguity. This theory would be consistent with prior research showing that TK animals pay less attention to cues of uncertain relevance when already engaged in a trained behavior (Schoenfeld et al., 2021; Weeden et al., 2019). Furthermore, it coincides with the finding in this chapter that TK rats were more likely than controls to persist with a lever following a win or a misleading loss. In the next chapter, I examine whether the effects of neurogenesis loss on PRL performance are preserved even in the face of added unpredictability, and therefore, increased task difficulty.

3.5 Figures

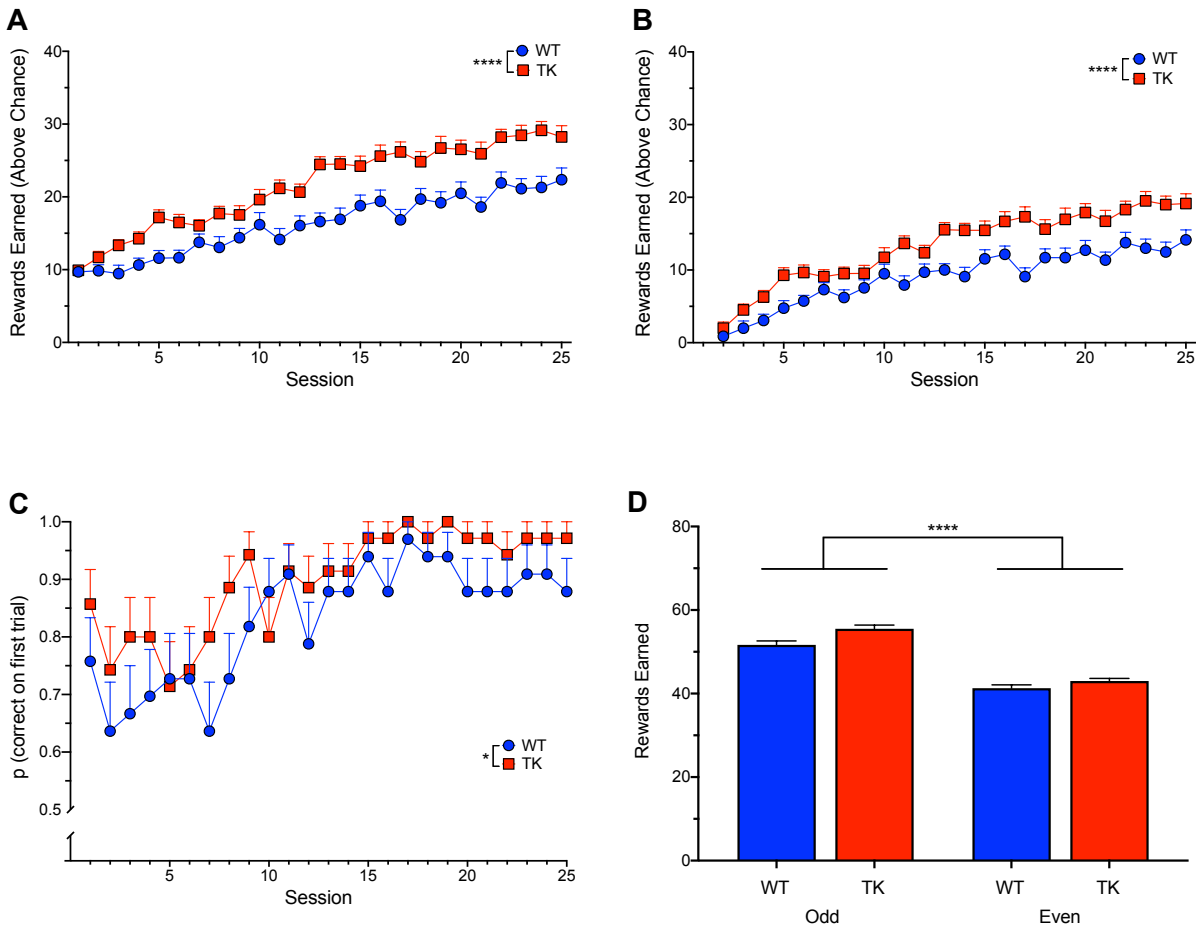

Figure 3.1. Rewards earned and first trial behavior. (A) All groups improved their performance with increasing sessions (effect of session: F (14.56, 960.9) = 48.12, p < 0.0001). However, TK rats earned significantly more rewards than WT controls, with that effect increasing over time (effect of genotype: F (1,66) = 21.84, p < 0.0001; session-by-genotype interaction: F (24, 1584) = 2.384, p = 0.0002). (B) TK rats also outperformed WTs even when only analyzing the final four blocks of each session, with the effect again becoming more pronounced over time -- suggesting that the genotype effect is not driven solely by superior performance during the first block (effect of genotype: F (1, 66) = 18.07, p < 0.0001; session-by-genotype interaction: F (23, 1518) = 1.796, p = 0.0117). (C) TK rats were significantly more likely than WTs to make a correct choice on the first trial (F (1, 66) = 6.424, p < 0.0136), and (D) outperformed WTs both on odd-numbered blocks (when the trained lever was correct) and on even-number blocks (when the non-trained lever was correct) on the final five days of testing (effect of genotype: F (1, 66) = 15.73, p = 0.0002). Furthermore, both WT and TK rats earned more rewards during odd blocks than during even blocks (F (1, 66) = 122.7, p < 0.0001). The data sets and statistical tests represented in this figure have grouped male and female rats together.

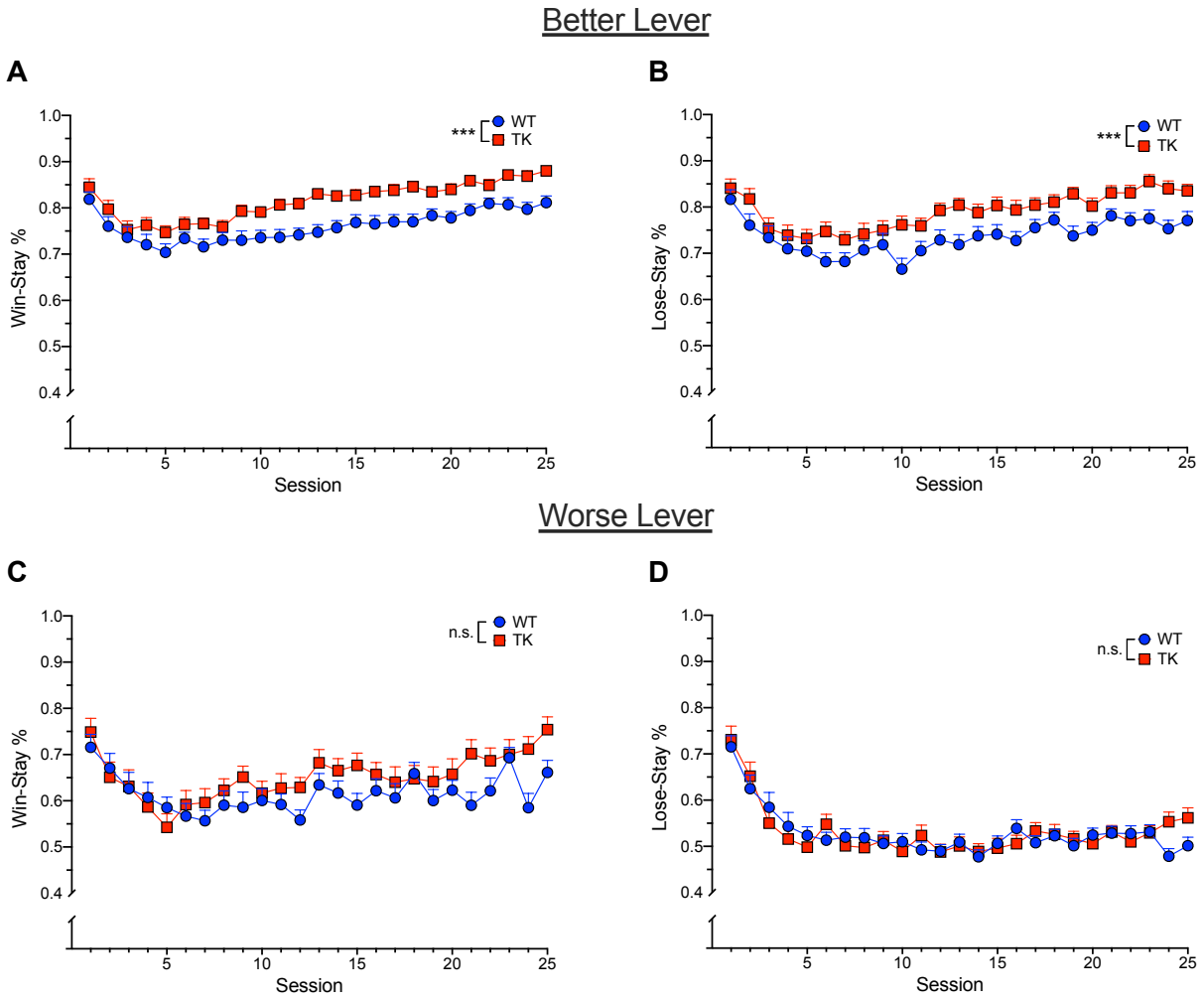

Figure 3.2. Reward strategy in response to accurate and misleading feedback varies with genotype. On the better lever, TK rats were significantly more likely to "stay" both following (A) accurate positive (effect of genotype: F (1,66) = 14.01, p = 0.0004) and (B) misleading negative feedback (effect of genotype: F (1,66) = 12.34, p = 0.0008). (C, D) A similar effect was not seen when analyzing behavior following a press on the worse lever, although TK rats trended toward increased win-stay behavior (effect of genotype: F (1,66) = 3.937, p = 0.0514). The data sets and statistical tests represented in this figure have grouped male and female rats together.

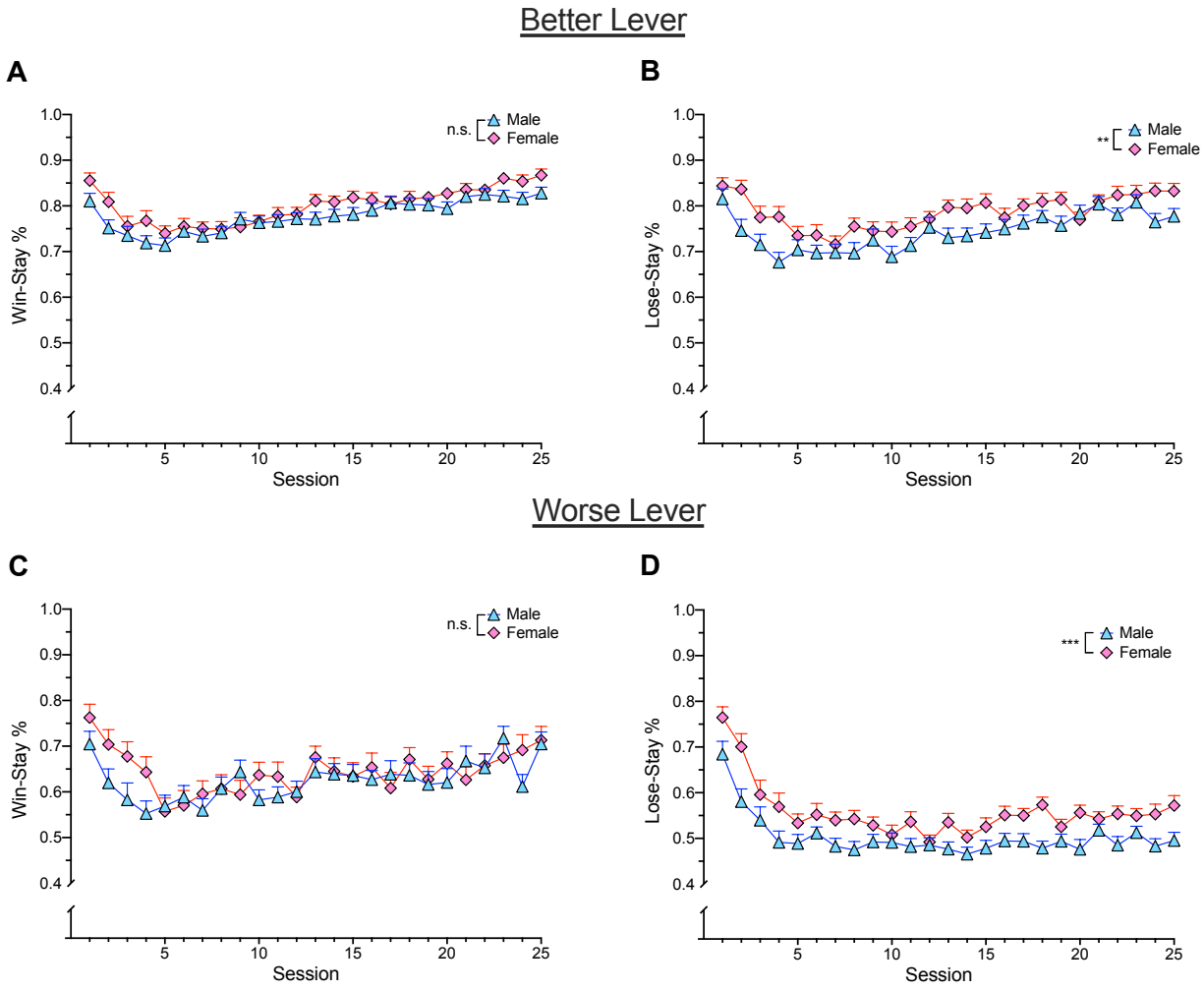

Figure 3.3. Reward strategy in response to accurate and misleading feedback varies with sex. Overall win-stay behavior in males and females did not differ significantly, either in response to (A) accurate positive feedback on the better lever, or (C) misleading positive feedback on the worse lever. However, female rats were significantly more likely than males to persist following a loss, both after (B) misleading negative feedback on the better lever (effect of sex: F (1, 66) = 7.072, p = 0.0098) and (D) accurate negative feedback on the worse lever (effect of sex: F (1, 66) = 15.97, p = 0.0002). The data sets and statistical tests represented in this figure have grouped WT and TK rats together.

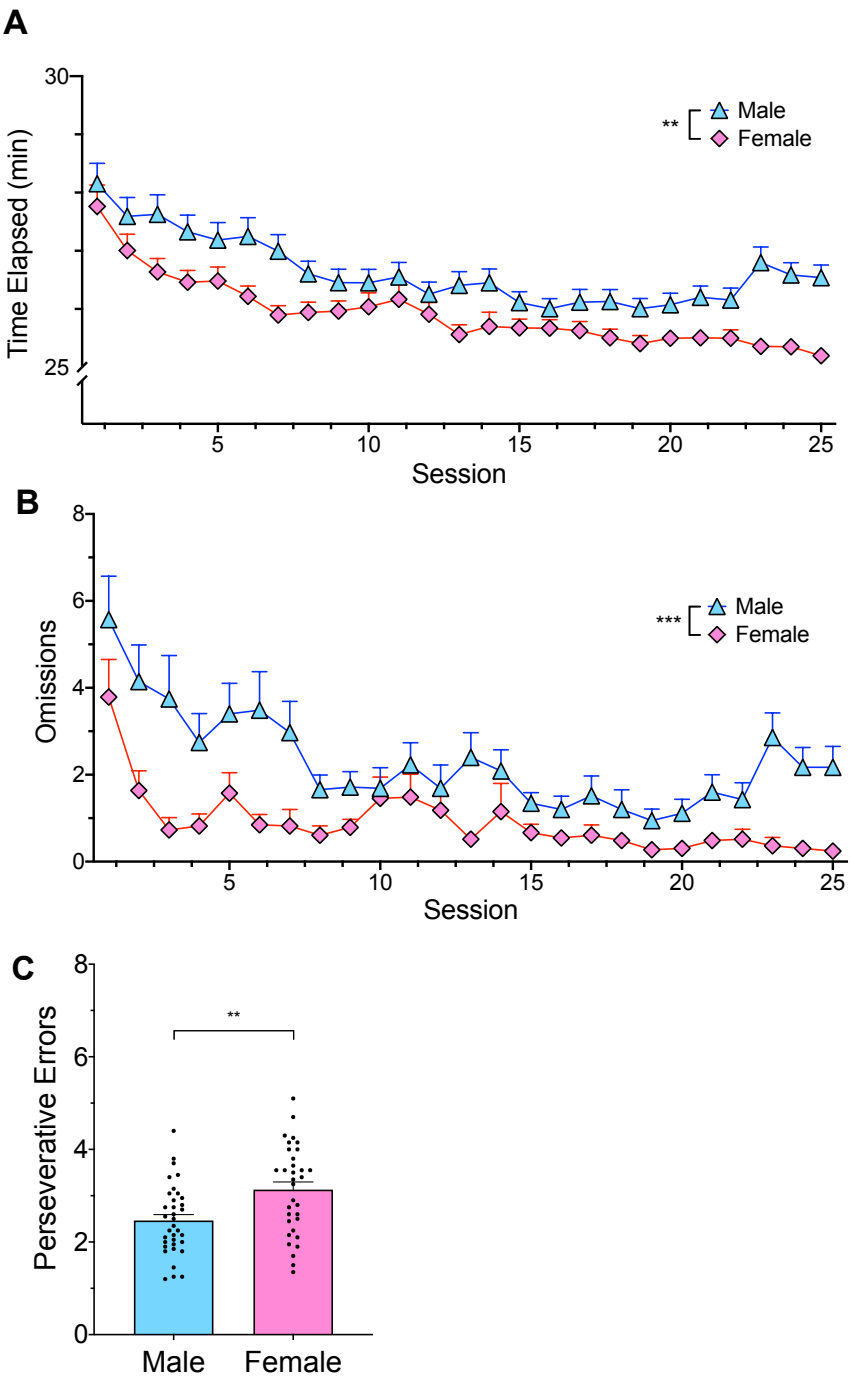

Figure 3.4. Further sex differences in PRL behavior. (A) With increasing sessions, rats became faster at completing all 200 trials. Additionally, female rats finished their sessions significantly faster than males, especially on later sessions (effect of session: F (7.615, 502.6) = 32.94, $p < 0.0001$; effect of sex: F (1, 66) = 8.252, $p = 0.0055$; session-by-sex interaction: F (24, 1584) = 2.556, $p < 0.0001$). (B) Females also committed significantly fewer omissions, especially on later sessions (effect of sex: F (1, 66) = 12.76, $p = 0.0007$; session-by-sex interaction: F (24, 1584) = 1.857, $p = 0.0071$). (C) On the final five days of testing, when rats were highly familiar with the task, females committed significantly more perseverative errors than males ($t_{3.167} = 60.66$, $p = 0.0024$). The data sets and statistical tests represented in this figure have grouped WT and TK rats together.

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Chapter 4:
Probabilistic Reversal Learning with Randomized First Blocks

4.1 Introduction

The previous chapter discussed the results from a PRL task in which the correct lever in the first block was fixed across sessions. Over time, rats learned which lever to press at the beginning of each session, and by the end of the study, over 90 percent of sessions began with a correct lever press. This type of predictability is often absent from PRL task design in the literature, where it is common either to randomize the identity of the initial better option (Rychlik et al., 2017; Seib et al., 2020) or to use entirely novel visual cues each session (Cools et al., 2002; Taswell et al., 2020). The present study investigates whether the trends observed in the previous chapter persist even when the correct lever in the first block is randomized, and therefore no longer predictable.

In humans, early judgments of task difficulty or self-aptitude can shape performance for an entire session (Aljamal et al., 2019; García et al., 2019; Peifer et al., 2020). It is possible that randomization of the first blocks could influence the rats' perception of overall task difficulty – or of their own competence, as they would now be more likely to fail during the first several trials. In general, we might expect performance to decline in response to randomization, which creates a more challenging task and introduces additional uncertainty. However, if new neurons are particularly sensitive to uncertainty or ambiguity, we might anticipate that the genotype effects would be exaggerated in the presence of added unpredictability. In the present chapter, I explore rat performance and decision-making strategy on a randomized version of the PRL task, with specific emphasis on the ways in which neurogenesis ablation and sex differentially influence reward-seeking behavior in response to additional uncertainty and increased task difficulty.

4.2 Abbreviated Methods

The methods employed in each study are highly similar, and a full description of the methods conserved across all experiments can be found in Chapter 2: Methods.

4.2.1 Animals

A total of 36 WT (19 male, 17 female) and 38 TK (17 male, 21 female) were used in this experiment. All rats were naïve and unhandled at the beginning of the study. Training began when rats were between 15 and 17 weeks of age and had been on drug treatment for at least seven weeks. One TK female died suddenly of unknown causes while on study, and her data was subsequently excluded from the analysis.

4.2.2 Reversal Task

The experimental protocol followed the standard outlined in Chapter 2: Methods, except that the "best lever" during the first reversal block was randomized across sessions. In the previous chapter, the "better" lever at the start of each session was the same as the lever used during each rat's probability training. In the present study, the "better" lever in the first block was randomly assigned each session.

If a rat omitted 25 or more trials in a single session, the data from that subject's session was excluded and replaced with the data from the subsequent session. However, if the same rat omitted 25 or more trials on five or more separate sessions, that rat (and all of its sessions) were removed from the analysis. In total, two rats (one WT female, one TK female) were eliminated under this criterion. I also excluded any rats that failed to press their non-trained lever at least 20 times in a session by the tenth reversal day. Four rats (two TK males, two TK females) were

excluded by this criterion. Out of the remaining 2023 rat-sessions, only 15 were eliminated for excessive omissions. (Another 15 rat-sessions were removed following box malfunction or researcher error.)

All rats were run on the reversal protocols for at least 28 sessions. To account for removed sessions, only the first 25 successful sessions for each rat are included in the data analysis for this chapter. Following the exclusions of the rats specified, data was collected from 35 WT (19 male, 16 female) and 32 TK (15 male, 17 female) rats.

4.3 Results

Females with intact neurogenesis earned fewer rewards than all other groups.

In the last chapter, TK rats earned significantly more rewards than WT controls when the "better" lever in the first reversal block was fixed across sessions. The present experiment tested a naïve cohort of rats on a new version of the PRL task in which the better lever in the first block was randomized. To assess task performance, I counted the total number of rewards earned by each rat in a single session. I used a three-way ANOVA (with sex and genotype as between-subjects factors, and session as a within-subject factor) to evaluate any between-group differences. Interestingly, WT females earned significantly fewer rewards than all other groups (*Fig. 4.1 A, 4.1 B*). The ANOVA revealed a significant sex-by-genotype interaction (F (1, 63) = 4.153, p = 0.0458) with a post-hoc Sidak test confirming the effect (WT males vs. WT females: $t_{63} = 3.973$, p = 0.0011, TK males vs. WT females: $t_{63} = 5.008$, p < 0.0001, TK females vs. WT females: $t_{63} = 4.167$, p = 0.0006). No other post-hoc sex-by-genotype comparisons were significant. The ANOVA also identified significant session-by-genotype (F (24, 1512) = 2.583, p

< 0.0001) and session-by-sex (F (24, 1512) = 3.007, p < 0.0001) interactions, possibly driven by the first several sessions, when sex and genotype effects had not yet stabilized.

Previous research has indicated that rats with ablated neurogenesis are less adept at making an initial discrimination between the better and worse levers at the beginning of a PRL session (Seib et al., 2020). To determine whether the underperformance of WT females could be driven solely by deficits in initial discrimination ability, I analyzed the number of rewards earned in the first block across all sessions (*Fig. 4.1 C*). The sex-by-genotype interaction was weaker than in the whole session analysis and no longer significant, suggesting that decisions in the first block likely did not alone drive the overall underperformance of WT females (F (24, 1512 = 4.252, p = 0.0631). Three-way ANOVA also revealed significant session-by-genotype (F (24, 1512) = 1.828, p = 0.0086) and session-by-sex (F (24, 1512) = 4.252, p < 0.0001) interactions. Post-hoc analysis identified no significant within-session genotype differences. However, a post-hoc Sidak test revealed that females, regardless of genotype, earned significantly fewer rewards than males in the first block of the second session and significantly more in the first block of the third session. These contradictory effect valences are not surprising given that many rats adopt a predominately "single-lever" strategy during the first few sessions before learning to adapt to the reversals. The randomization process did not guarantee that all groups were assigned the same first correct lever in equal proportions for each session – therefore, especially on early days, group performance is heavily influenced by the number of rats in each group whose preferred levers were "better" during the first block.

Performance and lever bias changed from early to late sessions.

In the previous chapter, TK rats outperformed WTs both on odd blocks (when their trained lever was "better") and even blocks (when their trained lever was "worse") during the last five testing sessions when rats had fully acclimated to the task. I chose to exclude the first block of each session from the analysis to control for any confounds arising from the initial discrimination. As a result of the initial lever randomization, odd-numbered blocks in this version of the task do not always reward the trained lever. Instead, I separated "preferred" blocks in which the trained lever was better, from "non-preferred" blocks in which the non-trained lever was better. Using this categorization, the previous findings was replicated: in the final five sessions, TK rats earned more rewards than WTs regardless of block type (*Fig. 4.1 E*: effect of genotype: F (1, 63) = 19.14, $p < 0.0001$). Although it failed to reach significance, a block-by-genotype interaction hinted that TK rats might outperform WT controls only on blocks in which their trained – but not their untrained – lever was better (block-by-genotype interaction: F (1, 63) = 3.893, $p = 0.0529$). In stark contrast to the findings in the previous chapter, rats in general performed no better on "preferred" blocks than on "non-preferred" blocks, suggesting that the randomization might weaken overall lever bias.

I next performed a similar analysis looking only at the first five sessions, when a robust preference for the trained lever might still be expected. Predictably, all groups earned significantly more rewards on "preferred" blocks in which their trained lever was more rewarding (*Fig. 4.1 D*: effect of block type: F (1, 63) = 182.1, $p < 0.0001$). I also found that TK rats, regardless of sex and block type, earned more rewards than WT controls (effect of genotype: F (1, 63) = 7.077, $p = 0.0099$). I did observe a significant effect of sex (F (1, 63) = 19.96, $p < 0.0001$) with a significant block-by-sex interaction (F (1, 63) = 9.592, $p = 0.0029$).

When comparing males to females within the same "block type," a post-hoc Sidak test revealed that males only outperformed females on blocks in which their non-preferred lever was better. On blocks in which their trained lever was better, there was no sex-based difference in performance.

When first blocks were randomized, the effect of neurogenesis loss on feedback sensitivity was blunted.

To investigate the causes of the underperformance among WT females, I next analyzed decision-making strategy through the calculation of win-stay and lose-stay ratios. In the previous chapter, TK rats exhibited significantly elevated win-stay behavior compared to WT controls – a tendency driven by an increased likelihood of persisting with a lever following accurate, but not misleading, positive feedback. However, in the present experiment, in which first blocks were randomized, the effect of genotype on win-stay behavior was no longer significant, though TK rats trended towards higher win-stay ratios (F (1, 63) = 1.605, p = 0.0685). In response to accurate, positive feedback, TK rats also trended towards elevated win-stay behavior compared to WTs (*Fig. 4.2 A:* F (1, 63) = 3.908, p = 0.0525), suggesting that the effect from the previous chapter may still be present, albeit attenuated. When analyzing only misleading wins, there was no effect of genotype (*Fig 4.2 C).* There was no genotype effect in lose-stay behavior in response to either accurate or misleading negative feedback (*Fig. 4.2 D:* accurate feedback: F (1, 63) = 0.6288, p = 0.4308; *Fig. 4.2 B:* misleading feedback: F (1, 63) = 1.815, p = 0.1827).

Male and female rats employed different decision-making strategies, particularly following losses.

The previous chapter showed that, compared to males, female rats exhibited an increased tendency to persist with a lever following a loss. In the current experiment, that effect was replicated: female rats had significantly higher lose-stay ratios than their male counterparts (F (1, 63) = 17.90, $p < 0.0001$). Next, I separated losses on the better lever (misleading feedback) and the worse lever (accurate feedback), running a three-way ANOVA with session and feedback veracity as within-subject factors and sex as a between-subjects factor. I again found a significant main effect of sex (F (1, 130) = 24.54, $p < 0.0001$) in addition to a near-significant block-by-sex interaction (F (1, 130) = 3.404, $p = 0.0673$), suggesting that this effect was stronger following accurate negative feedback (*Fig. 4.3 B, D*). However, all rats – regardless of sex – were less likely to switch levers when losses were misleading than when the losses represented accurate negative feedback (effect of feedback veracity: F (1, 130) = 24.54, $p < 0.0001$). Together with the findings from the previous chapter, these results suggest a generalized diminished sensitivity to negative feedback among female rats.

There was no simple effect of sex on overall win-stay behavior. Following accurate positive feedback (wins on the "better" lever), female rats were overall no more likely than males to persist with the lever from the previous trial, although this was true during early sessions (*Fig. 4.3 A*: session-by-sex interaction: F (24, 1512) = 3.304, $p < 0.0001$). After misleading positive feedback, however, females were significantly more likely to stay with the lever that produced a loss (*Fig. 4.3 C:* F (1, 63) = 4.732, $p = 0.0344$). Therefore, female rats in this experiment generally appear more perseverative than males on this task, being more likely to persist with a lever following both losses and misleading wins.

Female rats were slower to complete sessions and omitted more trials, but committed a similar number of perseverative errors when compared to males.

I next analyzed the total amount of time taken for rats to complete a single PRL session with 200 trials – generally between 25 and 30 minutes. In the previous chapter, I found that female rats completed their sessions significantly faster than their male counterparts. However, in the present cohort, I observed the opposite effect: that females took significantly longer to complete a single session, especially during early sessions (*Fig. 4.4 A*; effect of sex: F (1, 63) = 24.88, $p < 0.0001$; session-by-sex interaction: F (24, 1512) = 6.274, $p < 0.0001$). There was no significant main effect of genotype, although WT rats were slower than TKs during early sessions – an effect largely driven by the slowness of WT females (session-by-genotype interaction: F (24, 1512) = 1.671, $p = 0.220$).

Overall, females also committed more omissions than males – another reversal of the findings in the previous chapter (F (1, 63) = 19.94, $p < 0.0001$). This effect was more pronounced during early sessions (session-by-sex interaction: F (24, 1512) = 4.583, $p < 0.0001$). However, post-hoc testing on a significant sex-by-genotype interaction (F (24, 1512) = 6.318, $p = 0.0415$) revealed that WT females committed significantly more omissions than all other groups (WT males vs. WT females: $t_{63} = 5.044$, $p < 0.0001$, TK males vs. WT females: $t_{63} = 4.160$, $p < 0.0006$, TK females vs. WT females: $t_{63} = 2.919$, $p = 0.0289$). Omissions occur when a rat fails to make a response after the levers have been extended for ten seconds. During the first ten days, females committed an average of 3.2 more omissions per session than males, while requiring about 101 more seconds to complete their sessions. Therefore, heightened omissions among females – and WT females specifically – cannot fully account for the fact that females were slower to complete the task than males.

Despite the observation that females were more likely to persist with the previous lever following both losses and misleading wins, both sexes committed a similar number of perseverative errors following reversals. This fact stands in contrast with the finding from the previous chapter that females committed more perseverative errors, on average, than males.

3.4 Discussion

A PRL task by definition features an inherent level of uncertainty, derived from ambiguous feedback and (relatively) unpredictable reversal timing. One might expect that the effect of randomizing the first blocks – the addition of one extra layer of unpredictability – would be relatively minimal on a task with such high baseline uncertainty. However, I identified numerous differences between rat behavior on the randomized version of the task, compared to the original version featuring "fixed" first blocks. When first blocks were randomized, the behavioral effect of neurogenesis loss was no longer apparent in males, suggesting that (at least in males) new neurons may be sensitive to the added uncertainty or perhaps, the increased difficulty, of the task. Furthermore, I observed several other sex differences in the randomized task, some of which were not evident in the previous version. The effect of sex on probabilistic decision-making seems, if anything, exacerbated by the additional uncertainty.

Using total earned rewards as a proxy for overall task performance, I found that WT females performed more poorly than all other groups. (In the previous chapter, there was only a significant effect of genotype: TK rats of both sexes outperformed WT controls.) Sex effects in reversal learning have traditionally been mixed: in the fish genus *Poecilia*, for example, males have been found alternately to outperform (Fuss & Witte, 2019) and fall behind (Petrazzini et al., 2017) female conspecifics. Findings are also contradictory in mammalian literature. In mice,

females performing a PRL task learn probability associations faster than males, though males ultimately attain the same level of performance (Chen, Ebitz, et al., 2021). However, female marmosets appear impaired on reversals with more complex pairings, requiring more trials to reach learning criteria than males (LaClair et al., 2019).

In this experiment, the underperformance of WT females seems to originate from the confluence of two weak decision-making strategies. Females of both genotypes were more likely than males to persist with the worse lever following a loss – generally, a poor strategy. Similarly, I observed a near-significant trend of TK rats being more likely to persist with the better lever following a win. Thus, WT females appear to use the worst combination of the two strategies, being both more likely to persist with a lever following accurate negative feedback, but less likely to persist following accurate positive feedback. It is worth noting, however, that the male and female rats in this experiment came from different litters. Another explanation for the underperformance of WT females, although less intriguing, is that the effects were cohort-driven. Given that the male rats were sourced from three unique cohorts, and the female rats from another three cohorts, this explanation seems less likely.

Randomization of first blocks appears to reduce lever "preference"

Rats received two days of probability training prior to the start of testing. During this training, the same lever was "better" each day, consistently yielding a reward 80 percent of the time. As a result, rats developed a certain degree of side bias, entering testing with a strong preference for their trained lever, a tendency which waned as the study progressed. Unsurprisingly, when I analyzed the first five testing sessions, I found that rats earned significantly more rewards during blocks in which their trained lever was better. On the final five

days of testing, however, the effect was no longer significant: rats earned similar numbers of rewards when their "preferred" lever was better compared to when their "non-preferred" lever was better. (It is worth noting that a genotype-by-block type interaction approached significance, with TK rats appearing to outperform WTs only when their trained lever was better, or "correct.") Still, the attenuation of the effect is interesting given that it remained robust on final testing days in the previous iteration of the task, when the first correct lever was fixed across sessions. It is possible that starting each session with a randomly chosen "better" lever weakened the rats' lever bias. Perhaps a more likely explanation is that the rats' preferences shifted to match the overall lever probabilities. In the randomized experiment, both levers are equally likely to be correct when all blocks are viewed in aggregate: half of the blocks throughout the experiment rewarded a rat's trained lever, and the other half, its untrained lever. In the previous version of the experiment, however, a rat's trained lever was correct 60 percent of the time: every session featured three blocks rewarding the trained lever, and only two that rewarded the untrained one.

Effects of neurogenesis loss and sex on strategy, speed, and omissions

In the previous version of the task, rats with ablated neurogenesis (TKs) exhibited significantly higher win-stay ratios than WT controls – an effect that missed significance in the current study. In contrast, I did replicate the strategic sex effect from the previous chapter, finding that females had overall higher lose-stay ratios than males. Interestingly, when behavioral responses were separated based on feedback veracity, I found that female rats were generally more likely than males to persist with the "worse" lever following both wins and losses. Although this tendency represents poor strategy, it is likely that it is more indicative of a

general inclination among females to persist with their current lever, rather than a particular bias towards incorrect levers specifically.

Additionally, female rats were slower to complete their sessions than their male counterparts – the opposite of the sex effect observed in the previous chapter. Further, females committed more errors of omission than males, although their heighted omission rate cannot fully account for their slower session times. Many previous reversal learning studies have found faster reaction times among females (Chen, Knep, et al., 2021; LaClair & Lacreuse, 2016), but not on more complicated tasks (Bissonette et al., 2012; Chen, Ebitz, et al., 2021). The introduction of additional uncertainty at the beginning of each session renders the task markedly more difficult than the previous version, and it is possible that this additional level of difficulty differentially affected female reaction times compared to those of their male counterparts.

Reversal difficulty and overall task perception

In sum, the results from this experiment indicate that randomization of the first blocks does indeed affect rat decision-making. Many of the genotype effects seen in the previous, "easier" version of the task were either muted or absent. Furthermore, new sex differences emerged that were not apparent when the first blocks were "fixed." I hypothesize that increasing task difficulty obscures the behavioral effects of neurogenesis loss, which might surface only when tasks are not too challenging. Similarly, increasing task difficulty might exacerbate sex differences, amplifying effects not observed when tasks are easy. To that end, I designed the next experiment to verify this theory, employing a deterministic version of the task that is significantly easier than the two versions already described.

4.5 Figures

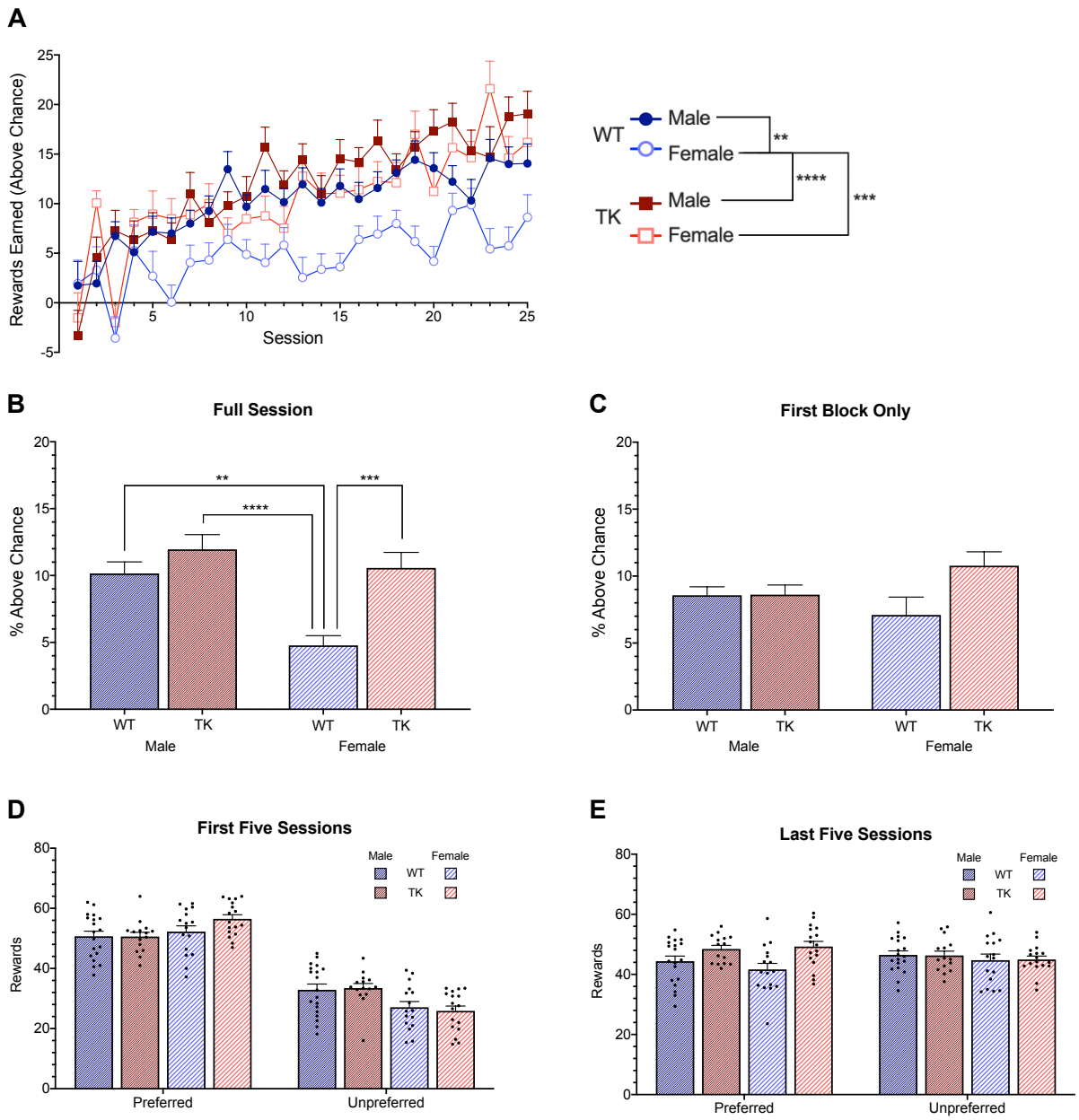

Figure 4.1. Reward performance across sex and genotype. (A) WT females earned significantly fewer rewards above chance (100 rewards) than all other groups, as revealed by three-way ANOVA and confirmed with a post-hoc Sidak test (sex-by-genotype effect: F (1, 63) = 4.153, p = 0.0458). (B) When data from all blocks was analyzed together, WT females significantly underperformed all other groups. (C) However, when only data from the first block was analyzed, no significant differences emerged. (D) During the first five days of testing, rats earned more rewards during blocks in which their preferred lever was "better" than during blocks in which their unpreferred lever was "better" (F (1, 63) = 182.1, p < 0.0001). Further, TKs generally outperformed WTs (F (1, 63) = 7.077, p = 0.0099), and males outperformed females on "unpreferred" blocks only (sex-by-preference interaction: F (1, 63) = 9.592, p = 0.0029), as confirmed by a post-hoc Sidak test. (E) During the final five day of testing, block type no longer predicted performance. However, TK rats still outperformed WTs (F (1, 63) = 19.14, p < 0.0001).

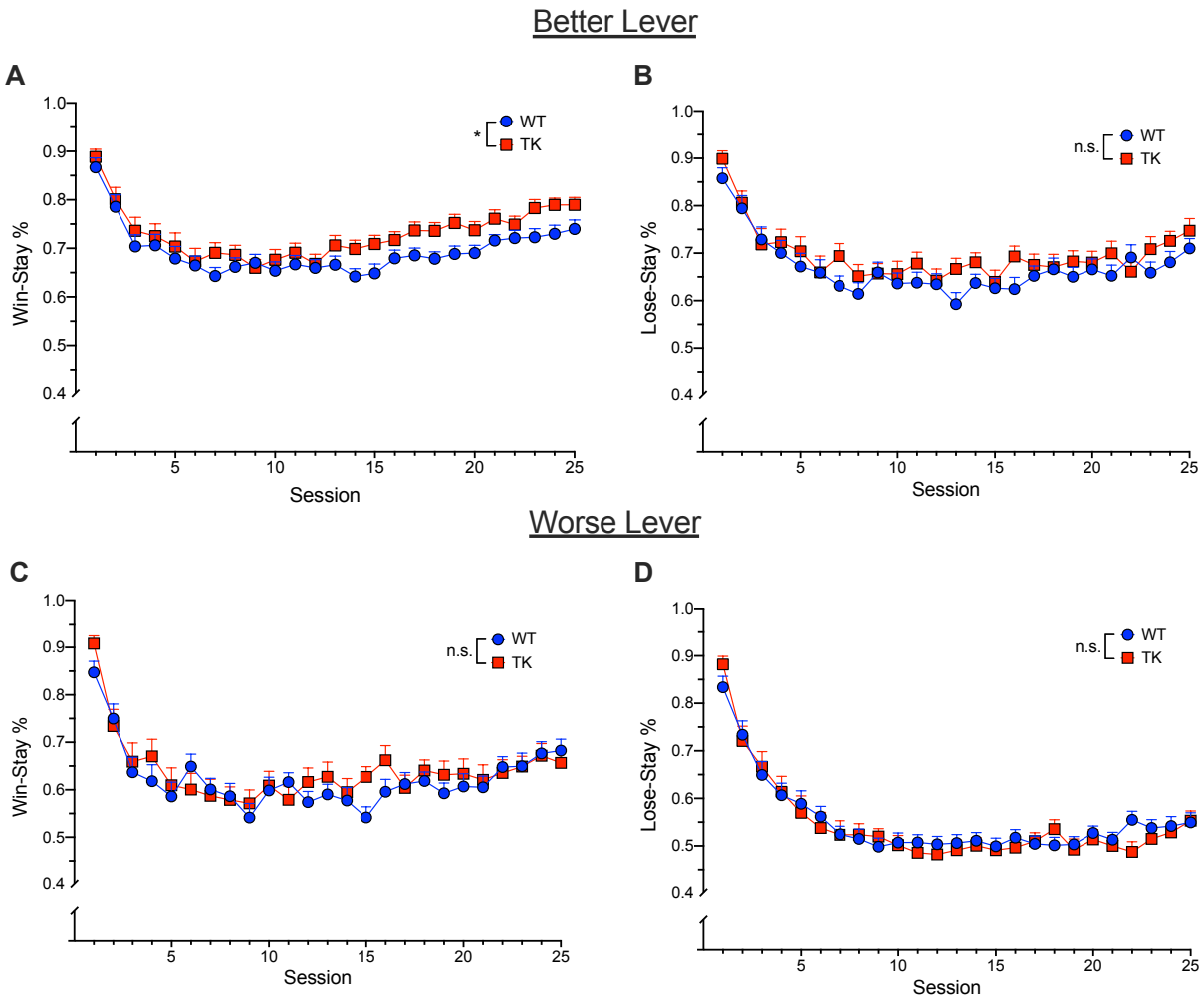

Figure 4.2. Reward strategy in response to accurate and misleading feedback varies with genotype. (A) TK rats were significantly more likely than WTs to persist with the better lever following a win (F (1, 65) = 4.159, p = 0.0455), but (B) not following a loss (misleading negative feedback). Following a press on the "worse" lever, WTs and TKs were (C) equally likely to persist following a win or (D) following a loss (accurate negative feedback). All of the datasets and statistical tests represented in this figure have grouped male and female rats together. Significance values in the figure represent the results of two-way ANOVA with genotype and session as factors and therefore may differ slightly from those in the text, which reflect three-way ANOVA results with sex, genotype, and session as factors.

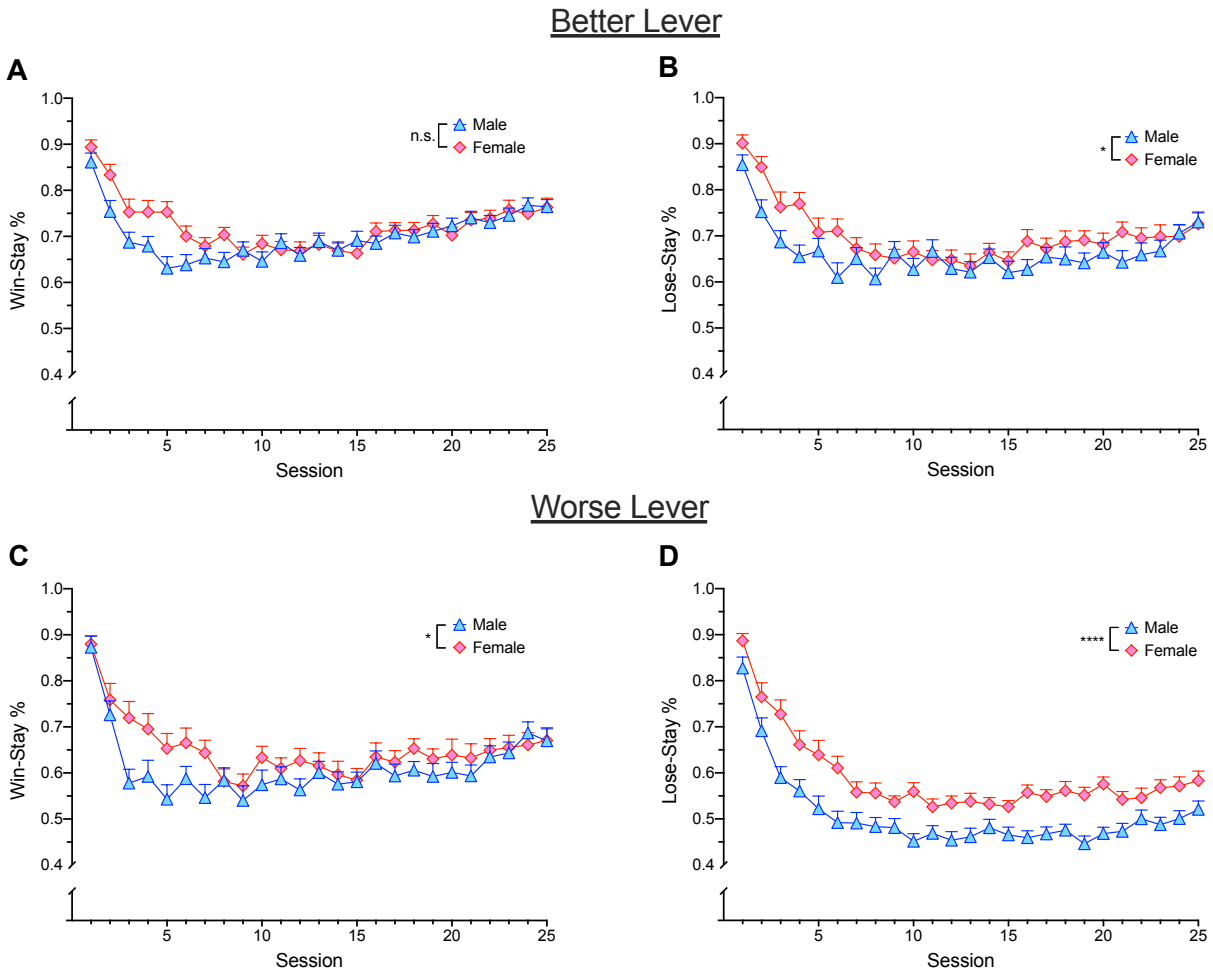

Figure 4.3. Reward strategy in response to accurate and misleading feedback varies with sex. (A) When analyzing win-stay behavior following accurate positive feedback, three-way ANOVA revealed a significant session-by-sex interaction (F (24, 1560) = 3.149, $p < 0.0001$) but no simple effect of sex (F (1, 65) = 1.512, $p = 0.2223$). (C) However, after receiving misleading positive feedback, females had significantly elevated win-stay ratios compared to males (effect of sex: F (1, 65) = 4.812, $p = 0.0318$). Similarly, females were significantly more likely to "stay" following a loss, both after (B) misleading negative feedback on the better lever (effect of sex: F (1, 65) = 4.067, $p = 0.0479$) and (D) accurate negative feedback on the worse lever (effect of sex: F (1, 65) = 28.45, $p < 0.0001$). All of the datasets and statistical tests represented in this figure have grouped WT and TK rats together. Significance values in the figure represent the results of two-way ANOVA with sex and session as factors and therefore may differ slightly from those in the text, which reflect three-way ANOVA results with sex, genotype, and session as factors.

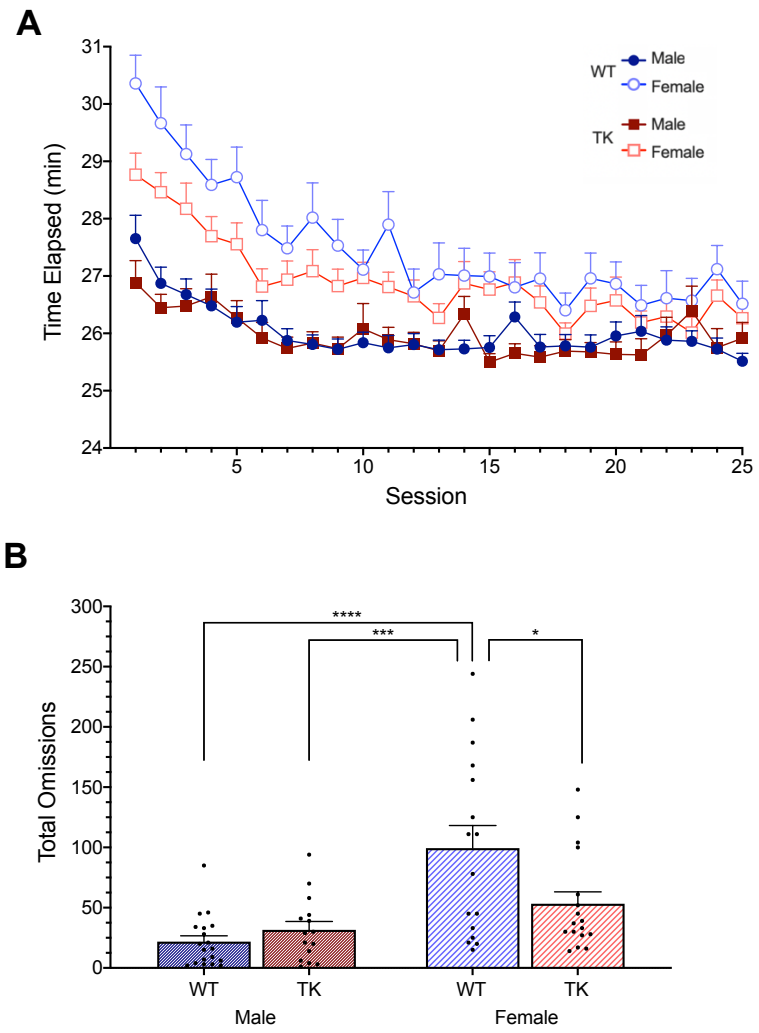

Figure 4.4. Further sex differences in PRL behavior. (A) Female rats were significantly slower to complete a full PRL session than their male counterparts, especially during early sessions (effect of sex: F (1, 63) = 24.88, $p < 0.0001$; effect of sex-by-session: F (24, 1512) = 6.274, $p < 0.0001$). (B) WT females committed more total omissions over the course of the experiment than any other group (effect of sex-by-genotype: F (24, 1512) = 6.318, $p = 0.0145$; WT males vs. WT females: $t_{63} = 5.044$, $p < 0.0001$, TK males vs. WT females: $t_{63} = 4.160$, $p < 0.0006$, TK females vs. WT females: $t_{63} = 2.919$, $p = 0.0289$).

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Chapter 5:
Deterministic Reversal Learning

5.1 Introduction

In the previous two chapters, I established that neurogenesis loss affects probabilistic reversal learning, but that the strength of that effect may depend upon the difficulty of the task or the level of situational uncertainty. The original experiment found that transgenic TK rats lacking neurogenesis outperform WT controls, earning more rewards per session and adopting superior strategies. The second experiment randomized which lever was correct during the first block of each session, making the task more difficult. In that study, the effects of genotype – and thus neurogenesis loss – were muted; however, I observed sex-dependent performance effects not apparent in the original experiment. These findings could be reconciled if the ways in which neurogenesis and sex affect behavior are linked to task difficulty, with some effects emerging when tasks are simple, and others when tasks are difficult or complex.

Therefore, I designed a new version of the two-armed bandit task in which lever reward contingencies were deterministic rather than probabilistic. Such a paradigm would test not only how rats perform on a noticeably easier task, but also whether the behavioral effects seen previously were in some way dependent upon probability or ambiguity: a phenomenon my lab has observed before. Mice with ablated neurogenesis respond differently to partially-predictive fear cues than intact controls, despite showing normal fear conditioning when cues are unambiguous (Glover et al., 2016). Similarly, TK mice and rats work less for rewards when the required effort level is unclear, but not when it is fully predictable (Karlsson et al., 2018). By employing a deterministic bandit task, I hoped to establish whether the effects of neurogenesis on reversal learning were, in fact, contingent upon probabilistic reward outcomes.

It is not unprecedented for the presence or absence of probability to alter observed behavioral effects. Unsurprisingly, animals respond more quickly to reversals when reward

probabilities are more deterministic than stochastic (Beron et al., 2022). Furthermore, administration of exogenous corticotropin-releasing factor (CRF) shifts rats' preference away from high-effort, high-reward options – but only when reward outcomes are deterministic (Bryce & Floresco, 2016). When rats must choose between risky (i.e., probabilistic) high-effort, high-reward choices and certain, low-effort, low-reward ones, the administration of CRF has no effect (Bryce et al., 2020). Additionally, when reward contingencies are probabilistic (80%/20%), female mice learn faster than males to select the more rewarding image out of a pair, but this effect is absent when contingencies are deterministic (Chen et al., 2021).

Neither is it unprecedented to study the effects of neurogenesis loss on deterministic reversal learning. Previously, my lab found that TK mice and rats showed normal reversal learning on a deterministic schedule, although the experimental protocol differed significantly from the ones discussed in this work and included only one reversal (Karlsson et al., 2018). Further, chemically-induced neurogenesis ablation was found to improve performance following reversals on a spatial avoidance task with deterministic outcomes (Brozka et al., 2017). However, to my knowledge, no prior studies have investigated the effects of neurogenesis loss on reversal learning in a deterministic bandit task.

In the present chapter, I detail the design and findings of the deterministic bandit task, comparing the results to those from previous iterations of tasks. Additionally, I discuss the greater implications of the study – and how task difficulty, probability, neurogenesis, and sex may differentially affect rodent decision-making and behavior.

5.2 Methods

Because the methods used in each study are highly similar, the full details of the methods conserved across all experiments can be found in Chapter 2: Methods.

5.2.1 Animals

A total of 35 WT (17 male, 18 female) and 31 TK (14 male, 17 female) rats were used in this experiment, all of which were naïve and unhandled at the beginning of the study. Training began when rats were between 15 and 17.5 weeks of age and had been on drug treatment for at least seven weeks. One rat, a TK female, became sick and was euthanized before study completion. Her data was subsequently excluded from the analysis.

5.2.2 Reversal Task

In general, the experimental protocol followed the outline presented in Chapter 2: Methods, except that the lever reward probabilities switched between 100% and 0% on reversal days rather than between 80% and 20%, creating a deterministic version of the task. Following basic lever training, rats received two days of "deterministic training" that replaced the "probability training" described in Chapter 2. During these training days, both levers were extended, but only one (pseudo-randomly assigned to each rat) delivered rewards, on an FR1 schedule. Pressing the other lever would not produce any rewards during deterministic training. Following these two days of training, rats progressed to reversal testing. For each rat, the lever that was rewarded during deterministic training was the same as the lever that was rewarded during the first block of testing on every reversal day. Thus, the identity of the initial correct lever was fixed across sessions.

If any rat committed 25 or more omissions in a single test session, the data for that rat-session was excluded from analysis and replaced with the data from the following session. The same procedure was followed if a session was compromised from researcher error or box malfunction. If five or more sessions from the same rat needed to be excluded, that rat was completely removed from the study. In sum, seven rats (two WT males, three WT females, and two TK females) were excluded under this criterion. In previous chapters, I excluded any rat that failed to press the non-trained lever at least 20 times in a single session by the tenth reversal day; however, no rats in the present study met that criterion. Out of the remaining 1685 rat-sessions, only fifteen were excluded as a result of high omissions (seven of which might have been related to a single instance of external noise). Two more sessions were excluded after equipment malfunction.

All rats were subjected to reversal protocols for at least 28 sessions. To accommodate removed sessions, only the first 25 successful sessions for each rat are included in the analysis for this chapter. After excluding the rats mentioned above, 30 WT (15 male, 15 female) and 28 TK (14 male, 14 female) rats provided data for this chapter.

5.3 Results

Rats with ablated neurogenesis earned more rewards than wild-type controls on a deterministic version of a two-armed bandit reversal learning task.

In Chapter 3, we saw that TK rats with ablated neurogenesis outperformed WT controls on a probabilistic reversal task in which the first blocks were predictable. To test whether the effect was dependent on the uncertainty that accompanies probabilistic reward outcomes, I repeated the experiment using a deterministic version of the task in which lever reward

probabilities oscillated between 100% and 0%. I found that the effect was preserved: TK rats earned significantly more rewards than WT controls (*Fig. 5.1 A*; effect of genotype: F (1, 54) = 18.38, $p < 0.0001$). (There was no main effect of sex or significant sex-by-genotype interaction in any of the rewards data for this chapter.)

I also noted a significant effect of session, with rats in all groups generally improving their performance as they became more experienced with protocol (F (13.13, 708.9) = 121.8, $p < 0.001$). Further, I identified a significant session-by-genotype interaction (F (24, 1296) = 1.805, $p = 0.0100$), with a post-hoc Sidak test revealing that TK rats significantly outperformed WTs in sessions ranging from the beginning to the end of the experiment, though the effect was diminished in the latest testing days. The overall main effect of session is unsurprising; it indicates that rats are improving over time, which means they are learning. Except where noted, the session effect is seen in every data measure, with rats adjusting their behavior and strategy as they become more familiar with task parameters.

When analyzing only performance in the first block, TK rats still outperformed controls (*Fig. 5.1 C*; effect of genotype: F (1, 54) = 13.63, $p = 0.0005$), although they were no more likely to press the correct lever on the very first trial (*Fig. 5.1 B*; F (1, 54) = 0.4741, $p = 0.4941$). I then analyzed the opposite measure: rewards earned per session, with the first block omitted. Again, TK rats earned more rewards than WTs, demonstrating that the overall effect was not driven solely by superior performance during the first block (*Fig. 5.1 D*; effect of genotype: F (1, 54) = 16.22, $p = 0.0002$). A post-hoc Sidak test on a significant session-by-genotype interaction revealed that TK rats significantly outperformed WTs in this measure on days at both early and late timepoints (session-by-genotype interaction: F (24, 1296) = 1.628, $p = 0.0285$).

Sensitivity to positive and negative feedback differed with neurogenesis ablation and sex.

On a bandit task in which reward outcomes are deterministic, the ideal strategy is a purely win-stay/lose-switch approach. In the absence of feedback ambiguity, reversals are (theoretically) immediately detectable and there are no obvious benefits of exploration. TK rats more closely emulated this approach: demonstrating both higher win-stay (*Fig. 5.2 A*; F (1, 54) = 13.15, p = 0.0006) and lower lose-stay (*Fig. 5.2 B*; F (1, 54) = 6.633, p = 0.0128) ratios than WT controls.

Interestingly, there was a significant main effect of sex in lose-stay behavior, in addition to the genotype effect mentioned above. Overall, females displayed elevated lose-stay ratios compared to males, a disadvantageous strategy when a loss unambiguously indicates an incorrect choice (*Fig. 5.2 D*; effect of sex: F (1, 54) = 16.26, p = 0.0002). Further, there was no significant effect of sex on win-stay behavior (*Fig. 5.2 C*).

Task performance and reward-seeking strategy differed according to "block type."

Next, I separately analyzed rewards data from Blocks 2 and 4 ("even" blocks) and Blocks 3 and 5 ("odd" blocks). The first block is inherently different than the other four because it precedes all reversals and is therefore the most predictable. For that reason and to remain consistent with other chapters, the first block was excluded from this analysis.

During deterministic pre-training, each rat was assigned a lever, which produced rewards on an FR1 schedule for two consecutive sessions. Then, on testing days, the "trained" lever was correct during odd blocks, producing a reward 100% of the time. Likewise, during even blocks, the "non-trained" lever was rewarded.

ODD BLOCKS:

When analyzing only behavior on odd blocks, WT females significantly underperformed all other groups (*Fig. 5.3 A*), as confirmed by a post-hoc Sidak test on a significant sex-by-genotype interaction (F (1, 54) = 4.824, p = 0.0324.)

To better understand the mediocre performance of WT females, I again turned to an analysis of their win-stay/lose-stay tendencies. WT rats, regardless of sex, displayed lower win-stay ratios on odd blocks than TKs, although this effect was most apparent during early testing days (*Fig. 5.3 C*; effect of genotype: F (1, 54) = 10.03, p = 0.0025; session-by-genotype interaction: F (24, 1296) = 1.604, p = 0.0327). On the other hand, female rats, regardless of genotype, were more likely than males to persist with a lever following a loss on odd blocks (*Fig. 5.3 E*; F (1, 54) = 11.36, p = 0.0014). On a deterministic task, persisting with a lever following a loss is a particularly disadvantageous strategy, for losses only occur after an incorrect choice. Interestingly, lose-stay behavior was not dependent on session – one of the only measures thus far not to change as rats became more familiar with the testing protocol.

EVEN BLOCKS:

When looking only at even blocks (in which the non-trained lever was rewarded), I noted a simple main effect of genotype: TKs earned significantly more rewards than WTs, regardless of sex (*Fig. 5.3 B*; F (1, 54) = 11.12, p = 0.0015). This seems largely attributable to the fact that, on even blocks, TKs are also more likely to persist with a lever following a win, regardless of session (*Fig. 5.3 D*; F (1, 54) = 6.318, p = 0.0150). On even blocks, females were again more likely to persist following a loss (*Fig. 5.3 F*; F (1, 54) = 10.22, p = 0.0023).

EFFECT OF BLOCK TYPE:

To determine conclusively whether "block type" affected behavior, I ran three-way ANOVAs with sex and genotype as between-subject factors, and "block type" as a within-subject factor. For two measures (rewards earned and win-stay ratio), there was a significant main effect of block type (rewards: F (1, 54) = 316.2, $p < 0.0001$; win-stay: F (1, 54) = 301.2, $p < 0.0001$), with rats generally earning more rewards and showing higher win-stay ratios during odd blocks. The effect of block type on lose-stay behavior just missed significance (F (1, 54) = 3.899, p = 0.0534). Further, when analyzing lose-stay behavior, there were significant main effects of both genotype and sex, without any secondary interactions. Regardless of block type, females had higher lose-stay ratios than males, being more likely to persist following a loss (F (1, 54) = 17.55, p = 0.0001). Additionally, WT rats in both block types were more likely to make lose-stay decisions than TKs (F (1, 54) = 7.832, p = 0.0071). I did not observe any significant sex-by-block type or genotype-by-block type interactions in any of the three measures, indicating that the identity of the correct lever did not appear to modulate behavior in a sex- or genotype-dependent fashion.

Female rats committed more perseverative errors than males, and more omissions than males during early sessions.

Feedback in this version of the bandit task is unambiguous: a win signals a correct choice, and a loss, an incorrect one. Theoretically, reversals are immediately decipherable. In the deterministic bandit, females committed more perseverative errors than males, making more consecutive wrong choices immediately following a reversal (*Fig. 5.4 A:* F (1, 54) = 6.892, p = 0.0112).

I observed no main effect of sex on omission rate or the total time taken to complete sessions, although female rats appeared to be slower and more omission-prone during early testing sessions (*Fig. 5.4 C:* time elapsed – session-by-sex interaction: F (24, 1296) = 4.731, $p < 0.0001$; *Fig. 5.4 B:* omissions – session-by-sex interaction: F (24, 1296) = 4.008, $p < 0.0001$). There was no significant main effect of genotype nor any sex-by-genotype interactions in either perseverative errors, omission rate, or elapsed time.

5.4 Discussion

The present chapter demonstrates that neurogenesis loss affects reversal learning even when reward contingencies are deterministic. I found that TK rats with ablated neurogenesis earned more rewards than WT controls on a deterministic two-armed bandit task, matching my previous findings noting the same performance effect when reward outcomes were probabilistic. Furthermore, the results are consistent with the hypothesis that the behavioral effects of neurogenesis loss would be preserved – or even exaggerated – on an easier version of the bandit task.

In fact, TK rats outperformed WT controls on the first blocks, before the onset of any reversal, despite the fact that rats of both genotypes were equally likely to choose the correct lever on the very first trial. This finding suggests that neurogenesis loss does not merely improve the rats' memories of how the sessions begin. Rather, it seems that rats with intact neurogenesis are equally skilled at identifying the correct lever, but more likely to stray from it – though whether from curiosity or genuine miscalculation remains to be determined. This theory is supported by the fact that WT and TK rats commit similar numbers of perseverative errors following reversals, suggesting that new neurons do not impair discrimination ability.

Furthermore, the idea that new neurons partially mediate exploration or curiosity would be consistent with research showing that mice with suppressed neurogenesis adopt more limited search patterns than intact controls (Garthe et al., 2009) and that mice with ablated neurogenesis struggle to learn that previously-learned shock zone has changed locations (Burghardt et al., 2012).

Underlying lever preference amid deterministic reward contingencies

As a result of the training structure, rats enter testing with a strong lever preference. The present version of the task is relatively easy, and feedback is unambiguous, so it is somewhat surprising that the lever preference is preserved even on later testing days. Rats earned more rewards on odd blocks (in which their trained lever is rewarded) than on even blocks (in which their non-trained lever was rewarded). Furthermore, rats were also more likely to persist with their trained lever after a win than when wins came on their non-trained lever – raising the possibility that the training permanently altered their confidence levels surrounding each lever.

Adding to the robustness of the observed performance effect, TK rats outperformed WTs on both odd and even blocks. TK rats were also more likely than WT controls to persist with their non-trained lever following a win, suggesting that neurogenesis loss can improve cognitive flexibility in certain contexts, and here, allow TK rats to better overcome their pre-established lever preference. This finding is interesting given the accumulation of opinion that neurogenesis ablation impairs cognitive flexibility (Anacker & Hen, 2017).

The role of sex in feedback sensitivity

Curiously, I have now noted more than one instance in which WT female rats underperform relative to other groups. In the previous chapter, WT females earned fewer overall rewards than all other sex/genotype groupings. Here, WT females underperformed other groups specifically on "odd" blocks, in which their trained lever was rewarded. This finding could simply be caused by the convergence of two unfortunate tendencies: that of female rats to be more likely to stay with a lever following a loss, and of WTs to be more likely to abandon a lever following a win.

Using similar tasks, others have found, compared to males, that female mice display a greater tendency to persist with their previous choice (Chen et al., 2021), that female rats are less sensitive to feedback in general (Bryce & Floresco, 2021), and that females are more likely to persist following a loss (Harris et al., 2021). I have replicated the last effect (greater tendency to "lose-stay") in all three versions of the two-armed bandit described thus far, indicating a robustness that withstands changes to task design and difficulty.

Task difficulty and reversal performance

In conjunction with findings from previous chapters, these results show that neurogenesis loss can actually improve reversal performance, regardless of whether reward contingencies are probabilistic. When first blocks were randomized, however, the effect of neurogenesis loss was muted (see: Chapter 4). The randomized version of the bandit task was arguably more challenging than the original, whereas the one described in this chapter was arguably simpler. Therefore, we might conclude that the effects of neurogenesis on reversal learning are exaggerated when tasks are "easy" and diminished when tasks are "hard." However, when

making comparisons between the studies, it is difficult to know which differences are attributable to "task difficulty," and which should be ascribed to inherent differences in the experimental protocols that might affect rat behavior or attitude. For example, rats in the present chapter received two days of deterministic training, potentially cementing their initial lever preference to a greater extent than rats in the prior two chapters that received probabilistic training.

In the next experiment, we aim to directly examine how task difficulty modulates the effects of neurogenesis on performance, this time using experimental protocols that are as similar as possible, to control for any extraneous factors that might influence behavior. If neurogenesis exerts a stronger effect when tasks are demonstrably simpler (and a weaker or absent effect when tasks are complex), my theory of the present findings would be confirmed. Furthermore, we could gain insight into other types of behaviors that might be modulated with altered neurogenesis levels, and ultimately, more insight into how exactly new neurons influence reversal learning and decision-making.

5.5 Figures

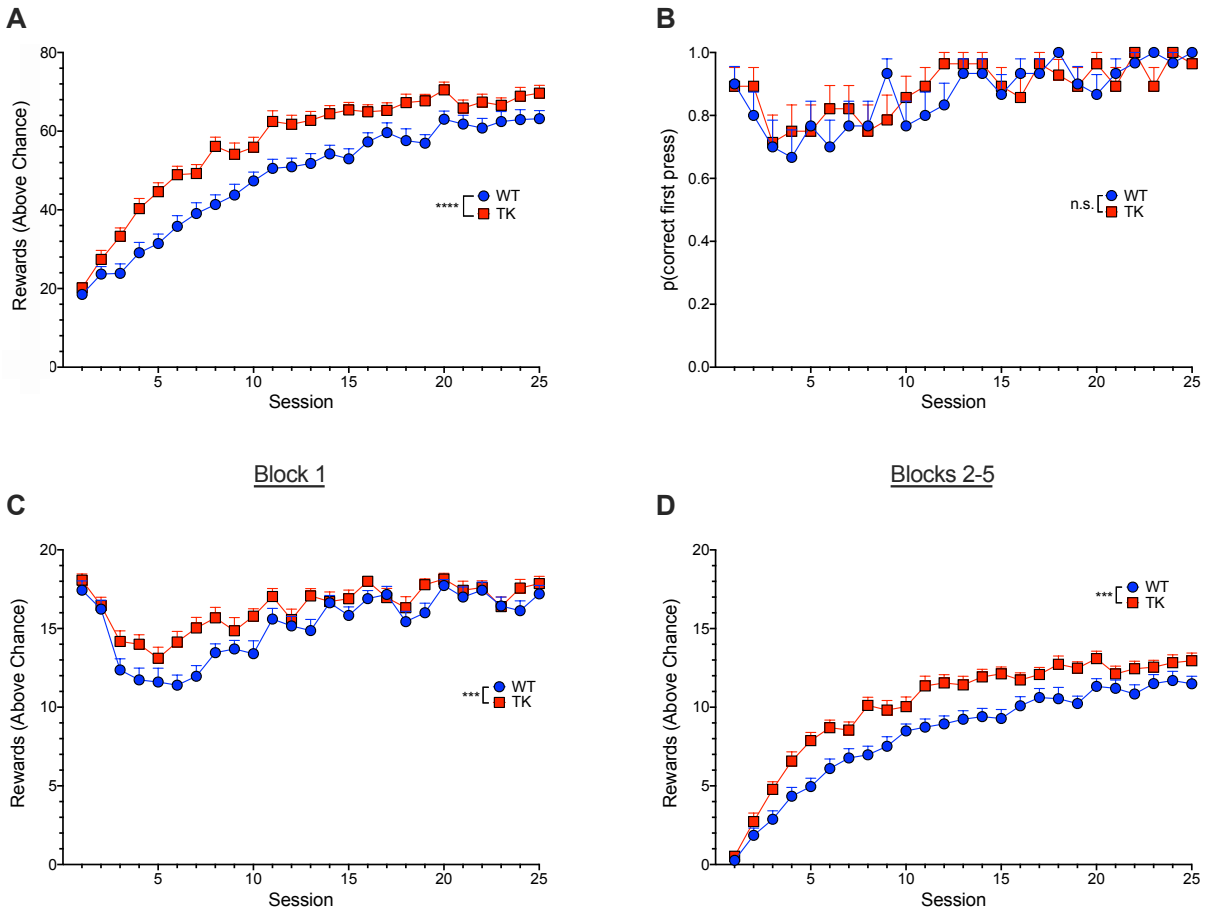

Figure 5.2. Reward acquisition on a deterministic two-armed bandit task. (A) Rewards earned above chance, per session. Male and female TK rats with ablated neurogenesis outperformed WT controls (effect of genotype: F (1, 56) = 17.68, $p < 0.0001$), despite (B) being equally likely to choose the correct lever on the very first trial. TK rats earned more rewards than WT controls both (C) during the first block, prior to any reversals (effect of genotype: F (1, 56) = 13.85, $p = 0.0005$) and (D) during the remainder of the session, while adapting to reversals (effect of genotype: F (1, 56) = 15.25, $p = 0.0003$). (Graph D shows the average number of rewards earned above chance per block.) All datasets and statistical analyses for this figure have grouped male and female rats together.

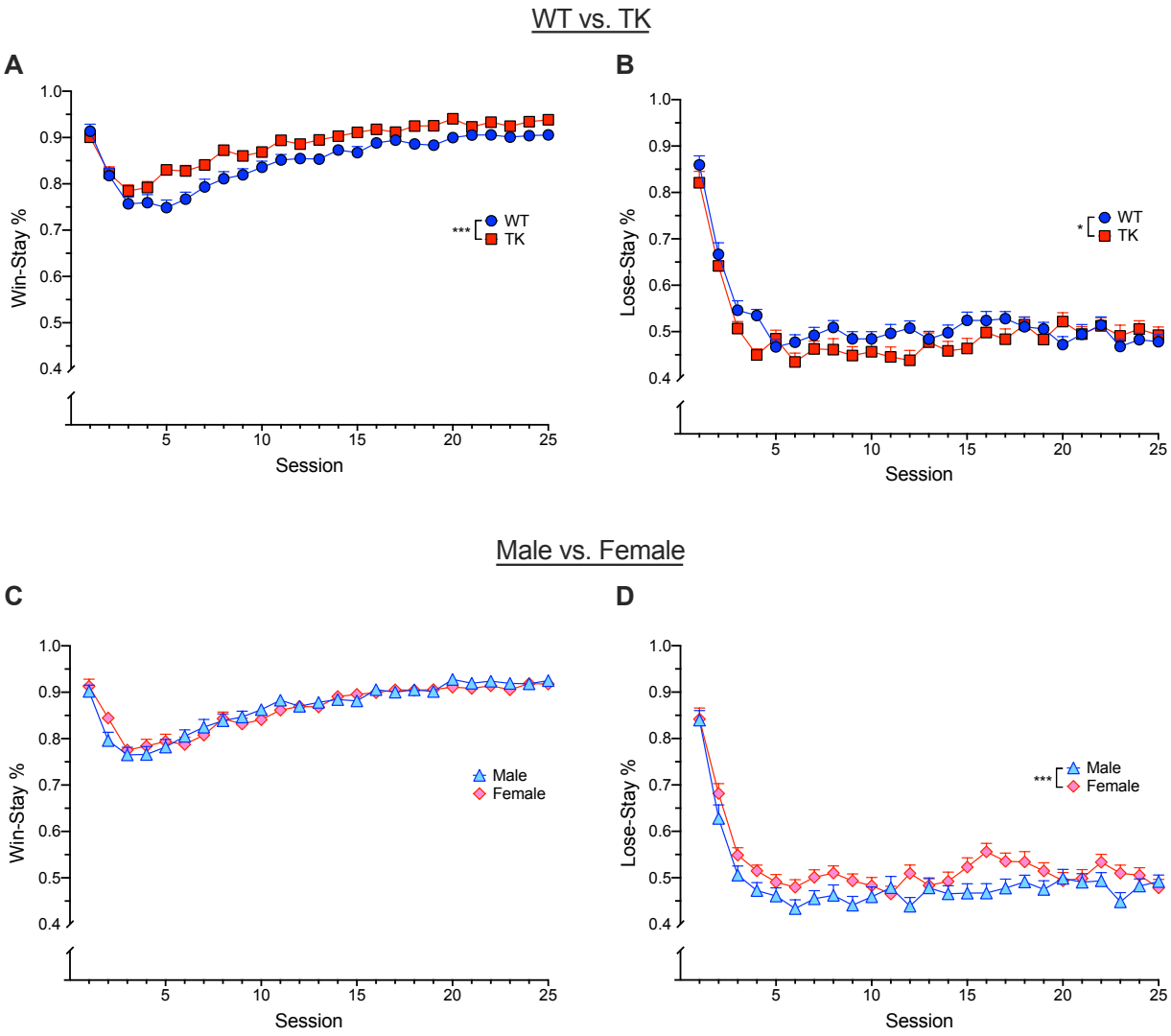

Figure 5.3. Win-stay/lose-stay behavior by genotype and sex. (A) TK rats had significantly higher win-stay (F (1, 56) = 13.41, p = 0.0006) and (B) lower lose-stay (F (1,56) = 5.286, p = 0.0252) ratios than WT controls. (C) Males and females did not differ in win-stay behavior, but (D) females had significantly higher lose-stay ratios, indicating that they were more likely to persist with a lever following a loss (F (1, 56) = 15.02, p = 0.0003).

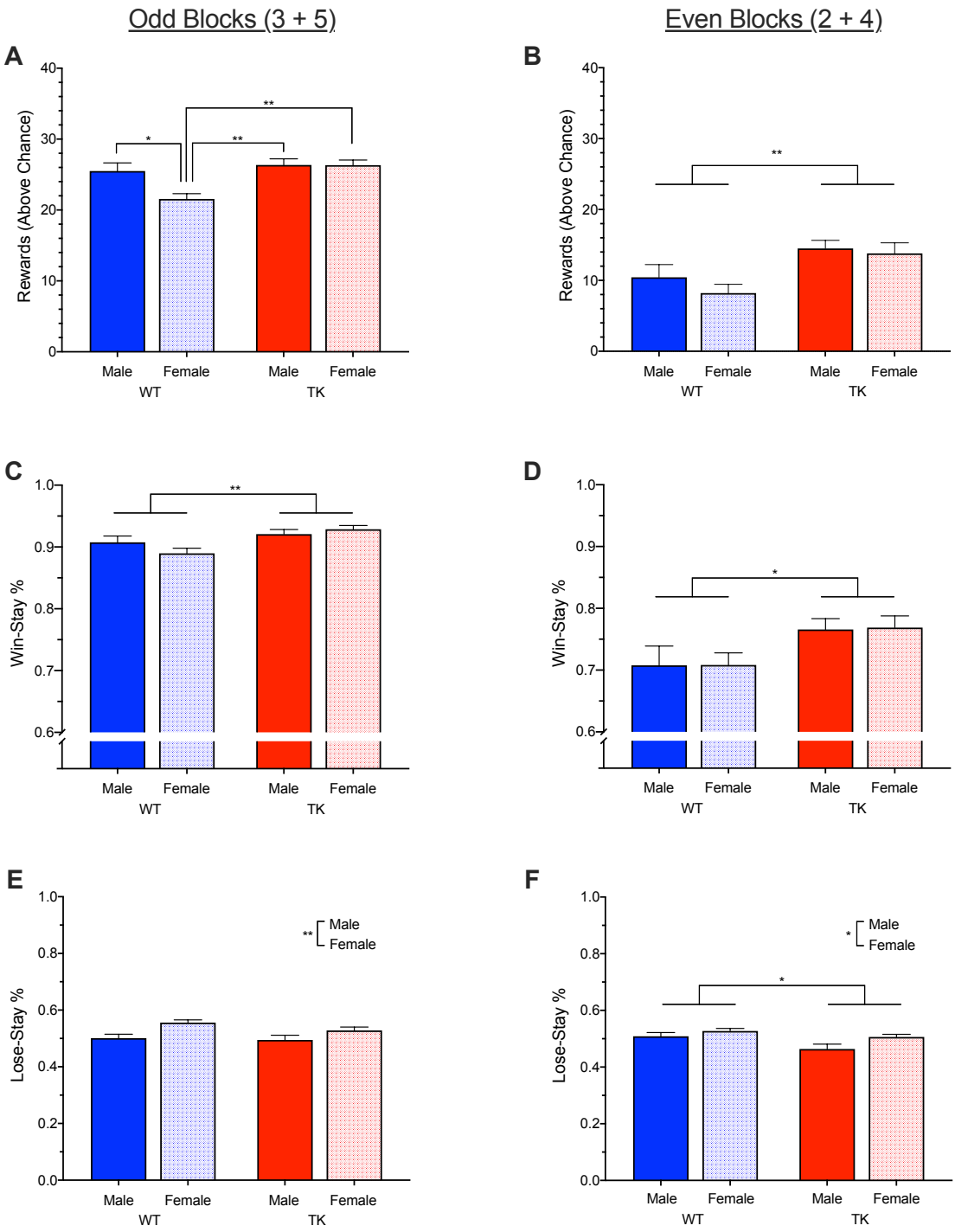

Figure 5.4. Reward-seeking behavior by "block type." (A) WT females earned significantly fewer rewards than all other groups on odd blocks, in which their trained lever was rewarded (sex-by-genotype interaction: F (1, 54) = 4.824, p = 0.0324). This underperformance was driven by the fact that (C) WT rats were significantly less likely to persist with a lever following a win (F (1, 54) = 10.03, p = 0.0025) and (E) female rats were more likely to persist with a lever following a loss (F (1, 54) = 11.19, p = 0.0015). (B) On even blocks, TK rats earned significantly more rewards than WTs, regardless of sex (F (1, 54) = 11.12, p = 0.0015). TK rats had both significantly (D) higher win-stay ratios (F (1, 54) = 6.749, p = 0.0121) and (F) lower lose-stay ratios than WTs (F (1, 54) = 6.797, p = 0.0118). Females were also significantly more likely than males to stay following a loss during even blocks (F (1, 54) = 5.901, p = 0.0185).

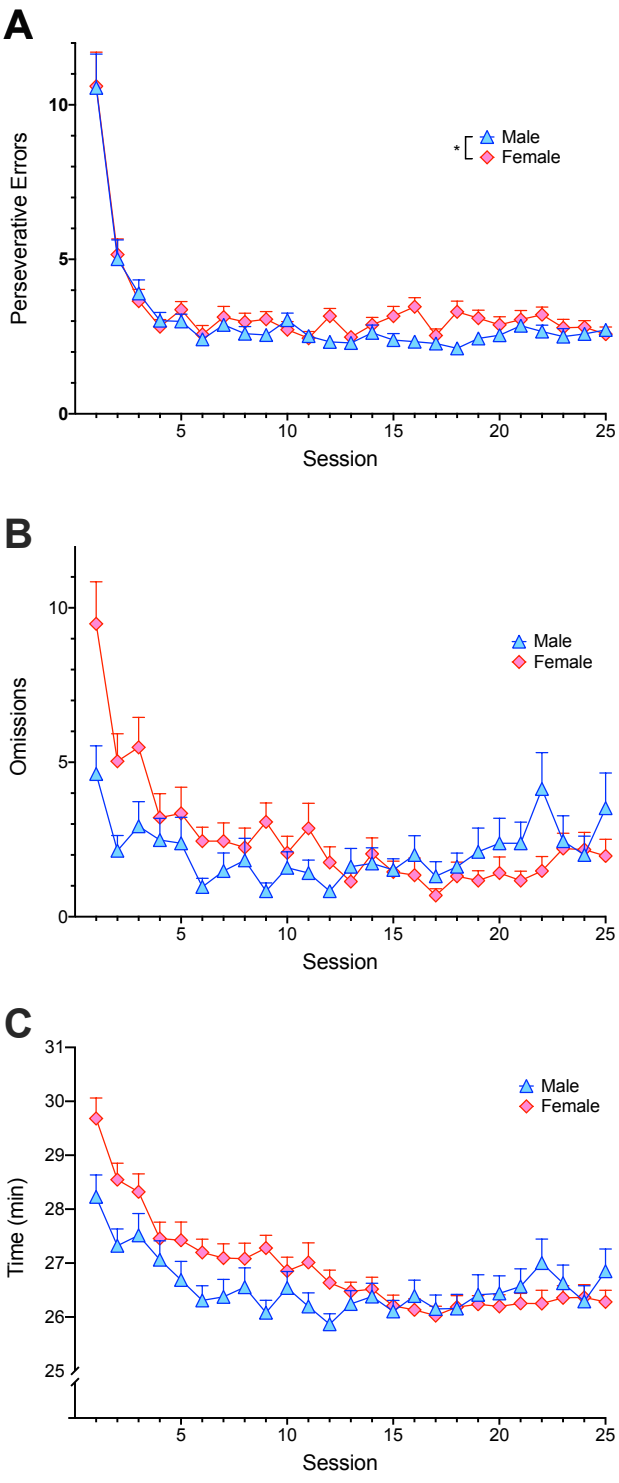

Figure 5.5. Further sex differences in PRL behavior. (A) Female rats committed significantly more perseverative errors than males ($F(1, 54) = 6.892$, $p = 0.0112$). (B) Females also committed more errors of omissions than males at the beginning of testing, but not at the end (session-by-sex interaction: $F(24, 1296) = 4.008$, $p < 0.0001$). (C) Similarly, female rats at the beginning of testing took longer to complete their sessions than did males, but this effect was not present on later testing days ($F(24, 1296) = 4.731$, $p < 0.0001$).

5.6 References

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Chapter 6:
Probabilistic Reversal Learning with Varying Reversal Frequency

6.1 Introduction

Thus far, the present work has provided evidence for a robust effect of neurogenesis upon reversal learning. In multiple versions of a two-armed bandit task, transgenic TK rats without adult neurogenesis outperform WT controls, earning more rewards per session. The previous chapter demonstrated that this effect is not probability-dependent and persists even when lever contingencies are deterministic. Previously, we also saw that the performance difference is attenuated when the lever identities are randomized at the beginning of each session – arguably creating a more challenging task. From these findings, it is tempting to conclude that the effects of neurogenesis emerge when tasks are relatively easy and diminish as tasks become more difficult. However, the prior experiments may not be directly comparable – rats on the deterministic bandit received a unique training protocol, and the effects of lever randomization could extend far beyond the initial discrimination.

To better study how task difficulty modulates the effects of neurogenesis on reversal learning, I designed two more versions of the two-armed bandit task: one with fewer reversals than the original, and the other with more. Aside from reversal frequency, the two new protocols were identical to each other and to the outline provided in Chapter 2: Methods. With increased reversal frequency, feedback in the bandit task becomes more ambiguous. Rats must decide, for instance, whether a loss on a previously lucrative lever represents solely the inherent unpredictability of probabilistic choice, or whether a (now more frequent) reversal has occurred.

In the context of our bandit tasks, reversals are a fairly low-probability event, occurring once every 40 trials in the original version. Misleading wins and losses are far more frequent: the better lever will fail to produce a reward pellet 20 percent of the time – just as often as the worse lever will successfully deliver one. Many have argued that the hippocampus mediates responses

to events of low probability (Cameron & Glover, 2015; Pigareva & Preobrazhenskaya, 1991; Simonov, 1974). Further, animals with lesioned hippocampi are less likely to attend to cues of dubious relevance (Honey & Good, 2000; Raphelson et al., 1965). Ablation of hippocampal neurogenesis may alter these behaviors, rendering animals less sensitive to events or cues that are ultimately unlikely to affect reward outcome.

Given that reversals are relatively uncommon in our tasks, overweighting their importance can prove a detrimental strategy. If rats are predisposed to assume that losses signal reversals, they will switch levers too often, missing out on valuable rewards. However, if rats underestimate the probability of reversal, they will persist with the incorrect lever for too long following a switch. In the original experiment, I established that TK rats outperformed WTs when reversals occurred every 40 trials. If new hippocampal neurons indeed modulate sensitivity to low probability events, we might expect the effects of neurogenesis on performance to depend on how frequently reversals occur.

In this chapter, I describe the findings from the two new bandit tasks, exploring how neurogenesis level influences learning and reward-seeking behavior when reversals are more, or less, frequent. Additionally, I discuss the greater implications of the study, and the ways in which task difficulty and neurogenesis level converge to shape behavior.

6.2 Methods

Because the protocols employed in each study closely resemble each other, the full details of the methods conserved across all experiments can be found in Chapter 2: Methods.

6.2.1 Animals

The data in this chapter was collected from 25 WT (14 male, 11 female) and 31 TK (14 male, 17 female) rats. All rats were naïve and unhandled at the beginning of the study, entering the training protocol between 14 and 17 weeks of age after having been on drug treatment for at least seven weeks. Two rats, both TK female, became sick and were euthanized before study completion. Their data was subsequently removed from the analysis. An additional rat (listed as WT male) was excluded when post-mortem histology did not match the results from PCR genotyping conducted following weaning. These three rats and 11 others excluded for failing behavioral criteria (described below) are not included in the total listed above.

6.2.2 Reversal Task

Generally, the experimental protocols used in this chapter followed the outline presented in Chapter 2: Methods, except that reversals were either more or less frequent. The first protocol in this chapter featured two reversals, each occurring every 66 trials; the second protocol featured six reversals, each occurring every 28 trials. Total session length remained unaltered at 200 trials, so the final block of each session was slightly longer by necessity.

At the beginning of the experiment, rats were pseudo-randomly assigned to the "two reversal" or "six reversal" protocol, in addition to being assigned a "trained lever" which was rewarded during the two sessions of probability pre-training that preceded reversal testing. As in the original version of the experiment, the best lever during the first block of each session was fixed across sessions, and matched the lever rewarded in each rat's probability training.

When a rat omitted 25 or more trials in a single test session, the data from that rat-session was removed and replaced with the data from the following session. (The same procedure was

followed when sessions were compromised by equipment malfunction.) If five or more sessions from the same rat were excluded, that rat was removed from the study. A total of five rats (two TK males and three WT females) were excluded under this criterion. Additionally, we excluded any rats that failed to press their non-trained lever at least 20 times by the tenth test session. Six rats (four WT males and two WT females) met that criterion and were subsequently excluded. Out of the remaining 1640 rat-sessions, 25 were excluded as a result of high omissions. Three more sessions were excluded due to equipment malfunction, and one more after the session was manually ended early by mistake. After exclusions, there were 27 rats that completed the "two reversal" experiment (7 WT males, 7 TK males, 4 WT females, and 9 TK females) and 29 rats that completed the "six reversal" experiment (7 WT males, 7 WT females, 7 TK males, and 8 TK females).

All rats received at least 28 sessions of testing. To accommodate removed sessions, only the first 25 successful sessions for each rat were included in the analysis for this chapter.

6.3 Results

TK rats with ablated neurogenesis earned more rewards than wild-type controls only when reversals were relatively infrequent.

In the original experiment (described in Chapter 3), TK rats outperformed WT controls, earning more rewards in our two-armed bandit task when reversals occurred four times per session, or every 40 trials. In the present experiment, I investigated whether this performance effect would persist when reversals happened either twice per session (every 66 trials), or six times per session (every 28 trials).

To assess performance, the number of rewards earned per session was counted and analyzed with three-way ANOVA, using sex and genotype as between-subject factors and session as a within-subject factor. When reversals occurred only twice per session, TK rats still earned more rewards than WT controls (*Fig. 6.1 A*; effect of genotype: F (1, 23) = 6.827, p = 0.0156). However, when reversals were more frequent, occurring six times per session, we no longer observed an effect of genotype, although a significant session-by-genotype interaction hinted at the same effect in later test sessions (*Fig. 6.1 B*; F (24, 600) = 1.669, p = 0.0242).

Previous chapters have demonstrated that learning on similar bandit tasks is not immediate, and performance on early testing days can differ greatly from performance on later sessions. To directly measure the effect of increasing reversal frequency at both early and late timepoints, I ran two more three-way ANOVAs, with sex, genotype, and reversal frequency (2, 4, or 6) as between-subject factors, using data from both the present and original experiments. First, I compared rewards data from the first five testing sessions, when rats were still acclimating to the protocol (*Fig. 6.1 C*). I found a strong effect of reversal frequency (F (2, 112) = 61.51, p < 0.0001), with rats struggling to earn rewards as reversals became more frequent and therefore, the task more challenging. Further, I identified a significant effect of genotype (F (1, 112) = 5.524, p = 0.0205), but no secondary interactions, indicating that TKs consistently outperformed WTs independent of sex and reversal frequency.

I observed similar results when analyzing the final five days of testing, when rats had reached a relatively stable level of performance (*Fig. 6.1 D*). Both the effect of reversal frequency (F (2, 112) = 58.01, p < 0.0001) and genotype (F (1, 112) = 11.43, p = 0.0010) were still significant, with no secondary interactions. Therefore, neurogenesis ablation appears to improve performance on a probabilistic two-armed bandit, independent of sex or reversal

frequency – although the effect becomes less pronounced, or even absent, at high levels of difficulty.

Sensitivity to feedback is not an immutable characteristic -- and changes with task difficulty and experience level.

As in prior chapters, I analyzed the probability that rats would persist with a lever that delivered a reward on the previous trial: their "win-stay" ratios. Previously, we observed that rats with ablated neurogenesis generally demonstrated higher win-stay ratios than WT controls, and that this tendency contributed to their overall improved performance. Surprisingly, I found no significant effect of sex or genotype when analyzing win-stay ratios in either the two- or six-reversal experiments individually. Next, I tested whether any genotype differences emerged when analyzing "misleading" and "accurate" wins separately: that is, whether the choice to stay following a win occurred on the current correct/better lever, or on the incorrect/worse one. For both the two- and six-reversal experiments, I observed no significant effects of either genotype or sex on win-stay behavior following either accurate or misleading feedback (*Figs. 6.2 A-D*). However, every analysis revealed a significant effect of session: further evidence that rats adjust their strategies as they become more experienced with the reversal task.

To assess the effect of increasing reversal frequency on win-stay tendencies during early testing days, I ran a three-way ANOVA with reversal frequency (2, 4, or 6), sex, and genotype as between-subject factors. The present experiment provided data for the two- and six-reversal protocols, whereas data from Chapter 2 was used for the four-reversal protocol. Win-stay data was averaged from sessions two through six, as it was unavailable for the first session of the original, four-reversal experiment. The ANOVA revealed only a significant effect of reversal

frequency (F (2, 112) = 4.565, $p < 0.0124$), suggesting no general effect of sex or genotype on win-stay behavior during early sessions. Further, no secondary interactions emerged, indicating that reversal number did not differentially affect win-stay behavior based on either sex or genotype (*Fig. 6.2 E*).

However, running the same analysis for final five testing days yielded different results (*Fig. 6.2 F*): the effect of reversal frequency was still significant (F (2, 112) = 13.64, $p < 0.0001$), but so was the simple effect of genotype (F (1, 112) = 4.659, $p = 0.0330$). On later testing sessions, TK rats exhibited higher win-stay ratios than WT controls, regardless of sex or reversal frequency. Taken together, these data show that win-stay behavior changes with experience level, reversal frequency, and genotype.

Next, I repeated the same analyses on lose-stay behavior to investigate how reversal frequency affects sensitivity to negative feedback. In both the two- and six-reversal protocols, experience level affected lose-stay behavior, with rats generally learning to switch more often following a loss as sessions progressed (effect of session – two reversals: F (4.921, 113.2) = 9.612, $p < 0.0001$; effect of session – six reversals: F (5.491, 137.3) = 15.28, $p < 0.0001$). However, there were no simple effects of sex or genotype for either reversal frequency. (A significant sex-by-session interaction will be discussed in the following section.) After separating lose-stay behavior according to whether losses occurred after a correct choice on the "better" lever, or an incorrect choice on the "worse" lever, there continued to be no effects of either sex or genotype on behavior, with one exception (*Figs. 6.3 A-D*). When rats experienced only two reversals, WTs were significantly more likely than TKs to persist with the worse lever following a loss (*Fig.* 6.3*B*; F (1, 23) = 7.763, $p = 0.0105$). Such a strategy would reduce earned rewards,

helping to explain the improved performance of TK rats compared to WTs on the two-reversal task.

Using the same procedure employed with the win-stay data, three-way ANOVAs were run to assess the influence of reversal frequency on lose-stay behavior at both early and late timepoints. On early testing days, there was a significant effect of reversal frequency on lose-stay behavior (*Fig. 6.3 E*; F (2, 112) = 4.220, p = 0.0171), which became even more pronounced on the final five days of testing (*Fig. 6.3 F*; F (2, 112) = 5.613, p = 0.0048). There were no significant simple effects of genotype or sex, indicating that the effects observed in previous chapters are not universal – the influence of genotype and sex on lose-stay behavior are not independent of experimental protocol. However, we did observe a significant reversal frequency-by-genotype interaction on later testing sessions (F (2, 112) = 4.126, p = 0.0187), driven by the finding that TK rats were more likely than WTs to stay after losses when experiencing four reversals, which was not replicated at other reversal frequencies. Across all reversal frequencies, the overall effect of genotype on lose-stay behavior was minimal; however, in selected instances, it still played an important role in determining rat performance.

The effects of sex on reward-seeking strategy and both high and low frequency of reversal

Previous chapters have described the effects of sex on feedback sensitivity in the two-armed bandit task. Specifically, females have seemed more likely than males to persist with their current lever following a loss, suggesting diminished sensitivity to negative feedback. In these two experiments, the effects of sex on win-stay and lose-stay tendencies were rather minimal. For both rats experiencing two and six reversals, we found no significant effects of sex on win-stay behavior, either in response to misleading wins on the worse lever, or accurate wins on the

better lever (*Figs. 6.4 A-D)*. The previous section established a significant effect of reversal frequency on win-stay behavior at both early and late timepoints. However, there were no secondary interactions with sex, indicating that reversal frequency influences win-stay behavior in a sex-independent manner (*Figs. 6.4 E-F)*.

Analysis of lose-stay behavior yielded similar results. In the two-reversal protocol, rats of both sexes demonstrated equivalent lose-stay behavior (*Figs. 6.5 A, B)*. For rats experiencing six reversals, I did find a significant sex-by-session interaction both following misleading losses on the better lever (*Fig. 6.5 C*; F (24, 600) = 1.587, p = 0.0382) and accurate losses on the worse lever (*Fig. 6.5 D*; *F* (24, 600) = 1.753, p = 0.0150). Female rats were generally more likely than males to persist with the current lever following a loss during early sessions, but this effect diminished as rats became more experienced on the task. When analyzing overall lose-stay behavior as a function of reversal frequency and sex, we found a near-significant frequency-by-sex interaction at late timepoints (*Fig. 6.5 F*; *F* (2, 112) = 2.479, p = 0.0884). This interaction is perhaps unsurprising given that the original experiment (with four reversals) found a pronounced sex effect on lose-stay behavior which was not replicated in either of the new protocols.

Altered reversal frequency attenuated sex effects previously seen in perseverative errors, omissions, and session time.

In the original, four-reversal version of the experiment, female rats completed sessions significantly faster than males, while committing fewer omissions but more perseverative errors. Surprisingly, few of these effects were preserved in the two- and six-reversal bandit tasks. Male and female rats experiencing two reversals committed similar numbers of perseverative errors and omissions (*Fig 6.6 A, C*) and completed sessions at an equivalent pace (*Fig. 6.6 E*). Male and

female rats on the six-reversal protocol also did not differ significantly in omissions or in the time required to complete a session (*Fig 6.6 D, F*). However, the effect of sex on perseverative errors was near-significant, with females trending towards committing more perseverative errors than males, mirroring the original effect observed in Chapter 3 (*Fig. 6.6 B*; effect of sex: F (1, 25) = 4.144, p = 0.0525). No genotype effects were observed in any of the measures mentioned above.

5.4 Discussion

Overall, the results from this chapter support the hypothesis that the influence of neurogenesis on bandit performance is modulated by task difficulty. When reversals occur either twice or four times per session, TK rats outperform WT controls, earning more rewards per session. When reversals occur six times per session – presumably, a more difficult task – the effect is no longer evident, although later testing sessions hint at its reemergence. Seemingly, the performance of TK rats is generally superior than that of controls, but only when tasks are not too challenging.

However, the relationship between neurogenesis and task difficulty may not be so simple. Previous chapters identified strategic differences between WT and TK rats that were not apparent after varying the reversal frequency. In particular, the effect of neurogenesis level on win-stay behavior was robust in both the original bandit and the deterministic version, with TK rats significantly more likely to persist with a lever following a win. This effect was significant in neither the two- nor the six-reversal bandit individually, but it reemerged in an analysis of all three experiments, with reversal frequency as a between-subjects factor.

It is worth noting that the group sizes in these two experiments were generally much smaller than in the rest of this work. The behavioral exclusion criteria removed eleven rats, with sickness or histological anomalies excluding another three. The disappearance or reduction of strategic differences between genotypes could simply result from overall reduced sample size. Decision-making between individual rats varies greatly, although robust group differences can still emerge with large sample sizes. With the increased noise of a small N, effects can be obscured or eliminated. However, a more intriguing explanation for the absent effects is that neurogenesis level affects behavior most strongly at moderate levels of ambiguity.

The hippocampus is suspected to play a role in mediating response to low-probability events (Pigareva & Preobrazhenskaya, 1991; Simonov, 1974, 1981). It is possible that newly-born granule cells modulate these responses, providing a mechanism for the behavioral results seen across this work. Reversals on the PRL task are relatively infrequent events, and the likelihood of their occurrence is overshadowed by the probability of receiving misleading feedback. Newly-born neurons might exert the most influence over behavior when reversals are of intermediate frequency – low-probability events that are neither vanishingly rare nor expressly anticipated. At intermediate frequencies, rats might feel the most uncertainty or curiosity surrounding impending reversals, and therefore, their decision-making might be most susceptible to the presence (or absence) of new hippocampal neurons.

Additionally, this chapter provided further evidence that learning and reversal strategy vary as rats become more familiar with the experiment, adapting to the repeated reversals. At all reversal frequencies, rats improved their performance over time, earning more rewards and adopting superior decision-making strategies. However, some group effects were apparent only

during early or late sessions, suggesting that rats of different sexes or neurogenesis levels learn to adapt to the task at different rates – opening an exciting avenue for future analysis.

Overall, the findings from this chapter support the idea that the effects of neurogenesis on performance are at least somewhat dependent on task difficulty. On arguably easy tasks (probabilistic bandits with two and four reversals, and a deterministic bandit with four reversals), TK rats outperform WTs, demonstrating that neurogenesis ablation can confer demonstrable behavioral advantages in certain contexts. On arguably more difficult tasks (probabilistic bandits with randomized starts or six reversals), the effect of neurogenesis is diminished or absent. Still, the extent to which task difficulty and reversal frequency are equivalent remains unclear. If new neurons are particularly sensitive to low probability events, variation in reversal frequency could alter behavior beyond the raw capability of rats to decipher the correct lever to press. Further, the general effects of neurogenesis on behavior might then depend less on choice-outcome probabilities, and more on the ambiguity of the task structure itself.

6.5 Figures

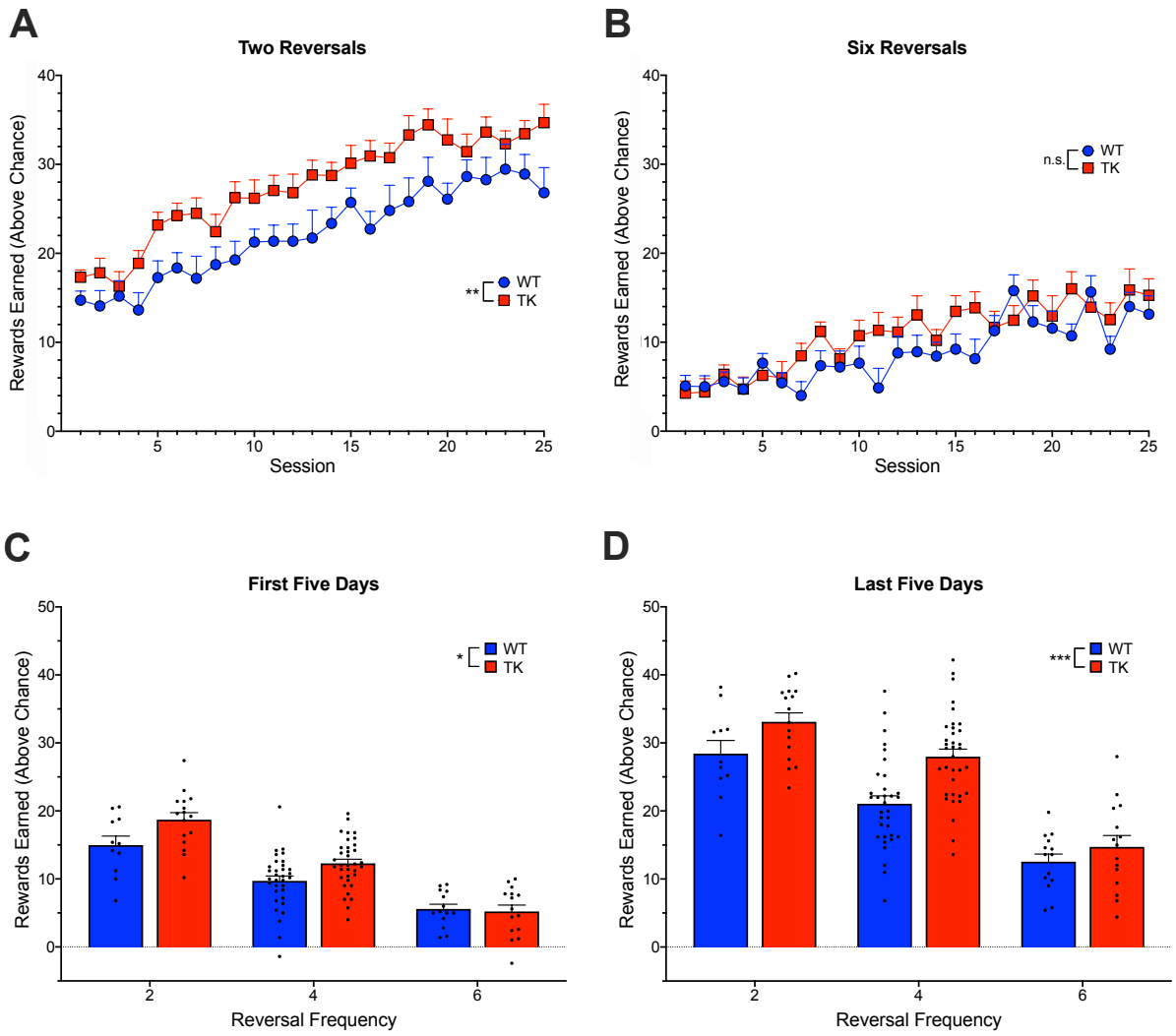

Figure 6.6. Bandit performance by reversal frequency. (A) When reversals occurred relatively infrequently (twice per session), TK rats outperformed WT controls, earning more rewards per session (F (1, 25) = 8.895, p = 0.0063). (B) However, when reversals occurred more frequently (six times per session), this effect was no longer significant (F (1, 27) = 1.586, p = 0.2186), although a significant session-by-genotype interaction (F (24, 648) = 1.716, p = 0.0185) hinted at an emerging difference on middle and late sessions. (C) Even on the first five days of testing, rat performance varied significantly with reversal frequency (F (2, 118) = 62.15, p < 0.0001). Overall, TK rats outperformed WTs in the first five sessions regardless of reversal frequency (effect of genotype: F (1, 118) = 6.853, p = 0.0100). (D) On the final five days of testing, performance still declined with increasing reversal frequency (effect of frequency: F (2, 118) = 55.64, p < 0.0001). Further, the effect of neurogenesis ablation on performance became more pronounced, with TK rats still outperforming WT controls (F (1, 118) = 14.17, p = 0.0003). There were no significant effects of sex, so all data sets and statistical tests presented in this figure have grouped male and female rats together.

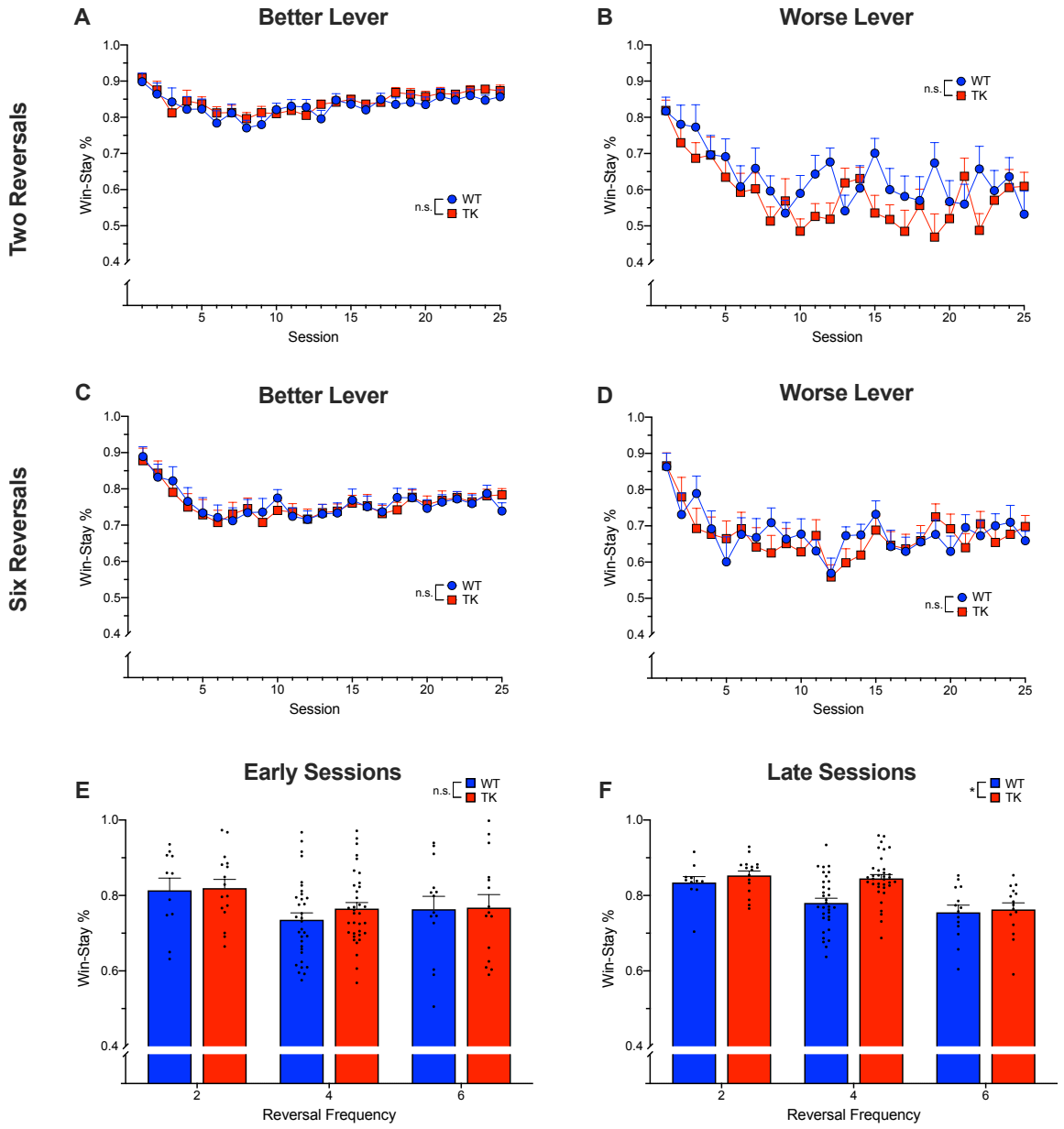

Figure 6.2. Win-stay behavior and reversal frequency, by genotype. WT and TK rats exhibited similar win-stay behavior following a win on the "better" lever (accurate feedback) both when reversals occurred (A) twice per session, and (C) six times per session. Similarly, win-stay behavior did not differ between genotypes following a win on the "worse" lever (misleading feedback), either when reversals occurred (B) twice per session, or (D) six times per session. (E) During early sessions, overall win-stay behavior (with accurate and misleading wins combined) varied with reversal frequency (F (2, 118) = 3.545, p = 0.0320) but not with genotype. (F) However, during the final five sessions, win-stay behavior varied both with reversal frequency (F (2, 118) = 12.85, p < 0.0001) and genotype (F (1, 118) = 5.889, p = 0.0168), with TK rats being more likely to stay on their current lever following a win, regardless of reversal frequency or sex. None of the first-line analyses revealed a significant effect of sex, so all of the data sets and statistical tests represented in this figure have grouped male and female rats together.

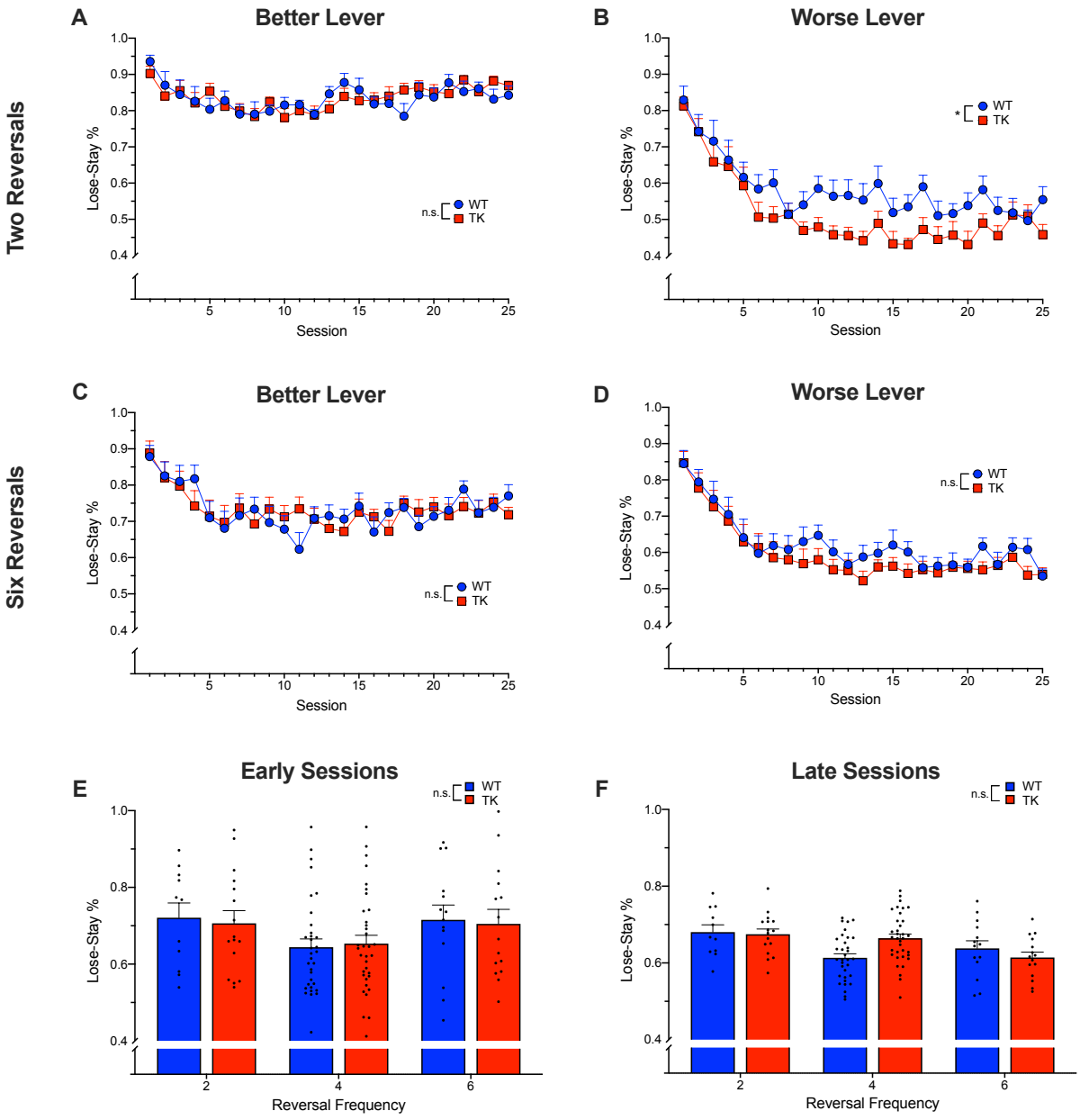

Figure 6.3. Lose-stay behavior and reversal frequency, by genotype. WT and TK rats exhibited similar lose-stay behavior following a loss on the "better" lever (misleading negative feedback) both when reversals occurred (A) twice per session, and (C) six times per session. (B) WT rats experiencing two reversals per session were more likely than TKs to persist with the "worse" lever following a loss (F (1, 25) = 7.729, p = 0.0102). (D) However, this effect was not observed when rats experienced six reversals per session. (E, F) During both early and late sessions, overall lose-stay behavior (with accurate and misleading losses combined) varied with reversal frequency (early: F (2, 118) = 3.491, p = 0.0337; late: F (2, 118) = 5.214, p = 0.0068), but not with genotype, although there was a significant reversal frequency-by-genotype interaction on later sessions (F (2, 118) = 4.412, p = 0.0142). The data sets and statistical tests represented in this figure have grouped male and female rats together. The effects of sex on lose-stay behavior were minimal; for more information, see Figure 5.5.

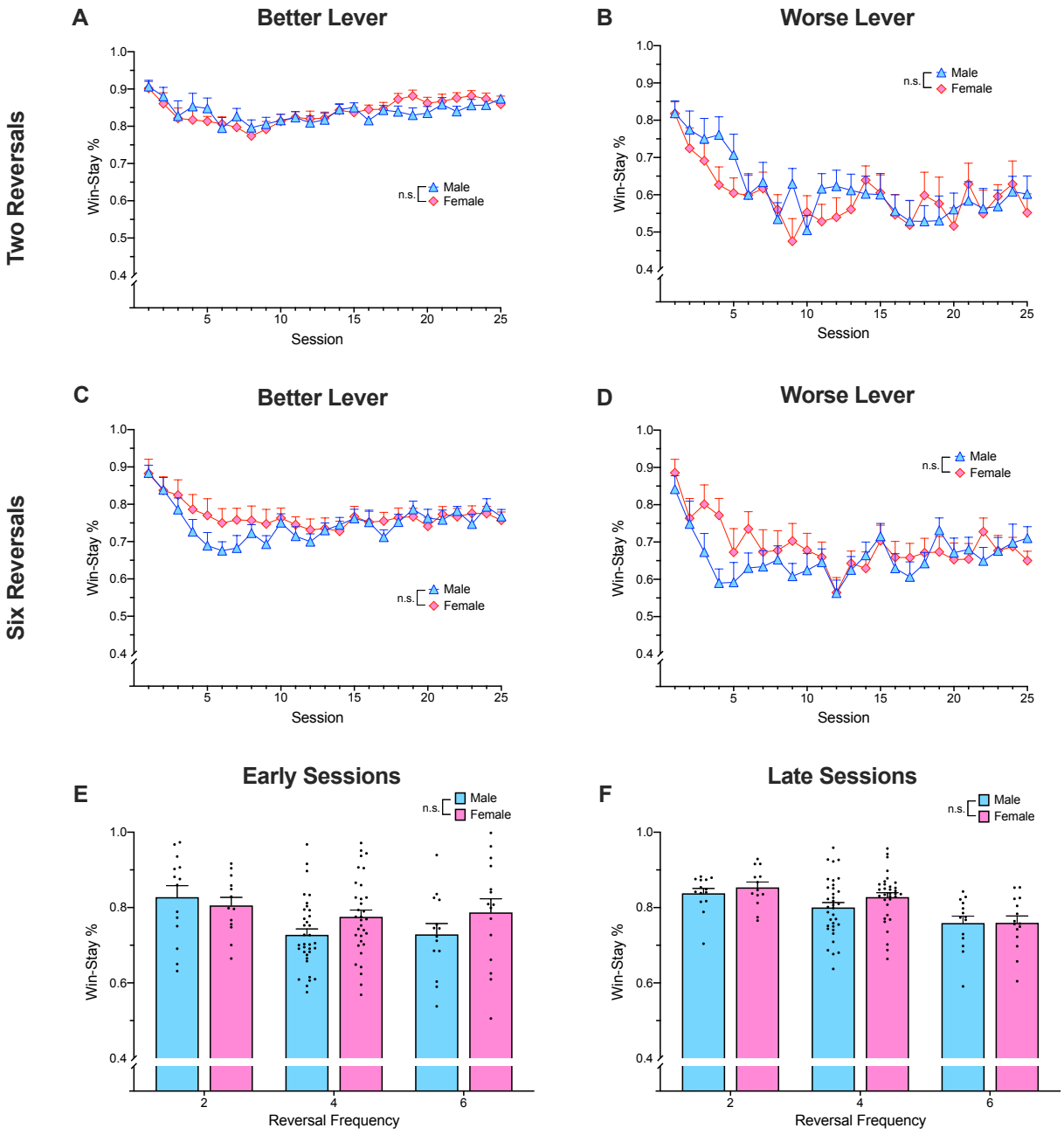

Figure 6.4. Win-stay behavior and reversal frequency, by sex. (A-D) Male and female rats exhibited similar win-stay behavior when on both two- and six-reversal bandit tasks, and following both misleading feedback on the worse lever, and accurate feedback on the better lever. (E, F) At both early and late timepoints, overall win-stay behavior (with accurate and misleading wins combined) varied with reversal frequency (early: F (2, 118) = 3.545, p = 0.0320; late: F (2, 118) = 12.85, p < 0.0001) but not significantly with sex. All of the datasets and statistical tests represented in this figure have grouped WT and TK rats together.

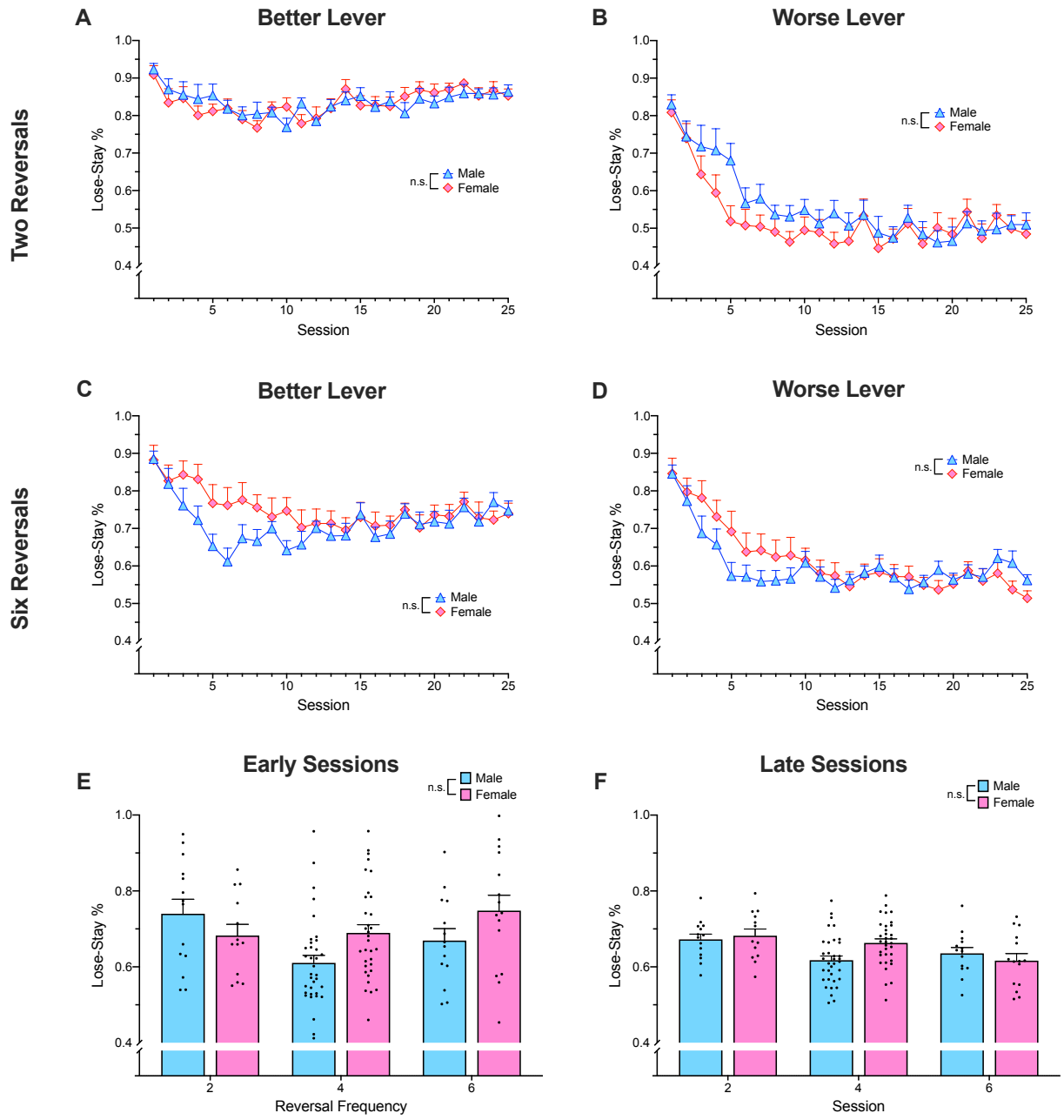

Figure 6.5. Lose-stay behavior and reversal frequency, by sex. (A, B) Male and female rats experiencing two reversals per session exhibited similar lose-stay behavior, following both losses on the "better" lever (misleading negative feedback) and on the "worse" lever (accurate negative feedback). (C, D) Male and female rats experiencing six reversals demonstrated similar lose-stay behavior overall; however, females appeared more likely to persist following losses during early sessions for both misleading losses (session-by-sex interaction F (24, 648) = 1.603, p = 0.0347) and accurate losses (session-by-sex interaction F (24, 648) = 1.833, p = 0.0092), with this effect waning with increasing sessions. (E, F) During both early and late sessions, overall lose-stay behavior (with accurate and misleading losses combined) varied with reversal frequency (early: F (2, 118) = 3.491, p = 0.0337; late: F (2, 118) = 5.214, p = 0.0068). On later sessions, we also noted a near-significant reversal frequency-by-sex interaction (F (2, 118) = 3.067, p = 0.0503), with the effect of sex appearing to adopt different directionality depending on reversal frequency. All of the datasets and statistical tests represented in this figure have grouped WT and TK rats together.

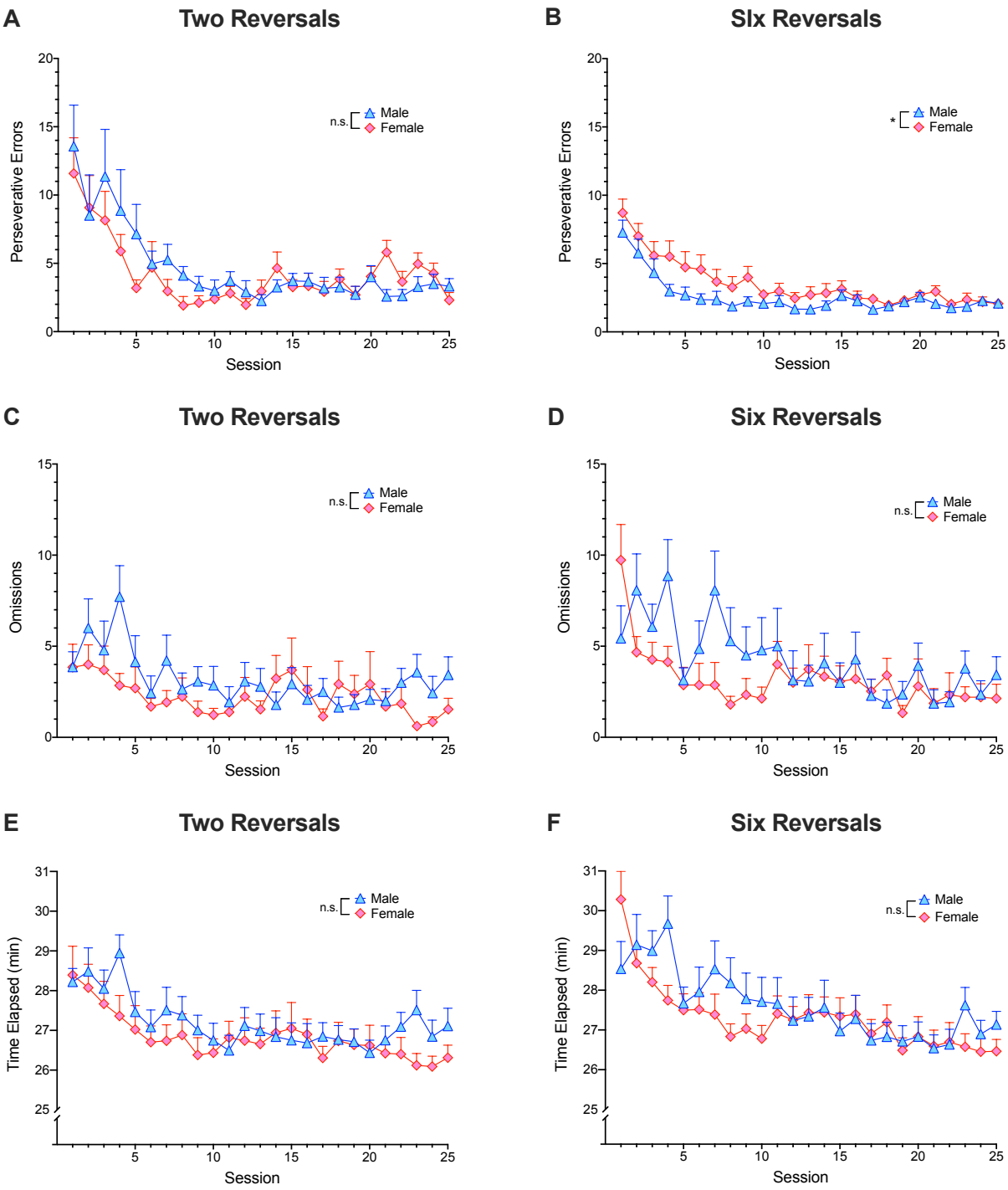

Figure 6.6. Perseverative error rate, omission rate, and total session time. (A) Male and female rats committed similar numbers of perseverative errors in the two-reversal protocol, but (B) females committed more than males when there were six reversal per session (F (1, 27) = 4.378, p = 0.0459). For both protocols, there was no effect of sex on either (C, D) the omission rate or (E, F) the amount of time required to complete a session. All of the datasets and statistical analyses represented in this figure have grouped WT and TK rats together. (No significant genotype effects were observed in any of the measures.)

6.6 References

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Chapter 7:
Discussion and Concluding Remarks

In life, we can seldom guarantee the consequences of our choices. Even the safest options carry with them the possibility – however infinitesimal – of risk. How we choose to interpret that uncertainty ultimately determines our choices, and therefore, our behavior. In this dissertation work, I provided evidence that neurogenesis ablation alters performance on a two-armed bandit reversal learning task, a test that emulates the probabilistic choices that all animals must make in the "real world." Furthermore, I showed that neurogenesis loss confers a distinct advantage on multiple forms of the bandit task, demonstrating that new hippocampal neurons do not always positively influence behavior. These findings hold clinical relevance: a connection between neurogenesis levels and depression has long been suspected (Jacobs et al., 2000; Sahay & Hen, 2007), and enhancement of neurogenesis has been suggested as a future therapy (Drew & Hen, 2007; Svoboda, 2022). This thesis work demonstrates that the behavioral effects of neurogenesis are not universal, and vary with sex, situational uncertainty, and specific task parameters.

7.1 Main Findings

Male and female TK rats without adult neurogenesis earned more rewards than WT controls on some – but not all – versions of an operant two-armed bandit reversal learning task. Specifically, TK rats of both sexes outperformed WT controls on a five-block, probabilistic bandit with a predictable start (Chapter 3). This finding was replicated when lever contingencies were deterministic (Chapter 5) and when reversal frequency was decreased (Chapter 6). When reversals became more frequent, the neurogenesis performance effect was no longer apparent (Chapter 6). Following randomization of the first blocks, WT and TK males performed at similar levels (Chapter 4). In females, however, the TK rats still outperformed WTs, a clear indication that neurogenesis ablation does not influence behavior independent of sex.

Furthermore, the generally elevated performance of TK rats could not be solely attributed to a better understanding of initial lever contingencies. Even with the first block removed, rats with ablated neurogenesis tended to outperform WT controls. Likewise, the performance difference was not caused by omission rates: WT and TK rats were equally likely to skip trials. Instead, the elevated performance of TK rats seemed to be the result of superior decision-making strategy. On many versions of the bandit task, TK rats without new neurons were significantly more likely than controls to persist with a lever following a win on the "better" lever – a sound strategy. Significantly, there was no effect of neurogenesis ablation on perseverative errors in any task version. Together with the finding that WT rats were generally more likely to abandon the "better" lever following a win, this result suggests that WTs may not be impaired in discerning which lever is more rewarding, but rather, may merely be more likely to deviate from it. Such a tendency could indicate noisier behavior overall or, perhaps, heightened curiosity and increased exploratory behavior.

Finally, a particularly robust finding was that female rats, regardless of genotype, were more likely than males to persist with the previous lever following a loss, whether misleading or accurate. Curiously, this strategic departure was not accompanied by any overall sex differences in performance, except in the randomized-start bandit, in which WT females significantly underperformed all other groups (see: Chapter 4). Effects of sex on task speed and omission rate were inconsistent between task versions.

Overall, the previous chapters demonstrate that neurogenesis ablation causes robust, oftentimes beneficial, performance effects on a two-armed bandit reversal learning task. These effects seem directly attributable to the different decision-making strategies employed by WT and TK rats while engaged in the task. The disparate strategies, which vary according to task

demands, could indicate different underlying biases and assumptions about the world. By better understanding the specific contexts in which neurogenesis ablation affects decision-making strategy, we can pinpoint the exact environmental and situational factors to which new neurons are sensitive – ultimately, coming one step closer to learning their functional role in determining animal behavior.

7.2 Greater Implications

7.2.1 Neurogenesis and Task Difficulty

The results suggest a relationship between task difficulty and the behavioral effects of neurogenesis ablation. On relatively easy versions of the task, including a particularly simple deterministic bandit, TK rats outperformed WT controls. In contrast, on tasks that were arguably more challenging (the randomized-start and increased-reversal bandit tasks), the effects of neurogenesis loss were weakened or absent. However, it is difficult to say whether the strength of the performance differences is directly correlated to task difficulty, or whether it is more dependent upon other related factors, such as general environmental uncertainty or reversal probability.

This work is far from the first to suggest that the behavioral effects of neurogenesis loss are modulated by task difficulty (Cameron & Schoenfeld, 2018; Shors et al., 2002). Twenty years ago, the Shors lab found that irradiated rats with ablated neurogenesis were not impaired in delayed conditioning tasks, in which rats learned that a long tone co-terminated with an electric shock. However, the rats did show impairments in trace conditioning, when the tone and shock were separated by a pause, or "trace interval" – a task proven to be more difficult (Beylin et al., 2001). Moreover, neurogenesis has long been implicated in pattern separation, or distinguishing

between highly similar cues. For example, mice with ablated neurogenesis struggle with spatial discrimination when cues are geographically close (and therefore difficult to distinguish) but not when they are further apart (Clelland et al., 2009). Conversely, mice with increased neurogenesis show improved pattern separation in a difficult contextual fear conditioning paradigm featuring highly similar environments (Clemenson et al., 2015). Interestingly, based on this thesis work, the opposite effect might be argued: that neurogenesis loss conferred a behavioral advantage when tasks were easy, but had no effect when tasks were more challenging.

In Chapter 6, we saw that increased reversal frequency (and therefore, task difficulty) eliminated the effect of genotype on performance. Strategic differences were most pronounced when reversals were moderately frequent, and largely absent when reversal frequency was altered in either direction. One interpretation of these findings is that new neurons exert the greatest influence on behavior when tasks are of moderate difficulty, biasing behavior when the correct path is unclear. When tasks are too easy, the answer becomes obvious; when too hard, animals veer into random guesswork. However, if tasks are moderately challenging, animals make relatively informed choices without being certain of their responses. Perhaps it is in these kinds of tasks that new neurons are able to bias behavior.

In contradiction of this theory, I found that the effects of neurogenesis ablation were still robust on arguably the easiest bandit: the deterministic version. TK rats were still more likely than WT controls to persist with a winning lever, despite the fact that reward feedback was never ambiguous. It is possible that our understanding of what makes a task "difficult" is flawed. General situational uncertainty, derived from factors like overall reversal frequency or the predictability of the first blocks, may ultimately influence the rats' perception of task difficulty or the activity of new granule cells more than the level of uncertainty inherent to each choice.

7.2.2 Neurogenesis and Low-Probability Events

Many have proposed a role for the hippocampus in the mediation of behavioral response to low-probability events (Pigareva & Preobrazhenskaya, 1991; Simonov, 1974). The hippocampus is also a suspected mismatch detector, identifying events or cues that contradict prior expectations (Duncan et al., 2012; Kumaran & Maguire, 2006). These two roles are closely linked: in order to identify surprising events, animals must set expectations, deciding which events are rare and which are commonplace.

Several behavioral studies support the involvement of the hippocampus – and new neurons specifically – in the processing of low-probability events. Compared to intact controls, animals with hippocampal lesions attend more to reliable cues than to less predictive, probabilistic stimuli (Honey & Good, 2000). Additionally, my lab has found that TK mice are less likely than WT controls to freeze in response to probabilistic, but not deterministic, threat cues (Glover et al., 2016). This dissertation provides further evidence that new hippocampal neurons may underlie behavioral response to unlikely events. In the bandit task, reversals are relatively infrequent, occurring only two to six times over a session of 200 trials. Optimal strategy would ignore the possibility of reversal until compelling evidence pointed to its occurrence. I found that WT rats with intact neurogenesis were generally more likely than TKs to switch away from the "better" lever – possibly, evidence that WT rats are more likely to over-anticipate reversals, and that new hippocampal neurons are particularly sensitive to low-probability events.

This theory may also explain the somewhat surprising finding that TK rats outperformed WTs even on a particularly simple deterministic bandit. In that task, reversals occurred four times per session: the same frequency as in the "original" version. However, the TK performance

effect was absent on a probabilistic version with six reversals per session. These results can be reconciled if the behavioral effects of neurogenesis loss are more dependent on reversal probability than on individual lever contingencies – very possible, if new neurons underlie the anticipation of rare events.

7.2.3 Neurogenesis and Curiosity

In this work, WT rats were generally more likely to switch away from the "better" lever following a win, thereby reducing their chances of receiving a reward on the next trial. This tendency does not necessarily indicate an impaired ability to determine which lever is currently more lucrative. In fact, we saw evidence to the contrary. On all task versions, WT and TK rats committed similar numbers of perseverative errors immediately following reversals. Further, when the starting lever contingencies were randomized, WT and TK rats earned equivalent numbers of rewards during the first testing block, suggesting that both genotypes were equally adept at determining which lever was "better" in the absence of any prior knowledge.

Even still, neurogenesis ablation conferred a distinct behavioral advantage in many of the experiments described within this dissertation. However, the same decision-making tendencies that earned TK rats more rewards on these highly regimented lab tasks might not be as advantageous in nature. The results suggest that rats with ablated neurogenesis are more likely to adhere to existing rules, while still maintaining the ability to learn new associations. Such a tendency might render TK rats less susceptible to distraction, but perhaps more likely to miss the relevance of novel stimuli.

The previous hypothesis fits with work from my lab showing that TK rats were less likely to become distracted by an irrelevant, aversive olfactory cue while running through a maze

(Schoenfeld et al., 2021). While WT and TK rats learned maze paths equally well in the absence of mint odor, WT rats were significantly slower to finish the maze in its presence. My lab also found that water-restricted TK rats were less likely to orient to a novel auditory cue while drinking (Weeden et al., 2019), again supporting the idea that TK animals are less likely to inhibit ongoing behavior in response to distractors. Similar results have been observed outside of our lab: over fifty years ago, researchers found that rats with hippocampal lesions retrieved rewards faster than intact controls when a novel, potentially distracting, tactile stimulus covered their path (Wickelgren & Isaacson, 1963).

In the present studies, there was no external, irrelevant cue to sidetrack the animals – although one might argue that the non-rewarding lever served as a distractor. However, TK rats were more likely to continue pressing a lever that was successful on the previous trial, indicating greater adherence to ongoing behavior and learned "rules." The opposite tendency of WT rats, while detrimental in this paradigm, could indicate heightened curiosity or exploration: both traits hypothesized to be evolutionarily advantageous (Byrne, 2013; Kidd & Hayden, 2015). Studies in humans show that peak curiosity occurs when subjects are only moderately unsure of a stimulus (Kang et al., 2008; Kinney & Kagan, 1976), dovetailing nicely with the idea that new neurons exert the greatest influence on behavior when tasks are of moderate difficulty and, perhaps, when rats are most curious.

Previously, others have suggested a connection between the hippocampus and curiosity (Gruber & Ranganath, 2019). The distraction studies mentioned above can also support hippocampal involvement in curiosity-like behaviors: intact animals were more easily distracted than those with lesioned hippocampi or ablated neurogenesis, potentially indicating heightened curiosity surrounding the novel cues. Additionally, fMRI data shows that hippocampal activity

changes according to how much an event or cue defies expectations (Duncan et al., 2012; Kumaran & Maguire, 2006). It is possible that these fluctuations in hippocampal activity mediate the behavioral expression of curiosity in response to surprising events.

7.2.4 The Hippocampus in Reversal Learning Circuitry

Traditional models of reversal learning networks do not include the hippocampus, usually focusing on a corticostriatal loop connecting the prefrontal cortex (PFC) and the basal ganglia (Frank et al., 2004; Hazy et al., 2007; O'Reilly & Frank, 2006). However, others have proposed the recruitment of a secondary, uncertainty-focused network during reversal learning, particularly when lever contingencies are probabilistic (Soltani & Izquierdo, 2019). This distributed network is theorized to include the hippocampus and amygdala: both brain regions implicated in probabilistic reversal learning (Costa et al., 2016; Vilà-Balló et al., 2017) but excluded from classic Go/No-Go dopaminergic circuit models. Additionally, Soltani & Izquierdo include the PFC, striatum, and mediodorsal thalamus in their model of the uncertainty network.

Prior work suggests a link between these two networks through attentional control. Attention-gated reinforcement learning models can successfully simulate human probabilistic reversal learning, suggesting modulation of the dopaminergic loop through top-down attentional control that modulates striatal excitability (Erdeniz & Atalay, 2010). Some evidence also implicates the ventral striatum and amygdala in altering attention in response to reward-prediction errors (Costa et al., 2016). The hippocampus is a key player in a recently proposed appraisal circuit, in which the hippocampus and anterior cingulate cortex work synergistically to elicit curiosity, as a function of prediction errors (Gruber & Ranganath, 2019). If curiosity is triggered, attention increases, thereby enhancing learning.

Expanding upon the theory put forth by Gruber & Ranganath, I suggest that the hippocampus and, particularly, new hippocampal granule cells, play an essential role in probabilistic reversal learning, mediating responses to "surprising" outcomes, as measured by reward prediction errors. Surprising outcomes would elicit heightened hippocampal response – a theory supported by fMRI studies indicating that hippocampal activity fluctuates with how well events match prior expectations (Duncan et al., 2012; Kumaran & Maguire, 2006). Further, I propose that newly-born neurons are particularly sensitive to expectation-defying events, explaining the finding that animals with higher levels of neurogenesis appear to exhibit more curiosity-like behaviors. Through hippocampal projections to the PFC (Barbas & Blatt, 1995; Hoover & Vertes, 2007), the curiosity signal might then pass indirectly to the striatal Go/No-Go cells via cortical top-down control of the dopaminergic action selection loop. Although further research is necessary to confirm the curiosity loop and its connections, behavioral data strongly supports the inclusion of the hippocampus – and new neurons specifically – in reversal learning circuits.

7.3 Future Directions

Repeated throughout this dissertation is the idea that bandit task learning occurs in phases, as rats gradually become more familiar with the task. Many sex or genotype effects were apparent during early – but not late – testing days, suggesting group differences in the ways in which animals learned to adapt to task demands, or the rate at which that adaptation occurred. This dissertation primarily adopted a bird's eye view of the experiments, examining group differences over all testing days. Future analyses could take a narrower approach, investigating each learning stage individually, with perhaps a specific focus on how neurogenesis and sex

affect learning and behavior during early sessions, when animals are still becoming familiar with task parameters.

Furthermore, the development of reinforcement learning models would lend valuable insight into how neurogenesis influences decision-making on a more granular level. Reinforcement learning models are used extensively in the analysis of bandit behavior and allow the calculation of distinct learning parameters that contribute to decision-making (Chen et al., 2021; Costa et al., 2016; Steyvers et al., 2009). In particular, quantification of choice consistency could prove useful, given the generally increased tendency of WT rats to switch away from the lucrative lever. Further, the calculation of learning rates could reveal whether neurogenesis loss or sex differentially affect learning in response to wins and losses. Additionally, hidden Markov models could identify latent goal states (i.e., exploration vs. exploitation) that could further explain trial-by-trial behavior (Costa et al., 2019; Ebitz et al., 2018). Generally, mathematical modelling techniques could provide a much deeper, nuanced understanding of the ways in which new neurons affect decision-making.

Finally, additional behavioral assays could further explore which testing contexts are most influenced by the presence (or absence) of new neurons. Previously, I hypothesized that the decision-making tendencies that reward TK rats on most versions of the PRL task might not be universally advantageous, potentially causing reduced curiosity and decreased awareness of novel cues. This theory could be confirmed with the identification of a PRL task in which curiosity is more rewarded, reversing the behavioral effect of neurogenesis so that TK rats would underperform compared to intact WT controls. To date, a pilot study using a restless bandit (Chen et al., 2021) has been so far unsuccessful. (The task was seemingly too difficult to note any behavioral differences within groups: all rats struggled equally to perform above chance.)

However, the eventual development of a similar task in which WT rats outperformed TKs would not only be exciting, but also incredibly useful in determining the functional relevance of neurogenesis ablation and the contexts in which it is beneficial or detrimental.

7.4 Conclusions

The mere existence of adult hippocampal neurogenesis was once a highly-contested issue; in fact, the field is not without controversy today. However, a growing wealth of evidence supports the. behavioral relevance of hippocampal neurogenesis and, specifically, the idea that new neurons play an important role in mediating responses to situational uncertainty or ambiguity. This dissertation adds to that evidence, demonstrating that adult neurogenesis plays a key role in probabilistic decision-making. I found that neurogenesis ablation conferred a distinct behavioral advantage in some, but not all, versions of a probabilistic reversal learning task, an effect driven primarily by superior decision-making strategy. Additionally, I demonstrated that the behavioral effects of neurogenesis ablation are not independent of sex, a finding of great clinical relevance given the long-suspected link between neurogenesis and depression, and the sex-specific clinical presentation of depression in humans. Furthermore, I proposed that new hippocampal neurons promote curiosity-like behaviors, increasing distractibility and decision noise, but allowing for greater exploratory behavior.

In summary, the exact function of newly-born granule cells – and the exact mechanisms by which they influence behavior – remain poorly understood. However, my dissertation work shows clearly that the behavioral effects of neurogenesis are not trivial. Future research can investigate the specific mechanisms by which newly-born neurons affect cognitive bias and decision-making and, further, the feasibility of harnessing neurogenesis as a therapeutic tool.

7.5 References

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