Social behavior and its psychopharmacological and genetic analysis in zebrafish

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Abstract

Zebrafish are a highly social species, almost never found outside a group. Social behaviors in zebrafish are complex and we have only begun to explore the mechanisms by which genes and environment modulate cognition and, in turn, drive behavior. Here, I focus on two areas that have been most intensely studied in zebrafish social behavior: collective movement (shoaling or schooling), and social choice, how fish choose a group to join. I explore the characteristics of each behavior and how it is affected by genetic or pharmacological manipulations. I discuss the central role played by the dopaminergic and serotonergic systems in maintaining social cohesion in zebrafish. I briefly discuss other social behaviors, such as social learning, individual recognition, and individual differences (or 'animal personality') that have been less well studied. The inherent complexity of social behaviors, involving several individuals of varying backgrounds and experiences, makes linking them to individual genes a major challenge. As this chapter shows, characterizing and manipulating social behaviors provides an excellent base of knowledge from which to explore genetic mechanisms of sociality.

The study of animal social behaviors is one of several fields that occupy the intersection of behavioral ecology and comparative psychology. Writers from each discipline have mostly focused on different types of explanations for social behaviors: ecologists on adaptive function; psychologists on (cognitive) mechanisms (Zentall, 2013). For example, behavioral ecology textbooks often classify social behaviors by the costs or benefits to the participants (e.g., Rubinstein & Alcock, 2019), whereas psychology textbooks tend to focus on the cognitive skills that might underlie specific social effects (e.g., Shettleworth, 2010). Questions about the genetics of behavior occupy a fruitful middle-ground between these two approaches: both the adaptive advantages that led to certain alleles being retained and the mechanisms by which genes influence behavior are key questions. Here, I attempt to provide a broad view of the behavior genetics of zebrafish social behavior that combines elements of both approaches.

Studying the genetics of social behaviors presents several unique challenges. Behavior emerges from the interacting effects of genes and the environment, mediated through cognition (Figure 1A). Since the contents of cognition are not directly observable – especially in non-verbal animals – hypotheses about genetic effects on cognition are necessarily based on a double inference: from genes to behavior, and then from behavior back to cognitive mechanisms. The difficulty of accurately making such inferences is further compounded in the case of social behaviors which, by definition, involve several individuals whose interactions may be shaped, for example, by kinship or previous shared experience. Members of groups may overlap genetically to different degrees (from eusocial insects to groups of unrelated individuals, such as those probably formed by zebrafish), may inhabit more or less shared environments, and may have varying degrees of influence on each other's behavioral decisions (Figure 1B). Finally, social behaviors are complex, sensitive to environmental conditions, and often change across animals' lifetimes (see below). They are often polygenic and qualitative traits, which introduces further challenges to their study (e.g., Wright, 2011). It has also been suggested that social behaviors in vertebrates derive from activity in a number of brain areas (sometimes called the social decision-making

network), rather than being the responsibility of any one brain region (O'Connell & Hofmann, 2012; Bshary et al., 2014).

For all these reasons, the study of the behavioral genetics of sociality is very much in its infancy. As a result, this chapter focuses mostly on social behaviors of zebrafish, and how they are modulated by genetic and environmental effects. Researchers have used a range of genetic and pharmacological manipulations to expose the mechanisms of social behavior, often in pursuit of disease models (Fontana et al., 2018). However, due to the complexity of social behaviors, linking them directly to the effects of one or two gene products is rarely possible. Though zebrafish have been at the forefront of many developments in this field, I will utilize data from other fish species to flesh out parts of our emerging understanding of this complex topic.
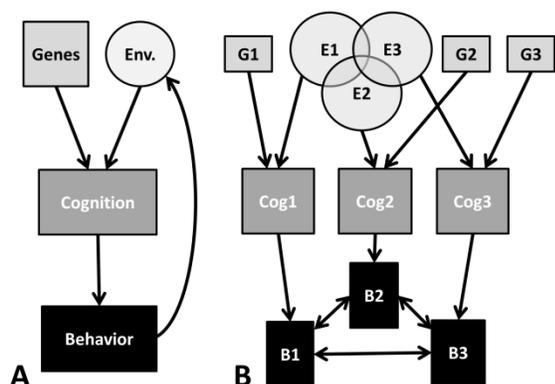

Figure 1. Genetic and environmental effects on behavior. **A**. Genetic and environmental effects combine to drive cognition, which creates behavior, which can then alter the experienced environment. **B**. In the case of social behaviors, individuals' behaviors are also affected by each other and their possibly partly shared environments and genetic backgrounds.

Social behavior may be sub-divided in several ways and encompasses a range of processes from social learning to communication, via collective decision-making, cooperation, and social facilitation (Shettleworth, 2010; Oliveira, 2013). Here, I focus mostly on two types of social behavior that have been intensively studied in zebrafish: collective movement and social choice.

Collective movement: schooling and shoaling

I first deal with collective movement, by far the most intensely studied and modeled social phenomenon in fish (Mirabet et al., 2007; Sumpter, 2010). Zebrafish are almost never found alone and, in still water, generally move and forage in small groups of about 10 individuals (Pritchard et al., 2001; Suriyampola et al 2016) which are referred to as either shoals or schools (Pitcher, 1983; Miller & Gerlai, 2012a). In gently flowing water, which they often inhabit in the wild, groups may be much larger (up to 300 individuals; Suriyampola et al., 2016).

Characterizing zebrafish shoaling

A large body of both empirical (e.g., Katz et al., 2011) and theoretical (e.g., Couzin et al., 2002) research exists on how movement in groups is modulated to avoid splitting of the group while maintaining sufficient distance between group-mates to avoid direct competition for resources (e.g., Miller & Gerlai, 2008). The costs and benefits of being in a shoal have been intensely studied (Krause & Ruxton, 2002). Individuals in a group are less likely to be predated because a large group is likely to detect a predator sooner (the 'many eyes effect'; e.g., Godin et al., 1988), because the risk of predation is diluted in larger groups (Pitcher & Parrish, 1993), and because predators may have difficulty tracking tightly packed phenotypically similar individuals ('predator confusion'; Landeau & Terborgh, 1986). These effects may have helped determine individual traits as well as group behaviors. For example, zebrafish's horizontal stripes may serve to enhance predator confusion (Hogan et al., 2016). Group membership may also confer foraging benefits, such as the ability to capture larger or more dangerous prey than a solitary individual could (Schmitt & Strand, 1982), or advantages in detecting shareable food resources (e.g., Reebs & Gallant, 1997). There are also costs to aggregating, such as the risk of increased parasite load or competition for limited local resources (Krause & Ruxton, 2002).

The movements of zebrafish shoals have been studied both in the lab (Miller & Gerlai, 2011; 2012a) and the wild (Suriyampola et al., 2016). Researchers usually characterize shoals on several measures: by

their size (i.e., number of members), though there is some debate as to the proper criteria for inclusion in a specific group (Miller & Gerlai, 2011; Quera et al., 2013); by the distances between members of the shoal, either the distance from a focal individual to its nearest neighbor (Clark & Evans, 1954) or the mean distance between all individuals (e.g., Miller & Gerlai, 2012b); and by the degree to which all the fish are facing in the same direction, called polarization. Highly polarized shoals are referred to as schools (Miller & Gerlai, 2012a). Both empirical and theoretical studies suggest that the motion of group members is affected primarily by local interactions with several of their nearest neighbors (e.g., Couzin et al., 2002; Partridge, 1981).

Zebrafish groups have several distinct characteristics. Small groups of zebrafish placed in a large bare tank (in a lab) initially spend about 1.5 times as much time schooling than shoaling, but with repeated exposure to the tank begin to shoal more (Miller & Gerlai, 2012a). Groups switch from one mode to the other every few seconds, reflecting the complex interplay of moment-to-moment individual decisions. The polarization of schools is relatively fixed, whereas shoals become progressively less polarized as the group habituates to its environment (Miller & Gerlai, 2012a). Fish in schools swim faster and are slightly further apart than when in shoals (Miller & Gerlai, 2012a; Parrish et al., 2002). Over repeated exposures to the tank, the group gradually dissolves as individuals make longer excursions away from the main body of the group (Miller & Gerlai, 2011; 2012b). In the lab, nearest neighbor distances within the group oscillate with a period between 5-15 seconds (Miller & Gerlai, 2008) around a value of 3-4 cm, about one body length (Dlugos & Rabin, 2003; Miller et al., 2013). These values are similar to those observed for groups of zebrafish in the wild in still, clear water (Suriyampola et al., 2016). However, habitat complexity such as vegetation in the water (e.g., roots in a rice paddy) may disperse shoals, and flowing water (e.g., in a stream) may increase group cohesion (Suriyampola et al., 2016). Zebrafish's ability to swim against a current may also depend on characteristics of the shoal (Wiwchar et al., 2018). It is

worthwhile to note that laboratory conditions rarely mimic the variability of environments fish experience in the wild, or the corresponding effects on their collective movement.

Environmental effects on shoaling

Collective movement in zebrafish develops between the ages of 12-40 days (Engeszer et al., 2007; Buske & Gerlai, 2011), though a preference for locations associated with conspecifics may emerge as early as one week post-hatching (Hinz et al., 2013). This preference increases in strength over the following two weeks, as zebrafish also develop a preference for viewing age-matched conspecifics at an angle (i.e., preferentially with one eye or the other; Dreosti et al., 2015). These aspects of early shoaling development may be considered the building blocks of eventual adult social behaviors. During development, shoals become increasingly cohesive (Buske & Gerlai, 2012; Mahabir et al., 2013), and fish acquire a visual preference for shoal mates similar to those amongst which they were raised (Engeszer et al., 2007). However, exposure during adulthood to a social environment consisting of a mixture of phenotypes can also affect shoaling preferences (Moretz et al., 2007).

There is a growing body of evidence to suggest that the maturation of shoaling behavior is driven by changes in dopamine and serotonin levels in the zebrafish brain. Amounts of both neurotransmitters increase dramatically during the period when shoaling emerges (Buske & Gerlai, 2012), and comparisons of different inbred zebrafish strains show an apparent correlation between the cohesion of the group and levels of dopamine (Mahabir et al., 2013). Additionally, dopamine antagonists have been shown to disrupt shoaling in adult zebrafish (Scerbina et al., 2012), and exposure to images of conspecifics increases dopamine levels in adult zebrafish brains (Saif et al., 2013). Zebrafish raised in isolation show increased inter-individual distances during shoaling and have decreased levels of DOPAC (a dopamine metabolite), but normal levels of serotonin, in their brains (Shams et al., 2018). However, prolonged isolation in adulthood decreases levels of serotonin, but not dopamine, in zebrafish brains (Shams et al.,

2015). Different inbred strains of zebrafish differ in the cohesion of their shoals (e.g., Seguret et al., 2016), and there are differences in the levels of expression of various neurotransmitter receptors between strains (Pan et al., 2012), which may contribute to these behavioral effects. Taken together, these findings suggest that an interplay of the dopaminergic and serotonergic systems in the developing zebrafish brain affect social behaviors in complex ways. It is also likely that other neurotransmitters, that have not received as much attention, play an important role, not to mention various hormonal effects (Oliveira & Gonçalves, 2008).

The distances between members of a shoal may reflect a compromise between competition for food and avoiding predation (Ruzzante, 1994). Guppies (*Poecilia reticulata*) and European minnows (*Phoxinus phoxinus*) descended from wild populations that experienced high levels of predation shoal more tightly in the lab, and tighten their shoals more in reaction to a predator cue, than fish from low-risk populations (Seghers 1974a, 1974b; Magurran & Seghers, 1991; Magurran & Pitcher, 1987; Huizinga et al., 2009). However, this population-genetic effect is modulated by experience (Magurran, 1990) and by rearing conditions, such that guppies from low predation stock raised with tank-mates derived from high-predation stock form tighter shoals, and vice versa (Song et al., 2011). In other words, guppies' genetic predisposition to shoal tightly can be modified by being reared with conspecifics that shoal less tightly, and vice versa. This effect has not, to the best of my knowledge, been directly observed in zebrafish, but some correlates of it have. For example, zebrafish may be able to hide from predators amongst vegetation in the water, which may partly explain why zebrafish shoals in vegetated water are more dispersed (Suriyampola et al., 2016), and members of zebrafish groups do swim closer together for a short while after the silhouette of a bird has passed overhead (Miller & Gerlai, 2007).

When food is concentrated in a defensible location, dominant zebrafish will attempt to monopolize the resource (Hamilton & Dill, 2002). When reared for several generations under conditions where food was

distributed in defensible patches, medaka (*Oryzias latipes*) shoals became tighter on exposure to a predator cue than those of fish reared under conditions where food was spread evenly across the tank surface (Ruzzante & Doyle, 1993). Fish from the former lines that displayed a higher 'social tolerance' – being more willing to remain close to a group of conspecifics – showed a higher growth rate (Ruzzante & Doyle, 1993), suggesting that social tolerance is heritable and may affect other behaviors, such as responses to predator-related cues. It has also been suggested that an increased tolerance for the presence of conspecifics is a prerequisite for the evolution of complex social structures in fish (White et al., 2017) and may be one of several factors supporting the appearance of cumulative culture in humans (Heyes, 2018).

Pharmacological and genetic effects

The large body of research on pharmacological manipulations of zebrafish behaviors includes many examples of how drugs alter shoaling in zebrafish (e.g., Echevarria et al., 2008). Many of these studies have focused on common drugs of abuse, such as alcohol, but an increasing number of studies examine the effects of more specific pharmacological agents, such as neurotransmitter agonists and antagonists.

Acute exposure to alcohol, in addition to its effects on a range of other behaviors, disrupts shoaling in a dose-dependent manner (Gerlai, 2000), a mechanism at least partially mediated by dopamine D1 receptors (Tran et al., 2015). However, repeated brief ethanol exposures over the course of several days tend to slightly *increase* the cohesion of a shoal, without reducing nearest-neighbor distances (Müller et al., 2017), possibly by altering the expression patterns of a range of genes involved in neurotransmitter function and production (Pan et al., 2011). Interestingly, zebrafish – like mammals – develop a tolerance to alcohol during chronic exposure, and show withdrawal effects when it is later removed (Gerlai et al., 2009). LSD and MDMA have also been shown to loosen zebrafish shoals (Green et al., 2012), as does

MK-801 – an NMDA receptor antagonist – even when only one fish in a group of four is exposed to the drug (Maaswinkel et al., 2013).

In some cases, drugs have been shown to have specific effects on different aspects of shoaling. For example, low concentrations of nicotine increase the distance between fish and their nearest neighbors but do not alter the polarization distribution of the shoal/school, whereas alcohol exposure has a smaller effect on distances within the shoal but disrupts polarization more (Miller et al., 2013). Results such as these suggest that there is a benefit to taking several measures of shoals and possibly attempting to link different aspects of collective movement to specific cognitive abilities. With the exception of the studies in the following section, that address one aspect of shoaling and its underlying cognition, this has not been attempted, as far as I am aware.

Several genes have been shown to have an effect on shoaling behavior. Zebrafish with a knockout of *dyrk1a*, a Down-syndrome and autism associated gene, shoal less tightly than wild-type fish and also spend less time close to a barrier behind which they can see a shoal (see below). It is noteworthy that these fish also show reduced stress in asocial assays (Kim et al., 2017). Zebrafish with a knockout of another autism-related gene, *shank3b*, also show increased distances between members of a shoal, as well as reduced social preference (see below), though these fish also display more general movement impairments (Liu et al., 2018).

Many of these pharmacological and genetic effects on social behavior are likely the result of non-specific effects on stress. Increased stress generally increases the cohesion of a shoal, and a range of anxiolytic drugs (such as ethanol, buspirone, or scopolamine; Hamilton et al., 2017) tend to disrupt or dissolve shoals, often in subtly different ways (Gebauer et al., 2011).

Collective movement is not a type of social cognition; it is a specific behavior. However, it emerges from a particular class of reactions to social stimuli. In other words, shoaling fish (and other animals that

move in closely coordinated herds or flocks) likely utilize a set of "traffic rules" (Parrish & Turchin, 1997), which are the cognitive mechanisms underlying their collective movement. This type of mechanism is generally absent from psychological discussions of social cognition, but could be considered a specific form of social facilitation (Zajonc, 1965; Oliveira, 2013) or contagion (Rosenthal et al., 2015). The following section deals with one behavior that contributes to shoaling, and which has been extensively tested independently of collective movement: social choice.

Social Choice

Groups of zebrafish, like those of many other small freshwater species, break up and reform over quite short timescales. In guppies, for example, membership in any given shoal may change approximately every 10 seconds (Croft et al., 2003). Fish must therefore make repeated decisions to join one of possibly several nearby shoals. Though it may seem that joining a group (social choice) and behaving within the group (shoaling) are the same thing, the two behaviors are functionally independent, and mediated by distinct genetic modules, at least in sticklebacks (*Gasterosteus aculeatus*; Greenwood et al., 2015). Several researchers have therefore attempted to document those characteristics of shoals that make them attractive, usually by giving a single test fish the option of approaching one of two shoals – generally located behind a transparent barrier – that differ on some dimension of interest. The cognitive mechanisms associated with this behavior have sometimes been labeled 'social preference' (e.g., Oliveira, 2013).

Zebrafish have been shown to prefer a shoal of conspecifics to an empty tank, even if the stimulus fish are of a different phenotype to the test individual (Sneckser et al., 2006), and different phenotypes of zebrafish prefer to shoal with conspecifics of a similar phenotype (Rosenthal and Ryan, 2005; Sneckser et al., 2010). In mixed-species shoals, zebrafish remain closer to their conspecifics than heterospecifics (Saverino and Gerlai, 2008). Many fish species prefer conspecifics of a similar size to themselves,

possibly because this will act to equate swimming speed and aid in keeping the shoal together (Krause et al., 2000; Croft et al., 2003). Shoals also assort by coloration (McRobert and Bradner, 1998), which increases the visual homogeneity of the shoal and may increase predator confusion. Zebrafish prefer to join more numerous shoals, although this preference also depends on the activity level of the stimulus shoal (Pritchard et al., 2001) and the sex of the stimulus fish (Ruhl and McRobert, 2005).

As with collective movement, various drugs have been shown to modify social choice in zebrafish. MK-801 – an NMDA receptor antagonist – disrupts social preferences in 3-week old zebrafish, as does alcohol (Dreosti et al., 2015). As noted above, knockout of *dyrk1a* – a gene associated with Down syndrome and autism – also impairs social preferences (Kim et al., 2017). Zebrafish exposed to low concentrations of alcohol during embryonic development show a dose-dependent decrease in social choice, an effect that has been suggested as an analogue for fetal alcohol syndrome (Fernandes & Gerlai, 2009).

The complexity of social behaviors, and the large variance in how researchers choose to measure social choice, have led to some conflicting results. For example, oxytocin and arginine-vasopressin, as well as their fish-specific homologs (isotocin and vasotocin), have been shown to increase preference for conspecifics of a similar phenotype in a dose dependent manner, and this effect was inhibited by antagonists for the corresponding receptors (Braida et al., 2012). However, vasotocin, at different dosages, has been shown to *decrease* preference for a shoal over an empty tank, and an antagonist for its receptor had the same effect (Lindeyer et al., 2015). These results point to some of the limitations of social choice paradigms in which test fish are physically separated from the social stimulus and in which the only measure is the proportion of time they spend within some distance of the partition (for other criticisms of one sub-type of this paradigm, and suggestions for improvement, see Agrillo et al., 2017).

Rodents – in what is known as the three-chamber assay – have frequently been shown to prefer

associating with a novel conspecific over an empty cage and over one that they are slightly more familiar

with (e.g., Yang et al., 2011). Zebrafish have recently been tested in a similar paradigm. Zebrafish of

different strains showed varying levels of preference for a novel conspecific over an empty blue box, and

a trend towards preferring a novel conspecific over a more familiar one (Barba-Escobedo & Gould,

2012). Exposure to both buspirone – a 5-HT$_{1A}$ receptor agonist – and to WIN 55,212 – a cannabinoid

receptor agonist – increased social preference, though in subtly different ways (Barba-Escobedo &

Gould, 2012). Note that this result is in some respects opposite to what might be expected, given that

buspirone functions as an anxiolytic drug in zebrafish (Gebauer et al., 2011) and might have been

expected to reduce social preference (see above). Fish exposed to ethanol – also an anxiolytic –

expressed normal preference for a novel conspecific over an empty tank, but did not show a preference

for a novel conspecific over a more familiar one, though control fish did (Ariyasiri et al., 2019).

Interestingly, a similar pattern of results was found for *sam2* knockout zebrafish, which have previously

been shown to display elevated anxiety (Choi et al., 2018). Taken together, these results show that

social choice in zebrafish is a complex phenomenon, affected by changes in anxiety levels (both

increases and decreases) and modulated by several different neurotransmitter systems.

Other paradigms

There are many other forms of social behavior not covered by the review above (see, e.g., Oliveira,

2013). Most of these have been much less studied than shoaling and social choice. There is, however,

interest in different social mechanisms in zebrafish. A growing literature exists on social learning – the

process by which animals learn about their environment by observing the behaviours of others

(reviewed in Nunes et al., 2017) – an area that has received a lot of attention in other fish species (e.g.,

Brown & Laland, 2003). For example, zebrafish can learn a conditioned response to odor or light cues

that predict the presentation of alarm pheromone – a substance released from damaged skin and thus indicative of the probable presence of a predator – and this response will spread to conspecifics in the same shoal that were never exposed to the alarm substance (Hall & Suboski, 1995), a classic example of social transmission.

There has recently been increased interest in the question of whether zebrafish can recognize individual conspecifics. It is known that zebrafish can detect kin, probably using olfactory cues (Mann et al., 2003), and it appears that they are able to distinguish familiar from unfamiliar unrelated conspecifics (Madeira & Oliveira, 2017). This suggests that membership in the aggregations formed by zebrafish in the wild may not be entirely random but may instead benefit from the advantages of shoaling with relatives (Krause & Ruxton, 2002). Individual identification may also play a role in the formation and maintenance of dominance hierarchies in zebrafish (e.g., Paull et al., 2010).

Zebrafish have also been shown to pay more attention to interacting conspecifics than to asocial stimuli or non-interacting fish, and some of the genes whose expression mediates this effect have been identified (Lopes et al., 2015). I note that increased attention to social stimuli is another mechanism (along with increased tolerance of nearby others, discussed above) that has been proposed to be important for the evolution of social structures and culture (Heyes, 2018).

Finally, there has been a recent surge in studies of consistent individual differences, sometimes referred to as 'animal personality', in zebrafish. Many of the early studies in this field focused on fish (Sih et al., 2004), and there is now a large body of research on individual differences in zebrafish (Khan & Echevarria, 2017) and how best to measure them (Toms et al., 2010; Teles & Oliveira, 2016). Individual differences are key to understanding social behaviors, both because the composition of a group may determine how its members react to various stimuli (e.g., Dingemanse et al., 2010), and because social contexts in many cases alter individual reaction norms (e.g., Guayasamin et al., 2017). Individual

differences thus have reproductive consequences, which will shape the genetic backgrounds of the fish

that are available to form groups (e.g., Vargas et al., 2018). There are strain differences in individual

differences in zebrafish (Roy & Bhat, 2018), and the molecular and genetic bases of these differences are

beginning to be elucidated (Laine & van Oers, 2017).

Conclusions

Many zebrafish social behaviors are comprised of and interact with the behaviors addressed in the other

chapters of this volume. For example, there is evidence that aggression and schooling are genetically

related behaviors in several fish species (Ruzzante, 1994), and I have noted above how stress levels

affect social interactions and are, in turn, affected by the availability of social stimuli. Social factors can

also modulate other behaviors. For example, the presence of alarm pheromone causes freezing, erratic

movement, and other stress-like responses in zebrafish (Speedie & Gerlai, 2009; Green et al., 2012), but

these reactions are subdued when within sight of unreactive conspecifics (i.e., conspecifics that are not

exposed to the alarm pheromone; Faustino et al., 2017). Many social behaviors, as a result, depend on

the activities of many genes involved in asocial processes, and often on several cognitive mechanisms,

and elucidating the genetic bases for these behaviors is a challenge.

In many of the studies cited above, dopaminergic and serotonergic systems play a prominent role in

shaping social behaviors. This may be partly due to the large amount of research conducted on these

systems. However, it has also been suggested that these two systems act in concert to create the

conditions necessary for social behavior to emerge (van den Bos, 2015): dopamine activation increases

the tendency of fish to aggregate, possibly by mediating the rewarding consequences of being close to

conspecifics (Saif et al., 2013); and serotonin activation decreases levels of aggression, increasing

tolerance for having others nearby. Our growing understanding of these two central systems, as well as

a growing list of other modulating factors, will eventually allow us to map out how genes shape social cognition and behavior in zebrafish and other vertebrates.

Acknowledgments

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