Early Olfactory Stimulation in Purpose-Bred Detection Dogs

by

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Abstract

Experiences during critical periods of early development have been shown to significantly impact cognitive development in dogs. However, the effect of early odor experiences on the ontogenetic development of cognition in dogs has yet to be explored despite the critical role of olfaction in a dog's perception of its environment. To address this question, we split purpose-bred detection dogs into three groups: 1) an early enrichment group, 2) an early imprinting group, and 3) a control group. Dogs in the early enrichment group received odor enrichment from 2-7 weeks of age while dogs in the early imprinting group received odor discrimination training from 2-6 months of age. All dogs were tested on the Delayed Search Task and the Detour Reversal Task at 3, 5, and 11 months of age to evaluate differences in cognitive development between the groups. Significant age effects were observed demonstrating development of cognitive abilities along with evidence suggesting that females outperform males on tasks measuring spatial discrimination abilities. A difference between the early imprinting group and the control group was also found suggesting that dogs in the early imprinting group have greater reward-driven arousal. Ultimately, larger sample sizes are needed to make conclusions about the effects of early odor exposure on canine cognitive development.

Table of Contents

List of Tables

List of Figures

Early Olfactory Stimulation in Purpose-Bred Detection Dogs

Olfaction is known to be the primary sensory modality utilized by dogs, allowing them to gather information about their surrounding environment (Hayes et al., 2018). While many species use olfaction to navigate their environment, dogs are specifically known for their high olfactory sensitivity (Walker et al., 2006), allowing them to be trained to detect a wide variety of stimuli including people, explosives, narcotics, and specific species of animals and insects (Furton & Myers, 2001). However, despite the importance of olfaction for dogs, little is known about how olfactory stimulation impacts other aspects of development, specifically cognitive development. Canine development has been shown to be influenced by many factors, including early life experiences (Foyer et al., 2013, 2014), handling procedures in post-natal development (Gazzano et al., 2008), and genetics (Persson et al., 2015). Specifically, when working with purpose-bred dogs, whether for service work or detection work, exposure tactics to various stimuli in early development are widely used in hopes of the dog developing a level of environmental soundness. Despite the benefit these puppy development programs provide for working dog and service dog training programs, many dogs still fail to graduate and perform the work they were bred to do, resulting in a loss of both time and resources (Lazarowski et al., 2018). These puppy development programs utilize a variety of methods, but one exposure tactic that has yet to be researched is olfactory enrichment. Given the fact that a dog's sense of olfaction is extremely powerful and plays a critical role in how dogs perceive their environment (Hayes et al., 2018), stimulation of this sensory modality in critical periods of early development may impact several facets of cognitive development.

Enrichment procedures are known to be beneficial for animals, specifically when the enrichment targets the dominant sensory modality for that species (Wells, 2009). Specifically, olfactory enrichment would be defined as the addition of odors to the environment of an animal to stimulate positive behaviors (Binks et al., 2018). Several studies have illustrated the positive impact of olfactory enrichment in adult dogs (Binks et al., 2018; Duranton & Horowitz, 2019; Graham et al., 2005), but no research has demonstrated the effect of olfactory enrichment in critical periods of early development. Critical periods for sensory development exist but vary based on the species, and alterations in sensory experiences during these critical periods can significantly impact sensory development (Berardi et al., 2000). Because the olfactory system tends to develop earlier than other sensory systems (Hudson & Distel, 1998), it is important to evaluate the effects of various olfactory inputs soon after birth to determine the critical period of development for the olfactory system. Several studies have demonstrated critical periods of development in the olfactory systems of rats and mice (Blais et al., 2006; Coopersmith & Leon, 1986; Meisami & Safari, 1981; Schmidt & Eckert, 1988), but none have been carried out in dogs. The purpose of this study will be to determine if exposure to odor at various points in the early development of purpose-bred detection dogs significantly impacts their cognitive development, which have both been shown to be indicators of career success in working dogs (Lazarowski et al., 2018; MacLean & Hare, 2018).

In the following sections, studies on the effects of early olfactory enrichment in rodents will be discussed. Research on olfactory enrichment in adult dogs as well as studies evaluating the impact of early life experiences during puppy development will also be discussed.

Early Olfactory Stimulation

Early research on the effects of early olfactory stimulation focused on variations in preferences related to odor enrichment, demonstrating that odor enrichment in early development can significantly impact the preferences of rodents. One study evaluated odor preferences preweaning, revealing that rats have a preference for maternal odor in a tilt-table odor preference test, which evaluates odor preference by measuring the time spent in the area of the table associated with a familiar odor compared to an area of the table with a non-familiar odor (Goldblatt, 1978). Another study analyzed the preferences of rats postweaning by presenting novel substances (i.e. sucrose and milk) to rats that experienced olfactory enrichment and rats that experienced standard laboratory rearing, demonstrating that olfactory enrichment caused rats to have a greater preference for novel foods than the control group (Hennessy et al., 1977).

Later studies moved past preference tests and evaluated other measures associated with early olfactory enrichment, including changes in odor sensitivity (Laing & Panhuber, 1980), physiological responses to odors (Coopersmith & Leon, 1986), and the effect of odor enrichment on stress behaviors (Dewaele et al., 2020). Other studies looked at neural responses and olfactory bulb development in response to odor enrichment. Schmidt & Eckert (1988) found that odor enrichment during the second week of development in mice significantly impacted the neural response of the olfactory bulb to the enrichment odor. Additional research demonstrated that early bulbar development benefits from olfactory enrichment (Martončíková et al., 2011) and is impaired by odor deprivation (Meisami & Safari, 1981).

Olfactory learning in early development was demonstrated to be one of the primary factors impacting odor responses, usually illustrated as a positive maternal association to the odor (Dewaele et al., 2020; Goldblatt, 1978; Sullivan & Leon, 1986). Blais et al. (2006) demonstrated that early maternal association with an odor not only impacted odor preference but also the rate at which the rats were conditioned and reconditioned to respond to the odor. Rat pups were exposed to aniseed odor from day 1 to day 20 by spraying the odors on the mothers. A preference test with aniseed was then conducted at day 21 and also at day 40. The rat pups exposed to aniseed approached the odor significantly more than the control group at day 21 but not at day 40, indicating that preference for an odor experienced during early development may be temporary. At day 41, both groups of rats were conditioned to choose aniseed in a Y-maze, and the experimental group learned the discrimination in the first test session whereas the control group did not. Once the rats met testing criteria, they were then reconditioned to respond to the odor either 5, 6, 7, 8, or 9 months after the initial training. A significant effect of group was found, indicating that the effect of early olfactory exposure is present at least 7 months from the early odor experience and 5 months from the last exposure to the odor. The experimental group was also significantly better at relearning the odor discrimination compared to the control group. This study not only demonstrates how evaluating the rate of reconditioning to an odor is preferable to odor preference tests but also that early olfactory enrichment and learning can have long-term effects.

Because neurogenesis in the olfactory bulb is continuous throughout the lifespan of an organism (Alonso et al., 2006; Johnson et al., 2013; Yamaguchi & Mori, 2005), it is beneficial to compare the effects of olfactory enrichment in adult rodents to the effects seen in rat pups to determine if any significant differences exist. The survival of neurons within the olfactory bulb of adult rodents was significantly dependent upon the level of olfactory input once the neurons had matured and formed synapses, but olfactory input did not impact the level of cell proliferation (Petreanu & Alvarez-Buylla, 2002; Rochefort et al., 2002; Yamaguchi & Mori, 2005). The level of olfactory stimulation also affected mitral cell area and density (Johnson et al., 2013). One study found that the survival of neurons in the olfactory bulb was only significantly altered when a learning task was associated with olfactory stimulation (Alonso et al., 2006). Rochefort et al. (2002) also evaluated the effects of olfactory enrichment on memory. Mice that experienced an odor enriched environment demonstrated behavioral responses indicative of odor recognition 4 times longer than the control group. The effect of interference was also analyzed by presenting the mice with one odor followed by a second odor and then presenting them with the first odor again. Mice in the experimental group spent less time investigating the first odor on the second exposure than they did on the first exposure whereas mice in the control group demonstrated no decline in investigation time on the second exposure. In another study, Rochefort & Lledo (2005) evaluated how long the increased odor memory due to olfactory enrichment was maintained once the enrichment was removed, and they determined that adult mice return to control levels after a period of 30 days without odor enrichment, demonstrating no long-term effects of olfactory stimulation in adults.

The plasticity of the olfactory system has led some researchers to question the relevance of a critical period of development in the olfactory bulb and conclude that olfactory development may be primarily dependent on current sensory experiences (Rochefort et al., 2002). Rochefort et al. (2002) claims that the survival and production of neurons in the olfactory bulb may switch from a preprogrammed state during development to being dependent on activation levels in adulthood. However, studies on early olfactory enrichment indicate that olfactory input in the first weeks of development demonstrated long-term effects (Coopersmith & Leon, 1986; Meisami & Safari, 1981; Schmidt & Eckert, 1988) in contrast to the short-term effects seen in adults (Rochefort & Lledo, 2005). These studies illustrate that the olfactory system most likely possesses a critical period early in development, and increased olfactory input during this period can significantly impact odor memory and neural development later in life.

Olfactory Stimulation in Dogs

Few studies have evaluated early odor experiences in dogs and their effect on development. One early study attempted to analyze the effect of anosmia on micturition behavioral development by placing cotton pellets in the nostrils of a puppy from 17 weeks to 24 weeks of age (Berg, 1944). However, the puppy only displayed minor differences from the control, likely due to the inability to properly control odor exposure. Later studies have evaluated the effect of early odor exposure on preferences in young puppies. One study found that puppies demonstrated a preference for familiar bedding (i.e. bedding that had been used by their litter) over unfamiliar bedding at around 2 weeks of age, but that this preference was no longer present when the puppies were older (Mekosh-Rosenbaum et al., 1994). Another study determined that a combination of prenatal and postnatal exposure to an odor resulted in odor preferences still being present 6 weeks after the last odor exposure (Hepper & Wells, 2006).

While few studies have been conducted on early olfactory stimulation in dogs, many studies have demonstrated the impact of early life experiences on behavioral development. Research has shown that factors such as parity, season of birth, litter size, and behavioral responses to various stimuli within the first year of development are significantly associated with temperament at a later age (Foyer et al., 2013, 2014). Other factors such as early environment and handling procedures during early development also significantly impact behavioral development (Gazzano et al., 2008). Auditory (Chaloupková et al., 2018) and audiovisual (Pluijmakers et al., 2010) enrichment procedures during early development have also been shown to impact behavioral development and fear responses in puppies. In working dogs, behavioral evaluations can demonstrate differences in successful dogs versus dropouts as early as 3 months of age (Lazarowski et al., 2018).

Several studies have also evaluated the effect of olfactory stimulation on adult dog behavior. Because a shelter environment can be an unstimulating and stress-inducing environment for a dog, several studies have illustrated the positive impact of olfactory enrichment on the behavior of shelter dogs, with several different odors increasing relaxing behaviors and decreasing vocalizations (Binks et al., 2018; Graham et al., 2005). Duranton & Horowitz (2019) looked at how participation in a nosework class altered pet dogs' emotional states. A group of pet dogs was split into groups, one receiving two weeks of nosework classes and the other receiving two weeks of heelwork classes. The classes were structured to have various levels to encourage owner participation, and materials were sent home with owners to allow for structured practice outside of class. The classes were balanced to control for the amount of owner interaction the dogs were receiving as well as the amount of food rewards the dogs were consuming. Before the classes began, all of the dogs were tested using the cognitive bias task to evaluate emotional state prior to the start of classes. The two groups did not differ significantly in their latency to approach the probe prior to class initiation. However, after two weeks of classes, the group of dogs participating in nosework classes displayed a significant decrease in their latency to approach the probe whereas the control did not differ significantly from their previous testing. These results demonstrate that increasing pet dogs' level of olfactory stimulation may cause them to have a more positive emotional state.

Other research has also demonstrated that scent-detection dogs show no significant difference in detection ability in high and low light intensity environments (Gazit & Terkel, 2003). Essentially, scent-detection dogs tend to rely strictly on olfaction when searching for odor even when visual cues are available. However, other studies have illustrated that pet dogs initially attempt to solve olfaction-based problems with alternative strategies rather than with olfaction (Polgár et al., 2015; Szetei et al., 2003). For example, even when visual cues were removed, pet dogs still tried to use the few visual cues that were available before they relied on olfaction to solve the problem (Polgár et al., 2015). Pet dogs also relied heavily on social cues and other strategies (i.e. win-stay strategies are seen when the dog chooses a certain location because they were rewarded there on the previous trial). However, research has shown that dogs receiving high levels of olfactory stimulation and odor training, such as detection dogs, tend to rely on olfaction to solve olfactory-based problems unlike pet dogs (Lazarowski et al., 2019).

The purpose of this study is to determine if various methods of odor presentations influence the cognitive development of a group of purpose-bred detection dogs. Dogs were split into three groups: early odor enrichment, early odor imprinting, and control group. Dogs in the early odor enrichment group received 25 sessions of odor enrichment from 2-7 weeks of age through experiencing odor presentations on Q-tips and on cotton rounds in aerated tins. Dogs in the early odor imprinting group received 36 sessions of odor discrimination training from 8-26 weeks of age and followed a set of training stages to ensure that dogs are imprinted on the target odor and consistently ignoring other distracting odors. Dogs in the control group did not experience odor enrichment or imprinting. All groups were tested on two spatial discrimination tasks at 3, 5, and 11 months of age to determine if the odor presentations impact cognitive development. The chosen tasks measure various aspects of executive function which is defined as the ability to appropriately respond and adapt to different environmental contexts (Olsen, 2018) and includes several components such as working memory, inhibitory control, attention, and cognitive flexibility (Diamond, 2013; Foraita et al., 2021). Figure 1 provides an overall timeline to illustrate the timing and duration of the two different types of early odor presentations and also demonstrate when testing occurs for each group. Because the experimental groups differ significantly from each other in experience, the early enrichment and early imprinting groups were separately compared to the control group and will not be compared to each other. For the early enrichment group, it is hypothesized that exposure to environmental stimuli during a critical period of development would positively impact environmental awareness and soundness, resulting in improved performance on spatial discrimination tasks relative to the control group. For the early imprinting group, it is hypothesized that experience on a discrimination task during adolescence would positively impact the development of executive function, leading to improved performance on the cognitive tasks relative to the control group.

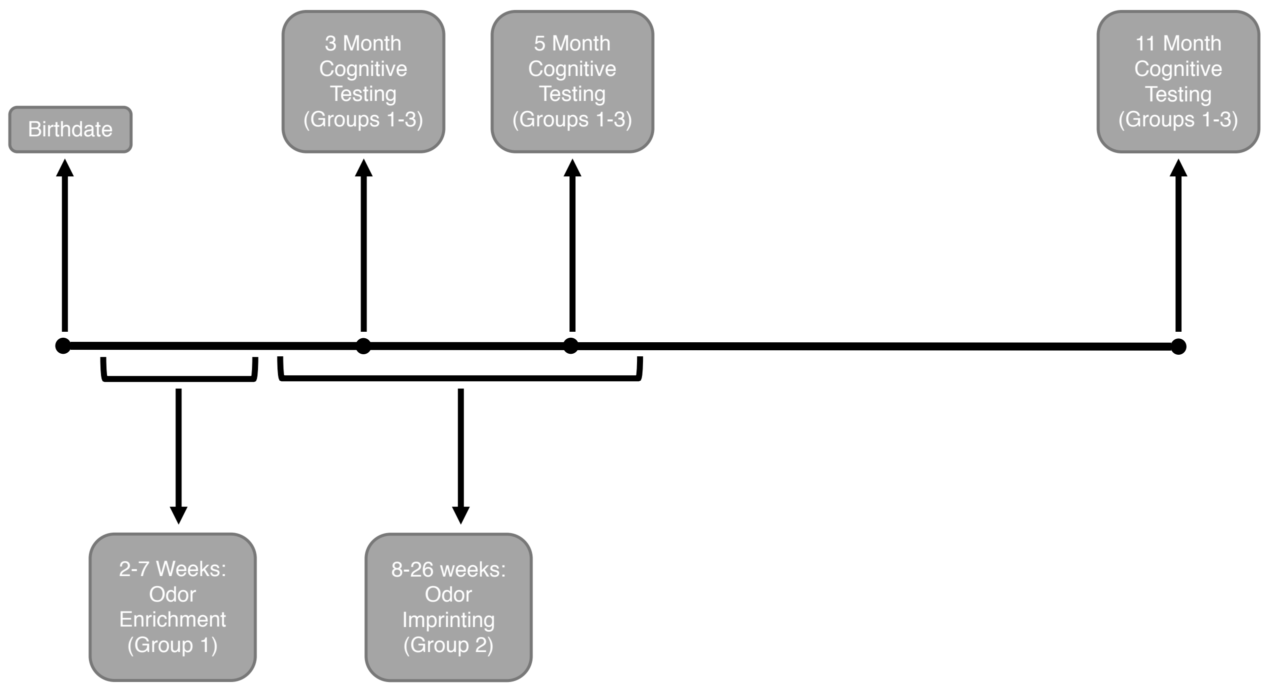

Figure 1: Timeline for odor presentations and testing for each group. Group 1 is the early odor enrichment group, Group 2 is the early odor imprinting group, and Group 3 is the control group.

Methods

Subjects

A group of 34 purpose-bred Labrador Retrievers bred and raised at Auburn University's Canine Performance Sciences (CPS) were used in this study. All study procedures were approved by the Auburn University Institutional Animal Care and Use Committee. The dogs were split into three different groups: 1) early odor enrichment group, 2) early odor imprinting group, and 3) control group. Dogs in the two experimental groups experienced various forms of odor presentations while the dogs in the control group received no odor presentations and advanced through the program normally. The enrichment (6 M, 5 F) and control groups (7 M, 4 F) are composed of 11 puppies while the imprinting group (7M, 5 F) consists of 12 puppies. However, one dog in the imprinting group was euthanized at 6 months of age due to medical complications so data was unable to be collected on him at the 11 month timepoint.

Stimuli

The 25 odors for the early olfactory enrichment group were presented separately on odorized Q-tips and on odorized cotton rounds in tins. The tins are 6.4 cm in diameter and are perforated with nine 0.2 cm holes to allow for odor dispersion (Figure 2). The Q-tips and cotton rounds were placed in airtight containers with each odor to ensure they are saturated with odor prior to testing. The odors used for enrichment are oils purchased from the Great American Spice Co. (Table 1). These odors were selected because our lab has frequently used these odors for odor learning and memory studies in adult dogs (Krichbaum et al., 2020).

Table 1

Odors used for olfactory enrichment.

Odor List				
Almond	Butter	Coffee	Maple	Pumpkin
Apricot	Butterscotch	Eggnog	Peach	Raspberry
Blackberry	Caramel	Grape	Peanut Butter	Strawberry
Black Walnut	Cherry	Lemon	Pecan	Tangerine
Blueberry	Chocolate	Lime	Pistachio	Watermelon

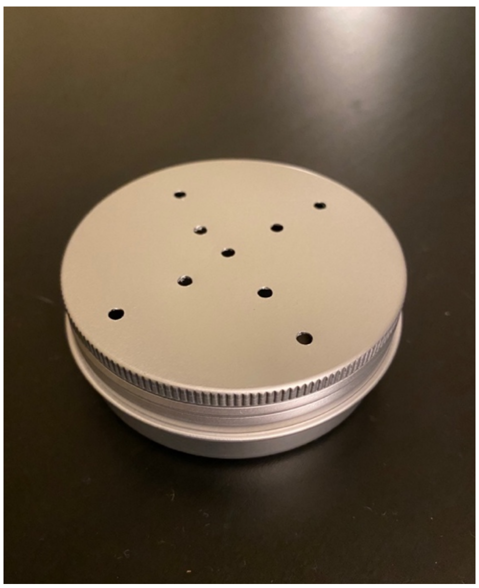

Figure 2: A perforated tin used for odor presentations.

For the early odor imprinting group, the target odor was double-base smokeless powder. In the later stages of training, distractor odors were used alongside the target odor and consisted of oils from the Great American Spice Co. along with other household products and chemical compounds (Table 2). The odors were stored in airtight containers and were presented to the dogs in perforated tins placed in PVC scent tube ports (Figure 3). The PVC scent tube ports utilize a 45 degree PVC elbow with a 10.2 cm diameter. The base of the scent tube is comprised of an 8.3 cm diameter open hub flange with ring drilled into a piece of wood that is 22.2 x 26.0 x

3.5 cm. The tin is placed in the bottom of the tube and a grate with an 8.3 cm diameter is placed over the tin.

Table 2

Odors used as distractor odors in the early imprinting group.

Distractor List				
Antacid	Black Walnut	Crayons	Lemon	Super Glue
Antibiotic Cream	Blueberry	Distilled White Vinegar	Peanut Butter	Tangerine
Aspirin	Butter	Eggnog	Pistachio	Watermelon
Baking Soda	Chocolate	Fish Oil Capsules	Raspberry	
Bicycle Tire Inner Tube	Coffee	Grape	Sodium Percarbonate	

Figure 3: PVC scent tube port used for odor imprinting.

Procedure

Early Odor Enrichment Group

The early odor enrichment group received passive presentations of odors from 2 weeks to 7 weeks of age (i.e. day 15 to day 49). Passive presentations of odor provide no feedback to the dog in the form of a reward while they are interacting with the odors and instead provide a form of olfactory enrichment for the puppies. Odor presentations occurred five times a week for a total of 25 enrichment sessions. Each session involved a randomly selected novel odor picked without replacement and two previously presented odors that were randomly selected with the exception of the first session which had one novel odor and the second session which had one novel odor and one previously presented odor. Previously presented odors were presented a maximum number of four times. The odors were presented to the puppies on Q-tips, and each odor was held a few centimeters in front of the puppy's nose for 10 – 15 seconds.

From 4 – 7 weeks of age, cotton rounds were placed into the perforated tins and presented to the puppies. The tins were placed in a 36.8 x 8.9 cm platform with 7.6 cm between each tin. Each puppy was tested separately in a 1.2 x 1.2 meter pen and was kept in the pen for 60 s. The experimenter stood inside the pen to decrease the chance that the puppy experienced separation anxiety, but if the puppy demonstrated extreme agitation (i.e. whining, yelping, and frantic movement in excess of 30 seconds), the experimenter knelt in the pen to avoid any undue stress to the puppy. The presentation of the tins occurred after the odors had been presented to the puppies on Q-tips and provided an opportunity for independent investigation of specific odors (e.g. visiting specific odors more often or spending more time at certain odors). Q-tips were changed in between puppies and two different tins were used to avoid the potential confound of odor marking.

Puppies in the control group and the early odor imprinting group were handled for the mean duration of the early odor enrichment sessions. These puppies did not experience odor presentations, but instead, the experimenter held the puppy to ensure that handling time is controlled for all subjects.

Early Odor Imprinting Group

Dogs in the early odor imprinting group received odor discrimination training from 8 weeks to 26 weeks of age (i.e. day 57 to day 182). The imprinting process involves the initial stages of teaching an odor discrimination (Helton, 2009) and usually involves training a behavioral response to a specified stimulus using operant conditioning. The other two groups of dogs (i.e. early olfactory enrichment and control groups) are not imprinted on the target odor until they are 10 months old. Puppies in the early odor imprinting group received biweekly training, for a total of 36 training sessions. Handling time for puppies in the control group and the early odor enrichment group was yoked to the duration of the sessions for each puppy in the early imprinting group to account for any variability in the session time experienced by the puppies in the experimental group. Puppies in the control group and the early odor enrichment group did experience odor presentations, but instead, the experimenter played or interacted with the puppy to ensure that handling time is controlled for all the puppies.

The early odor imprinting process occurred in several stages, and the dogs advanced through each stage once they met the criteria for their current stage. Dogs were rewarded with kibble throughout each session, and the amount of food given to the dog during each session was recorded and subtracted from their PM meal to ensure each dog was receiving the same amount of food. Each stage and associated criteria are listed below. A description of each stage can also be found in Table 3.

Stage 1 – Chamber: At 8 weeks of age, puppies were placed in a chamber made of wood (Figure 4). The box is 0.7 x 1.2 x 0.6 m with a PVC scent tube port secured 1.3 cm from the front of the box. A 57.2 x 70.0 cm sheet of plexiglass was cut to slide into the front of the front of the box to allow for a clear view of the dog during testing. An 8.5 cm diameter circle was cut out of the plexiglass and a 5.1 cm diameter circle was cut out of the front of the scent tube to allow the experimenter to have access to the inside of the scent tube during testing for the presentation of the food reward.

Stage 1 was divided into three substages. Stage 1a consisted of one session with the goal of creating an initial association between the target odor and the reward (i.e. kibble). The target odor was placed in the scent tube port at the front of the box, and a trial involved the puppy approaching the tube, hearing a click from a tin box clicker, and being rewarded with several pieces of kibble through the opening in the tube by Experimenter 1 (E1). After the puppy was rewarded, it was pulled back by Experimenter 2 (E2) to the opposite end of the box and released to approach the odor again. The session concluded after 5 trials.

Stage 1b followed a similar procedure as Stage 1a with the inclusion of a behavioral criteria, and each session consisted of 10 trials. In the second Stage 1b session, if the puppy was demonstrating targeted movement towards the port upon release (i.e. running towards the port, not sniffing the floor or the walls of the box as they move towards the port) in 8 out 10 trials, the puppy moved onto Stage 1c. If not, the puppy received additional sessions until criteria was met.

In Stage 1c, the dog was reinforced using a PetSafe Treat & Train® Remote Reward Dog Trainer placed in the front of the box next to the scent tube port to ensure the puppy learns that its reward will be delivered from the remote reward dog trainer and also to begin to shape a response to the odor (i.e. sticking head in scent tube port). The remote reward dog trainer releases pieces of kibble when a button on a remote is pressed by the experimenter, ensuring that the dog does not associate the reward with the experimenter. Each session consisted of at least 20 trials, with each trial involving the dog sticking its head in the tube, hearing a tone from the remote reward dog trainer, and being reinforced with kibble delivered by the remote reward dog trainer. More trials may be given in a session based on the discretion of the trainer to shape the response to the odor, but only the first 20 trials were used to determine if the dog met criteria for the session. In the second Stage 1c session, if the puppy was demonstrating a focused response of placing its head in the tube after receiving reinforcement and was not distracted by the remote reward dog trainer for 16 out of 20 trials, the puppy moved onto Stage 2. If not, the puppy received additional sessions until criteria was met.

Figure 4: The chamber used for the initial stages of odor imprinting.

Stage 2 – One Port: The puppy was placed in an enclosed and empty 3 x 5 m room with a single PVC scent tube port containing the target odor. The remote reward dog trainer was placed 1 m away from the scent tube port, and the starting position was placed between the remote reward dog trainer and the scent tube port so that the puppy was 0.5 m away from both. The puppy was released from a starting location to investigate the port and upon investigation was rewarded with food. After being rewarded, the puppy was allowed to return to the port, and upon investigation, the puppy was rewarded again. Each session included at least 20 trials, with each trial consisting of the puppy sticking its head in the tube and being rewarded from the remote reward dog trainer. More trials were given in a session based on the discretion of the trainer to shape the response to the odor, but only the first 20 trials were used to determine if the dog met criteria for the session. If the puppy immediately returned to the port after being rewarded for 16 out of 20 trials over 3 consecutive sessions, the puppy moved onto Stage 3. If not, the puppy received additional sessions until criteria was met.

Stage 3 – Two Ports: The set-up was similar to Stage 2, but in this stage, two scent tube ports were placed on the ground 0.5 m from each other. One port held the target odor while the other held a blank cotton round. The ports were arced so they were both 1 m away from the remote reward dog trainer. This stage is necessary to conclude that the puppies can differentiate between the odor of the cotton round (i.e. method of odor delivery for the target odor and distractor odors) and the target odor and that the puppies understand that the behavioral response is specifically associated with the target odor. When the puppy was released from the starting position, it was allowed to investigate both ports, but a reward immediately followed investigation of the port with the target odor. The position of the ports switched after three correct responses in one position, and a session consisted of 10 total switches. The number of trials in each session varied based on the dog's performance throughout the session, but only the first 20 trials were used to determine if the dog met criteria for the session. If the puppy remained at the port with the target odor upon investigation and ignored the other port for 16 out of 20 trials for 3 consecutive sessions, the puppy moved onto Stage 4. If not, the puppy received additional sessions until criteria was met.

Stage 4 – Three Ports: In this stage, three scent tube ports were used to increase searching behaviors and to introduce novel distractor odors (Table 2). The ports were placed in an arch so that they were all 2 m away from the starting point. The remote reward dog trainer was placed 0.5 m behind the starting point, and the ports were placed 1 m away from each other. One port held the target odor, another held a novel distractor, and the last held a blank cotton round. The puppy was released from the starting position and was allowed to investigate each port. Upon a significant change in behavior, defined as a reflexive-like response to a conditioned odor (Lazarowski, Krichbaum, DeGreeff, et al., 2020), at the target port, the puppy was rewarded. Each session included 24 trials, and the location of the port with the target odor was balanced for each position throughout the session. If the puppy displayed a freezing or hovering behavior at the target port (i.e. remains still or stares at target port for up to 2 seconds) and ignores the other ports for 20 out of 24 trials for 3 consecutive sessions, the puppy moved onto Stage 5. If not, the puppy received additional sessions until criteria was met.

Stage 5 – Five Ports with Food: In this stage, 10 pieces of kibble were placed in an aerated tin as a distractor in one of the ports to ensure that the puppy was able to inhibit responding to food. Five ports were present, with one port holding the target odor, one holding a novel distractor, one holding the kibble, one holding a blank cotton round, and the other port was empty. The ports were placed in an arch so that they were all 2 m away from the starting point and 1 m away from each other. The remote reward dog trainer was placed 0.5 m behind the starting point. The puppy was released from the starting point and was rewarded after displaying a freezing behavior (i.e. final response) at the target port. Some puppies developed a nose-poking behavior (i.e. shoving their head in and out of the tube rapidly) as their final response and were rewarded for this instead of the freezing behavior. Each session included 24 trials, and the location of the port with the target odor was balanced for each position throughout the session. If the puppy displayed a final response at the target port and ignored the other ports for 20 out of 24 trials for 3 consecutive sessions, the puppy moved onto Stage 6. If not, the puppy received additional sessions until criteria was met.

Stage 6 – 5 Ports: The set up for this stage was similar to Stage 5 except that food was removed as a permanent distractor and a previously experienced distractor odor (Table 2) was used instead. Food was used as the previously experienced distractor odor every 5th session to ensure the puppies continued to ignore food throughout training. The ports were placed in an arch so that all of them were 2 m away from the start point and 1 m away from each other. The remote reward dog trainer was placed 0.5 m behind the starting point. The puppy was released from the starting point and was rewarded after displaying a final response at the target port. Each session included 24 trials, and the location of the port with the target odor was balanced for each position throughout the session. The puppy continued to receive sessions at this stage until it reached 26 weeks of age.

Table 3

Descriptions of the odor imprinting stages

	Number of Ports	Odors Present	Criteria
Stage 1	1, in chamber	Target odor	Substages: 1a (initial imprinting), 1b (focused on tube, handfeeding); 1c (focused on tube, remote reward dog trainer)
Stage 2	1	Target odor	3 consecutive sessions, running to port for 16/20 trials
Stage 3	2	Target odor, blank cotton round	3 consecutive sessions, focusing on target odor for 16/20 trials
Stage 4	3	Target odor, novel distractor, blank cotton round	3 consecutive sessions, final response at target odor for 20/24 trials
Stage 5	5	Target odor, novel distractor, food, blank cotton round	3 consecutive sessions, final response at target odor for 20/24 trials
Stage 6	5	Target odor, novel distractor, previously experienced distractor, blank cotton round	None, maintenance training until 26 weeks old

Testing

To determine the impact of the various odor presentations on cognitive development, the dogs were tested on two cognitive tasks at 3, 5, and 11 months of age. The tasks used to measure the impact of early odor exposure on cognitive development were the delayed search task measuring attention (Krichbaum et al., 2021; Lazarowski, Krichbaum, Waggoner, et al., 2020) and the detour reversal task measuring behavioral flexibility and inhibitory control (Osthaus et al., 2010).

Delayed Search Task. The delayed search task (DST) involved an experimenter hiding a toy reward in one of three containers as the dog observed. The containers were placed in a 3 x 3 m arena with the containers placed equidistant from the start point (2 m) and 1 m apart from each other. E1 placed the reward in one of the containers and then positioned herself behind the center container facing the back wall. The dog was then released from the start point by E2 and the dog's choice was recorded. A stair-step procedure was used to incrementally increase the delay in which the dog had to wait before making a choice based on the dog's ability to meet an established criteria in a 6-trial block. Each dog started out at a 0 s delay, and if it chose correctly in at least 4 out of 6 consecutive trials, it advanced to a 15 s delay. Trials at a 0 s delay functioned as warm-up trials and demonstrated the dog's ability to perform at the most basic level of the task. If the dog was unable to meet criteria within the 6-trial block at a certain delay, the dog remained at the same delay for an additional 6-trial block. If criteria was not met at a certain delay during the second trial block at that delay, the task ended. Each session consisted of a maximum of 36 trials, and delays of 0, 15, 30, 45, 60, and 90 s were used. The location of the reward was balanced across the 3 positions in each trial block. Dependent measures for this task are maximum delay reached and number of trials to meet the warm-up criteria. If the dog met the warm-up criteria at the 0 s delay, it also completed an odor control to ensure that the dog was not using olfaction to find the reward. The odor control consisted of 6 trials in which the dog was removed or distracted from the arena while the reward was placed in one of the containers.

Average performance on the odor control at each age was compared to chance using one-sample *t*-tests.

Detour Reversal Task. The detour reversal task (DRT) involved the dog navigating around a barrier to reach an experimenter holding a reward. The dog was placed in a 4.6 x 4.6 m arena with a barrier placed 2 m from the starting point. The barrier was 3.6 m in length and 0.9 m in height, and it was positioned so that an opening was present between the barrier and the arena wall on either the right or left side. The starting side for the opening was balanced between dogs. E1 held the dog at the starting point which was marked off with a 1 x 1 m square on the ground while E2 sat behind the barrier with a toy reward. Once E2 called out to the dog, E1 released the dog from the starting point. A correct choice was marked as the dog stepping out of the starting point in the direction corresponding to the side related to the barrier opening. A criteria of 3 correct consecutive choices must be met during the acquisition phase of the task, and then the dog was removed from the arena while the barrier opening was switched to the opposite side of the room. The dog was then tested on the reversal phase of the task until a criteria of 3 correct consecutive choices was met. On either phase of the task, the dog was given a maximum of 15 trials to meet criteria. If a dog failed to meet criteria in 15 trials, the task ended. In addition, if a dog timed out (i.e. failed to navigate around the barrier in 30 s) 5 times on either phase of the task, the task ended. Choice was live-coded during the task but latency to cross the barrier opening, physical engagement with the barrier, and vocalizations were coded using video recordings (GoPro Hero 8). Dependent measures for this task are number of trials to criteria on the acquisition and reversal phases, total number of correct trials in the reversal phase, trial number of the first correct reversal, the difference score (i.e. first reversal trial latency – last acquisition trial latency), and the percentage of trials in which the dog touched the barrier or vocalized for both the acquisition and reversal phases.

Statistical Analyses

To determine if early odor exposures affect cognitive development, a series of linear mixed-effects models (LMERs) were used to evaluate the effect of sex, age, and group on the dependent measures associated with the DST and DRT. All interactions between group, age, and sex were also included as fixed effects. Subject ID nested within litter was included as a random effect, and an interaction between group and litter was also included as a random effect to account for variation in the group effect between litters.

For the dogs in the early imprinting group, Spearman's rank-order correlations were run between the number of training sessions to meet the Stage 5 criteria and dependent measures from the DST and DRT at the 5-month and 11-month timepoints. These correlations were conducted to determine if the ability to learn an odor discrimination is related to performance on cognitive measures during adolescence. Bonferroni corrections were not used due to preplanned hypotheses that less training sessions to meet the Stage 5 criteria would be directly correlated with better performance on both cognitive tasks (Armstrong, 2014). Data analyses were conducted using the R statistical program (RStudio, Version 1.1.456, Boston, MA, U.S.A) and SPSS Statistics (v.28, IBM, Armonk, NY, U.S.A).

Results

Early Odor Enrichment

Data was collected on 11 dogs in the enrichment group (6 M, 5 F) at 3 and 5 months of age from 6 litters and 7 dogs (4 M, 3 F) at 11 months of age from 4 litters. For the control group, data was collected on 11 dogs (7 M, 4 F) at 3 and 5 months of age from 5 litters and 6 dogs (4 M, 2 F) at 11 months of age from 3 litters.

Delayed Search Task

Figure 5 shows the relationship between age and maximum delay reached on the DST. For maximum delay reached, there was a significant effect of age found (LMER: $t(50.4) = 7.76$, $p < .01$) such that the maximum delay reached increases as dogs get older. Figure 6 demonstrates the difference between males and females on maximum delay reached. The effect of sex was significant (LMER: $t(49.7) = -2.09$, $p < .05$) demonstrating that females reach higher delays than males. All interactions were not statistically significant (ps > .05) and were removed from the model. The effect of group was not significant ($p > .05$).

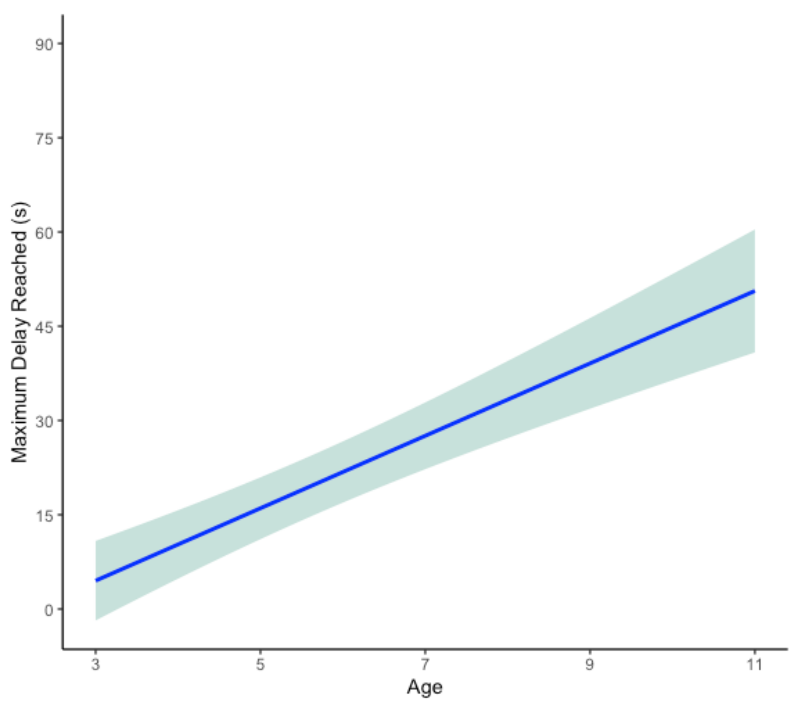

Figure 5: Effect of age on maximum delay reached on the DST for dogs in the early enrichment group and control group. Confidence intervals represent 95% confidence intervals (CI).

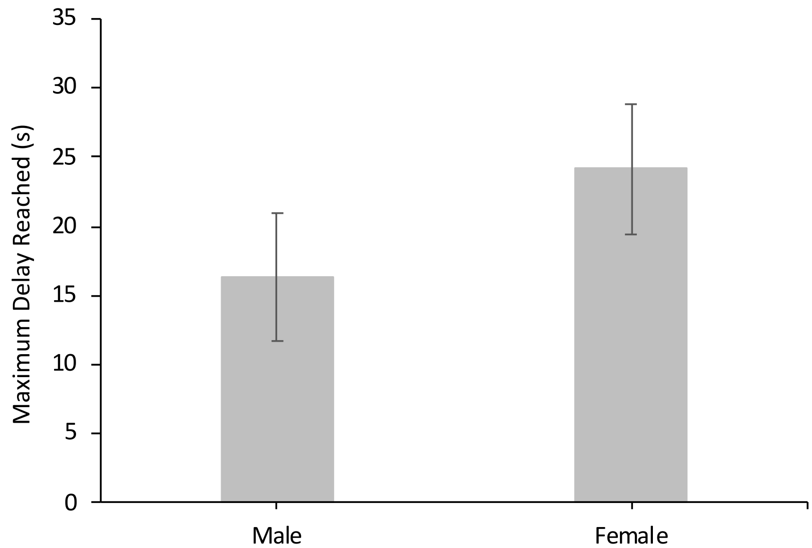

Figure 6: Difference between males and females on maximum delay reached on the DST for dogs in the early enrichment group and the control group. Confidence intervals represent standard error (SE).

In regard to the number of trials to meet the warm-up criteria, there was an effect of sex (LMER: $t(48.1) = 3.24$, $p < .01$) such that males require more trials ($M = 9.82$, SEM = 0.68) to meet the warm-up criteria than females ($M = 8.13$, SEM = 0.78). However, as illustrated in Figure 7, there was an interaction between age and sex ($p < .05$) so the effect of age on number of trials to meet the warm-up criteria was analyzed separately for males and females. The effect of age was significant in males (LMER: $t(22.8) = -6.68$, $p < .01$) such that the number of trials decreases as males get older. There was not a significant effect of age for females ($p > .05$). The other interactions were not significant (ps > .05) and were removed from the model. The effect of group and age were not significant (ps > .05).

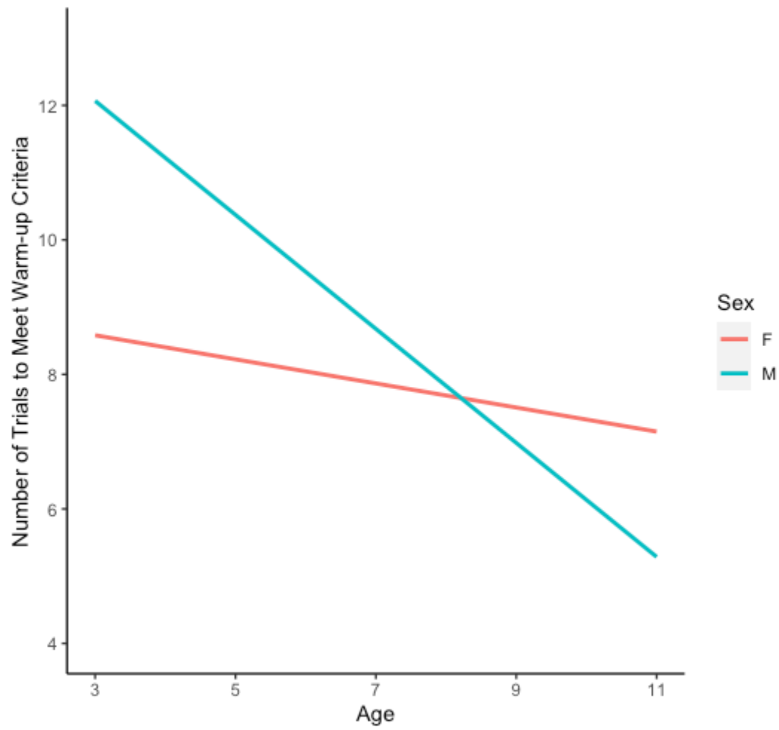

Figure 7: Interaction between age and sex for number of trials to meet the warm-up criteria on the DST for dogs in the early enrichment group and the control group.

Performance on the odor control trials was compared to chance, indicating that dogs were not performing significantly above chance on these trials at any age (ps > .05).

Detour Reversal Task

One dog in the enrichment group at 3 months of age met the maximum number of timeouts in the acquisition phase of the task so the data was removed from analyses. Two dogs in the enrichment group at 5 months of age were unable to meet criteria during the acquisition phase of the task so they were only included in analyses using dependent measures from the acquisition phase of the task. An error in the video recording for one dog in the enrichment group at 3 months of age prevented the calculation of the percentage of trials touched and vocalized during the reversal phase of the task so this dog was excluded from analyses involving those dependent measures.

Figure 8 shows the relationship between age and number of trials to criteria in both phases of the DRT. There was a significant effect of age on number of trials to criteria in both the acquisition phase (LMER: $t(47.7) = -2.53$, $p < .05$) and the reversal phase (LMER: $t(40.9) = -2.35$, $p < .05$) demonstrating the number of trials to criteria in both phases decreases as dogs get older. There was also a significant effect of age for total number of correct trials in the reversal phase (LMER: $t(44.2) = -2.91$, $p < .01$) such that the number of trials decreases as dogs get older. In all models, none of the interactions were significant (*p*s > .05) so they were removed from the models. The effect of group and sex were also not significant in any of the models (*p*s > .05).

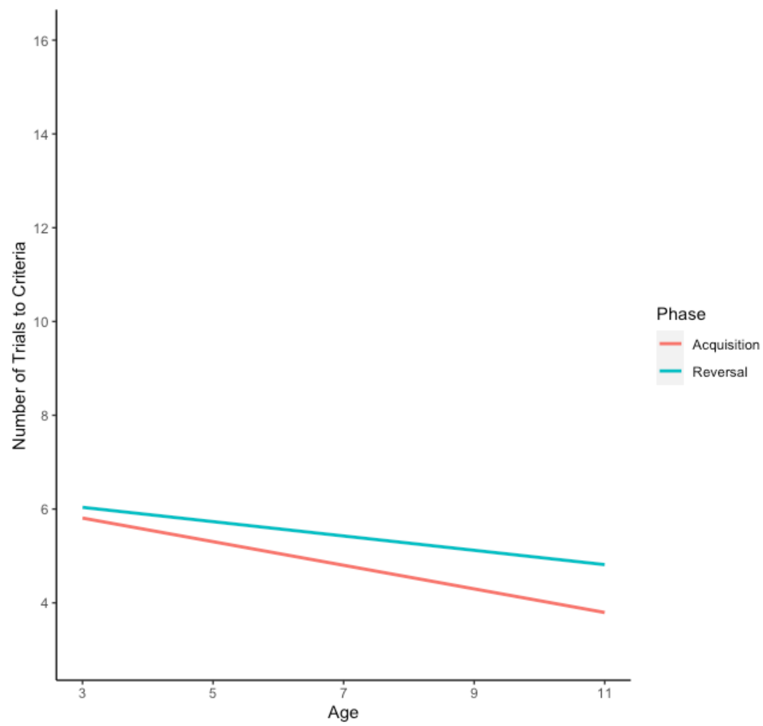

Figure 8: Effect of age on number of trials to criteria on the acquisition (red line) and reversal (blue line) phases of the DRT for dogs in the early enrichment group and control group.

Figure 9 shows the relationship between age and the percentage of trials when the barrier was touched in both phases of the DRT. There was a significant effect of age for percentage of trials when the barrier was touched in the acquisition phase (LMER: $t(45.1) = -3.87$, $p < .01$) and the reversal phase (LMER: $t(40.3) = -3.40$, $p < .01$) such that both percentages decrease as dogs get older. None of the interactions were significant in either model (*p*s > .05) and were removed from both models. The effect of sex and group were not significant for either dependent measure (*p*s > .05).

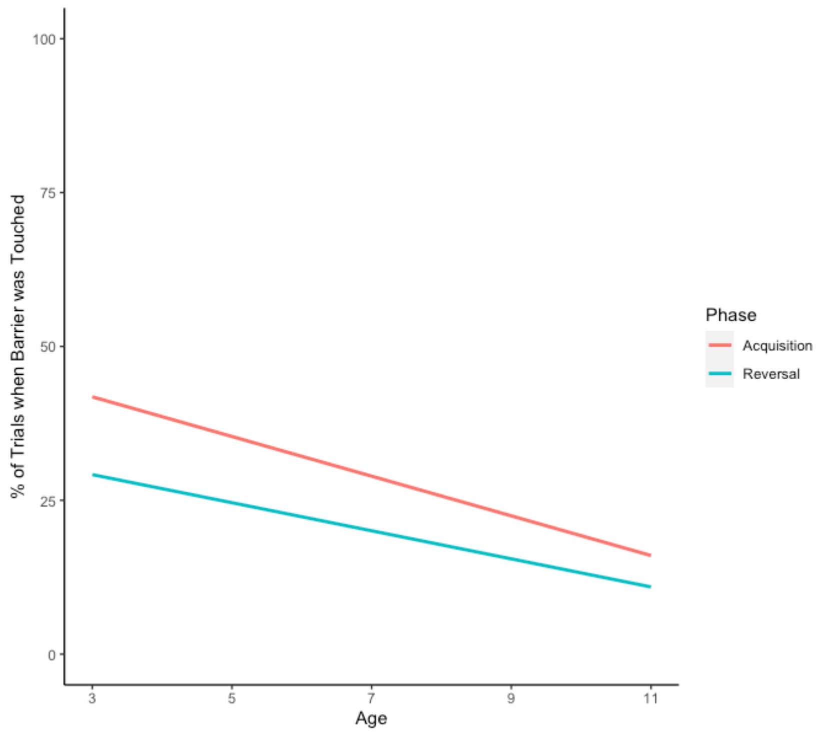

Figure 9: Effect of age on percentage of trials when the barrier was touched for both the acquisition (red line) and reversal (blue line) phases of the DRT for dogs in the early enrichment group and control group.

Figure 10 shows the relationship between age and the percentage of trials when vocalizations occurred in both phases of the DRT. There was a significant effect of age on the percentage of trials when vocalizations occurred in the acquisition phase (LMER: $t(42.4) = -2.38$, $p < .05$) and the reversal phase (LMER: $t(29.6) = -2.79$, $p < .01$) demonstrating that the percentage of vocalizations decreased in both phases as dogs aged. All interactions in both models were not statistically significant (*p*s > .05) and were removed from the models. The effect of group and sex were not significant in either model (*p*s > .05).

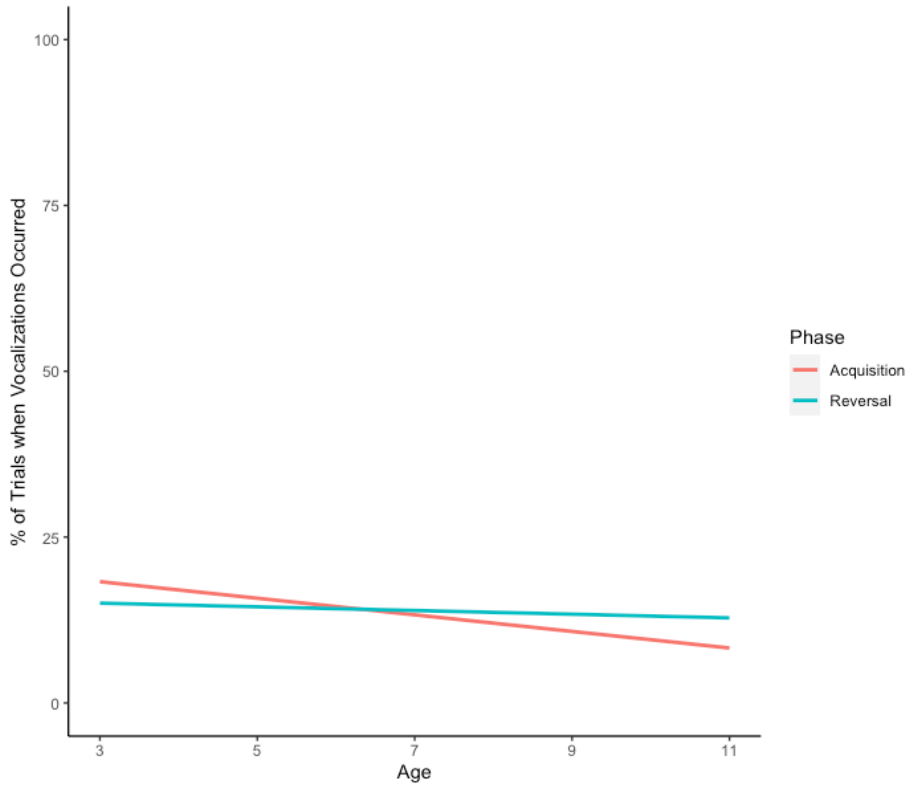

Figure 10: Effect of age on percentage of trials when vocalizations occurred for both the acquisition (red line) and reversal (blue line) phases of the DRT for dogs in the early enrichment group and control group.

For the difference score (first reversal trial latency – last acquisition trial latency), the interaction between age and sex was significant ($p < .05$) so the effect of age was analyzed separately for males and females. Figure 11 shows the interaction between age and sex on the difference score for the DRT. There was a significant effect of age on the difference score for males (LMER: $t(27.1) = 2.09$, $p < .05$) such that the difference score increases as males get older. There was no significant effect of age for females ($p > .05$). All other interactions were not significant (*p*s > .05) and were removed from the model. The effect of group, age, and sex were not significant (*p*s > .05).

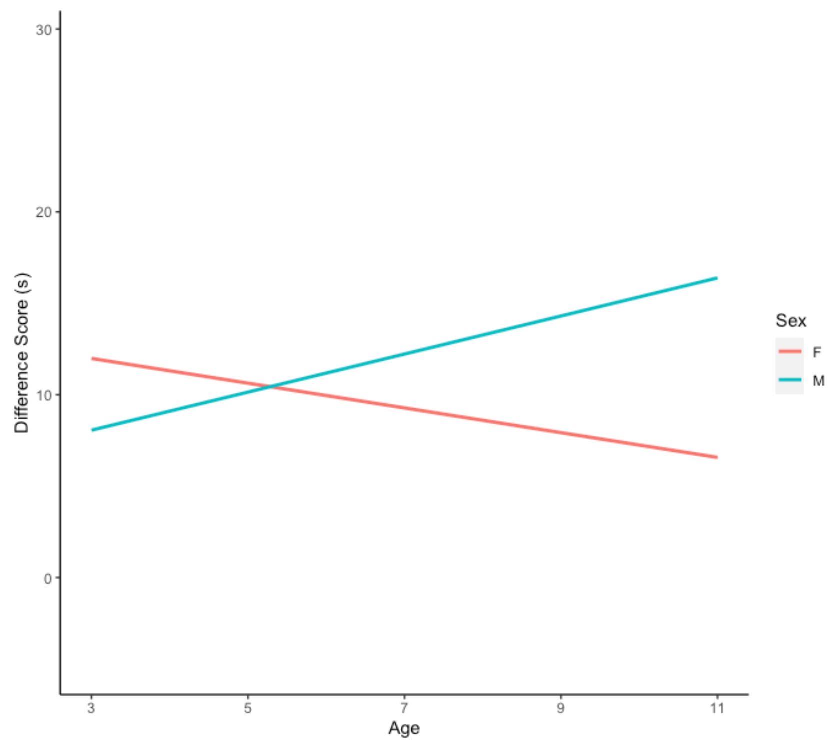

Figure 11: Interaction between age and sex for the difference score on the DRT for dogs in the early enrichment group and the control group.

For the first correct reversal trial number, no interactions were significant (*p*s > .05) so they were removed from the model. The effect of age, sex, and group were not significant (*p*s > .05).

Early Odor Imprinting

In the imprinting group, data was collected on 12 dogs (7 M, 5 F) at 3 and 5 months of age from 5 litters and 6 dogs (4 M, 2 F) at 11 months of age from 3 litters. The control group used in the analyses with the enrichment group was also used in analyses with the imprinting group.

Early Imprinting Training Stages

Number of training sessions to meet the Stage 5 criteria was not significantly correlated with maximum delay reached from the DST, number of trials to criteria in the reversal phase of the DRT, or the difference score from the DRT at the 5-month timepoint (*p*s > .31). First correct reversal trial number was negatively correlated with number of training sessions to meet the Stage 5 criteria at the 11-month timepoint ($r(4) = -.836$, $p < .05$) but was not significantly correlated at the 5-month timepoint ($p = .19$). Table 4 provides descriptive information regarding the average number of training sessions required at each stage for dogs in the early imprinting group.

Table 4

Average number of training sessions at each stage for dogs in the early imprinting group

	Average	SE	Range
Stage 1 (Minimum 5 sessions)	5.4	0.15	5 - 6
Stage 2 (Minimum 3 sessions)	3.6	0.29	3 - 6
Stage 3 (Minimum 3 sessions)	5.7	0.41	4 - 9
Stage 4 (Minimum 3 sessions)	4.4	0.45	3 - 8
Stage 5 (Minimum 3 sessions)	4	0.28	3 - 6

Delayed Search Task

Figure 12 shows the relationship between age and maximum delay reached on the DST. There was a significant effect of age for maximum delay reached (LMER: $t(53.2) = 5.72$, $p < .01$) indicating that as dogs get older, the maximum delay reached increases. Figure 13 demonstrates the difference between males and females on maximum delay reached. The effect of sex was significant (LMER: $t(39.2) = -2.22$, $p < .05$) such that females reach higher delays than males. All interactions were not significant (*p*s > .05) and were removed from the model. The effect of group was not significant ($p > .05$).

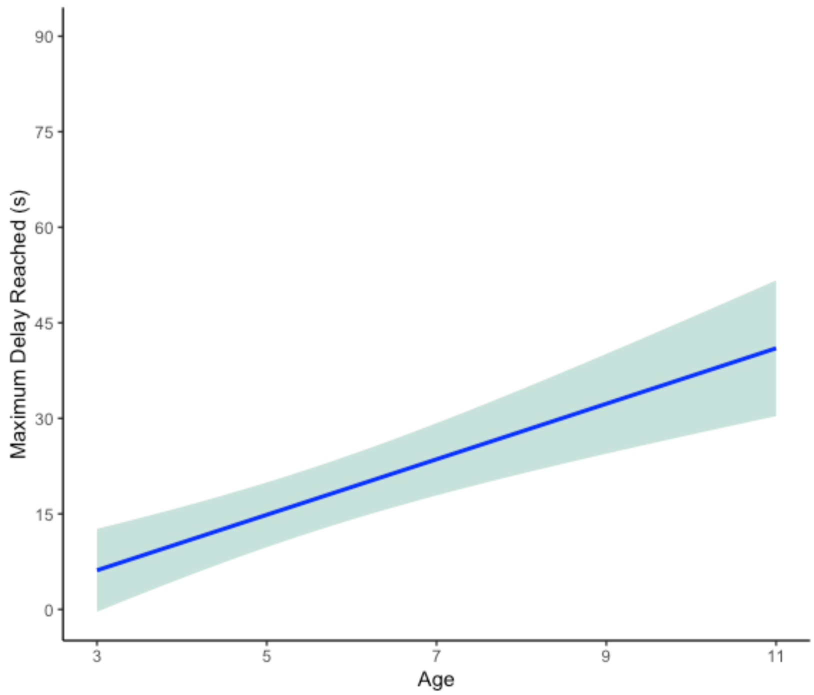

Figure 12: Effect of age on maximum delay reached on the DST for dogs in the early imprinting group and control group. Confidence bands represent 95% CI.

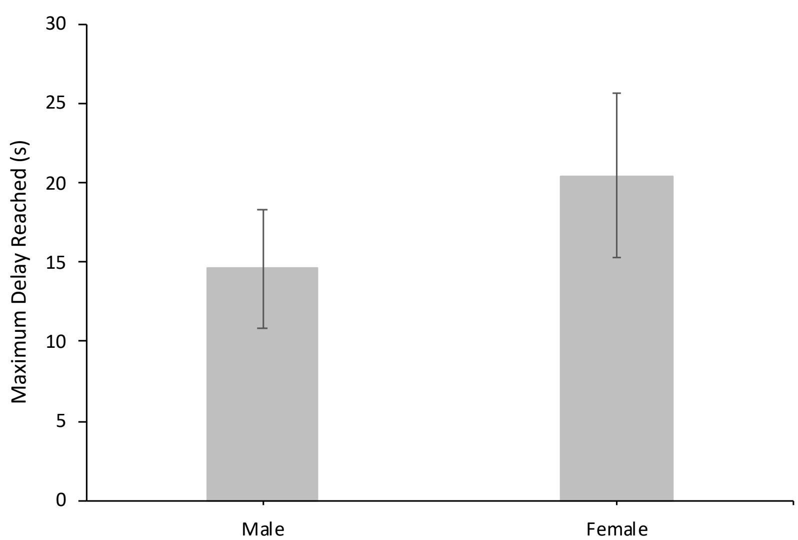

Figure 13: Difference between males and females on maximum delay reached on the DST for dogs in the early imprinting group and the control group. Confidence intervals represent SE.

Figure 14 shows the relationship between age and number of trials to meet the warm-up criteria on the DST. The effect of age was significant (LMER: $t(41.5) = -3.33$, $p < .01$) such that as dogs get older, the number of trials to criteria decreases. None of the interactions were significant (*p*s > .05) so they were removed from the model. The effect of group and sex were not significant (*p*s > .05).

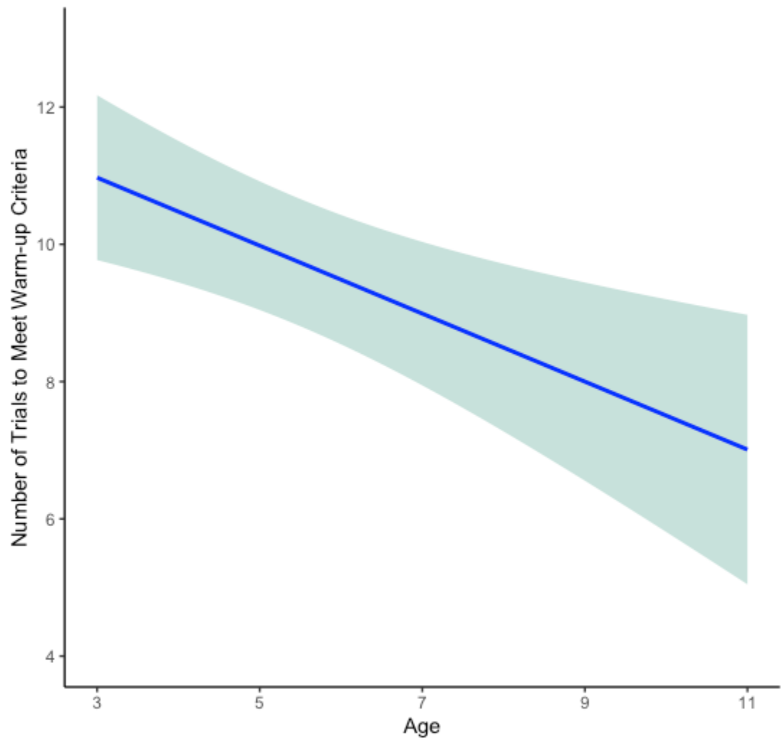

Figure 14: Effect of age on number of trials to meet the warm-up criteria on the DST for dogs in the early imprinting group and control group. Confidence bands represent 95% CI.

One-sample *t*-tests indicated that dogs were not performing significantly above chance on the odor control trials at any age (*p*s > .05).

Detour Reversal Task

Two dogs in the imprinting group at 3 months of age were unable to complete the acquisition phase of the task due to meeting the timeout maximum so their data was removed from analyses. Two other dogs in the imprinting group were unable to complete the reversal phase of the task, one at 3 months of age as a result of meeting the timeout maximum and another at 5 months of age that was unable to meet criteria in the allotted number of trials, so both dogs were excluded from analyses involving dependent measures associated with the reversal phase.

Figure 15 shows the relationship between age and the number of trials to criteria in both phases of the DRT. There was a significant effect of age on the number of trials to criteria in the acquisition phase (LMER: $t(32.3) = -2.95$, $p < .01$) and the reversal phase (LMER: $t(43.4) = -2.75$, $p < .01$) demonstrating that as dogs get older, they require less trials to meet criteria in both phases. The effect of age was also significant for the total number of correct trials in the reversal phase (LMER: $t(42.8) = -2.15$, $p < .05$) such that the number of trials decreases as dogs age. In all of the models, none of the interactions were significant (ps > .05) so they were removed from the models. The effect of group and sex were not significant in any of the models (ps > .05).

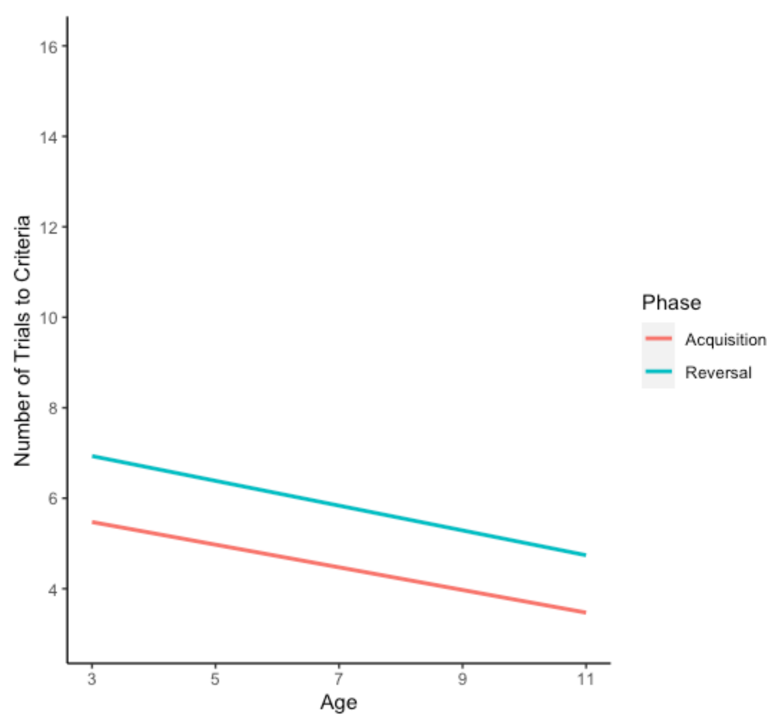

Figure 15: Effect of age on number of trials to criteria on the acquisition (red line) and reversal (blue line) phases of the DRT for dogs in the early imprinting group and control group.

Figure 16 shows the relationship between age and the percentage of trials when the barrier was touched in both the acquisition and reversal phases of the DRT. The effect of age was significant for the percentage of trials when the barrier was touched in the acquisition phase (LMER: $t(50.1) = -5.17$, $p < .01$) and the reversal phase (LMER: $t(47.8) = -4.10$, $p < .01$) such that as dogs get older, the percentage of trials in which the barrier is touched decreases in both phases. The effect of age was also significant for the percentage of trials in which vocalizations occurred in the reversal phase (LMER: $t(40.2) = -2.27$, $p < .05$) demonstrating that the percentage decreases as dogs get older. In all of the models, none of the interactions were significant (*p*s > .05) so they were removed from the models. The effect of sex and group were not significant in any of the models (*p*s > .05). In addition, for the percentage of trials in which vocalizations occurred in the acquisition phase, no interactions were significant (*p*s > .05) and the effect of age, sex, and group were not significant (*p*s > .05).

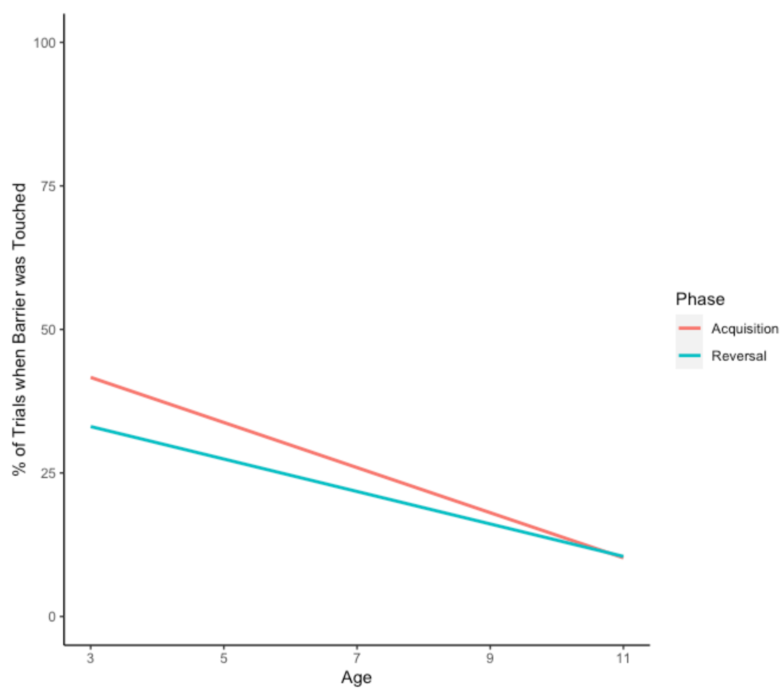

Figure 16: Effect of age on percentage of trials when the barrier was touched for both the acquisition (red line) and reversal (blue line) phases of the DRT for dogs in the early imprinting group and control group.

Figure 17 shows the difference between the imprinting group and the control group on the trial number of the first correct reversal on the DRT. There was a significant effect of group for first correct reversal trial number (LMER: $t(50.0) = -3.51$, $p < .01$) such that dogs in the control group required less trials to make their first correct reversal than dogs in the imprinting group. All of the interactions were not statistically significant (*p*s > .05) and were removed from the model. The effect of age and sex were not significant (*p*s > .05).

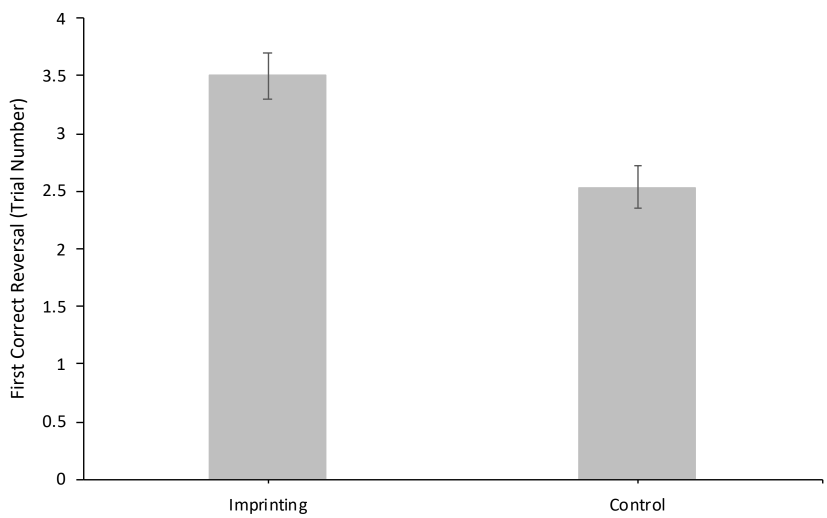

Figure 17: Difference between the imprinting group and the control group for first correct reversal. Confidence intervals represent SE.

For the difference score (first reversal trial latency – last acquisition trial latency), none of the interactions were significant (*p*s > .05) so they were removed from the model. The effect of age, sex, and group were not significant (*p*s > .05).

Discussion

In this study, our primary goal was to utilize various types of odor stimulation during early development in a group of purpose-bred detection dogs and evaluate their effect on cognitive development. Specifically, we wanted to see if passive exposure to odor enrichment in a critical period of early development positively impacted the development of spatial discrimination abilities, and we wanted to see if learning an odor discrimination task during adolescence significantly affected the development of executive function. Our results indicate that performance on both the DST and the DRT is significantly affected by the age of the dogs at the time of testing, demonstrating clear development of cognitive abilities within the first year of development. However, early odor experience did not appear to significantly influence performance on either task, with only one dependent measure indicating an effect of group membership when comparing the early imprinting group to the control group. In addition to these findings, we also found evidence for developmental differences between sexes in our sample. We discuss our results with respect to the different types of early odor experiences for each task along with the limitations associated with our findings.

Delayed Search Task

Comparison of the early enrichment group to the control group indicated that the ability to attend to the location of a hidden reward improved as dogs aged, demonstrated by the increase in the maximum delay reached on the DST as dogs got older. A similar developmental effect was observed in the early imprinting group and control group comparison, illustrated by an increase in the maximum delay reached along with a decrease in the number of trials required to meet the warm-up criteria for the DST as dogs aged. These results match previous findings associated with the development of canine cognition and the ability to attend to visually displaced objects over various delays (Bray et al., 2021; Lazarowski, Krichbaum, Waggoner, et al., 2020).

When comparing the early enrichment group to the control group, we found that females outperformed males on both dependent measures associated with the DST, with females reaching higher delays and requiring less trials to meet the warm-up criteria than males. While no studies have reported sex differences on this task specifically, another study utilizing a T-maze paradigm found that female dogs outperform males on a spatial learning and memory task (Mongillo et al., 2017). Female dogs also demonstrate better performance relative to males on tasks evaluating aspects of physical cognition and object permanence (Müller et al., 2011; Rooijakkers et al., 2009; Scandurra, Alterisio, et al., 2018). We observed a difference in developmental trajectory for males and females on this task as well. Males demonstrated a significant decrease in the number of trials to meet the warm-up criteria as they got older. While females required less trials to meet the warm-up criteria for the DST than males across all ages, they did not demonstrate a significant decrease for this dependent measure within the first year of development. This is likely due to the fact that females took less trials to meet criteria at the 3 month timepoint compared to males, so although the developmental trajectory for females on this dependent measure demonstrates a decrease in the number of trials as they get older, it is not statistically significant. Comparison of the early imprinting group to the control group also illustrated a similar differences between males and females as demonstrated by females reaching higher delays than males on the DST.

Detour Reversal Task

The number of trials to criteria in both phases of the DRT significantly decreased as age increased in the results comparing the early enrichment group and the control group. This same result was also observed in the comparison of the early imprinting group and the control group. This finding demonstrates that as dogs get older, they not only learn how to navigate around the barrier faster in the acquisition phase of the task, but they demonstrate greater behavioral flexibility and inhibitory control in the reversal phase of the task by requiring less trials to reach criteria. These results support other studies regarding the development of inhibitory control and reversal learning in dogs (Bray et al., 2021; Lazarowski, Krichbaum, Waggoner, et al., 2020). We also found in both the comparison between the early enrichment group and the control group and the comparison between the early imprinting group and the control group that the total number of correct trials in the reversal phase of the DRT decreases as age increases. This finding is likely due to the fact that dogs require less trials to reach criteria on the reversal phase as they get older, and as a result of this, the total number of correct trials decreases accordingly.

Comparison of the early enrichment group and the control group revealed that the percentage of trials in which dogs physically engaged with the barrier and vocalized in both phases of the tasks decreased as dogs aged. This finding illustrates that dogs are not only demonstrating greater inhibitory control with their ability to make a correct choice on the DRT as they get older, but they are also demonstrating it in the behaviors they exhibit during the trials. In a detour task utilizing a V-shaped fence, touching the barrier is considered inhibitory failure as dogs were required to navigate around the barrier to receive the reward (Bray et al., 2015). While touching the barrier was not considered an inhibitory failure in the DRT, dogs that do not physically engage with the barrier are ultimately demonstrating greater inhibitory control by instead navigating around the barrier. Barrier contact and vocalizations could also be considered a measure of frustration demonstrated by the dog completing the task. Upon learning how to navigate around the barrier, dogs usually go straight to the barrier opening, exhibiting a learned motor response (Osthaus et al., 2010). However, dogs that did not understand the task or how to navigate around the barrier would often jump on the barrier, paw the barrier, or express vocalizations. Therefore, a decrease in barrier contact and vocalizations suggests greater understanding of the task corresponding to the development of inhibitory control and spatial learning during the first year of development. The results associated with the comparison of the early imprinting group and the control group also reveal similar findings, demonstrating a decrease in the percentage of trials when the barrier was touched in both phases of the DRT and a decrease in the percentage of trials when vocalizations occurred in the reversal phase.

When comparing the early enrichment group to the control group, an interaction between age and sex demonstrated that the difference score for males increases as they age whereas there is no age effect observed for females. Because the difference score is calculated by subtracting the latency to cross the barrier opening for the last acquisition trial from the first reversal trial latency, a larger score indicates that a dog demonstrated longer perservation on the previously reinforced route. This finding suggests that performance on the DRT may be differentially affected by sex within the first year of development, with males displaying less behavioral flexibility and greater preservation as they reach 1 year of age. Alternatively, age does not significantly affect performance in females. Sex differences in spatial navigation have been observed in mammals, with males utilizing an allocentric navigation strategy (i.e. referring to positions of environmental landmarks) and females utilizing an egocentric navigation strategy (i.e. referring to motor responses) (Herman & Wallen, 2007; Scandurra, Alterisio, et al., 2018). While one study demonstrated better spatial learning and memory in female dogs compared to males, there was no effect of sex on the reversal learning portion of the task (Mongillo et al., 2017). In regards to spatial navigation strategies, male dogs demonstrate greater flexibility in switching from a preferred to a non-preferred strategy on a social learning task (Fugazza et al., 2017). However, a study using a plus-shaped maze required dogs to undergo a reversal-learning phase to evaluate flexibility in switching to a non-preferred navigation strategy (Scandurra, Marinelli, et al., 2018). Results indicated that the probability of successfully switching to a non-preferred navigation strategy decreases with age in males but increases with age in females. In another study with rats using a water maze task, experimenters tested navigation strategies used by both males and females when the platform was either submerged or visible (Kanit et al., 1998). They found that the strategy used by males was more efficient when the task remained unchanged, but the strategy used by females allowed for increased adaptivity in changing conditions. Temperament differences in dogs have also been observed between males and females (Scandurra, Alterisio, et al., 2018) and can ultimately impact cognitive performance (Bray et al., 2015, 2017; Marshall-Pescini et al., 2008). Specifically, differences in reward motivation have been observed in this population of dogs, with males exhibiting greater reward motivation than females (Lazarowski et al., 2021). An increase in reward motivation can subsequently elevate arousal for the reward, which could lead to a decrease in performance on tasks measuring inhibitory control (Bray et al., 2015). Therefore, this finding may be indicative of either developmental differences in temperament or behavioral flexibility associated with spatial learning abilities. This result was specific to the comparison between the early enrichment group and the control group and was not observed in the comparison between the early imprinting group and the control group.

In the comparison between the early imprinting group and the control group, dogs in the control group required less trials to make their first correct reversal than dogs in the imprinting group. This finding is opposite of what we hypothesized, suggesting a decrease in behavioral flexibility for the early imprinting group relative to the control group despite evidence indicating environmental enrichment, including experience with cognitively demanding tasks and environments, promotes cognitive and behavioral flexibility (Gelfo, 2019). One potential factor that could account for this group difference could be variations in reward-driven arousal. In a previous study using the DRT to measure cognitive development in this population of dogs, an increase in the latency to detour at 3 months was predictive of selection as a detection dog at 12 months (Lazarowski, Krichbaum, Waggoner, et al., 2020). This relationship was attributed to arousal associated with a higher motivation to obtain the reward, a trait selected for in detection dogs (Rooney et al., 2004). Dogs with a higher reward motivation would experience an increase in arousal for the reward, specifically when the detour route was reversed and the dog was initially prevented from obtaining the reward. As elevated levels of arousal have been shown to reduce inhibitory control as task difficulty increases (Bray et al., 2015), the difference between the groups could suggest that early imprinting dogs have higher reward motivation, as increased experience receiving a reward in a training scenario could elevate reward motivation in dogs selected for this trait. Training experience in general has also been shown to influence cognitive performance (Barrera et al., 2019; D'Aniello et al., 2015; Foraita et al., 2021; Marshall-Pescini et al., 2008, 2009; Scandurra et al., 2015), but the effect of training on performance appears to be a factor of the type of training dogs receive (Lazarowski, Thompkins, Krichbaum, et al., 2020).

Early Imprinting Training Sessions

Dogs in the early imprinting group demonstrated variability in the number of sessions they took to meet criteria at each of the training stages. On average, dogs reached criteria at Stages 1, 2, 4, and 5 within 1 session of the minimum number of training sessions required at that stage (i.e. if the minimum number of sessions at a specific stage was 3, dogs took around 4 sessions to meet criteria on average). In contrast, the average number of training sessions for Stage 3 was almost twice the minimum number of required training sessions. This finding is understandable considering Stage 3 is the first stage that requires dogs in the early imprinting group to make an active discrimination between two different stimuli. In general, it appears that dogs learning an odor discrimination at an early age can quickly learn how to shape a specific behavioral response in conjunction with a target stimuli (i.e. Stages 1 and 2), and although they initially require more training sessions to accurately discriminate between the target stimuli and a distractor (i.e. Stage 3), they rapidly learn how to discriminate their target odor from other novel distractors (i.e. Stages 4 and 5).

To determine if there was a relationship between the ability to learn an odor discrimination and other tasks measuring cognitive abilities, we ran correlations between dependent measures from the DRT and DST at 5 and 11 months of age and the number of training sessions it took each dog to reach the final training criteria (i.e. Stage 5 criteria) before transitioning into maintenance training (i.e. Stage 6). We found a negative correlation between the first correct reversal trial number on the DRT at the 11-month timepoint and the number of training sessions, demonstrating that dogs requiring more trials to make their first correct reversal on the DRT took less training sessions to reach criteria at Stage 5. Learning an odor discrimination task requires dogs to inhibit responding to any distracting odors and only provide a final response on their target odor. However, our results seem to indicate that better performance on an odor discrimination task is related to less inhibitory control as measured by the DRT. This result is likely due to other factors that influence performance on the DRT. Performance on cognitive tasks is known to be influenced by temperamental differences, including arousal and motivation for the reward (Bray et al., 2015; Lazarowski, Thompkins, Krichbaum, et al., 2020). Dogs with higher motivation for a reward would perform better on a discrimination task that rewards correct responses to specific stimuli. However, reward-driven arousal could lead to a decrease in performance on a task that requires inhibition of a response that has previously been rewarded. This finding could provide additional insight into the difference between the early imprinting group and the control group that was also observed for this dependent measure. While purpose-bred detection dogs experiencing odor discrimination training during adolescence may develop greater levels of reward motivation as a whole, additional within-group differences in reward motivation can also be observed.

Limitations

The major limitation of this study is the number of dogs in each group. In this study, all subjects, apart from group differences in early odor experience, experienced the same rearing and training experience. The use of whole litters in this study also allowed for some control of genetic variation in individuals and consequently, heritability of different traits. However, the subtle distinctions that could result from different experiences with odor during early development still require adequate sample sizes to conclude whether group differences truly exist.

The results of this study provide interesting insight into the effects of age and sex on canine cognitive development. While we observed some indication of an effect of odor discrimination training during adolescence on cognitive performance, our sample sizes are not large enough to make concrete conclusions about the effects of early odor exposure on the development of cognition in purpose-bred detection dogs. Ultimately, more dogs need to be added to each group to increase sample sizes and allow for better conclusions regarding the effects of early odor exposure.

Conclusions

In sum, our results extend the current findings on the ontogenetic development of canine cognition. By testing dogs on cognitive tasks multiple times during the first year of development, we observed significant increases in cognitive abilities as dogs reach 12 months of age. Our findings also illustrated several sex differences related to cognitive performance, with females outperforming males on tasks utilizing spatial discrimination abilities. While we did not find many effects of early odor exposure on cognitive development in dogs, our results suggest that dogs in the early imprinting group may demonstrate higher motivation for the reward relative to the control group. Variations in reward motivation also seemed to differentially affect dogs within the early imprinting group as demonstrated by a relationship between the number of training sessions required to meet criteria and the number of trials until the first correct reversal on the DRT. Ultimately, larger sample sizes are necessary to conclusively determine if early odor exposure affects cognitive development in dogs.

References

Alonso, M., Viollet, C., Gabellec, M.-M., Meas-Yedid, V., Olivo-Marin, J.-C., & Lledo, P.-M. (2006). Olfactory Discrimination Learning Increases the Survival of Adult-Born Neurons in the Olfactory Bulb. *Journal of Neuroscience*, *26*(41), 10508–10513. https://doi.org/10.1523/JNEUROSCI.2633-06.2006

Armstrong, R. A. (2014). When to use the Bonferroni correction. *Ophthalmic and Physiological Optics*, *34*(5), 502–508. https://doi.org/10.1111/opo.12131

Barrera, G., Alterisio, A., Scandurra, A., Bentosela, M., & D'Aniello, B. (2019). Training improves inhibitory control in water rescue dogs. *Animal Cognition*, *22*(1), 127–131. https://doi.org/10.1007/s10071-018-1224-9

Berardi, N., Pizzorusso, T., & Maffei, L. (2000). Critical periods during sensory development. *Current Opinion in Neurobiology*, *10*(1), 138–145. https://doi.org/10.1016/S0959-4388(99)00047-1

Berg, I. A. (1944). Development of behavior: The micturition pattern in the dog. *Journal of Experimental Psychology*, *34*(5), 343–368. https://doi.org/10.1037/h0055459

Binks, J., Taylor, S., Wills, A., & Montrose, V. T. (2018). The behavioural effects of olfactory stimulation on dogs at a rescue shelter. *Applied Animal Behaviour Science*, *202*, 69–76. https://doi.org/10.1016/j.applanim.2018.01.009

Blais, I., Terkel, J., & Goldblatt, A. (2006). Long-term impact of early olfactory experience on later olfactory conditioning. *Developmental Psychobiology*, *48*(7), 501–507. https://doi.org/10.1002/dev.20176

Bray, E. E., Gruen, M. E., Gnanadesikan, G. E., Horschler, D. J., Levy, K. M., Kennedy, B. S., Hare, B. A., & MacLean, E. L. (2021). Dog cognitive development: A longitudinal study across the first 2 years of life. *Animal Cognition*, *24*(2), 311–328. https://doi.org/10.1007/s10071-020-01443-7

Bray, E. E., MacLean, E. L., & Hare, B. A. (2015). Increasing arousal enhances inhibitory control in calm but not excitable dogs. *Animal Cognition*, *18*(6), 1317–1329. https://doi.org/10.1007/s10071-015-0901-1

Bray, E. E., Sammel, M. D., Seyfarth, R. M., Serpell, J. A., & Cheney, D. L. (2017). Temperament and problem solving in a population of adolescent guide dogs. *Animal Cognition*, *20*(5), 923–939. https://doi.org/10.1007/s10071-017-1112-8

Chaloupková, H., Svobodová, I., Vápeník, P., & Bartoš, L. (2018). Increased resistance to sudden noise by audio stimulation during early ontogeny in German shepherd puppies. *PLoS ONE*, *13*(5). https://doi.org/10.1371/journal.pone.0196553

Coopersmith, R., & Leon, M. (1986). Enhanced neural response by adult rats to odors experienced early in life. *Brain Research*, *371*(2), 400–403. https://doi.org/10.1016/0006-8993(86)90384-7

D'Aniello, B., Scandurra, A., Prato-Previde, E., & Valsecchi, P. (2015). Gazing toward humans: A study on water rescue dogs using the impossible task paradigm. *Behavioural Processes*, *110*, 68–73. https://doi.org/10.1016/j.beproc.2014.09.022

Dewaele, A., Badonnel, K., Persuy, M.-A., Durieux, D., Bombail, V., Favreau-Peigné, A., & Baly, C. (2020). Effect of environmental exposure to a maternally-learned odorant on anxiety-like behaviors at weaning in mice. *Animal Cognition*. https://doi.org/10.1007/s10071-020-01393-0

Diamond, A. (2013). Executive Functions. *Annual Review of Psychology*, *64*(1), 135–168. https://doi.org/10.1146/annurev-psych-113011-143750

Duranton, C., & Horowitz, A. (2019). Let me sniff! Nosework induces positive judgment bias in pet dogs. *Applied Animal Behaviour Science*, *211*, 61–66. https://doi.org/10.1016/j.applanim.2018.12.009

Foraita, M., Howell, T., & Bennett, P. (2021). Environmental influences on development of executive functions in dogs. *Animal Cognition*, *24*(4), 655–675. https://doi.org/10.1007/s10071-021-01489-1

Foyer, P., Bjällerhag, N., Wilsson, E., & Jensen, P. (2014). Behaviour and experiences of dogs during the first year of life predict the outcome in a later temperament test. *Applied Animal Behaviour Science*, *155*, 93–100. https://doi.org/10.1016/j.applanim.2014.03.006

Foyer, P., Wilsson, E., Wright, D., & Jensen, P. (2013). Early experiences modulate stress coping in a population of German shepherd dogs. *Applied Animal Behaviour Science*, *146*(1), 79–87. https://doi.org/10.1016/j.applanim.2013.03.013

Fugazza, C., Mongillo, P., & Marinelli, L. (2017). Sex differences in dogs' social learning of spatial information. *Animal Cognition*, *20*(4), 789–794. https://doi.org/10.1007/s10071-017-1099-1

Furton, K. G., & Myers, L. J. (2001). The scientific foundation and efficacy of the use of canines as chemical detectors for explosives1Invited paper for the special issue of Talanta 'Methods for Explosive Analysis and Detection'.1. *Talanta*, *54*(3), 487–500. https://doi.org/10.1016/S0039-9140(00)00546-4

Gazit, I., & Terkel, J. (2003). Domination of olfaction over vision in explosives detection by dogs. *Applied Animal Behaviour Science*, *82*(1), 65–73. https://doi.org/10.1016/S0168-1591(03)00051-0

Gazzano, A., Mariti, C., Notari, L., Sighieri, C., & McBride, E. A. (2008). Effects of early gentling and early environment on emotional development of puppies. *Applied Animal Behaviour Science*, *110*(3), 294–304. https://doi.org/10.1016/j.applanim.2007.05.007

Gelfo, F. (2019). Does Experience Enhance Cognitive Flexibility? An Overview of the Evidence Provided by the Environmental Enrichment Studies. *Frontiers in Behavioral Neuroscience*, *13*. https://www.frontiersin.org/article/10.3389/fnbeh.2019.00150

Goldblatt, A. (1978). Effects of early olfactory experience on a tilt table odor preference. *Behavioral Biology*, *22*(2), 269–273. https://doi.org/10.1016/S0091-6773(78)92335-0

Graham, L., Wells, D. L., & Hepper, P. G. (2005). The influence of olfactory stimulation on the behaviour of dogs housed in a rescue shelter. *Applied Animal Behaviour Science*, *91*(1), 143–153. https://doi.org/10.1016/j.applanim.2004.08.024

Hayes, J. E., McGreevy, P. D., Forbes, S. L., Laing, G., & Stuetz, R. M. (2018). Critical review of dog detection and the influences of physiology, training, and analytical methodologies. *Talanta*, *185*, 499–512. https://doi.org/10.1016/j.talanta.2018.04.010

Helton, W. S. (Ed.). (2009). *Canine ergonomics: The science of working dogs*. CRC Press/Taylor & Francis.

Hennessy, M. B., Smotherman, W. P., & Levine, S. (1977). Early olfactory enrichment enhances later consumption of novel substances. *Physiology & Behavior*, *19*(4), 481–483. https://doi.org/10.1016/0031-9384(77)90221-9

Hepper, P. G., & Wells, D. L. (2006). Perinatal Olfactory Learning in the Domestic Dog. *Chemical Senses*, *31*(3), 207–212. https://doi.org/10.1093/chemse/bjj020

Herman, R. A., & Wallen, K. (2007). Cognitive Performance in Rhesus Monkeys Varies by Sex and Prenatal Androgen Exposure. *Hormones and Behavior*, *51*(4), 496–507. https://doi.org/10.1016/j.yhbeh.2007.01.005

Hudson, R., & Distel, H. (1998). Induced Peripheral Sensitivity in the Developing Vertebrate Olfactory System. *Annals of the New York Academy of Sciences*, *855*(1), 109–115. https://doi.org/10.1111/j.1749-6632.1998.tb10552.x

Johnson, M. C., Biju, K. C., Hoffman, J., & Fadool, D. A. (2013). Odor enrichment sculpts the abundance of olfactory bulb mitral cells. *Neuroscience Letters*, *541*, 173–178. https://doi.org/10.1016/j.neulet.2013.02.027

Kanit, L., Taşkıran, D., Furedy, J. J., Kulali, B., McDonald, R., & Pöğün, Ş. (1998). Nicotine interacts with sex in affecting rat choice between “look-out” and “navigational” cognitive styles in the Morris water maze place learning task. *Brain Research Bulletin*, *46*(5), 441–445. https://doi.org/10.1016/S0361-9230(98)00008-2

Krichbaum, S., Rogers, B., Cox, E., Waggoner, L. P., & Katz, J. S. (2020). Odor span task in dogs (Canis familiaris). *Animal Cognition*, *23*(3), 571–580. https://doi.org/10.1007/s10071-020-01362-7

Krichbaum, S., Smith, J. G., Lazarowski, L., & Katz, J. S. (2021). Controlling for dogs’ (Canis familiaris) use of nonmnemonic strategies in a spatial working memory task. *Journal of Experimental Psychology: Animal Learning and Cognition*, *47*(3), 364–370. https://doi.org/10.1037/xan0000293

Laing, D. G., & Panhuber, H. (1980). Olfactory sensitivity of rats reared in an odorous or deodorized environment. *Physiology & Behavior*, *25*(4), 555–558. https://doi.org/10.1016/0031-9384(80)90121-3

Lazarowski, L., Haney, P. S., Brock, J., Fischer, T., Rogers, B., Angle, C., Katz, J. S., & Waggoner, L. P. (2018). Investigation of the Behavioral Characteristics of Dogs Purpose-Bred and Prepared to Perform Vapor Wake® Detection of Person-Borne Explosives. *Frontiers in Veterinary Science*, *5*. https://doi.org/10.3389/fvets.2018.00050

Lazarowski, L., Krichbaum, S., DeGreeff, L. E., Simon, A., Singletary, M., Angle, C., & Waggoner, L. P. (2020). Methodological Considerations in Canine Olfactory Detection Research. *Frontiers in Veterinary Science*, *7*. https://doi.org/10.3389/fvets.2020.00408

Lazarowski, L., Krichbaum, S., Waggoner, L. P., & Katz, J. S. (2020). The development of problem-solving abilities in a population of candidate detection dogs (Canis familiaris). *Animal Cognition*, *23*(4), 755–768. https://doi.org/10.1007/s10071-020-01387-y

Lazarowski, L., Rogers, B., Krichbaum, S., Haney, P., Smith, J. G., & Waggoner, P. (2021). Validation of a Behavior Test for Predicting Puppies' Suitability as Detection Dogs. *Animals*, *11*(4), 993. https://doi.org/10.3390/ani11040993

Lazarowski, L., Rogers, B., Waggoner, L. P., & Katz, J. S. (2019). When the nose knows: Ontogenetic changes in detection dogs' (Canis familiaris) responsiveness to social and olfactory cues. *Animal Behaviour*, *153*, 61–68. https://doi.org/10.1016/j.anbehav.2019.05.002

Lazarowski, L., Thompkins, A., Krichbaum, S., Waggoner, L. P., Deshpande, G., & Katz, J. S. (2020). Comparing pet and detection dogs (Canis familiaris) on two aspects of social cognition. *Learning & Behavior*, *48*(4), 432–443. https://doi.org/10.3758/s13420-020-00431-8

MacLean, E. L., & Hare, B. (2018). Enhanced Selection of Assistance and Explosive Detection Dogs Using Cognitive Measures. *Frontiers in Veterinary Science*, *5*. https://doi.org/10.3389/fvets.2018.00236

Marshall-Pescini, S., Passalacqua, C., Barnard, S., Valsecchi, P., & Prato-Previde, E. (2009). Agility and search and rescue training differently affects pet dogs' behaviour in socio-cognitive tasks. *Behavioural Processes*, *81*(3), 416–422. https://doi.org/10.1016/j.beproc.2009.03.015

Marshall-Pescini, S., Valsecchi, P., Petak, I., Accorsi, P. A., & Previde, E. P. (2008). Does training make you smarter? The effects of training on dogs' performance (Canis familiaris) in a problem solving task. *Behavioural Processes*, *78*(3), 449–454. https://doi.org/10.1016/j.beproc.2008.02.022

Martončíková, M., Lievajová, K., Orendáčová, J., Blaško, J., & Račeková, E. (2011). Odor enrichment influences neurogenesis in the rostral migratory stream of young rats. *Acta Histochemica*, *113*(3), 326–332. https://doi.org/10.1016/j.acthis.2010.01.002

Meisami, E., & Safari, L. (1981). A quantitative study of the effects of early unilateral olfactory deprivation on the number and distribution of mitral and tufted cells and of glomeruli in the rat olfactory bulb. *Brain Research*, *221*(1), 81–107. https://doi.org/10.1016/0006-8993(81)91065-9

Mekosh-Rosenbaum, V., Carr, W. J., Goodwin, J. L., Thomas, P. L., D'Ver, A., & Wysocki, C. J. (1994). Age-dependent responses to chemosensory cues mediating kin recognition in dogs (Canis familiaris). *Physiology & Behavior*, *55*(3), 495–499. https://doi.org/10.1016/0031-9384(94)90106-6

Mongillo, P., Scandurra, A., D'Aniello, B., & Marinelli, L. (2017). Effect of sex and gonadectomy on dogs' spatial performance. *Applied Animal Behaviour Science*, *191*, 84–89. https://doi.org/10.1016/j.applanim.2017.01.017

Müller, C. A., Mayer, C., Dörrenberg, S., Huber, L., & Range, F. (2011). Female but not male dogs respond to a size constancy violation. *Biology Letters*, *7*(5), 689–691. https://doi.org/10.1098/rsbl.2011.0287

Olsen, M. R. (2018). A case for methodological overhaul and increased study of executive function in the domestic dog (Canis lupus familiaris). *Animal Cognition*, *21*(2), 175–195. https://doi.org/10.1007/s10071-018-1162-6

Osthaus, B., Marlow, D., & Ducat, P. (2010). Minding the gap: Spatial perseveration error in dogs. *Animal Cognition*, *13*(6), 881–885. https://doi.org/10.1007/s10071-010-0331-z

Persson, M. E., Roth, L. S. V., Johnsson, M., Wright, D., & Jensen, P. (2015). Human-directed social behaviour in dogs shows significant heritability. *Genes, Brain and Behavior*, *14*(4), 337–344. https://doi.org/10.1111/gbb.12194

Petreanu, L., & Alvarez-Buylla, A. (2002). Maturation and Death of Adult-Born Olfactory Bulb Granule Neurons: Role of Olfaction. *The Journal of Neuroscience*, *22*(14), 6106–6113. https://doi.org/10.1523/JNEUROSCI.22-14-06106.2002

Pluijmakers, J. J. T. M., Appleby, D. L., & Bradshaw, J. W. S. (2010). Exposure to video images between 3 and 5 weeks of age decreases neophobia in domestic dogs. *Applied Animal Behaviour Science*, *126*(1), 51–58. https://doi.org/10.1016/j.applanim.2010.05.006

Polgár, Z., Miklósi, Á., & Gácsi, M. (2015). Strategies Used by Pet Dogs for Solving Olfaction-Based Problems at Various Distances. *PLoS ONE*, *10*(7). https://doi.org/10.1371/journal.pone.0131610

Rochefort, C., Gheusi, G., Vincent, J.-D., & Lledo, P.-M. (2002). Enriched Odor Exposure Increases the Number of Newborn Neurons in the Adult Olfactory Bulb and Improves Odor Memory. *The Journal of Neuroscience*, *22*(7), 2679–2689. https://doi.org/10.1523/JNEUROSCI.22-07-02679.2002

Rochefort, C., & Lledo, P.-M. (2005). Short-term survival of newborn neurons in the adult olfactory bulb after exposure to a complex odor environment. *European Journal of Neuroscience*, *22*(11), 2863–2870. https://doi.org/10.1111/j.1460-9568.2005.04486.x

Rooijakkers, E. F., Kaminski, J., & Call, J. (2009). Comparing dogs and great apes in their ability to visually track object transpositions. *Animal Cognition*, *12*(6), 789–796. https://doi.org/10.1007/s10071-009-0238-8

Rooney, N., Bradshaw, J., & Almey, H. (2004). Attributes of Specialist Search Dogs—A Questionnaire Survey of UK Dog Handlers and Trainers. *ASTM International*, *49*(2). https://www.astm.org/jfs2003048.html

Scandurra, A., Alterisio, A., Di Cosmo, A., & D’Aniello, B. (2018). Behavioral and Perceptual Differences between Sexes in Dogs: An Overview. *Animals : An Open Access Journal from MDPI*, *8*(9), 151. https://doi.org/10.3390/ani8090151

Scandurra, A., Marinelli, L., Lõoke, M., D’Aniello, B., & Mongillo, P. (2018). The effect of age, sex and gonadectomy on dogs’ use of spatial navigation strategies. *Applied Animal Behaviour Science*, *205*, 89–97. https://doi.org/10.1016/j.applanim.2018.05.010

Scandurra, A., Prato-Previde, E., Valsecchi, P., Aria, M., & D’Aniello, B. (2015). Guide dogs as a model for investigating the effect of life experience and training on gazing behaviour. *Animal Cognition*, *18*(4), 937–944. https://doi.org/10.1007/s10071-015-0864-2

Schmidt, U., & Eckert, M. (1988). The influence of early odour experience on the neural response of the olfactory bulb in laboratory mice. *Journal of Comparative Physiology A*, *163*(6), 771–776. https://doi.org/10.1007/BF00604054

Sullivan, R. M., & Leon, M. (1986). Early olfactory learning induces an enhanced olfactory bulb response in young rats. *Developmental Brain Research*, *27*(1), 278–282. https://doi.org/10.1016/0165-3806(86)90256-7

Szetei, V., Miklósi, Á., Topál, J., & Csányi, V. (2003). When dogs seem to lose their nose: An investigation on the use of visual and olfactory cues in communicative context between dog and owner. *Applied Animal Behaviour Science*, *83*(2), 141–152. https://doi.org/10.1016/S0168-1591(03)00114-X

Walker, D. B., Walker, J. C., Cavnar, P. J., Taylor, J. L., Pickel, D. H., Hall, S. B., & Suarez, J. C. (2006). Naturalistic quantification of canine olfactory sensitivity. *Applied Animal Behaviour Science*, *97*(2), 241–254. https://doi.org/10.1016/j.applanim.2005.07.009

Wells, D. L. (2009). Sensory stimulation as environmental enrichment for captive animals: A review. *Applied Animal Behaviour Science*, *118*(1), 1–11. https://doi.org/10.1016/j.applanim.2009.01.002

Yamaguchi, M., & Mori, K. (2005). Critical period for sensory experience-dependent survival of newly generated granule cells in the adult mouse olfactory bulb. *Proceedings of the National Academy of Sciences*, *102*(27), 9697–9702. https://doi.org/10.1073/pnas.0406082102