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# The Effects of Dorsal Lateral Telencephalon Lesions on Zebrafish Social Behaviour

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**Abstract**

Zebrafish are extremely social and aggregate in groups to form shoals. This social behaviour has been studied in the wild and in a laboratory setting, yet the mechanisms underlying the behaviour are unknown. There is evidence to suggest that the dorsal lateral telencephalon might play a role in shaping shoaling behaviour, being involved in modulating social behaviours and social reward associated with shoaling. In the current thesis, I adapted and combined several existing methods for performing lesions on the dorsal lateral telencephalon to create my own method to measure the role of the dorsal lateral telencephalon in social reward and shoaling behaviour. I predicted that the social reward associated with shoaling would be reduced by the lesions, thus that experimental fish would swim farther away from controls, which would disrupt the collective moment of the group.

Two experimental groups, a control group, and a sham-lesioned group were behaviourally tested. The groups involved one focal fish that either received a lesion, sham-lesion or randomly picked control swimming with four other fish that did not have surgery. The group's movements were tracked and recorded for 20 minutes over three consecutive days. Common measures of shoaling such as the nearest neighbour distance, inter-individual distance, and polarization were computed. Overall, as predicted, both experimental groups appeared to swim farther away from sham and control groups, although this effect was only seen in the first trial. There was no difference in polarization across groups. Overall, I found that the lesions to the dorsal lateral telencephalon have an effect on shoaling behaviour under certain conditions.

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# Chapter 1: Introduction

Zebrafish are a shoaling fish, meaning that they spend the majority of their time in groups, and are increasingly commonly used to study collective behaviour (Miller & Gerlai, 2011). They are also a common model organism in genetics and developmental biology (Howe et al., 2013). However, there has been very little study of the mechanisms that underlie social behaviours, such as shoaling, in fish. The purpose of this thesis was to explore one area of the zebrafish brain, the dorsal lateral telencephalon, and its role in shaping shoaling behaviour.

## 1.1 Social Learning and Social Cognition

Sociality is common across a wide variety of species and is defined as the degree to which animals interact or form long-term associations and complex social relationships with one another (Brakes, 2019; Wey, Blumstein, Shen, & Jordán, 2008). Sociality is found wherever the costs are exceed by the total benefits of association (Silk, 2007). Sociality provides both advantages and disadvantages to individuals, such as increased mating opportunities or enhanced foraging time due to a decrease in required vigilance. One disadvantage of grouping is increased competition between group members (Silk, 2007).

By being social, animals can acquire information about their environment by observing another conspecific or by directly interacting with them; this is called social learning (Hoppitt & Laland, 2013). Individuals that acquire information from conspecifics this way are known as ‘observers’, while their conspecifics and other group members are referred to as ‘demonstrators’ (Brown & Laland, 2003), though this distinction may mean little in the wild, where all individuals are likely to both

demonstrate and observe most of the time. Social learning has been widely studied in animals and is observed in almost every species that interacts socially (Brown & Laland, 2003). Social learning is beneficial for transferring environmental information between individuals and may minimize the costs, in time and effort, that an individual pays for the acquiring that information (Geng & Peterson, 2019). Social cognition, on the other hand, refers to the set of mechanisms used to develop, process, and react to the newly acquired or updated information derived from social interactions (Shettleworth, 2010; Frith, 2008).

**1.2 Zebrafish**

The zebrafish (*Danio rerio*) is a freshwater teleost, part of the Cyprinid family (Spence, Gerlach, Lawrence, & Smith, 2008). Zebrafish are found mostly in Myanmar, Bangladesh and northeastern India, but can also be found in southeast and south Asia (Engeszer, Patterson, Rao, & Parichy, 2007). *Danio* originates from *dhani*, which is Bengali and translates to “of the rice field” (Spence et al., 2008). Zebrafish are commonly found in shallow bodies of water next to rice paddies, where the water depth lowers the chances of predation (Spence et al., 2006). Zebrafish in the wild have been found in environments with a wide range of temperatures, pH levels, vegetation cover, and water flow (Graham, Keyserlingk, & Franks, 2018). They are most commonly found in slow moving waters that range from 24.6℃ to 38. 6℃ (Engeszer et al., 2007). Zebrafish are considered to be omnivorous and consume zooplankton, nematodes and a wide variety of insects that fall onto the surface of the water (Spence, Fatema, Ellis, Ahmed, & Smith, 2007b; McClure et al., 2006). In the wild, Zebrafish are often found in groups and the size of the group may depend on the speed of the water flow (Graham, Keyserlingk, & Franks, 2018). In fast-flowing water, large groups of ~300 fish have been observed; in

slow-moving water, groups of 6 to 7 fish are more common; and in still water, groups of up to 22 individuals are observed (Graham, Keyselingk, & Franks, 2018).

Zebrafish are usually less than 40 mm long (from the snout to the fork of the tail; Spence et al., 2008). The striped pattern seen on adult zebrafish starts to form during the juvenile stage, when melanophores, which form the dark stripes, and xanthophores and iridophores, which form the lighter areas, arrange themselves into a striped like pattern (McGown & Barsh, 2016). Zebrafish display some sexual dimorphism. Males are torpedo-shaped, leaner and their fins and belly are gold in colour; females have a more rounded belly and usually have very little gold on their undersides, sometimes none at all (Wixon, 2000; Fontana, Mezzomo, Kalueff, Rosemberg et al., 2018).

Within the past 40 years, zebrafish have rapidly become a useful model for research in vertebrates. They are inexpensive, easy to experimentally manipulate, small, and breed in large quantities (Kalueff & Cachat, 2016). Zebrafish have been used as a model to study development, as their embryo and early hatchling stage is transparent (Teame et al., 2019; Meyers, 2018). For example, zebrafish have been used as a model to study fetal alcohol syndrome (Bilotta, Barnett, Hancock, & Saszik, 2004) and cardiac development associated with human heart disease (Bakkers, 2011). Zebrafish have also been used to study regenerative properties. Unlike mammals, that have restricted regenerative properties for only a few organs and tissues, zebrafish are capable of fully regenerating several organs and tissues, including the heart (Raya et al., 2003; Poss et al., 2002), kidney (Reimschuessel, 2001), and spinal cord (Becker et al., 1998). They have also been used to study neurodegenerative diseases, such as scoliosis (Boswell & Ciruna, 2017) and Parkinson’s disease (Matsui & Takashi, 2017). Zebrafish are also a common

model organism in genetics; the zebrafish genome has been sequenced (Howe et al., 2013) and a range of studies using several genetic tools have followed (Howe et al., 2013, 2017). Zebrafish have also been used in behavioural studies to examine locomotion (Müller & van Leeuwen, 2004), and learning and memory in both larvae (Roberts, Bill, & Glanzman, 2013; Best et al., 2008) and adults (Gerlai, 2011, 2016; Sison & Gerlai, 2010).

Zebrafish utilize social learning and transmit social information in a number of different ways. For example, zebrafish that observe conspecifics show improved foraging success and specific place preference (Zala & Määttänen, 2013), faster escape times and a preference for the escape route from an artificial predator (Lindeyer & Reader, 2010), and faster learning in observational conditioning tasks (Suboski, Bain, Carty, McQuoid, Seelen & Seifert, 1990). Examples of social interactions that have been researched in zebrafish are social relationships (Larson, Malley & Melloni, 2006), social learning (Lindeyer & Reader, 2010), and social recognition and preference (Madeira & Oliveira, 2017; Al-Imari & Gerlai, 2007).

**1.3 Shoaling**

Shoaling is when a group of fish swim together in an unstructured way (Faucher et al., 2010). This behaviour in zebrafish is innate and a preference for shoaling with conspecifics is determined early on by visual and olfactory experience (Spence et al., 2008; Friedrich et al., 2010). Shoaling facilitates social learning (Moretz et al., 2007). Fish generally prefer to shoal to with other individuals that are of the same species, phenotype (such as a scale pattern), colour, size, and that are parasite free (Krause et al., 2000; Peichel 2004; Miller & Gerlai, 2011). For example, when zebrafish were presented with computer-animated conspecifics that varied in stripe pattern, they preferred to shoal

with the pattern that best matched their own. Therefore, stripes function as a shoaling cue (Rosenthal & Ryan, 2004). Zebrafish can also learn a preferred stripe pattern and prefer to shoal with conspecifics that have the same pattern as those they were raised with, independent of their own colouration (Spence et al., 2008). Qin, Wong, Seguin, & Gerlai, (2014) found that zebrafish shoaled with live stimulus fish and animated computer-generated zebrafish, but not with moving objects that were the same dimensions as a zebrafish but lacked specific features of a fish. Zebrafish can also recognize kin and prefer to shoal with familiar over unfamiliar kin, and with unfamiliar kin over conspecifics that are not kin (Spence et al., 2008). Kin are most likely recognized through olfactory cues (Spence et al., 2008). In addition, the attractiveness of a shoal – how motivated an individual is to join it – is influenced by how active the shoal is and its size (Spence et al., 2008). Zebrafish prefer to shoal with groups that are more numerous, though that preference is modulated by the activity level of the group (Pritchard, Lawrence & Krause, 2001). Male zebrafish prefer to shoal with females; females prefer to shoal with the larger group, independent of sex (Spence et al., 2008).

Shoaling is beneficial: it provides better mating selections and enhanced foraging opportunities (Engeszer, Ryan, & Parichy, 2004; Wright, Ward, Croft, & Krause, 2006). Shoaling also provides protection against predators by several mechanisms. One is the ‘dilution effect’: in a larger group, each individual has a lower chance of being targeted by a predator (Inman & Krebs, 1987). Similar-looking fish in large groups also confuse predators by making it challenging for the predator to focus on a single individual (‘predator confusion’; Krakauer, 1995). Finally, larger shoals have a better chance of detecting a predator, and detecting them earlier (the ‘many eyes’ hypothesis). However,

the detection rate eventually plateaus as group size increases (Roberts, 1995). Being in a group may also have costs, such as having to share any resources the group finds, direct competition for food (kleptoparasitism) or mates, and the possibility that predators preferentially target larger groups (Krause & Ruxton, 2002).

Zebrafish develop a tendency to shoal as soon as 6 days post-fertilization (Meshalkina et al., 2018), though there are some conflicting findings (Buske & Gerlai, 2011). Shoal cohesion intensifies between the first and third month of life, which means that distances between shoal members decrease over this time (Meshalkina et al., 2018; Buske & Gerlai, 2011). Larval zebrafish are able to shoal, but only show preferences for specific traits (see above) when they are juveniles; these preferences do not change once they are determined (Engeszer, Barbiano, Ryan & Parichy, 2007). When two-week old larvae were placed in a U-shaped area with a compartment at either end – a control compartment containing no fish, and an experimental compartment with fish of the same age – the larvae showed a weak preference for the compartment containing conspecifics (Dreosti et al., 2015). However, when the larvae were tested just one week later, at three weeks old, the preference for the social compartment was very robust (Dreosti et al., 2015). The adaptive reasons for shoaling being age-dependent are not known (Buske & Gerlai, 2011). It is possible that an increase in body size during development makes individuals more susceptible to detection by predators, or increases their need to forage on richer food patches, both of which would tend to promote shoaling (Buske & Gerlai, 2011).

### 1.4 Measures of Shoaling

In the lab, shoaling is commonly measured in one of two ways. The first technique is to allow the focal fish to freely swim with a group of conspecifics and measure their distances from one another (Qin et al., 2014); this is the method adopted for behavioural testing in the current thesis. The second technique is to separate a test fish from a group of conspecifics which acts as a social stimulus by a transparent barrier, and quantify the focal individual's response in terms of their distance from the conspecific compartment (Qin et al., 2014). This procedure has the advantage that the behaviour of the focal individual is easier to track and control, but is obviously far less natural than the alternative.

There are several standard measures of shoal cohesion in freely-interacting groups. The most common three in the literature, also used in this thesis, are the nearest neighbour distance (NND), inter-individual distance (IID), and polarization. While NND and IID are highly correlated, because they are both measurements of distances between group-members, they can vary independently, for example if a small internally cohesive subgroup splits off from the main shoal (Miller & Gerlai, 2011). The NND is the distance between a focal fish and the shoal member closest to them (Buske & Gerlai, 2011). Each fish only has one nearest neighbour, so this calculation is independent of shoal size (Buske & Gerlai, 2011). Generally, shoaling species swim approximately one body length away from their nearest neighbour (Faucher et al., 2010). The IID is the mean distance between a focal fish and all the other fish in the group; thus, this measurement is dependent on shoal size as well as on the distribution of the group (Buske & Gerlai, 2011). In the current thesis, all conditions consisted of 5 individuals, so IIDs can be directly compared between conditions and conditions. Both NND and IID measure the

tightness of a shoal (Miller & Gerlai, 2007). Finally, polarization is the degree to which all the members of the group are facing in the same direction (Miller & Gerlai, 2012). It is technically defined as the magnitude of the summed vector of the bearings of all the fish, and ranges from 0 to 1, with 1 indicating that the individuals in the group are all facing the same direction and 0 indicating the opposite. Polarization distributions of zebrafish groups tend to be bimodal (see below) and larger groups tend to be less polarized than smaller groups (Miller & Gerlai, 2012).

### 1.5 Schooling

Schooling is when a group of fish swim together in a highly polarized and synchronized manner (Faucher et al., 2010). Therefore, by definition, all schools are shoals (Faucher et al., 2010). Schooling increases protection from predation by, for example, enhancing predator confusion, and also improves the ability of the group to stay together while traveling (Kasumyan & Pavlov, 2018). Fish use their lateral line to coordinate their movements and spatial orientation while schooling (Kasumyan & Pavlov, 2018; Ghysen & Dambly-Chaudière, 2004; Montgomery, Coombs, & Baker, 2001). The lateral line is a sensory system found in all fish that allows them to sense changes in water pressure, such as those produced by other fish's movements, at close range, approximately 1-2 body lengths away (Butler & Maruska, 2016). The lateral line is separate from but connected to the fish auditory system, which can detect changes in pressure from a greater distance (Butler & Maruska, 2016).

Fish strongly prefer to be part of a school in which all the members are able to swim at a similar top speed. If a fish is too slow, it risks being separated from the group (Killen, 2017). Schools swim at a faster speed than shoals, but the fish are further apart

from each other (Gerlai, 2014). As noted above, groups' switching between schooling and shoaling leads to polarization distributions that are usually bimodal – one mode representing schooling (high polarization), the other shoaling (low polarization; Miller & Gerlai, 2012). There may be benefits to moving in one mode over the other under various circumstances, such as increased predator confusion or faster predator avoidance when in a school, or decreased foraging interference when in a shoal (Miller & Gerlai, 2007, 2008).

**1.6 Telencephalon Anatomy**

For this thesis, I focused on the dorsal lateral telencephalon of the zebrafish brain, a region often assumed to be homologous to the mammalian hippocampus, and explored its role in social behaviour.

The teleost brain is typically 0.4 - 2 mm thick and 4.5 mm long (Kotrschal, Van Staaden & Huber, 1998; Vernier, 2017; Friedrich, Jacobson, & Zhu, 2010). It can be divided into three main areas: the midbrain, the forebrain and the rhombencephalon. The telencephalon is located in the forebrain, along with the diencephalon (Yamamoto, 2009). The telencephalon itself can be divided into two regions: area ventralis and area dorsalis. There are five regions to the area ventralis: the supracommissural, the ventral, dorsal, lateral and commissural areas (Yamamoto, 2009). There are six regions to the dorsalis area: the nucleus taenia, central, ventral, dorsal and lateral, posterior areas (Yamamoto, 2009). The telencephalon plays an important role in vision, olfaction, memory, ingestion, and mating behaviour (Menke, Spitsbergen, Wolterbeek & Woutersen, 2011). Several studies have shown that the telencephalon is capable of regenerating after being lesioned

(Marz, Schmidt, Rastegar, Strahle, 2011; Kishimoto, Shimizu, Sawamoto, 2012; Ohnmacht et al., 2016; Skaggs et al., 2014).

Bshary et al. (2014) note that the dorsal lateral part of the telencephalon could be both homologous to the mammalian hippocampus, as well as being part of a larger social decision-making network (SDMN). The SDMN is thought to regulate social behaviour and decision-making in vertebrates and consists of two main components: the social behaviour network and the mesolimbic reward system (Bshary, Gingins, & Vail, 2014; Cabrera-Álvarez et al., 2017). The SDMN is composed of two main sub-networks: the social behaviour network and the basal forebrain reward system. Two structures are involved in both networks; the supracommissural part of the ventral pallium and the ventral part of the ventral telencephalon, which are homologous to the extended amydgala and lateral septum, respectively. Teles et al. (2015) presented two possible explanations of how social information is represented in the brain. The first theory, "functional specialization", hypothesizes that specific regions throughout the brain are necessary for specific cognitive behaviours (Teles et al., 2015). The second theory, "functional connectivity", hypothesizes that different cognitive abilities are subserved by networks of specific regions (Teles et al., 2015). Both theories could possibly be working in parallel.

Both *c-fos* and *egr-1* (both immediate early genes) are expressed in the dorsal lateral telencephalon, ventral telencephalic area, and the preoptic area when zebrafish are engaged in ostensibly social tasks, such as interacting with a single conspecific or a mirror, providing some evidence that these structures work in parallel to support social interactions, and supporting the "functional connectivity" hypothesis (Teles et al., 2015).

The dorsal lateral telencephalon has also been identified as part of the basal forebrain reward system, itself part of the mesolimbic reward system in teleost fish, which the hippocampus is part of in mammals (Bshary et al., 2014; O'Connell & Hofmann, 2011). The dorsal lateral telencephalon both receives and outputs information through structures that are also involved in the basal forebrain reward system. These are the posterior tuberculum, the medial part of the dorsal telencephalon, the central part of the ventral telencephalon and the dorsal part of the ventral telencephalon (Bshary et al., 2014). These areas are thought to be homologous to the mammalian ventral tegmental area, basolateral amygdala, striatum and nucleus accumbens (Bshary et al., 2014). In mammals, the hippocampus is often considered to contain a cognitive map of the environment (O'Connell & Hofmann, 2011). It is possible that the dorsal lateral telencephalon functions in a similar way, by modulating the memory of a rewarding stimulus. This is not yet known and will be examined in the current thesis. It has been argued that visual contact with a conspecific (Sison & Gerlai, Gómez-Laplaza & Gerlai, 2010, Al-Imari & Gerlai, 2008), locating a conspecific (Scerbina, Chatterjee, & Gerlai, 2012), or shoaling with conspecifics (Buske & Gerlai, 2011; Al-Imari & Gerlai, 2007) are all rewarding to isolated zebrafish, which is why reward-related areas of the brain are important in the regulation of social behaviours. It has also been noted that the dorsal telencephalon and related areas (the supracommissural nucleus of the ventral telencephalon, the periventricular nucleus of the posterior tuberculum, and the anterior tuberal nucleus) serve to process sensory information from the lateral line about the motions of nearby conspecifics (Butler & Maruska, 2016).

In mammals, the hippocampus is considered to be required for relational learning, such as spatial learning (Shapiro & Eichenbaum, 1999; Floresco, 2014). When the hippocampus is lesioned, mammals are impaired on spatial memory tasks (Morris, 1981; Clark, Broadbent, & Squire, 2007). In addition, the CA2 region of the hippocampus is specifically engaged in regulating social behaviour and memory (Hitti & Siegalbaum, 2014; Stevenson & Caldwell, 2014). Despite lacking a hippocampus that shares key anatomical features with mammals, specifically the tri-synaptic circuit (dentate gyrus-CA3-CA1; Salas et al., 2006), zebrafish can nonetheless successfully perform tasks that are hippocampus-dependent in mammals, such as spatial learning (Sison & Gerlai, 2010), place conditioning (Eddins, Petro, Williams, Cerutti, & Levin, 2009), episodic-like memory (Hamilton et al., 2016), and alternation memory tasks (Cognato et al., 2012). Goldfish with lesions of the lateral telencephalon show similar impairments in a trace-conditioning task to those produced by hippocampal lesions in mammals (Portavella, Torres, & Salas, 2004). Blocking NMDA receptors in the goldfish telencephalon, using the antagonist MK-801, leads to impairments in spatial learning (Gómez et al., 2006), paralleling similar findings in mice (Morris et al., 1986). Similarly, in zebrafish MK-801 reduces shoal cohesion and disrupts shoaling behaviour (Meshalkina et al., 2018; Echevarria, Hammack, Pratt, & Hosemann, 2008).

### 1.7 The current thesis

To the best of my knowledge, the role of the dorsal lateral telencephalon in shoaling and schooling of zebrafish has not been studied previously. In one of the few existing related studies, Shinozuka & Watanabe (2004) aspirated the dorsal lateral telencephalon of goldfish and measured shoaling behaviour using a common paradigm

(see above) in which the test fish is separated from a conspecific group by a transparent barrier. The researchers found no difference in shoaling tendency between fish with lesions of the dorsal lateral telencephalon, fish with lesions of the dorsal medial telencephalon, and sham-lesioned conditions.

My research focused on examining the effects of dorsal lateral lesions of the telencephalon and underlying mechanisms associated with shoaling behaviour in groups of zebrafish. Specific lesions of this area of the brain have only very rarely been performed in such small fish (e.g., Skaggs, Goldman, & Parent, 2014), and so a large part of my work consisted in developing and perfecting a robust lesion method (Chapter 2). I then placed single lesioned fish in groups of non-lesioned conspecifics and quantified their social behaviour over several days (Chapter 3).

**1.8 Summary**

Zebrafish spend a large amount of their time shoaling and schooling, yet the mechanisms associated with these behaviours remain poorly understood. There is evidence to suggest that the dorsal lateral telencephalon plays a role in managing and maintaining social behaviours in fish, and thus is likely to be involved with shoaling, possibly by modulating the social reward from shoaling. I hypothesize that lesioning the dorsal lateral telencephalon will reduce the social reward associated with shoaling, reducing zebrafish's motivation to shoal. I therefore expect to see lesioned fish swimming further away from their groups than controls. This may also lead to a broader disruption of the collective movement of the group, which may result in differences in schooling,

## Chapter 2: Development of Lesion Methods

The methods I developed for performing lesions of the dorsal lateral telencephalon are a mix of several methods found in the literature (see below). Some of these have been previously applied to zebrafish but most come from work on other, larger, fish (mostly goldfish), and had to be adapted for the much smaller zebrafish brain. Zebrafish were used in the current because they are extremely social, while goldfish have a weaker tendency to shoal (Magurran & Pitcher, 1982). Several different approaches – for example, how to properly restrain the fish during surgery – were tried and abandoned. Here, I present the final method, which was used for all the fish that then progressed to the behavioural experiments (Chapter 3).

### 2.1 Subjects

Wild-type adult zebrafish (*Danio rerio*) were obtained from a local pet store (Big Al's, Kitchener, ON). Before the experiment began, fish were housed in an automated high-density fish rank (Pentair Aquatic Habitats) in groups of 10. Adult zebrafish were used because of their tendency to naturally form tight and cohesive shoals. Fish were fed once or twice a day with either brine shrimp or commercially bought fish flakes. Lesioned and sham-lesioned fish were subcutaneously injected with unique colour combinations of visible elastomer tags (VIE, Northwest Marine Technologies) 5 days before the experiment began. Sex was not determined before testing. Fish were placed on a 12-hour light/dark cycle. The temperature of the water for housing was 25°C ± 2°C and the salinity was 500 - 600 ppm.

### 2.2 Surgery

Fish were food deprived for 24 hours before surgery began. On the day of surgery, fish were netted into a 5.1” x 2.8” x 2.6” knockout tank containing 200 ml of 125 mg/L MS-222 (Sigma-Aldrich) for approximately 10 minutes, one at a time. A tail pinch was used to test if the subject was anaesthetized. Once the fish was unresponsive to a tail pinch, they were moved to a 1.9” x 3” x 1.3” surgical chamber that was held in a stereotaxic apparatus. The chamber was packed with 30 g of aquarium sand wetted with the MS-222 solution to hold the fish in place. Each lesioned fish was injected over the course of 52 seconds with either 2 $\mu$L or 2.5 $\mu$L of 15 mM Quinolinic Acid (QA; amount based on Skaggs, Goldman, & Parent, 2014) into both their left and right dorsal lateral telencephalons. Sham-lesioned fish were injected with either 2 $\mu$L or 2.5 $\mu$L of sterile saline at the same locations. Injection location was determined relative to the position of the eyes as well as using the *Neuroanatomy of the Zebrafish Brain: A Topological Atlas* (Wullimann et al., 1996) as a guide. Injections were given at a depth of 2 mm below the surface of the skin (Skaggs et al., 2014). After injection, the fish was netted into a 20” x 10” x 12” recovery tank, which was half filled with system water and 60 g of ocean salt (Instant Ocean) to increase the salinity of the water to 1000 ppm. Following surgery, the subject’s behaviour was observed closely for recovery. Fish were netted back into their home tanks and watched for 5 days following surgery. All procedures were approved by the Wilfrid Laurier University Animal Care Committee (Animal Use Protocol R14007).

**2.3 Lesions**

Though exocitoxic brain lesions in fish are not common, they have been performed before in a few teleosts such as goldfish and zebrafish. Satio & Watanabe (2003) outlined procedural methods for precision stereotaxic surgery in goldfish. They

used ear bars fitted with rubber plates to hold the fish in place. To adapt this for zebrafish, because they are significantly smaller than goldfish, we used a custom surgery chamber and sand to hold the fish in place. In addition, Satio & Watanabe (2003) inserted a mouthpiece into the goldfish's mouth, to administer a constant flow of MS-222. My adaptation was to combine 5 ml of MS-222 with the sand in the surgery chamber, so that the sand to not only acted as an agent to hold the fish in place but also ensured that the MS-222 solution continued to flow through their gills. Several other options for doing lesions were considered, such as aspiration and electrical lesions, but we ultimately decided on an excitotoxic lesion given the easy accessibility of the required materials and the time restrictions. In addition, we believed that excitotoxic lesions were easier to perform. The novelty of excitotoxic lesions in zebrafish allowed us to adapt previous methods to construct a new method that worked efficiently for us.

Lesions were performed by injecting 2,3 Pyridinedicarboxylic acid (quinolinic acid, QA, Sigma-Aldrich) into the fish's brains. QA is an N-methyl-D-aspartate (NMDA) receptor agonist (Lugo-Huitón et al., 2013). QA causes neurotoxicity by increasing glutamate concentration, inhibiting its reuptake by astrocytes; this results in cell death (Lugo-Huitón et al., 2013; Vaz, Outeiro, & Ferreira, 2018). The mammalian hippocampus contains numerous NMDA receptors, making it susceptible to QA neurotoxicity; this is also true for the dorsal lateral telencephalon of teleost fish (Lugo-Huitón et al., 2013; Skaggs et al, 2014). In addition, we chose to inject QA because Skaggs et al. (2014) indicated an amount that made the most accurate lesion within the dorsal lateral telencephalon, which we used as a reference.

Skaggs et al. (2014) found that 2 days post-surgery a blood clot begins to form at the site of the lesion; at 7 days post-surgery, the brain tissue begins to repair itself, and by 14 days post-surgery, the site is significantly repaired. Their staining showed that radial glial stem cells migrate to the lesion site; this signifies the beginning of neurogenesis. Because of this ability of the zebrafish brain to regenerate lesioned areas, some of the fish in my experiment (group E2) were tested behaviourally between 4 and 6 days post-surgery and were euthanized on the 6$^{th}$ day following testing, to avoid imaging lesions that had begun to repair. I note that it is not known whether the regeneration of brain lesions in fish also restores the function of those brain areas.

**2.4 Conditions**

A total of 195 fish were run under one of four conditions (E1, E2, control or sham). We divided our experimental conditions into two separate conditions, E1 and E2. In condition E1 (n=7), the fish received 2 $\mu$L of 15 mM QA to their left and right dorsal lateral telencephalons to a depth of approximately 2 mm. During the three days of behavioural testing, these fish were paired with a group of conspecifics drawn each day from a tank containing approximately 10 fish. Thus, they were probably tested with at least some different fish each day. Fish in group E1 underwent behavioural testing about one month after surgery, making it possible that their lesion sites had regenerated by then.

In the second experimental condition, E2 (n=11), the fish received 2.5 $\mu$L of 15 mM QA into their left and right dorsal lateral telencephalons to a depth of approximately 2 mm. Each fish was housed in a tank with 4 control fish for 24 hours before the group was behaviourally tested, so that these fish underwent all their behavioural testing with

the same 4 conspecifics for all three trials. The first trial occurred on the 4$^{th}$ day post-lesion; trials continued until the 6$^{th}$ day post-lesion.

Fish in the sham condition (n=10) received an injection of sterile saline into their left and right dorsal lateral telencephalons to a depth of approximately 2 mm. The sham fish were also put into groups with 4 other fish that did not receive surgery 24 hours before they were behaviourally tested, and went through all their behavioural testing with those conspecifics. Their first trial occurred on the 4$^{th}$ day post sham-lesion; trials continued until the 6$^{th}$ day post sham-lesion.

The control condition was composed of 5 fish that did not undergo surgery. The first batch of control groups (n=6) were run similarly to group E1: fish were tested with partners from the same tank, but the tank contained more than 5 fish, making it impossible to know if the same fish were run in all three trials. The second batch of control groups (n=5) were run similarly to the fish in group E2: group of 5 fish were formed 24 hours before the fish were tested and went through all behavioural tests together.

# Chapter 3: Behavioural Experiments

## 3.1 Introduction

In my behavioural testing, groups of 5 fish each were placed into a circular tank and allowed to swim freely together for 20 minutes on three consecutive days. Groups of 5 fish were used because groups of 6 to 7 fish are commonly seen in the wild (see above). This approach, which allows fish to interact freely and swim together, has several obvious advantages when compared to other methods commonly used in the literature on shoaling, such as allowing a stimulus fish to interact with a group of conspecifics only through a transparent divider (Ferandes, Rampersad, Jones, & Eberhart, 2018), using replicas such as a robotic fish (Butail, Bartolini & Portfiri, 2013), or displaying computer generated conspecifics on a monitor (Saverino & Gerlai, 2008). These alternative methods are sometimes used when individuals cannot be tracked when swimming together. However, by tagging the fish and using automated tracking software, we were able to identify each focal fish in the group. I characterized movement within the group by several common measures: the nearest neighbour distance (NND), the interindividual distance (IID), and the polarization of the group.

## 3.2 Methods

### 3.2.1 Apparatus

Fish were housed in groups of 5 for at least 24 hours before behavioural testing began. Fish were assigned to one of three possible conditions: lesioned, sham-lesioned, or control. Lesioned conditions included one fish that had undergone the lesion surgery (see Chapter 2) and four other fish which did not have surgery. Sham-lesioned groups included one fish that had undergone the sham-lesion surgery (see Chapter 2) and four

other fish that did not have surgery. Finally, the control condition consisted of five fish that did not have surgery.

The testing arena was a circular white tank, 60 cm in diameter, filled to a depth of 9 cm with system water drawn from the housing rack. Trials were recorded using a video camera (Sony handycam FDR-AX100) mounted directly above the tank. A UV lamp was placed to one side of the testing tank, to make the fluorescent tags more visible. The test apparatus was surrounded by a white curtain and white walls. A TV monitor connected to the camera was placed outside the curtain, to monitor the trials. During the last 5 minutes of the trial, I videotaped the group for about 30 seconds with my iPhone and pointed with my finger to which fish was tagged, while doing so it is possible that I scared the fish. The videos helped identify which fish was the lesioned or sham-lesioned (tagged) fish; these videos were correlated to the data videos and made identifying the tagged fish for later coding much easier.

### 3.2.2 Procedure

Each day, for 3 consecutive days, fish were gently netted from their home tank into a bucket filled with system water drawn from the housing rack. They were then immediately netted into the testing tank and left there to interact for 20 min. At the end of the trial, the fish were netted from the testing tank into the bucket and then returned to their home tanks. Fish were fed at the end of the day, after all trials were complete. All trials were run between 10:00 and 17:00 h, during the light cycle.

### 3.2.3 Analysis

Videos were imported to a desktop computer and converted to shorten the video to 10 minutes, eliminating the first 5 minutes to account for the habituation period, and

the last 5 minutes. Videos were then tracked using *idTracker* (Perez-Escudero et al., 2014). The tracking software assigns unique identities to each fish. The tagged (lesioned or sham-lesioned) fish was then identified by eye with the help of the videos that I took on my iPhone during the trials. Specific characteristics, for example, body length and size were noted to assist in correct identification. I then noted the identity assigned by the tracking software to the tagged fish. In the control condition, where none of the fish were tagged, I chose a random individual to serve as the focal fish in each file. The data files containing trajectories were imported into RStudio and a custom script was used to extract our measures of shoaling (see Appendix A). NND and IID were calculated for each fish in each frame and then averaged across frames for each trial, giving a single score per individual per trial. Polarization was calculated for each frame of each trial and the distribution of values for each trial was saved. Movement in the third dimension (depth) was not tracked, which is common in such experiments (e.g., Miller & Gerlai, 2007). We did not predict that our lesions would alter fish's swimming depth.

Statistical analyses on these measures were conducted in R, Microsoft Excel, and *Mathematica* (*Wolfram Research*).A significance value of 0.01 was used for all analyses.

In the control condition, two trials had NND and IID values that appeared to be outliers. I used the influence.ME package (Nieuwenhuis, Pelzer & Grotenhuis, 2017) in R to calculate Cook's distance and used that to estimate the influence these data points might have on the mixed linear model. The appropriate cut-off distance was determined by dividing 4 by the number of trials across all conditions (117), which equals 0.03. The two data points under consideration both had distances exceeding this value, and were therefore removed from all further analyses.

Polarization distributions, which are usually bimodal (Miller & Gerlai, 2012), were subjected to a Maximum Likelihood Estimation procedure, to extract the modes of the distribution. Each distribution was fit with a model consisting of a single, two, or three, normal distributions. The best model was selected using the Akaike Information Criterion (which penalizes models that have more parameters). The best-fit values for the mean, variance, and amplitude of each component were saved and used for further analysis.

### 3.2.4 Histology

Once the third and final day of behavioural testing was completed, fish were euthanized with 250 mg/L of MS-222. 15 minutes after the last opercular movement, they were removed from the solution and sacrificed by decapitation just in front of their gills. The entire head of the fish was then post-fixed in 4% paraformaldehyde for 24 hours at $4°C$. Tissue was then preserved in increasing sucrose solutions (15%, 30%) for 72 hours in a fridge at $4°C$. After 96 hours the tissue was sectioned at 40, 50 and 60 microns using a cryostat at -20$°C$ onto slides that had been coated with gelatin. Slides were put in a $4°C$ fridge overnight to allow the optimal cutting temperature compound (OCT compound) on the slides to fully dry. The slides were stained approximately 24 hours later with a 0.5% cresyl violet solution and immediately cover-slipped with Permount (Fisher Chemical). Slides were then left to dry in a fume hood for 7 days before imaging. Photographs of the tissue were taken by microscope (Olympus BX43, with an attached XM10 camera and *cellSens* imaging software), at a magnification of 10x. Brains of fish from the control condition were also stained and imaged for comparison. Figure 3.1 shows sample slices from a lesioned, sham-lesioned, and control brain, as well as the

corresponding labeled slice from the *Neuroanatomy of the Zebrafish Brain: A Topological Atlas* (Wullimann et al., 1996). Appendix B gives sample slices from 7 individual lesioned fish (all from group E2). We could not identify a clear lesion in the remaining 3 fish.

**3.3 Results**

As noted above, two different batches of control and experimental conditions were treated differently from one another in the time between surgery and testing, the composition of conspecific conditions, and in details of the surgery. However, because the behavioural test all the conditions went through was the same, I wanted to see if it would be possible to combine the experimental and/or control groups into one condition. A Wilcoxon test was used, because of our small sample sizes, and because the data were not normally distributed. I found a significant difference between the experimental condition (E1 vs. E2) in both NND ($z = -3.38$, $p < 0.001$) and IID ($z = -3.11$, $p < 0.01$). There was no significant difference between the control condition for either NND ($z = -0.80$, $p = 0.42$) or IID ($z = -0.92$, $p = 0.36$). Due to this, data from the two experimental groups, E1 and E2, were analyzed separately; the control condition were combined into one group.

It was hypothesized that lesioned individuals would either be less motivated or less capable of maintaining normal movement patterns, and thus likely keep a greater distance from the other four fish in their group. It is also possible that the altered behaviour of the manipulated fish would have general effects on the collective movement of the group.

I first examined whether groups had different movement patterns as a function of condition (Experimental, Control, or Sham), or day (trial). Differences in NND and IID across conditions were tested using repeated measures mixed models. A total of 115 trials were available for analysis after removing two outliers (see methods). Trial and condition were entered into the model as fixed effects and group identity as a random effect. The best-fit models for both NND and IID did not include an interaction effect between trial and condition (comparison of AIC scores. NND: with interaction, 981.12; without interaction, 990.77; IID: with interaction, 1153.3; without interaction, 1162.3). For pairwise comparisons, all conditions were compared to controls, and trials 2 and 3 were compared to trial 1.

In the model for NND (Figure 3.2, left panel), condition E1 was significantly different from the control condition ($t(36) = 3.27$, $p = 0.002$), as was the sham condition ($t(36) = -2.69$, $p = 0.01$). Thus, lesions had an effect on NND: fish in the E1 condition were generally farther away from their nearest neighbours, compared to the control condition. Conversely, conditions containing sham-lesioned fish swam closer to their nearest neighbours than fish in the control condition. Condition E2 was not significantly different from control ($t(36) = -0.77$, $p = 0.44$). In addition, there was a significant effect of the trial, with trials 2 and 3 being different from trial 1 (Trial 1 vs. 2: $t(73) = 2.43$, $p = 0.002$; trial 1 vs. 3: $t(73) = 3.48$, $p = 0.0008$) trials. Thus, as trials progressed, fish moved farther away from their nearest neighbors, as expected. Post-hoc testing revealed a significant difference between the two experimental conditions ($t(35) = 3.98$, $p = 0.002$) and between group E1 and the sham-lesioned condition ($t(35) = 5.63$, $p < 0.0001$).

In the model for IID (Figure 3.2, right panel), condition E1 was significantly different from controls ($t(37) = 3.34$, $p = 0.002$) but the sham condition was not ($t(37) = -2.07$, $p = 0.046$), and neither was the E2 condition ($t(37) = -0.26$, $p = 0.79$) . The third trial was significantly different from the first ($t(74) = 3.04$, $p = 0.003$), but trial two was not ($t(74) = 1.87$, $p = 0.06$). Post-hoc tests revealed, as for the NND, a significant difference between the two experimental conditions ($t(35) = 3.60$, $p = 0.005$) and between group E1 and the sham-lesioned condition ($t(35) = 5.15$, $p = 0.0001$).

Next, I explored differences in polarization between conditions. The polarization of a group is a measure of the degree to which all the fish are moving in the same direction. It is therefore not calculated at the individual level. Polarization ranges from 0 (everyone is facing in different directions) to 1 (everyone facing the same direction). I constructed distributions of polarization for each trial (examples are shown in figure 3.3).

Since measures of polarization are not independent of each other (because the polarization frequently changes slowly; Miller & Gerlai, 2012), comparing distributions of polarization to each other tends to artificially inflate the significance of differences between them. Instead, since the majority of the polarization distributions in our data appeared, from visual inspection, to consist of three distinct components (see Figure 3.3), we used a Maximum Likelihood Estimation procedure (Miller & Gerlai, 2012) to detect whether the distribution really had three distinct components, and to extract the parameters of these components (see Methods for details). One file from group E1 had to be excluded as it could not be fit. All tested distributions, in all four conditions, were tri-modal, as identified by the three-component model having the lowest AIC score. Table 3.4 gives the means of the modes of the three distributions for each condition.

To identify differences between conditions in their polarization, I calculated the time each group spent in each mode of their polarization distribution. The intersections between the best-fit components were used as thresholds and all the data were placed into one component or another. From this, I calculated, for each trial, what proportion of the trial the group spent in their high, medium, or low polarization mode. Figure 3.4 shows a sample distribution with its modes and thresholds. I then constructed distributions of the time spent in each mode by fish in each condition of the experiment (Figure 3.5). In all conditions, fish spent about half their time in the medium mode (Control: 47.8%; E1: 51.5%; E2: 50.4%; Sham: 50.1%) and the rest of their time approximately equally divided between the high and low polarization modes. I compared the time spent in each mode across groups (Table 3.4). There were no significant differences between any groups on any mode.

The analysis above deals with group-wide effects. It is possible, however, that manipulated (lesioned or sham-lesioned) fish behave differently than control fish, but that their behaviour does not alter the behaviour of the group as a whole. Therefore, to further analyze the NND and IID scores, I ranked each individual by their NND and IID scores relative to those of their group-mates in each trial. So, each individual in each trial received a ranking of 1-5 for both NND and IID, 1 indicating the greatest distance within the group. I then compared the mean rankings of the focal individuals in each condition (in E1 and E2, the lesioned fish; in the Sham groups, the sham-lesioned fish; and in the Control groups, a randomly selected individual; Figure 3.6). Rank frequencies were computed by dividing the total number of occurrences of a ranking by the total number of data points in each condition (i.e., the frequencies represent the proportion of all trials on

which the focal fish had that ranking). The ranking data were compared across conditions using Fisher's exact test (which is preferable to the chi-squared test when the expected counts in some cells of the contingency table are less than 5). Table 3.5 shows the test results for the NND rankings; Table 3.6 shows the IID tests. There were no significant differences between any two groups.

Finally, I created another measure of the focal fish's behaviour relative to its non-manipulated group-mates by dividing their NND or IID by the mean of the other 4 fish. For this measure, the NND- or IID-ratio, values larger than 1 indicate that the focal fish was farther from the group than the rest of the fish in the group were from each other. The ratio is therefore normalized within each group, allowing me to test any effects of my manipulations (lesions) while controlling for any variation introduced by the specific context within which that fish was tested (the other individuals in its group). This specific measurement allows us to quantify the focal fish's social behaviour in relation to the other members of their group, instead of quantifying overall group behaviour. Figure 3.7 shows the NND (left) and IID (right) ratios for all conditions and trials. A mixed-model repeated measures ANOVA revealed, for both the NND and IID ratios, a significant effect of trial (NND: $F(2, 105) = 9.85$, $p = 0.0001$; IID: $F(2, 105) = 6.99$, $p = 0.001$), but not of condition (NND: $F(3, 105) = 1.18$, $p = 0.32$; IID: $F(3,105) = 1.77$, $p = 0.16$). There was no significant interaction between trial and condition in either case (NND: $F(6, 105) = 0.93$, $p = 0.48$; IID: $F(6, 105) = 1.05$, $p = 0.40$). To further explore the effect of trial on the distance ratios, I ran a one-way ANOVA for the effect of trial only on the data for each condition separately. Though no results were significant, for both NND and IID, groups E1 and E2 had strongly trending trial effects (NND: E1, $F(1, 19) = 6.17$, $p = 0.02$;

E2, $F(1, 31) = 5.75$, $p = 0.02$; IID: E1, $F(1, 19) = 8.06$, $p = 0.01$; E2: $F(1, 31) = 5.41$, $p = 0.03$), whereas the control and sham-lesioned groups showed no effects (NND: control, $F(1, 31) = 3.45$, $p = 0.07$; sham, $F(1, 28) = 0.15$, $p = 0.70$; IID: control, $F(1, 31) = 1.72$, $p = 0.20$; sham, $F(1, 28) = 0.04$, $p = 0.85$). These results, combined with an examination of figure 3.7, suggest that the lesioned focal fish, but not the sham-lesioned or control fish, swam farther from the rest of the group than the other fish did, but only on the first trial. I discuss these results further in the next chapter.

**Tables:**

Table 3.1: Means and standard deviations (in brackets) for NND as a function of condition (rows) and testing trial (columns).

| Condition/Trial | 1 | 2 | 3 |
|---|---|---|---|
| Control | 70.61 (± 40.29) | 62.74 (± 23.13) | 71.11 (± 24.07) |
| E1 | 73.33 (± 17.14) | 85.24 (± 23.00) | 88.90 (± 21.62) |
| E2 | 53.14 (± 16.72) | 61.23 (± 14.84) | 63.01 (± 18.46) |
| Sham | 45.19 (± 8.56) | 50.83 (± 9.21) | 50.28 (± 9.52) |

Table 3.2: Means and standard deviations (in brackets) for IID as a function of condition (rows) and testing trial (columns).

| Condition/Trial | 1 | 2 | 3 |
|---|---|---|---|
| Control | 146.66 (± 89.01) | 117.46 (± 46.31) | 138.00 (± 47.06) |
| E1 | 143.00 (± 36.21) | 167.28 (± 46.62) | 178.27 (± 46.20) |
| E2 | 108.56 (± 34.09) | 122.55 (± 30.20) | 127.95 (± 42.58) |
| Sham | 93.12 (± 18.55) | 105.65 (± 21.58) | 100.98 (± 21.29) |

Table 3.3: Means and standard deviations (in brackets) of the modes of the three components of the polarization distribution for each condition.

| Condition/Component | 1 | 2 | 3 |
|---|---|---|---|
| Control | 0.24 (± 0.02) | 0.56 (± 0.04) | 0.90 (± 0.05) |
| E1 | 0.24 (± 0.05) | 0.57 (± 0.05) | 0.92 (± 0.04) |
| E2 | 0.24 (± 0.01) | 0.56 (± 0.03) | 0.89 (± 0.02) |
| Sham | 0.24 (± 0.02) | 0.56 (± 0.02) | 0.92 (± 0.03) |

Table 3.4: T-tests comparing time spent in the low, medium, and high polarization modes by condition. The table gives the test statistic followed by the p-value in brackets for each comparison. No values were significant at $\alpha = 0.01$.

| Group | E2 | | | Control | | | Sham | | |
|---|---|---|---|---|---|---|---|---|---|
| | Low | Med | Hi | Low | Med | Hi | Low | Med | Hi |
| E1 | 2.62 (0.01) | 0.52 (0.61) | 2.64 (0.01) | 1.16 (0.25) | 1.43 (0.16) | 0.96 (0.34) | 1.26 (0.21) | 0.74 (0.46) | 0.64 (0.53) |
| E2 | | | | 1.50 (0.14) | 0.86 (0.39) | 1.58 (0.12) | 1.79 (0.08) | 0.13 (0.90) | 2.47 (0.02) |
| Control | | | | | | | 0.16 (0.87) | 0.91 (0.37) | 0.48 (0.63) |

Table 3.5. Comparisons of NND rankings across conditions. The table gives p-values for the Fisher's exact tests comparing every two conditions to each other.

| Condition | E1 | E2 | Sham |
|---|---|---|---|
| Control | 0.62 | 0.90 | 0.22 |
| E1 | | 0.65 | 0.41 |
| E2 | | | 0.05 |

Table 3.6. Comparisons of IID rankings across conditions. The table gives p-values for the Fisher's exact tests comparing every two conditions to each other.

| Condition | E1 | E2 | Sham |
|---|---|---|---|
| Control | 0.22 | 0.36 | 0.37 |
| E1 | | 0.95 | 0.16 |
| E2 | | | 0.14 |

**Figures:**

Figure 3.1: Brain section examples. Sections from **A**) a control fish; **B**) a lesioned fish; and **C**) a sham-lesioned fish. Images taken at 10X magnification. Slices sectioned at 50 $\mu$m. Orange arrows in **B** and **C** show lesion sites. **D**) the corresponding slice shown in the zebrafish brain atlas (Wulliman et al., 1996; slice #71). *Dl*, dorsal lateral telencephalon (T); *Dc*, dorsal central T; *Dm*, dorsal medial T; *Dp*, posterior dorsal T; *LOT*, lateral olfactory tract; *MOT*, medial olfactory tract; *Vc*, central nucleus of the ventral telencephalon (VT); *Vd*, dorsal nucleus of the VT; *Vl*, lateral nucleus of the VT; *Vv*, ventral nucleus of the VT.

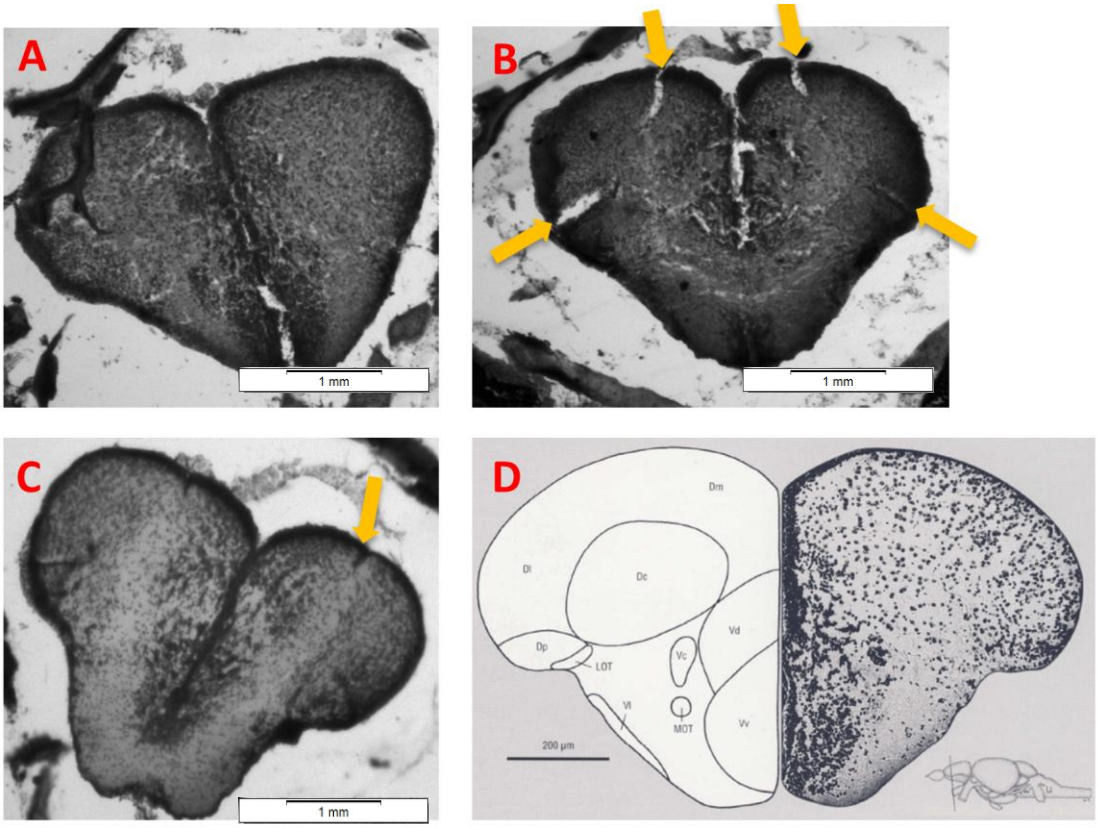


Figure 3.2: Boxplots of mean NND (left) and IID (right) by condition and trial number. Colours represent trials (red = trial 1; green = trial 2; blue = trial 3). C = control; E1 = experimental group 1; E2 = experimental group 2; Sh = sham-lesioned.

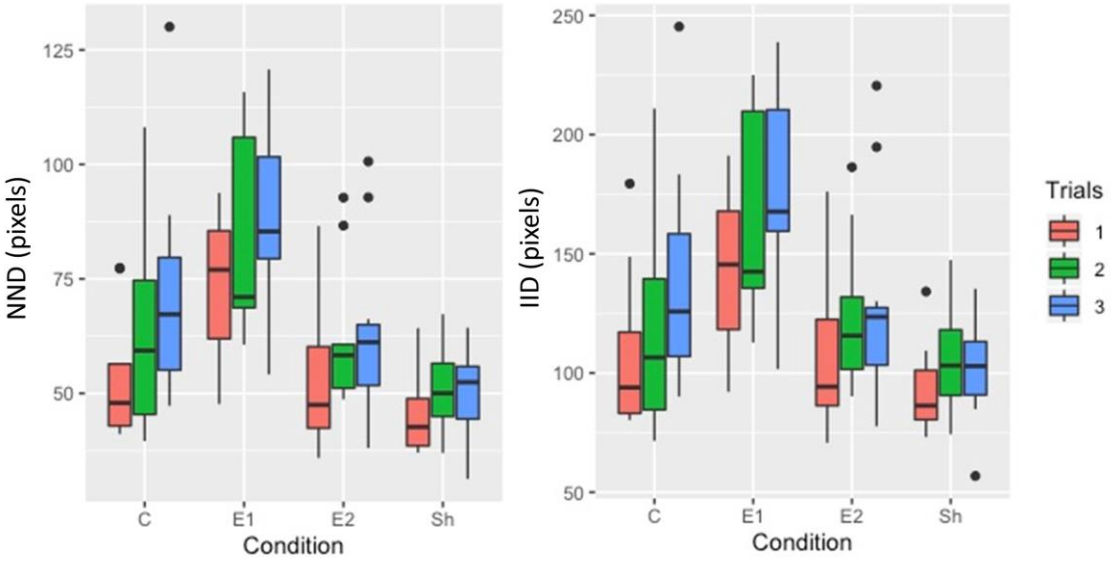


Figure 3.3: Sample polarization distributions from the four conditions (one file from each condition): Control (green), Experimental 1 (red), Experimental 2 (orange), and Sham-lesioned (blue). All the distributions appear to be tri-modal (see text for details).

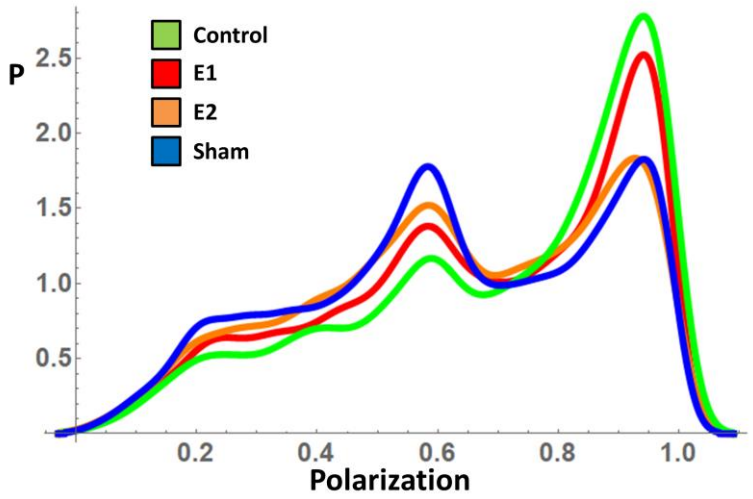


Figure 3.4: A sample polarization distribution (in black; from condition E2; this is the same distribution as is shown in figure 3.3 in orange), with its best-fit model components (red, green, and blue). The intersections between the components, shown as vertical grey lines, were used to partition the data into modes.

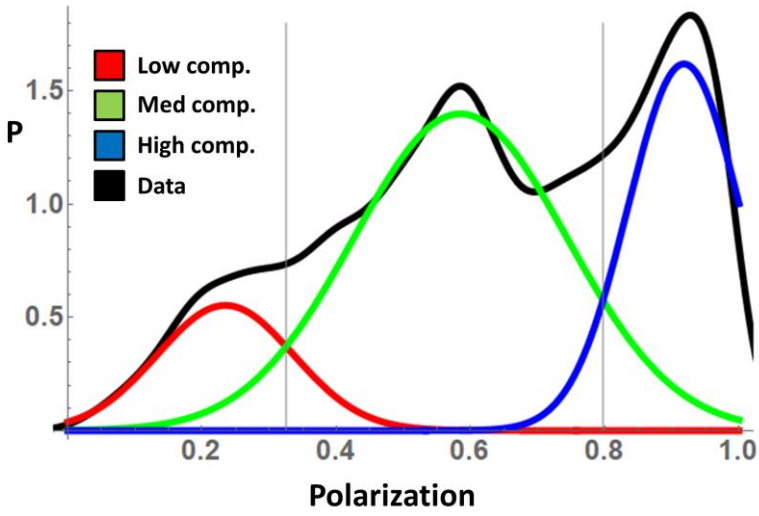


Figure 3.5: Distributions of time spent in each mode of the polarization distribution, by condition. **A**: E1; **B**: E2; **C**: Control; **D**: Sham-lesioned. The distributions show the proportion of the trial that the group spent in each mode of their polarization distribution.

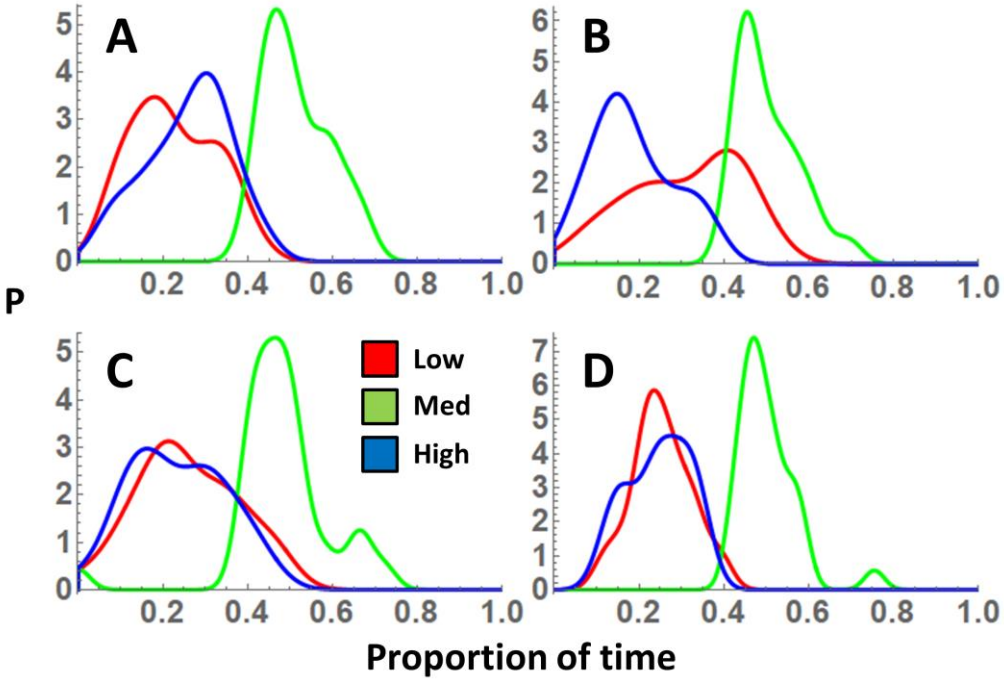


Figure 3.6: Frequency graphs of NND (left), and IID (right). The figure shows how often the focal fish occupied each rank, compared to the other group members. Rank 1 indicates the greatest distance in the group, rank 5 the smallest.

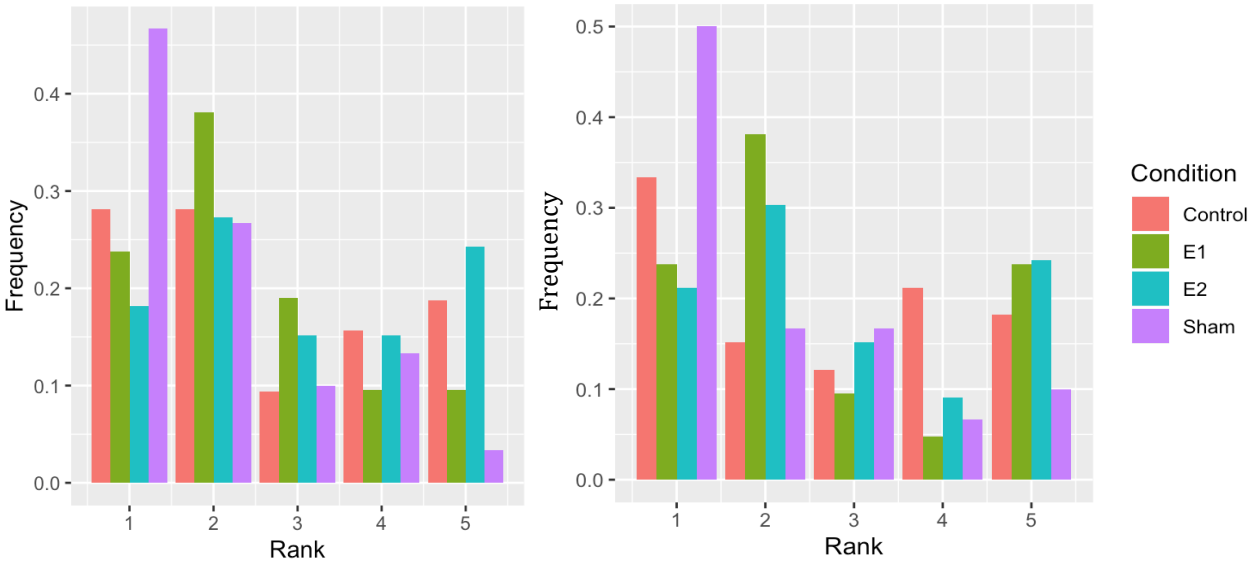
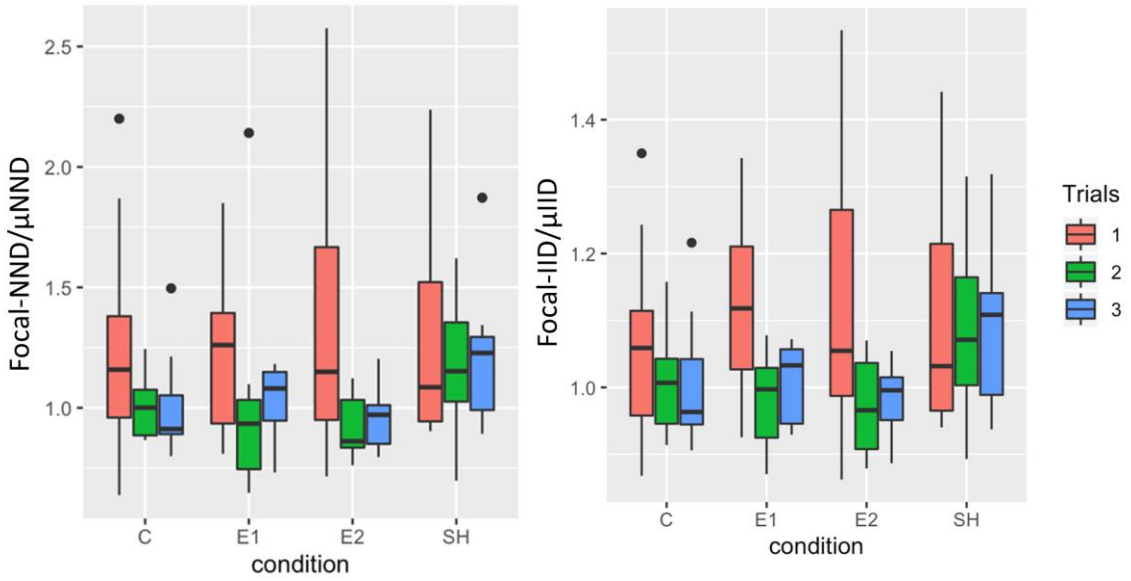


Figure 3.7. Focal fish distances relative to the mean of the group. The figure shows the ratio of the NND (left) or IID (right) of the focal fish, divided by the mean NND or IID of the other 4 fish in its group. Values larger than 1 indicate that the focal fish was farther from the group than the average. Colours represent trials (red = trial 1; green = trial 2; blue = trial 3). C = control; E1 = experimental group 1; E2 = experimental group 2; Sh = sham-lesioned.



## Chapter 4. Discussion

In this thesis, I set out to quantify the effects of lesions to the dorsal lateral telencephalon on shoaling behaviours in zebrafish. A widely accepted framework for considering the neural basis of social behaviours in fish, the social decision-making network (SDMN) approach (Bshary et al., 2014) suggests that this area of the brain is a component in the fish reward system, most likely mediating the rewarding effects of being close to conspecifics – being part of a group. For this reason, I considered it the most likely site at which to perform a lesion that would have a disruptive effect on shoaling.

First, I developed, from scratch, a method for performing these lesions in zebrafish. Brain lesions have only rarely been performed in such small fish. The method I developed will allow for lesions to any region of the brain. Figure 3.1B shows a sample brain slice from a lesioned fish in which the damage to the dorsal lateral telencephalon is clearly visible. The sample brain slice is from the E2 condition. E1 was our first batch of fish, in which the sections were too thin and we were not able to properly transfer them to the slide.

To test the effects of these lesions, I performed a behavioural experiment in which groups of 5 fish, one of which was manipulated (either lesioned or sham-lesioned) were allowed to swim together in a circular tank for 20 min on three consecutive days. These groups were compared to groups in which all the fish were not lesioned. Since lesions of the zebrafish brain can regenerate over the course of a few weeks (e.g., Ohnmacht et al., 2016), some of the experimental fish were tested just 4 days after their surgeries (E2), while others were given a full month to recover from the surgery (E1), but also possibly

enough time for the lesion site to grow back. I note that it is not known whether the areas of the brain that grow back also recover their function, or how long that might take (for example, it is possible that although glial cells can migrate into the site and differentiate into neurons within about 3 weeks, it takes them much longer to form functional synapses onto other brain regions).

In my behavioural tests, the manipulated fish were tagged so that they could be identified in the videos and their behaviour compared to their group mates. For control groups, a randomly selected individual in each trial was selected as the focal fish. The collective motion of the group was quantified using common measures: the distance between an individual and its nearest neighbor (NND), the mean distance between an individual and all the other fish (IID), and the polarization of the group. I explored the effects of having a lesioned fish in the group on the overall group dynamics, as well as comparing each focal fish to the behaviour of its group-mates.

Overall, fish in condition E1 – whose manipulated member had a longer time between surgery and behavioural testing – showed a larger NND and IID than groups in any other condition (Figure 3.2). This suggests that lesions of the telencephalon do have a disruptive effect on the dynamics of shoaling, causing shoals to become less cohesive.

The fact that I found this result in condition E1 but not in condition E2, who received their behavioural testing very shortly after surgery, suggests that behaviour takes some time to settle down after surgery as the short time between surgery and behavioural testing in the E1 condition might have an effect on overall behaviour. It is also possible that other details of the procedure that differed between the groups led to the behavioural differences.

As expected based on earlier literature (e.g., Miller & Gerlai, 2012), over the course of the three trials, fish moved further apart from each other, as reflected in increasing NNDs and IIDs across all conditions. However, when I compared the behaviour of the focal fish to that of its group-mates, I found a nearly significant effect of trial on both NND and IID only in groups E1 and E2, not in the control or sham groups. In other words, lesioned fish appeared to swim further from their group than the group average distance, but only on the first day. On the second and third trials, there was no difference in distance between the lesioned fish and their group-mates, in any condition. This might suggest that fish are able to somehow compensate for the loss of function produced by the lesion. It is also possible that the group as a whole began to shoal less tightly (as seen in figure 3.2), and the lesioned fish simply did not adjust their distances, bringing them into line with the rest of the group. A third alternative is that the effects of the lesion may only appear under conditions of stress. It has been hypothesized that the loosening of the shoal with repeated exposure to the same arena are a result of habituation (Miller & Gerlai, 2012). Thus, if the fish were more stressed on the first trial, this might have caused the lesioned fish to increase their distance from the group. It is not clear why the lesion would only affect the fish when highly stressed, but I note that our understanding of the neural bases of fish behaviour is overall very poor, a point also emphasized by Bshary et al. (2014). In addition, it is not clear why there is a ranking effect (as seen in figure 3.6), where the sham focal fish is ranked first most often, meaning that the focal fish was the farthest distance away from the rest of their group the most often. It is possible that the lesion could have caused this effect; though it is likely this effect would go away if the groups were tested more. As mentioned in chapter 2, sex

was not determined during our study. Sex differences are not a common measure in studies that examine shoaling and schooling in zebrafish, although it has been noted that both female and male zebrafish fish prefer to shoal with females (Spence et al., 2008). It is possible that in shoals that were a closer distance, such as the sham group, the focal fish was a female, while other groups such as the E1 and E2 group; the focal fish was a male.

Another potential reason for the results that we found could be due to the dorsal lateral telencephalon being homologous to the hippocampus. While the hippocampus is necessary for spatial orientation, it is not likely that the lesion affected the fish's ability to orient themselves. Zebrafish also use their lateral line to orient themselves in relation to others around them. However, my apparatus did not direction measure spatial ability in relation to an individual's external environment.

Finally, I also examined the polarization of the groups as they were swimming. In all my data, polarization distributions were tri-modal, with groups spending about half of their time in a medium-polarization state and dividing the remaining time approximately equally between schooling (a highly polarized state) and disorder (very low polarization). This is different from what I expected based on the literature: polarization distributions are usually bimodal (Miller & Gerlai, 2011). It is possible that the size of my tank, which was smaller than those used in past work to allow for tracking of individual identities, accounts for this. I found no differences between any conditions in any aspect of their polarization. This suggests that however lesions of the telencephalon affect distances between the members of a group, they do not impact the polarization of the group. Such a separation between the mechanisms of cohesion and polarization has been observed

before in the effects of some drugs on zebrafish shoaling (Miller, Greene, Dydinski & Gerlai, 2013).

In summary, though I did not find as clear-cut an effect of lesions on shoaling behaviour as I would have liked, I did show that lesions of the telencephalon disrupt social behaviours under some conditions. Thus, the dorsal lateral telencephalon does have a role in modulating social behaviours, such as shoaling in zebrafish. This thesis did not directly test memory, although because the dorsal lateral telencephalon plays a role in different aspects of memory it is possible the lesion could have affected this. Future work could examine various memory tasks in which the lesion may show an effect in zebrafish such as an episodic-like memory task, spatial memory task or a recognition memory task. I also developed a robust method for performing lesions of the zebrafish brain that will be useful in future studies.

## References

Al-Imari, L., & Gerlai, R,. (2008). Sight of conspecifics as reward in associative learning in zebrafish (Danio rerio). *Behav Brain Res, 189*(1), 216–219.

Bakkers, J. (2011). Zebrafish as a model to study cardiac development and human cardiac disease. *Cardiovasc. Res.*, *91*, 279–288.

Becker, T., Bernhardt, R., Reinhard, E., Wullimann, M. F., Tongiorgi, E. & Schachner, M. (1998). Readiness of zebrafish brain neurons to regenerate a spinal axon correlates with differential expression of specific cell recognition molecules. *J Neurosci*, *18*, 5789–5803.

Best, J., Berghmans, S., Hunt, J. et al. (2008). Non-Associative Learning in Larval Zebrafish. *Neuropsychopharmacol*, *33*, 1206–1215.

Bilotta, J., Barnett, J. A., Hancock, L., & Saszik, S. (2004). Ethanol exposure alters zebrafish development: A novel model of fetal alcohol syndrome. *Neurotoxicology and Teratology*, *26*(6), 737–743.

Boswell, C. W., & Ciruna, B. (2017). Understanding Idiopathic Scoliosis: A New Zebrafish School of Thought. *Trends in Genetics*, *33*(3), 183–196.

Brakes, P. (2019). Sociality and wild animal welfare: future directions. *Frontiers in Veterinary Science, 6,* 1-7.

Brown, C., & Laland, K. N. (2003). Social learning in fishes: A review. *Fish and Fisheries*, *4*(3), 280–288.

Bshary, R., Gingins, S., & Vail, A. L. (2014). Social cognition in fishes. *Trends in Cognitive Sciences*, *18*(9), 465–471.

Buske, C., & Gerlai, R. (2011). Shoaling develops with age in Zebrafish (Danio rerio).

*Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *35*(6), 1409–1415.

Butail, S., Bartolini, T., & Porfiri, M. (2013). Collective Response of Zebrafish Shoals to a Free-Swimming Robotic Fish. *PLoS ONE*, *8*(10).

Butler, J. M., & Maruska, K. P. (2016). The mechanosensory lateral line system mediates activation of socially-relevant brain regions during territorial interactions. *Frontiers in Behavioural Neuroscience*, *10*, 1–18.

Cabrera-Álvarez, M. J., Swaney, W. T. and Reader, S. M. (2017). Forebrain activation during social exposure in wild-type guppies. *Physiology & Behavior*, *182*, 107–113.

Clark, R. E., Broadbent, N. J., & Squire, L. R. (2007). The hippocampus and spatial memory: Findings with a novel modification of the water maze. *Journal of Neuroscience*, *27*(25), 6647–6654.

Cognato, G. de P., Bortolotto, J. W., Blazina, A. R., Christoff, R. R., Lara, D. R., Vianna, M. R., & Bonan, C. D. (2012). Y-Maze memory task in zebrafish (Danio rerio): The role of glutamatergic and cholinergic systems on the acquisition and consolidation periods. *Neurobiology of Learning and Memory*, *98*(4), 321–328.

Dreosti, E., Lopes, G., Kampff, A. R., & Wilson, S. W. (2015). Development of social behavior in young zebrafish. *Frontiers in Neural Circuits*, *9*, 1–9.

Echevarria, D. J., Hammack, C. M., Pratt, D. W., & Hosemann, J. D. (n.d.). A novel behavioral test battery to assess global drug effects using the zebrafish. *International Journal of Comparative Psychology*, *21*(1), 19–34.

Eddins, D., Petro, A., Williams, P., Cerutti, D. T., & Levin, E. D. (2009). Nicotine effects on learning in zebrafish: The role of dopaminergic systems. *Psychopharmacology*,

*202*(1–3), 103–109.

Engeszer, R. E., Patterson, L. B., Rao, A. A., & Parichy, D. M. (2007). Zebrafish in The Wild: A Review of Natural History And New Notes from the Field. *Zebrafish*, *4*(1).

Faucher, K., Parmentier, E., Becco, C., Vandewalle, N., & Vandewalle, P. (2010). Fish lateral system is required for accurate control of shoaling behaviour. *Animal Behaviour*, *79*(3), 679–687.

Fernandes, Y., Rampersad, M., Jones, E. M., & Eberhart, J. K. (2018). Social deficits following embryonic ethanol exposure arise in post-larval zebrafish. *Addiction Biology*, 898–907.

Floresco, S. B. (2015). The Nucleus Accumbens: An Interface Between Cognition, Emotion, and Action. *Annual Review of Psychology*, *66*(1), 25–52.

Fontana, B. D., Mezzomo, N. J., Kalueff, A. V., & Rosemberg, D. B. (2018). The developing utility of zebrafish models of neurological and neuropsychiatric disorders: a critical review. *Exp Neurol*, *299*(Pt A), 157–171.

Friedrich, R. W., Jacobson, G. A., & Zhu, P. (2010). Circuit Neuroscience in Zebrafish. *Current Biology*, *20*(8), R371–R381.

Frith, C. D. (2008). Social cognition. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *363*(1499), 2033–2039.

Geng, Y., & Peterson, R. T. (2019). The zebrafish subcortical social brain as a model for studying social behavior disorders. *Disease Models & Mechanisms*, *12*(8).

Gerlai, R. (2016). Chapter 17- Learning and memory in zebrafish (Danio rerio). *Methods in Cell Biology*, *134*, 551–586.

Ghysen, A., & Dambly-Chaudière, C. (2004). Development of the zebrafish lateral line.

*Current Opinion in Neurobiology*, *14*(1), 67–73.

Gómez L, et al. (2006). Immobilization of chitosan-invertase neoglycoconjugate on carboxymethylcellulose-modified chitin. *Prep Biochem Biotechnol*, *36*(3), 259–271.

Graham, C. A., Keyserlingk, M. V., & Franks, B. (2017). Zebrafish welfare: Natural history, social motivation and behaviour. *Applied Animal Behaviour Science*, *200*, 13–22.

Hamilton, T. J., Myggland, A., Duperreault, E., May, Z., Gallup, J., Powell, R. A., … Digweed, S. M. (2016). Episodic-like memory in zebrafish. *Animal Cognition*, *19*(6), 1071–1079.

Hitti, F. L., & Siegelbaum, S. S. (2014). The hippocampal CA2 region is essential for social memory. *Nature*, *508*(7494).

Hoppitt, W., & Laland, K. (2013). *Social Learning: An Introduction to Mechanisms, Methods and Models*. Princeton: Princeton University Press.

Howe, D. G., Bradford, Y. M., Eagle, A., Fashena, D., Frazer, K., Kalita, P., Mani, P., Martin, R., Moxon, S. T., Paddock, H., Pich, C., Ramachandran, S., Ruzicka, L., Schaper, K., Shao, X., Singer, A., Toro, S., Van Slyke, C., & Westerfield, M. (2017). The Zebrafish Model Organism Database: new support for human disease models, mutation details, gene expression phenotypes and searching. *Nucleic Acids Research*, *45*(D1), 758–768.

Howe, K., et al. (2013). The zebrafish reference genome sequence and its relationship to the human genome,. *Nature*, *496*, 498–503.

Iman, A., & Krebs, J. (1987). Predation and group living. *Trends Ecol. Evol.*, *2*(2), 31–32.

Kalueff A. V., Gebhardt M., Stewart A. M., Cachat J. M., Brimmer M., Chawla J. S., et al. . (2013). Towards a comprehensive catalog of zebrafish behavior 1.0 and beyond. *Zebrafish, 10*, 70–86.

Kasumyan, A. O., & Pavlov, D. S. (2018). Evolution of schooling behavior in fish. *Journal of Ichthyology*, *58*(5), 670–678.

Killen, S. S., Marras, S., Nadler, L., & Domenici, P. (2017). The role of physiological traits in assortment among and within fish shoals. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1727).

Kishimoto, N., Shimizu, K., & Sawamoto, K. (2012). Neuronal regeneration in a zebrafish model of adult brain injury. *Disease Models & Mechanisms*, *5*(2), 200–209.

Kotrschal, K., Staaden, M. J., & Huber, R. (1998). Fish Brains: Evolution and Anvironmental Relationships. *Reviews in Fish Biology and Fisheries*, *8*, 373–408.

Krakauer, D. C. (1995). Groups confuse predators by exploiting perceptual bottlenecks: a connectionist model of the confusion effect. *Behavioral Ecology and Sociobiology*, *36*(6), 421–429.

Krause, J., Butlin, R. K., Peuhkuri, N., & Pritchard, V. L. (2000). The social organization of fish shoals: A test of the predictive power of laboratory experiments for the field. *Biological Reviews*, *75*(4), 477–501.

Larson, E. T., O'Malley, D. M., & Melloni, R. H. (2006). Aggression and vasotocin are associated with dominant-subordinate relationships in zebrafish. *Behavioural Brain Research*, *167*(1), 94–102.

Lindeyer, C. M., & Reader, S. M. (2010). Social learning of escape routes in zebrafish

and the stability of behavioural traditions. *Animal Behaviour*, *79*(4), 827–834.

Lugo-Huitrón, R., Ugalde Muñiz, P., Pineda, B., Pedraza-Chaverrí, J., Ríos, C., & Pérez-De La Cruz, V. (2013). Quinolinic acid: An endogenous neurotoxin with multiple targets. *Oxidative Medicine and Cellular Longevity*, *2013*.

Madeira, N., & Oliveira, R. F. (2017). Long-Term Social Recognition Memory in Zebrafish. *Zebrafish*, *14*(4), 305–310.

Magurran, A. E., & Pitcher, T. J. (1983). Foraging, timidity and shoal size in minnows and goldfish. *Behavior Ecology and Sociobiology*, *12*, 147-152.

März, M., Schmidt, R., Rastegar, S., & Strahle, U. (2011). Regenerative response following stab injury in the adult zebrafish telencephalon. *Developmental Dynamics*, *240*(9), 2221–2231.

Matsui, H., Gavinio, R., & Takahashi, R. (2012). Medaka fish Parkinson's diease model. *Experimental Neurobiology*, *21*(3), 94–100.

McClure, M. M., McIntyre, P. B., & McCune, A. R. (2006). Notes on the natural diet and habitat of eight danionin fishes, including the zebrafish Danio rerio. *Journal of Fish Biology*, *69*(2), 553–570.

McGowan, K. A., & Barsh, G. S. (2016). How the zebrafish got its stripes. *eLife*, *5*.

Menke, A. L., Spitsbergen, J. M., Wolterbeek, A. P. M., & Woutersen, R. A. (2011). Normal anatomy and histology of the adult zebrafish. *Toxicologic Pathology*, *39*(5), 759–775.

Meshalkina, D. A., Kizlyk, M. N., Kysil, E. V, Collier, A. D., Echevarria, D. J., Abreu, M. S., … Kalueff, A. V. (2018). Zebrafish models of autism spectrum disorder. *Experimental Neurology*, *299*, 207–216.

Meyers, J. R. (2018). Zebrafish: Development of a Vertebrate Model Organism. *Current Protocols Essential Laboratory Techniques*, *16*(1).

Miller, N.Y., & Gerlai, R. (2007). Quantification of shoaling behavior in zebrafish (Danio rerio). *Behavioural Brain Research, 184*: 157-166.

Miller, N.Y., Gerlai, R. (2008). Oscillations in shoal cohesion in zebrafish (*Danio rerio*). *Behavioural Brain Research, 193*: 148-151.

Miller, N., & Gerlai, R. (2012). From Schooling to Shoaling: Patterns of Collective Motion in Zebrafish (Danio rerio). *PLoS ONE*, *7*(11), 8–13.

Miller, N. Y., & Gerlai, R. (2011). Shoaling in zebrafish: What we don't know. *Reviews in the Neurosciences*, *22*(1), 17–25.

Miller, N., Greene, K., Dydinski, A., & Gerlai, R. (2013). Effects of nicotine and alcohol on zebrafish (Danio rerio) shoaling. *Behav Brain Res.*, *240*, 192–196.

Moretz, J. A., Martins, E. P., & Robison, B. D. (2007). Behavioral syndromes and the evolution of correlated behavior in zebrafish. *Behavioral Ecology*, *18*(3), 556–562.

Morris, R. G. M. (1981). Spatial localization does not require the presence of local cues. *Learning and Motivation*, *12*(2), 239–260.

Müller, U. K. & van Leeuwen, J. L. (n.d.). Swimming of larval zebrafish: ontogeny of body waves and implications for locomotory development. *J. Exp. Biol.*, *2007*, 853–868.

Nieuwenhuis, R., Grotenhuis, M. te, & Pelzer, B. (2012). influence.ME: Tools for Detecting Influential Data in Mixed Effects Models. *The R Journal*, *4*(2), 38–47.

O'Connell, L. A., & Hofmann, H. A. (2011). The Vertebrate mesolimbic reward system and social behavior network: A comparative synthesis. *Journal of Comparative*

*Neurology*, *519*(18), 3599–3639.

Ohnmacht, J., Yang, Y., Maurer, G. W., Barreiro-Iglesias, A., Tsarouchas, T. M., Wehner, D., … Becker, T. (2016). Spinal motor neurons are regenerated after mechanical lesion and genetic ablation in larval zebrafish. *Development (Cambridge)*, *143*(9), 1464–1474.

Peichel, C. L. (2004). Social behavior: How do fish find their shoal mate? *Current Biology*, *14*(13), 503–504.

Pérez-Escudero, A., Vicente-Page, J., Hinz, R. et al. (2014). idTracker: tracking individuals in a group by automatic identification of unmarked animals. *Nat Methods*, *11*, 743–748.

Portavella, M., Torres, B., & Salas, C. (2004). Avoidance response in goldfish: emotional and temporal involvement of medial and lateral telencephalic pallium. *J Neurosci*, *24*(9), 2335–2342.

Poss, K. D., Wilson, L. G., & Keating, M. T. (2002). Heart regeneration in zebrafish. *Science*, *298*(5601), 2188–2190.

Pritchard, V. L., Lawrence, J., Butlin, R. K., & Krause, J. (2001). Shoal choice in zebrafish, Danio rerio: The influence of shoal size and activity. *Animal Behaviour*, *62*(6), 1085–1088.

Qin, M., Wong, A., Seguin, D., & Gerlai, R. (2014). Induction of social behavior in zebrafish: Live versus computer animated fish as stimuli. *Zebrafish*, *11*(3), 185–197.

Reimschessel, R. (2001). A fish model of renal regeneration and development. *ILAR J*, *42*, 285–291.

Raya, A., Koth, C. M., Buscher, D., et al. (2003). Activation of Notch signaling pathway

precedes heart regeneration in zebrafish. *Proc. Natl. Acad. Sci*, 11889–11895.

Roberts, A. C., Bill, B. R., & Glanzman, D. (2013). Learning and memory in zebrafish larvae. *Frontiers in Neural Circuits, 7*(126).

Roberts, G. (1996). Why individual vigilance declines as group size increases. *Animal Behaviour*, *51*(5), 1077–1086.

Rosenthal, G. G., & Ryan, M. J. (2005). Assortative preferences for stripes in danios. *Animal Behaviour*, *70*, 1063–1066.

Saverino, C., & Gerlai, R. (2008). The social zebrafish: behavioral responses to conspecific, heterospecific, and computer animated fish. *Behav Brain Res*, *191*(1), 77–87.

Scerbina, T., Chatterjee, D., & Gerlai, R. (2012). Dopamine receptor antagonism disrupts social preference in zebrafish: A strain comparison study. *Amino Acids*, *43*(5), 2059–2072.

Shapiro, M. L., & Eichenbaum, H. (1999). Hippocampus as a memory map: Synaptic plasticity and memory encoding by hippocampal neurons. *Hippocampus*, *9*(4), 365–384.

Shettleworth, S. J. (2010). *Cognition, evolution, and behavior* (2nd ed.). Oxford University Press.

Shinozuka, K., & Watanabe, S. (2004). Effects of telencephalic ablation on shoaling behavior in goldfish. *Physiology and Behavior*, *81*(1), 141–148.

Silk J. B. (2007). The adaptive value of sociality in mammalian groups. Philosophical transactions of the Royal Society of London. Series B, *Biological sciences*, *362*(1480), 539–559.

Sison, M., & Gerlai, R. (2010). Associative learning in zebrafish (Danio rerio) in the plus maze. *Behavioural Brain Research*, *207*(1), 99–104.

Skaggs, K., Goldman, D., & Parent, J. M. (2014). Excitotoxic brain injury in adult zebrafish stimulates neurogenesis and long-distance neuronal integration. *Glia*, *62*(12), 2061–2079.

Spence, R., Fatema, M. K., Ellis, S., Ahmed, Z. F., & Smith, C. (2007). Diet, growth and recruitment of wild zebrafish in Bangladesh. *Journal of Fish Biology*, *71*(1), 304–309.

Spence, R., Gerlach, G., Lawrence, C., & Smith, C. (2008). The behaviour and ecology of the zebrafish, Danio rerio. *Biological Reviews*, *83*(1), 13–34.

Stevenson, E. L., & Caldwell, H. K. (2014). Lesions to the CA2 region of the hippocampus impair social memory in mice. *European Journal of Neuroscience*, *40*(9), 3294–3301.

Suboski, M. D., Bain, S., Carty, A. E., McQuoid, L. M., Seelan, M. I., & Seifert, M. (1990). Alarm reaction in acquisition and social transmission of simulated- predator recognition by zebra danio fish (Brachydanio rerio). *Journal of Comparative Psychology*, *104*(1), 101–112.

Teame, T., Z. Zhang, C. Ran, H. Zhang, Y. Yang, Q. Ding, M. Xie, C. Gao, R.E. Olsen, Y. Ye, et al. (2019). The use of zebrafish (Danio rerio) as biomedical models. *Anim. Front*, *9*(3), 68–77.

Teles, M. C., Almeida, O., Lopes, J.S., & Oliveira, R. F. (2015). Social interactions elicit rapid shifts in functional connectivity in the social decision-making network of zebrafish. *Proceedings. Biological Sciences*, *282*(1816).

Vaz, R. L., Outeiro, T. F., & Ferreira, J. J. (2018). Zebrafish as an animal model for drug discovery in Parkinson's disease and other movement disorders: A systematic review. *Frontiers in Neurology, 9*.

Vernier, P. (2017). *The Brains of Teleost Fishes*. *Evolution of Nervous Systems*.

Wey, T., Blumstein, D.T., Shen, W., & Jordán, F. (2008). Social network analysis of animal behaviour: a promising tool for the study for sociality. Animal Behaviour, 75, 333-344.

Wixon, J. (2000). Danio rerio, the zebrafish. *Yeast*, *17*(3), 225–231.

Wright, D., Ward, A. J., Croft, D. P., & Krause, J. (2006). Social organization, grouping, and domestication in fish. *Zebrafish*, *3*(2), 141–155.

Wullimann, M. F., Rupp, B., Reichert, H. (1996). *Neuroanatomy of the Zebrafish Brain: a Topological Atlas*. Birkhäuser.

Yamamoto, N. (2009). Studies on the teleost brain morphology in search of the origin of cognition. *Japanese Psychological Research*, *51*(3), 154–167.

Zala, S. M., & Määttänen, I. (2013). Social learning of an associative foraging task in zebrafish. *Naturwissenschaften*, *100*(5), 469–472.

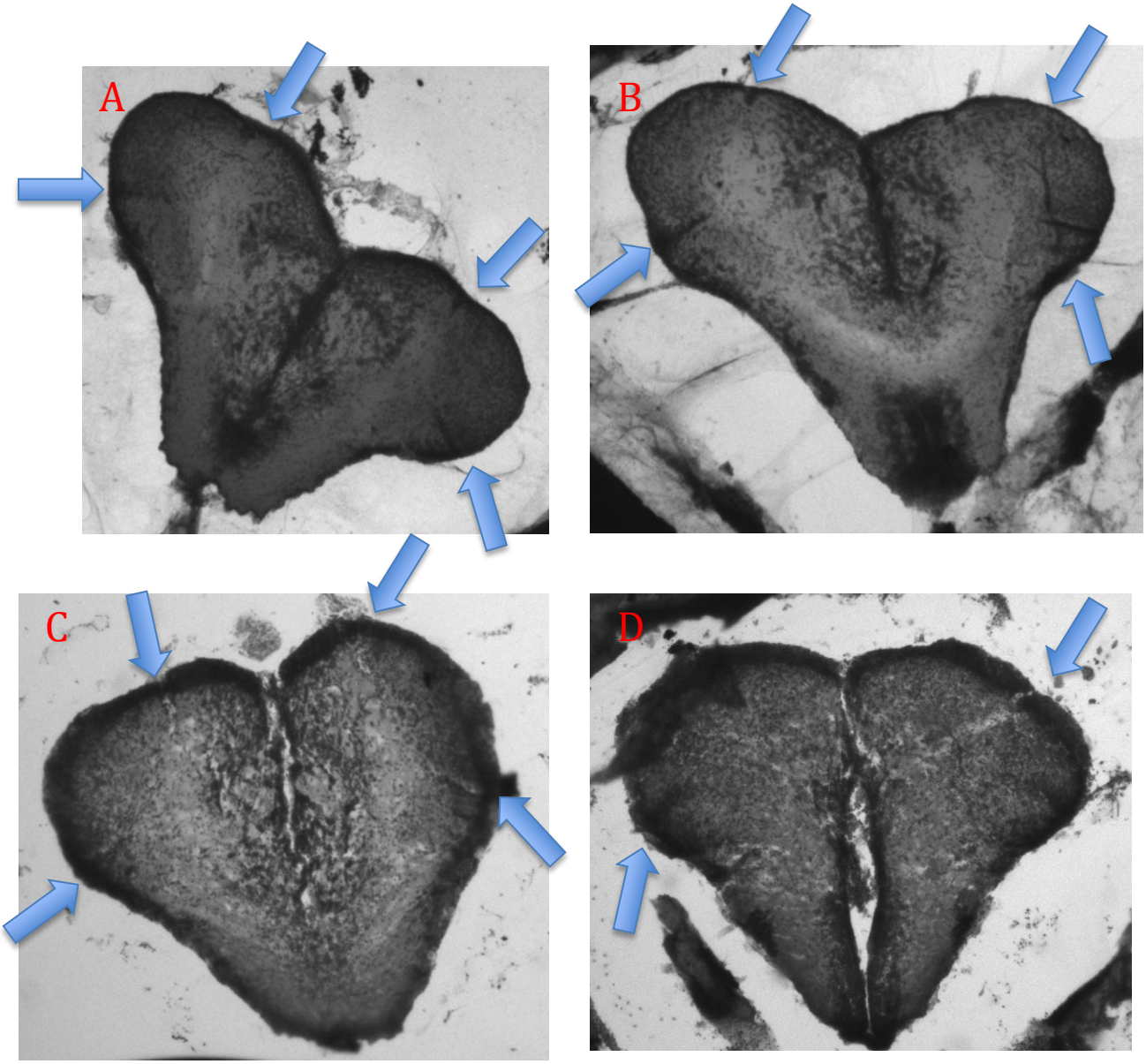
## Appendix A: R code

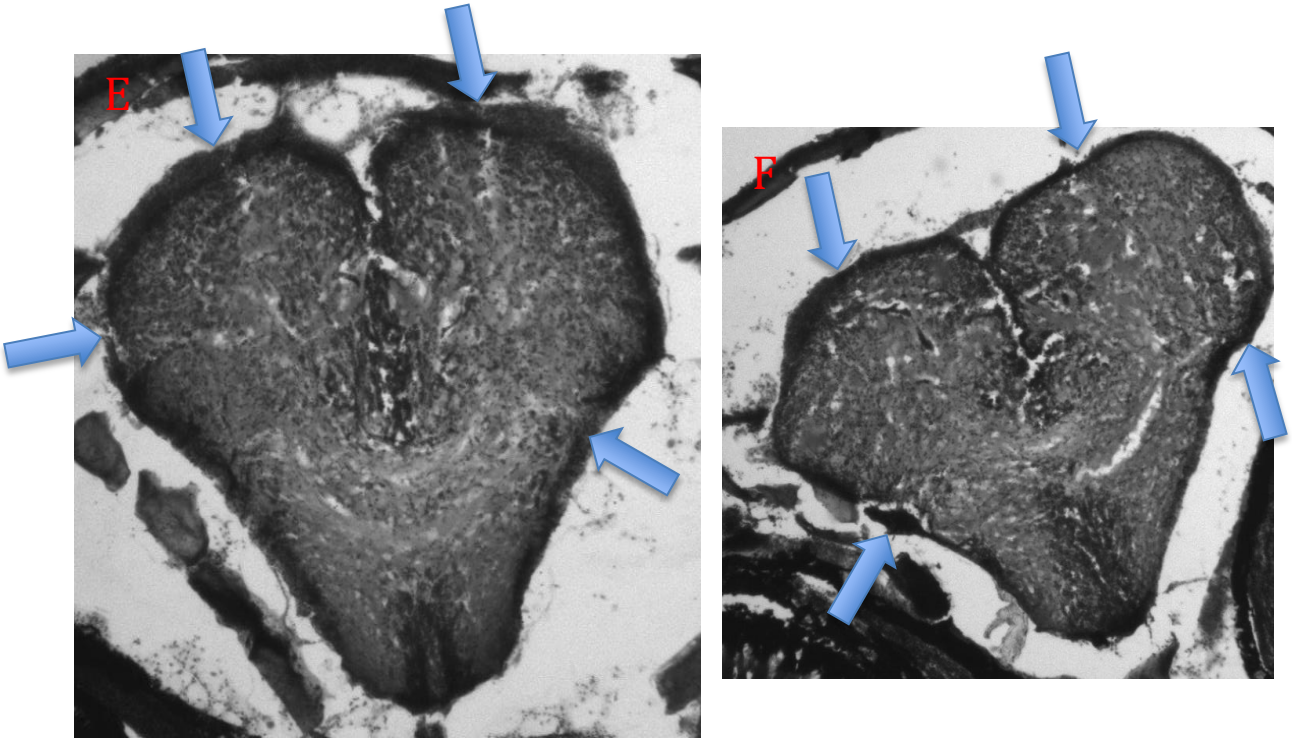
```
# This R script takes input from IDTracker and calculates:
# nearest neighbour distances, interindividual distances, and polarization.

# The data from IDTracker gives coordinates for each target. Each row is in the form:
# X1, Y1, P1, X2, Y2, P2... Xn, Yn, Pn
# where Xi and Yi are the coordinaes of the ith target, and Pi is the probability of it
 having been
# tracked correctly. n is the number of targets present.

# This script is designed for experiments where one focal individual is different from
 the others;
# it compares that individual to all the others (in my experiments, one fish had a brain
 lesion).
# The ID of the focal individual can be set at the top of the script, or the script can
 choose one at random.
# The script assumes the video was filmed at 10 fps, for downsampling. You can change
 this below.

# Free to use and modify. Hailey Katzman, 2019.


library("readxl") #package for reading in Excel files


setwd("mydrive/mypath/")                #enter the path to the tracking file
mydata <- read.csv("datafile.csv")      #enter the file name

numtargets <- length(mydata[1,])/3      #returns the number of animals tracked
if(numtargets != round(numtargets)){    #if number of columns in data is not divisible by
 3 - something wrong.
  print("Error in data file - wrong number of columns")
}

mylesion <- sample(1:numtargets, 1)  #randomly selects one target to compare (see above).
 replace with number for manual choice
myframes <- length(mydata[[1]])      #returns the total number of frames in the video
indep <- round((myframes*2)/(600))   #downsampling constant. If the video was 10 fps,
 this is 1 per 2 sec.


#find the nearest neighbour distance (NND):

mydist <- function(x1, y1, x2, y2) {  #returns the distance between two points
  a=(x2-x1)^2
  b=(y2-y1)^2
  c=sqrt(a+b)
  return(c)
}

nn <- function(ID, frame) {              #returns NND for one individual (by index) in
 one frame
  colx1 = paste("X", ID, sep="")  #X coordinate of ID
  coly1 = paste("Y", ID, sep="")  #Y coordinate of ID
  coordx1 = mydata[[frame,colx1]] #X coordinates of all
  coordy1 = mydata[[frame,coly1]] #Y coordinates of all
  everyone = c(1:numtargets)
  others = everyone[-ID]          #eliminate ID from list to compare

  dlist <- c()   #list of distances from all the other targets
  for(val in others){
    dlist <- c(dlist, mydist(coordx1, coordy1, mydata[[frame,paste("X",val,sep="")]],
 mydata[[frame,paste("Y",val,sep="")]]))
  }
  nnd <- min(dlist) #return the shortest distance
  return(nnd)
}

allnns <- vector("list", numtargets)   #all the NND values
```

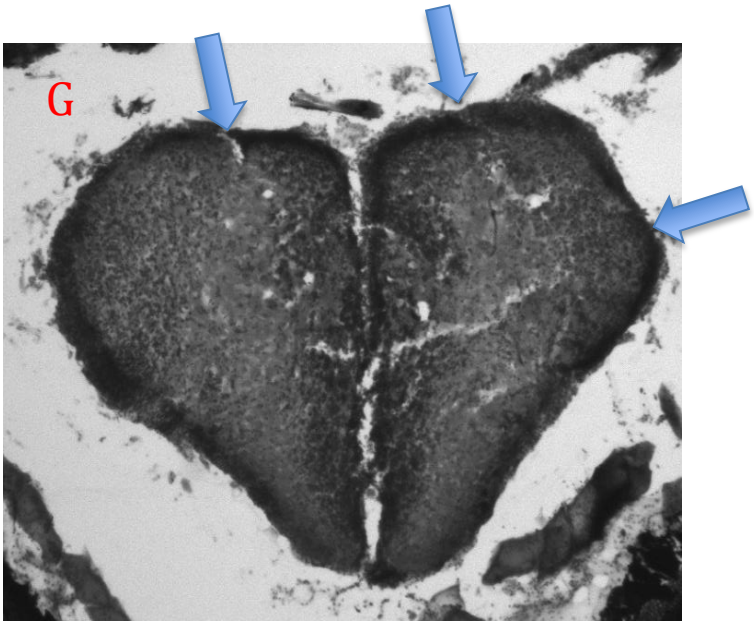
```
nnmeans <- c()                  #mean NND for each target
for(i in 1:numtargets){
  temp = mapply(nn, i, 1:myframes)  #NND for target i in all frames
  allnns[i] = list(temp)            #add to list
  nnmeans = c(nnmeans, mean(temp, na.rm=TRUE))  #save the mean
}

dsnns <- vector("list", numtargets)   #all the NND values, downsampled
for(i in 1:numtargets){
  temp = allnns[[i]][seq(1, length(allnns[[i]]),indep)]  #downsampled NND list for target
 i
  dsnns[i] = list(temp)               #add to list
}

# compare the NND of the focal target to the others, using a KS test (on downsampled
 data).
nonlesionnn <- unlist(dsnns[-mylesion])  #NNDs of everyone but the focal target
nncomparison <- ks.test(dsnns[[mylesion]], nonlesionnn)

#plot the distributions of NND:
plot1 <- density (dsnns[[mylesion]], na.rm = TRUE)  #distribution for focal target (black
 line)
plotnon <- density(nonlesionnn, na.rm = TRUE)      #distribution for others (red line)
plot(plot1, col="black")
lines(plotnon, col="red")

gmNND <- mean(nnmeans)  #gran mean of NND values (mean across targets)


#Calculate inter-individual distance (IID)

iid<-function(ID, frame) {     #calculates IID of one target in one frame
  colx1 = paste("X", ID, sep="") #X coordinate of ID
  coly1 = paste("Y", ID, sep="") #Y coordinate of ID
  coordx1 = mydata[[frame,colx1]] #X coordinates of all targets
  coordy1 = mydata[[frame,coly1]] #Y coordinates of all targets
  everyone = c(1:numtargets)
  others = everyone[-ID]       #eliminate ID from list to compare

  dlist <- c()   #list of distances from all the other targets
  for(val in others){
    dlist <- c(dlist, mydist(coordx1, coordy1, mydata[[frame,paste("X",val,sep="")]],
 mydata[[frame,paste("Y",val,sep="")]]))
  }
  ii <- mean(dlist) #return the mean distance
  return(ii)
}

alliis <- vector("list", numtargets)   #all the IID values
iimeans <- c()                  #mean NIID for each target
for(i in 1:numtargets){
  temp = mapply(iid, i, 1:myframes)  #IID for target i in all frames
  alliis[i] = list(temp)            #add to list
  iimeans = c(iimeans, mean(temp, na.rm=TRUE))  #save the mean
}

dsiis <- vector("list", numtargets)   #all the IID values, downsampled
for(i in 1:numtargets){
  temp = alliis[[i]][seq(1, length(alliis[[i]]),indep)]  #downsampled NND list for target
 i
  dsiis[i] = list(temp)               #add to list
}

# compare the IID of the focal target to the others, using a KS test (on downsampled
 data).
nonlesionii <- unlist(dsiis[-mylesion])  #IIDs of everyone but the focal target
iicomparison <- ks.test(dsiis[[mylesion]], nonlesionii)

#plot the distributions of IID:
plot1 <- density (dsiis[[mylesion]], na.rm = TRUE)  #distribution for focal target (black
 line)
```

```
plotnon <- density(nonlesionii, na.rm = TRUE)      #distribution for others (blue line)
plot(plot1, col="black")
lines(plotnon, col="blue")

gmIID <- mean(iimeans)  #gran mean of NND values (mean across targets)


#calculate polarization of the group

myangle <- function(x1, y1, x2, y2){ #returns a heading from 2 datapoints
  dx<-c(x2-x1)
  dy<-c(y2-y1)
  theta<-atan2(dy,dx)
  return(theta)
}

myvector <- function(X1,Y1,X2,Y2){  #returns a unit vector pointing to heading, from 2
 points
  ang <- myangle(X1,Y1,X2,Y2)
  dx <- cos(ang)
  dy <- sin(ang)
  return(c(dx,dy))
}

frameangle <- function(frame){   #find the polarization in one frame
  mynumerator <- numtargets   #number of angles that could be found

  vecs <- vector("list", numtargets)   #movement vectors for all targets
  for(i in 1:numtargets){
    temp =
 myvector(mydata[[frame,paste("X",i,sep="")]],mydata[[frame,paste("Y",i,sep="")]],mydata[[
 frame-1,paste("X",i,sep="")]],mydata[[frame-1,paste("Y",i,sep="")]])
    vecs[[i]] = temp
    if(is.na(temp[1])){   #no movement or missing values can lead to missing vectors
      mynumerator <- mynumerator - 1
    }
  }

  if(mynumerator <= 1){  #return vector mgnitude, if it can be calculated
    return(NaN)
  } else {
    dxsum <- Reduce("+", unlist(vecs)[c(TRUE,FALSE)])  #sum of the dxs
    dysum <- Reduce("+", unlist(vecs)[c(FALSE,TRUE)])  #sum of the dys
    sumsize<-sqrt((dxsum^2)+(dysum^2))   #final vector
    return(sumsize/mynumerator)
  }
}

allpols <- mapply(frameangle,2:myframes)   #find the polarization in all frames

#plot the distribution of polarization:
plot1 <- density (allpols, na.rm = TRUE)
plot(plot1, col="black")


acff<-acf(allpols, na.action = na.pass, lag.max = 200)   #find the autocorrelation
 function
downconst <- which(acff$acf<=0.1)[1] - 1   #new downsampling constant: first lag where
 ACF <= 0.1

#downsample the polarization (for stats) using the ACF results
polpts <- c(2:myframes)[seq(1, myframes, downconst)]
pol <- mapply(frameangle, polpts)
pol <- write.csv(pol,"outfile.csv", row.names=FALSE)  #save the data to file
```

**Appendix B: QA lesions:** Brain section examples. Sections from **A**) ID1, Scale: 200 $\mu$m **B**) ID2, Scale: 200 $\mu$m C) ID3, Scale: 1 mm D) ID4, Scale: 1 mm E) ID5, Scale: 1 mm F) ID6, Scale: 1 mm G) ID7, Scale: 1mm. Images taken at 10X magnification. Slices sectioned at 60 $\mu$m. Blue arrows indicate lesion sites.




E
F


G