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Motivated State Control in Larval Zebrafish: Behavioral Paradigms and Anatomical Substrates

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Abstract

Over the course of each day, animals prioritize different objectives. Immediate goals may reflect fluctuating internal homeostatic demands, prompting individuals to seek out energy supplies or warmth. At other times, the environment may present temporary challenges or opportunities. Homeostatic demands and environmental signals often elicit *persistent* changes in an animal's behavior to meet needs and challenges over extended periods of time. These changes reflect the underlying motivational state of the animal. The larval zebrafish has been established as an effective genetically tractable vertebrate system to study neural circuits for sensory-motor reflexes. Fewer studies have exploited zebrafish to study brain circuits that control motivated behavior. In part this is because appropriate conceptual frameworks, anatomical knowledge, and behavioral paradigms are not yet well established. This review sketches a general conceptual framework for studying motivated state control in animal models, how this applies to larval zebrafish and the current knowledge on neuroanatomical substrates for state control in this model.

Keywords

Motivation; behavior; zebrafish; neuromodulator

Introduction

Animal behavior is governed by both signals from the environment, and internal state systems that greatly influence how those signals are interpreted (Baerends, 1971). A major class of internal state systems control behaviors that promote goal-directed activities such as feeding, aggression, social behaviors, and responses to threats. Collectively, we refer to these as motivational state systems. Motivational states reflect the activity of neuromodulatory systems that shape the activity of neuronal circuits over timeframes of minutes to hours (Pfaff et al., 2008). Neuromodulation has been extensively studied at molecular and cellular levels in many systems revealing that amines, neuropeptides and small molecules powerfully

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Declaration of Interest

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influence the activity and output of neurons in most brain regions (Marder and Calabrese, 1996; Marder, O'Leary and Shruti, 2014; Wester and McBain, 2014). Many neuromodulators act broadly, allowing coordinated tuning of disparate circuits and providing flexibility in the function of groups of neurons as computational needs change (Robbins, 1997). However, there is a pressing gap in our understanding of the specific behavioral roles of diverse neuromodulatory systems. The most detailed account of the action of neuromodulators has been provided by studies on the crustacean stomatogastric nervous system (Stein, 2009). Even in this simple model, little is known about the behavioral contexts that drive neuromodulator release; indeed the functional consequences of neuromodulation are only now being explored (Diehl et al., 2013). The strongest evidence that neuromodulators exert context-specific functions has come from experiments in invertebrates. In *Drosophila*, specific serotonergic and dopaminergic neurons that regulate aggressive behavior have been identified (Alekseyenko et al., 2013, 2014), while in *C elegans*, identified neurons use serotonin and pigment dispersing factor to promote roaming and dwelling states respectively (Flavell et al., 2013). Although there is a vast literature on the action of neuromodulators in mammals, many experiments rely on pharmacological manipulations of uncertain specificity, and behavioral tests that do not clearly prove a link to motivational control (Paredes and Agmo, 2004). Genetic and transgenic techniques now permit far more precise manipulations. An area that is ripe for exploration, therefore, is the study of how specific neuromodulators adaptively regulate behavior during different motivated states in vertebrates.

An ideal vertebrate system for parsing the context-specific functions of neuromodulators would have a simple nervous system and discrete repertoire of behaviors. At larval stages, zebrafish have a manageable repertoire of behaviors and a brain that comprises around 100,000 neurons (Fero, Yokogawa and Burgess, 2011). Adults possess a much broader behavioral repertoire, but this sophistication is accompanied by a much larger and less tractable brain. Larvae show circadian changes in behavior with characteristic features of sleep and wakefulness, confirming that at least long-term internal states are maintained (Zhdanova et al., 2001; Prober et al., 2006; Yokogawa et al., 2007). Larvae have already been widely used to decode the neuronal circuitry underlying stereotyped sensory-motor reflexes, using a sophisticated arsenal of tools that facilitate non-invasive monitoring and manipulation of neurons in the intact animal (reviewed in (Chiu and Prober, 2013)). These methods are ideal for studying the neuronal basis of motivated states, where invasive manipulations may irrevocably bias internal state control. This review discusses evidence that larval stage zebrafish exhibit modifications of behavior consistent with motivational states, in the context of a general conceptual framework for studying motivational control in animals. As we are ultimately interested in applying functional insights from fish to understanding the state-dependent use of neuromodulators in humans, we also discuss the neuroanatomical substrates for motivation in fish and the extent to which these systems are conserved.

Defining motivated behavior in animal models

In this review, we will focus on internal states that in humans are experienced as changes in motivational drive. Defining an apparent change in motivational state in animals from

observations of behavior or physiology is difficult - our skill as humans at reading the intentions of other people, arises from experience and cognition, and also uses hard-wired mechanisms that mirror the activation of corresponding neuronal circuits during our observation of human motor actions (Baldwin and Baird, 2001; Bonini, Ferrari and Fogassi, 2013). While zebrafish and humans share fundamental neuroanatomical similarities, evolutionary divergence over the last 400 million years has also lead to significant differences, with many regions of the brain differentially elaborated, as discussed below. Thus, anthromorphic interpretation of behavior, that fails to take into account differences in neuroanatomical function may be extremely misleading. Indeed, Theodore Schneirla famously asserted that "...approach and withdrawal are the only empirical, objective terms applicable to all motivated behavior in all animals" (Schneirla, 1959). Nevertheless, laboratory studies allow the life history and environment of animals to be tightly controlled, ensuring that changes to the environment produce reproducible behavioral responses whose contribution to the well-being and survival of the individual can be sometimes be clearly interpreted. For example, when a nutrient-deprived rat shows greater responsiveness to food odors, it is reasonable to suggest that its behavior is affected by an internal state similar to 'hunger'. Unfortunately, clues to adaptive function from overt behavior are often more ambiguous. For instance many animals show a propensity to locomote near the walls of an arena. This is frequently interpreted as evidence for a wall-hugging behavior known as thigmotaxis, and presented as evidence for anxiety (Schnörr et al., 2012). However, wall-following also arises trivially from predominantly straight movement paths in arenas with concave walls. When tested with convex walls, wall-following may disappear or be greatly diminished (Creed Jr and Miller, 1990). Thus, interpretations of the functional significance of a behavior, especially with respect to the motivational state of the animal, require an abundance of caution.

There is no single definition for motivated behavior. Such states include "the internal proximate causes of behavior, variously labeled as drives, instincts or causal systems" (Colgan, 1993), that "enable the organism to regulate the availability, probability, or proximity of stimuli" (Salamone, 1992).

In addition, motivational states are persistent, lasting minutes to hours, and terminated after an objective has been met (Murray, 1964). Most work focuses on just four types of state: hunger, fear, sex and aggression, broad categories that have the merit of being applicable to most species, but there are many other relevant internally driven goal-directed activities (e.g. parental behaviors, salt-appetite and thirst). Research into sleep using zebrafish and other model organisms has been facilitated by the adoption of criteria that operationally define 'behavioral sleep'. Similarly, a set of criteria that are measurable in animals may assist as we probe neuronal mechanisms for motivated behavior. We propose that an operational definition of a motivated state in animal models should include at least these factors: (1) A change in behavior or physiology that is maintained by internal mechanisms. (2) The change is reversible, persisting temporarily even after a trigger stimulus is no longer present in the environment, or terminating after a specific goal is met. (3) Behavior should be goal-directed, promoting either the acquisition or avoidance of a specific objective. We will consider each of these elements and contrast them with processes that also produce persistent changes in behavior but which do not fulfill these criteria.

First, behavioral changes should be driven by internal processes, as opposed to being continuously triggered by stimuli that persist in the environment. In such cases, it is obviously unnecessary to postulate an enduring change in the animal's internal state, although potentially, behaviors may reflect a mix of ongoing sensorimotor processing and autogenic actions. In larvae, an example is heightened locomotor activity in response to increased water temperature. Here, elevated activity may be directly driven by the continuing presence of noxious heat, but possibly also reflect a motivational drive to escape from the aversive environment (Prober et al., 2008; Curtright et al., 2015). Even sophisticated goal-directed behaviors do not necessarily need to be driven by an internal drive. In *Drosophila*, male courtship behavior can be parsed into a series of discrete steps, sequentially triggered by cues from the female (Bastock and Manning, 1955; Greenspan and Ferveur, 2000). Similarly, during phototaxis, larval zebrafish deploy a series of stereotyped swimming movements for navigating toward a light source (Burgess, Schoch and Granato, 2010). Although these events may appear goal-directed and require coordinated motor actions sustained over several minutes, the behavior can be completely described as a series of motor events acutely triggered by sensory cues; an underlying change in internal state is not essential to explain the behavior. We also distinguish persistent changes in behavior that involve simple sensory or motor plasticity that involve forms of learning (Colgan, 1993). Repeated exposure to abrupt acoustic stimuli induces habituation of the escape response in larvae, reflecting sensory or sensorimotor adaptation (Wolman et al., 2011). Motor responses are also plastic: larval zebrafish, for instance, adaptively regulate the vigor of swim movements in response to feedback on forward propulsion, with persistent changes in motor performance (Portugues and Engert, 2011). Such forms of sensory or motor learning do not necessarily implicate a change in goal-directed activity.

Second, motivational states are usually quickly terminated when an objective has been achieved. Thus, within minutes of nutrient ingestion, food-deprived rats no longer show elevated levels of feeding behavior (Kohn, 1951). Other states typically persist for a limited duration, abating after minutes or hours: after a single foot shock, crouching behavior is initially elevated in rats, then gradually declines over several hours (Blanchard and Blanchard, 1969). This is different from autogenic processes that are regulated over longer time periods such as circadian oscillations. Even at early larval stages, behavior in zebrafish is strongly influenced by an internal circadian clock that drives sleep and wake-like changes in locomotor activity (Cahill, Hurd and Batchelor, 1998; Hurd and Cahill, 2002). We conceptualize circadian changes as providing a baseline for internal state, on which short-term changes in motivated behavior are superimposed. However there may be considerable overlap between systems that govern wakefulness and motivated behavior, discussed below.

Goal-directed behavior during motivated states

Our third criterion is that a motivated state should promote the acquisition or avoidance of a specific objective. Distinct motivated states equip animals to cope with different challenges. Nevertheless, studies on motivated states have distinguished 'activational' and 'directional' components (Salamone, 1988; Bailey et al., 2015; Salamone et al., 2015). The directional component relates to goal-directed behaviors whereby sensory processing and motor programs are tuned to advance homeostatic needs or prepare for anticipated challenges. In

contrast, the activational component refers to a quantitative invigoration of movement, such as the frequency, duration, speed or amplitude of motor activity, that in isolation may not selectively promote acquisition of a goal. We will discuss activational and directional components of motivation in turn, first outlining the connection between motivation and net motor activity, then discussing changes in goal-directed sensory and motor behaviors.

The close connection between motivational drive and movement activation has long been recognized, indeed these processes have been referred to as ‘twin galaxies’ of the brain (Mogenson, Jones and Yim, 1980). This is supported by the intimate link between neural substrates for drive and motor activity through dopaminergic signaling (Wise and Bozarth, 1987; McGinty et al., 2013; Kõks, 2015). Thus, food-restriction triggers an increase in ambulatory activity in many mammals, presumably to facilitate foraging behavior (Koubi et al., 1991; Day, Kyriazakis and Lawrence, 1995; Weed et al., 1997). However, heightened motor activity is not an essential feature of motivated states (Murray, 1964). In mammals, threatening stimuli such as predator odors may trigger flight, but in other cases solicit a freezing response or activate stress pathways without changing motor activity (Perrot-Sinal, Ossenkopp and Kavaliers, 1999; Blanchard, Blanchard and Griebel, 2005; de Oliveira Crisanto et al., 2015). In other cases, movement may be clearly goal-directed: sociosexual stimuli drive the expression of behaviors like grooming and pursuit that show a clear connection to a motivational objective (Paredes and Agmo, 1989). Larval zebrafish show a temporary increase in motor activity following several different types of stimulus, possibly representing behavioral activation during motivated states. After loss of illumination larvae show a persistent increase in locomotor activity, that facilitates swimming back into illuminated regions of the environment (Prober et al., 2006; Burgess and Granato, 2007; Emran et al., 2007; Fernandes et al., 2012). Additionally, exposure to a few seconds of water-flow causes an increase in locomotor activity that persists for several minutes (Yokogawa, Hannan and Burgess, 2012). Flow-induced hyperactivity is accompanied by changes in sensory responsiveness that are consistent with goal-directed behavior, suggesting that it is one component of a motivated state that anticipates additional challenges in a turbulent environment.

The activational component of motivated behaviors is often described as a state of arousal. The term ‘arousal’ has had a shifting meaning in the literature, applied to stages of sleep and wakefulness, and also to phasic periods within the wake state that are characterized by an energization of behavior (Robbins, 1997). The degree to which these phenomena are separable is unclear. For instance, is the arousal that accompanies the sight of a dangerous predator simply an extreme activation of part of the same neural mechanism that governs sleep-wake transitions? Or are mechanisms that govern sleep-wake transitions distinct from those involved in phasic arousal? The latter possibility is seen in *Drosophila*, where dopamine acts at distinct sites within the nervous system to regulate sleep-wake transitions, and episodic arousal induced by an air-puff stimulus (Lebestky et al., 2009). On the other hand, in vertebrates, the neuropeptide hypocretin is required to maintain wakefulness, but also motivates appetite and feeding behavior. It has recently been suggested that tonic hypocretin neuron activity maintains wakefulness, whereas bursts of activity stimulate motivated behaviors like feeding (Mahler et al., 2014).

At least some of the behavioral modifications during a motivational state should promote a specific goal. We will focus on the two most frequently characterized behavioral changes during motivated states: (1) responsiveness to sensory stimuli, and (2) patterns of movement in the environment. However, more complex behaviors may also be modified: in shoaling fish species, hunger decreases shoal cohesion permitting more individualistic foraging patterns to emerge at the expense of increased predatory risk (Sogard and Olla, 1997).

Motivational states strongly increase the salience of relevant sensory signals, improving the ability of animals to select appropriate behavioral responses to the constant flood of competing information reaching the brain. For instance, in mammals, fear elevates sensitivity to threatening stimuli, potentiating startle responses (Anderson, Crowell and Brown, 1985, p. 19; de Sá et al., 2014) whereas hunger significantly increases olfactory sensitivity enabling energy-deprived animals to more readily locate food (Aimé et al., 2007; Ramaekers et al., 2016). During motivated states, changes in sensory responsiveness may be confined to ethologically relevant modalities. Thus, hunger does not alter acoustic startle thresholds in rats or humans, presumably because threatening auditory cues do not convey useful information for locating food (Anderson et al., 1985, p. 19; de Sá et al., 2014). Comparable results have been obtained from work in fish. Starvation increases the responsiveness of several fish species to prey, but actually suppresses escape from predatory threats, possibly reflecting elevated risk-tolerance during food-search behavior (Ware, 1972; Croy and Hughes, 1991; Munk, 1995). Intriguingly, similar results have already been obtained in zebrafish larvae: unfed larvae show reduced avoidance of a simulated visual threat (Clift et al., 2014). Experiments in zebrafish confirm that changes in sensory responsiveness can be highly modality specific. During arousal induced by water-flow, elevated locomotor activity is accompanied by increased sensitivity to visual motion, while other sensory thresholds are not altered (Yokogawa et al., 2012). During water flow, fish are exposed to whole field visual motion, and use this information to orient against the current (Lyon, 1904), thus sensitization to visual motion during water-flow induced arousal prepares fish to respond more quickly in turbulent water. Experiments using controlled expression of neuropeptides in larvae further suggest that motivational states selectively regulate sensory systems. For instance, after increasing hypocretin levels, larvae show increased responsiveness to dark-flash visual stimuli, but no change in responses to acoustic or thermal stimuli (Woods et al., 2014). Changes in responsiveness to sensory stimuli do not necessarily reflect altered sensitivity of the sensory apparatus. Central gating of sensory signals may be even more critical, because changes in behavioral responsiveness are in some cases decoupled from changes in the sensitivity of sensory organs (reviewed in (van Swinderen and Andretic, 2003)).

The goal-directed component of motivated state control also includes changes in the spatial pattern of autogenic locomotor activity, as distinct from the overall level of activity. Changes in the spatial pattern of activity include alterations in where an animal prefers to spend time (place preference), and in its movement path. Place preference reflects goal-directed behavior, in the active avoidance of threats or proximity to incentives. For instance, in mouse, the open field is a standard test for measuring anxiety through comparison of time spent in the perimeter and center of the arena (Crawley, 2007). Anxious mice spend more time in the perimeter of the environment, presumably thereby reducing exposure to threats.

Movement trajectories are also significantly affected by behavioral state. Early studies in *Drosophila* and *Coccinella septempunctata* (ladybird beetle) larvae demonstrated that starvation resulted in an increased rate of re-orientation and period of spatially restricted locomotion after encountering food, consistent with a search state for additional nutrition in the local environment (Carter and Dixon, 1982; Bell et al., 1985). The locomotor dynamics described in these studies were subsequently observed in many other species, including vertebrates, establishing a paradigm for investigating regulation of foraging behavior by hunger (reviewed in (Bell, 1990)).

Finally, motivated states have been suggested to include a component of 'generalized arousal' that encompasses a global increase in responsiveness to sensory stimuli, elevated motor activity and enhanced emotional responsiveness (Pfaff et al., 2008). The existence of a generalized arousal state has been inferred from a statistical factor that accounts for a substantial fraction of the variability in spontaneous locomotion and sensory responsiveness in mice during different arousal experiments (Garey et al., 2003), as well as from the success of selective breeding in generating high and low arousal strains of mice (Weil et al., 2010). A difficulty with this concept is the considerable evidence, as outlined above, that changes in sensory responsiveness are often highly selective, confined to a single ethologically relevant modality. One possibility is that the factor from principal component analysis that has been interpreted as supporting the existence of generalized arousal, in fact relates to a process that governs part of the sleep/wake continuum, rather than contributing to energized behavior during short-term motivated states.

In summary, we suggest that motivated states manifest three distinct and quantifiable changes in behavior: (1) net motor activity, (2) responsiveness to sensory stimuli and (3) the spatiotemporal organization of movement. These distinct processes can be parsed by carefully designed behavioral tests. Studies that differentiate elevated motor activity from goal-directed changes in sensory or motor processing use tasks that measure performance accuracy to distinguish simple hyperactivity from goal-directed movements (Moses et al., 1995). For instance, a task in which mice must continuously depress a lever can distinguish accidental lever-pressing due to hyperactivity and goal-directed lever pressing as part of reward-seeking behavior (Bailey et al., 2015). Similarly, in zebrafish, the rapid orienting of larvae to whole-field visual motion during flow-induced arousal is not simply due to hyperactivity, because the turning movements that cause a change in orientation are more reliably made in the direction of visual motion than during the baseline state (Yokogawa et al., 2012). Behavioral assays like these that distinguish different effects of motivated states are critical for dissecting the context-specific roles of neuromodulatory systems (Paredes and Agmo, 2004).

Neuroanatomical substrates for motivated state control

Current knowledge of the neurophysiologic substrates that serve goal-directed behaviors in vertebrates is mostly derived from studies on mammals, most often rodents. To use zebrafish as a model to dissect mechanisms underlying motivation, similarities and differences to mammalian neuroanatomy need to be understood. The teleostean brain, while constructed according to the common vertebrate bauplan (Striedter, 2005), also diverges in important

ways, with varying degrees of anatomical conservation of transmitter and neuropeptidergic systems most critical for motivation and internal state control in mammals (Kaslin and Panula, 2001; Tay et al., 2011; Herget et al., 2014). An important caveat is that the functional organization of the teleostean forebrain remains incompletely understood, leading to conflicting interpretations regarding the homology or functional equivalence of specific structures with mammals. Notably, information about particular brain structures has been obtained in a piecemeal manner, either from studies using the larval or the mature zebrafish brain. Larval brains can be efficiently investigated using *in situ*-hybridization and epi-fluorescence immunohistology in conjunction with high-throughput imaging technologies (Ronneberger et al., 2012; Marquart et al., 2015; Randlett et al., 2015). For example, the molecular factors that define preoptic and hypothalamic regions, including the ascending dopaminergic systems, are much better investigated in the larval zebrafish brain (Tay et al., 2011; Herget et al., 2014). The mature teleostean brain, in contrast, shows better histological differentiation and visible brain nuclei facilitating annotation of gene expression patterns and brain function. Zebrafish has been added as a model system recently, but due to the rich availability of transgenic lines, there is a rapidly growing literature on the molecular organization and development of the brain and how it compares to tetrapods. As goldfish is a related carp-like (cyprinid) species that shares many similarities in brain organization (Rupp, Wullimann and Reichert, 1996), valuable information is also derived from studies on this model. Goldfish have been used in numerous classical tracing and behavioral studies because of their large size, which facilitates tracing and manipulation with non-genetic methods.

Critical structures for motivational control in mammals include the ascending dopaminergic system of the ventral tegmental area and substantia nigra, the ventral striatum including the nucleus accumbens, the lateral septum, the basal cholinergic septum (basal nucleus of Meynert) and the amygdaloid complex (Swanson and Petrovich, 1998; Phelps and LeDoux, 2005; Zahm, 2006; Salamone et al., 2007; Zahm et al., 2013).These forebrain structures each contain or are influenced by neuromodulatory transmitters including dopamine, serotonin, acetylcholine and the neuropeptides vasopressin and oxytocin, which play central roles in motivation, particularly in the context of social and reproductive behavior (Stoop, Hegoburu and van den Burg, 2015). In addition, the hypothalamus is a vital neuropeptidergic relay station, central to motivational drives that subserve physiological homeostasis (Puelles and Rubenstein, 2015). Recent progress in neuroanatomical findings regarding the corresponding basal forebrain and hypothalamic structures in zebrafish provides a key to understanding neural systems underlying motivation and goal-directed behaviors in teleosts. For instance, neurons in the medial domain of the adult zebrafish pallium are activated during motivated behaviors, supporting a conserved functional role with the mammalian amygdala (von Trotha, Vernier and Bally-Cuif, 2014).

The dopaminergic system exemplifies the mosaic of peculiarities and similarities of neuroanatomical organization between teleosts and mammals (Fig. 1). Teleosts, including cyprinids lack the clusters of dopaminergic neurons that are present in the ventral midbrain of mammals and other tetrapods. Instead, diencephalic clusters of dopaminergic neurons with ascending projections into a subpallial striatal-like area represent candidate homologs of the mammalian VTA/SNc dopaminergic clusters, possibly resembling an ancestral

condition (Rink and Wullimann, 2001, 2002). Supporting this hypothesis, the basal ganglia in fish show significant developmental and structural similarities to mammals, consistent with a conserved functional organization (Wullimann and Mueller, 2004; Mueller and Wullimann, 2009). Expression patterns of conserved regulatory genes during development have greatly facilitated the identification of basal ganglia structures in zebrafish. In mammals, these structures arise from subpallial ganglionic eminences, and expression patterns of *nkx.2.1*, *nkx2.4*, *lhx6* and *lhx7* orthologs reveal a ventrally located primordial pallidum in zebrafish that is homologous to the mammalian medial ganglionic eminence, and a more dorsally located striatal primordium corresponding to the lateral ganglionic eminence (Mueller, Wullimann and Guo, 2008; Manoli and Driever, 2014). Furthermore, the distribution of dopamine receptors and substance P in the teleostean striatopallidum suggest a largely conserved functional organization of the basal ganglia (Sharma, Berthoud and Breckwoldt, 1989; Kapsimali et al., 2000). However, separate pallidal and septal elements have not been identified with certainty in the mature zebrafish (Mueller and Guo, 2009; Ganz et al., 2012) and the expression patterns of *orthopedia* transcription factors and other molecular markers, suggests that the dopaminergic groups projecting to the zebrafish subpallium represent A11 neurons derived from the alar plate pretectal and thalamic regions rather than from the basal plate diencephalon (Kastenhuber et al., 2010; Tay et al., 2011). This finding appears to contradict the notion that these dopaminergic neurons are truly homologous to the ascending dopaminergic system of other vertebrates as claimed earlier (Rink and Wullimann, 2001). Nevertheless, given the conserved organization of the basal ganglia proper, it seems likely that ascending dopaminergic neurons serve similar functions (Lange et al., 2012). Indeed, pharmacological and genetic experiments support this idea. In mammals, the dopaminergic system plays a key role in initiating activity during motivated states (Salamone, 1988; Bailey et al., 2015) and accordingly multiple studies have linked dopaminergic signaling to motor behavior in zebrafish larvae (Souza, Romano-Silva and Tropepe, 2011; Farrell et al., 2011; Irons et al., 2013; McPherson et al., 2016).

The unsolved questions regarding ascending dopaminergic systems in zebrafish suggest that we need a more sophisticated understanding of the posterior tubercular and preoptic-hypothalamic regions. These areas also contain key neuropeptidergic systems including oxotocin- and vasopressin-like neuropeptides. Notably, this region differentiates earlier in zebrafish compared to that of the larval basal ganglia and amygdaloid complex, and may therefore play a more significant role in motivational control at larval stages. The neuropeptidergic systems of the preoptic-hypothalamic area and the posterior tuberculum are characterized by widespread tangential and radial migration during early development, precluding a direct comparison with mammals (Wullimann and Puelles, 1999; Mueller and Wullimann, 2002). However, recent molecular and developmental gene expression pattern analyzes have revealed detailed correspondences between zebrafish and mammals (Machluf, Gutnick and Levkowitz, 2011; Cheung et al., 2013). The preoptic and hypothalamic regions are now seen as highly conserved across vertebrates, most likely because of their central role in maintenance of physiological homeostasis. A dorsal part of the preoptic region in teleosts topologically corresponds to the mammalian paraventricular nucleus involved in appetite and sexual behavior (Forlano and Cone, 2007; Herget et al., 2014). The ventromedial hypothalamus, known to regulate male-male aggression in mammals, also shows conserved

developmental gene expression profile in zebrafish (Kurrasch et al., 2007, 2009). Finally, orthologs of mammalian vasopressin and oxytocin, whose functions are linked to motivated social behaviors, are expressed in larvae at around 5 days post-fertilization (Herget et al., 2014; Caldwell and Albers, 2015). Intriguingly, around this stage, larvae begin to express olfactory guided social recognition, hinting at the possible functional involvement of these neuropeptides (Gerlach et al., 2008; Hinz et al., 2012).

The hypothalamus also plays a central role in coordinating responses to adverse environmental conditions that act as stressors. The hypothalamic-pituitary-adrenal (HPA) axis controls the release of glucocorticoids which act on many cell types to protect the body against threats. The major corticosteroid in teleosts is cortisol and larval zebrafish have a general stress response to changes in the environment, as shown by the many stimuli that trigger cortisol release (Alsop and Vijayan, 2008; Yeh, Glöck and Ryu, 2013). Consistent with the conservation of the HPA axis (actually in fish, the hypothalamic-pituitary-interrenal (HPI) axis) genetic ablation of hypothalamic neurosecretory cells in zebrafish impairs cortisol release in response to stress (Gutierrez-Triana et al., 2014). In mammals, stress drives behavioral adaptations, including appetite suppression (Morton, Meek and Schwartz, 2014), and similarly, after activation of the HPI axis by environmental stressors, larvae show a temporary reduction in predation behavior that recovers around the same time that cortisol levels normalize (De Marco et al., 2014). Responses to aversive cues may additionally be mediated by the locus coeruleus, which whole-brain activity mapping has shown is also activated by different types of aversive stimulus (Randlett et al., 2015).

Finally, brainstem reticular neurons may be required to initiate motivated states. Reticular neurons in the nucleus gigantocellularis (NGC) have been proposed to act as central elements that drive arousal states (Pfaff, Martin and Faber, 2012). In this model, NGC neurons act as first responders for diverse environmental and homeostatic perturbations. NGC neurons have widespread ascending projections through which they may activate neuromodulator systems involved in motivational states and may elevate motor activity through projections to the spinal cord. Provocatively, it has been suggested that NGC neurons evolved from a primitive network of giant medullary neurons, that in fish are represented by reticulospinal neurons such as the Mauthner cell. Like NGC neurons, the Mauthner cell is activated by multiple sensory pathways and activates a vigorous motor activity, the escape response. Curiously, a similar hypothesis was advanced by Landis and Hunt in their seminal book that described the startle response in humans (Landis, 1939). They noted that perturbations that attract attention, may also produce a very mild experience of startle, followed by an emotional response. However, in humans, the NGC does not play a central role in mediating startle responses (Leitner, Powers and Hoffman, 1980) and so the relevant neurons in fish are likely reticulospinal neurons other than the Mauthner cell. Indeed, Mauthner cell activation is accompanied by network activity in many other medullary neurons, providing a possible substrate for persistent arousal (Gahtan et al., 2002).

Prospects for studies on motivation using zebrafish

Thus, zebrafish show substantial neuroanatomical conservation of systems implicated in behavioral state control in mammals, and evidence is accumulating that by 6 days post fertilization, larvae show persistent changes in behavior consistent with the presence of motivational states. Why use larvae to dissect motivational circuits? Work in the crustacean stomatogastric ganglion system has highlighted the remarkable degree to which circuit dynamics are tuned by a large number of different neuromodulators (reviewed in (Marder, 2012)). Neuromodulators thus profoundly influence computation and information processing by neuronal circuits during internal states. However, at present, there is limited information on the functional contribution of individual neuromodulator systems to defined motivational states in vertebrates. There is much evidence that neuromodulators regulate circuit function in a manner consistent with a role in motivation, but we know much less about how the endogenous release of neuromodulators selectively regulates behavior. Distinct motivated states result from the coherent activity of multiple neuromodulator systems, which likely have both overlapping and unique functions in each state. Adding to the complexity, individual neurons may co-release multiple neuromodulators and achieve appropriate modulation of target circuit activity through their coordinated activities (reviewed in (Nusbaum et al., 2001)). It is therefore critical to delineate the circumstances in which neuromodulator secreting neurons are activated, thereby altering the function of target circuits. For instance, there is considerable evidence that serotonergic signaling from the dorsal raphe has a state-dependent role in modulating responses to sensory cues. Activation of dorsal raphe neurons suppresses neuronal responses to acoustic, olfactory and mechanosensory stimuli (Andersen and Dafny, 1982; Sheibani and Farazifard, 2006; Petzold, Hagiwara and Murthy, 2009; Dugué et al., 2014), although sub-groups of sensory neurons also show increased or prolonged sensory responses during serotonin application (Ebert and Ostwald, 1992; Hurley and Pollak, 1999). Such studies are typically performed without actively controlling the internal state of the animal. A full accounting requires that we understand the functional consequences of endogenous neuromodulator activity during well-controlled motivational states. Zebrafish larvae constitute a valuable system because genetic and transgenic tools allow neuronal circuitry to be probed at the cellular level. For instance, in zebrafish, ablation of dorsal raphe neurons prevents sensitization to visual motion that normally occurs during flow-induced arousal, showing how serotonergic neurons normally contribute to the maintenance of a defined behavioral state (Yokogawa et al., 2012).

Still, motivational states that are manifest at larval stages remain incompletely characterized, and thus the richness of the motivational ‘repertoire’ is not yet clear. Important forms of motivation that are widely studied in mammals drive social behaviors such as aggression and mating. Current evidence however, suggests that social behaviors appear in zebrafish only after the first week of development (Engeszer et al., 2007; Dreosti et al., 2015), although sensory imprinting involved in social recognition may occur during this period (Gerlach et al., 2008). Thus a limitation of studies using larvae is that motivational control of social behavior likely appears only at juvenile or adult stages and thus may not be amenable to analysis using the simpler larval nervous system.

A final question concerns the dominant motivational state of larvae at 5–7 days post-fertilization, a stage commonly used for behavioral studies. Motivational state can influence almost any aspect of physiology or behavior, but is ignored in many behavioral studies. Without any intervention, what is the ‘baseline’ motivational state of larvae at this stage? In fact, this likely varies greatly between experiments and laboratories. Simply moving a larva from a holding area into the testing arena produces a distinctive temporal pattern of locomotor activity: at first larvae show strongly reduced movement, followed by several minutes of heightened activity before activity settles into a steady state (Yokogawa et al., 2012). These periods may well reflect changes in motivational drive. Understanding the baseline state becomes increasingly important as we attempt to manipulate motivational drives. One possibility is that by 5 days post fertilization, larvae are primarily motivated to consume food. At this stage, yolk supplies are almost depleted, suggesting a need to replenish energy supplies and nutrients. Around the same time, larvae first begin to show well coordinated predation behavior (Budick and O’Malley, 2000; McElligott and O’Malley, 2005; Bianco and Engert, 2015), and feeding modifies several larval behaviors (Clift et al., 2014). Thus, one possibility is that after 5 days, larval behavior is adjusted by motivational systems to maximize chances of consuming food. This might include regulation of visual systems for improved recognition of prey items, and modification of movement patterns for foraging behavior. A resolution to this question will be important for correctly interpreting studies on motivation.

The notion of motivational drive was introduced by the American psychologist Robert Woodworth as part of his quest to introduce the study of ‘motivology’ (Woodworth, 1918; Graham, 1967). Woodworth considered the case of a baseball pitcher, describing the problem of drive as “why he is engaged in this exercise at all, why he pitches better on one day than on another, why he rouses himself more against one than against another batter” He observed that science regards ‘Why’ questions with great suspicion, preferring to substitute ‘How’ questions. Almost 100 years later, mechanisms that maintain motivated states and translate objectives into actions are starting to be unraveled. Genetic tractability, neuroanatomical simplicity and a growing range of suitable behavioral tests make the larval zebrafish an appealing system for these studies. Thus, at this very old frontier of neuroscience, one of the newest model organisms to be exploited by neuroscientists, is poised to finally provide compelling answers to the question of ‘How’.

Zebrafish dopaminergic cell groups sending ascending projections to the teleostean striatopallidum are located in the posterior tuberculum of the diencephalic basal plate. The nature of these neurons is still under debate. For example, they may be derived from ascending dopaminergic groups of ancestral vertebrates and homologous to the ventral midbrain (ventral tegmental area and substantia nigra pars compacta corresponding to A9–11) neurons of mammals (Rink and Wullimann, 2001). Alternatively it has been suggested that they are homologous to A11 dopaminergic neurons. Despite the fact that these neurons are located in the alar plate pretectum and thalamus in adult rodents (as indicated in this figure), they may be derived from alar plate hypothalamic or posterior tubercular sites based on their expression of otp and nkx2.1 during development (Ryu et al., 2007). However, otp may not specifically label these neurons making other explanations possible. Therefore, it remains currently an open question if zebrafish possess dopaminergic neurons that are

homologous and/or functionally equivalent to the mammalian ascending ventral midbrain dopaminergic neurons.

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References

Aimé P, Duchamp-Viret P, Chaput MA, Savigner A, Mahfouz M and Julliard AK (2007) Fasting increases and satiation decreases olfactory detection for a neutral odor in rats. Behavioural Brain Research, 179, 258–264. [PubMed: 17367877]

Alekseyenko OV, Chan Y-B, Fernandez M de la P, Bülow T, Pankratz MJ and Kravitz EA (2014) Single serotonergic neurons that modulate aggression in Drosophila. Current biology: CB, 24, 2700–2707. [PubMed: 25447998]

Alekseyenko OV, Chan Y-B, Li R and Kravitz EA (2013) Single dopaminergic neurons that modulate aggression in Drosophila. Proceedings of the National Academy of Sciences of the United States of America, 110, 6151–6156. [PubMed: 23530210]

Alsop D and Vijayan MM (2008) Development of the corticosteroid stress axis and receptor expression in zebrafish. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology, 294, R711–R719.

Andersen E and Dafny N (1982) Dorsal raphe nucleus modulates sensory evoked responses in caudate and septum. Int J Neurosci, 17, 151–5. [PubMed: 6302021]

Anderson DC, Crowell CR and Brown JS (1985) Startle potentiation and heart rate as affected by fear and hunger. Psychological Reports, 56, 95–106. [PubMed: 3983328]

Baerends GP (1971) The Ethological Analysis of Fish Behaviour. Fish Physiology pp. 279–370. Academic Press, New York.

Bailey MR, Jensen G, Taylor K, Mezias C, Williamson C, Silver R, et al. (2015) A novel strategy for dissecting goal-directed action and arousal components of motivated behavior with a progressive hold-down task. Behavioral Neuroscience, 129, 269–280. [PubMed: 26030428]

Baldwin DA and Baird JA (2001) Discerning intentions in dynamic human action. Trends in Cognitive Sciences, 5, 171–178. [PubMed: 11287271]

Bastock M and Manning A (1955) The Courtship of Drosophila melanogaster. Behaviour, 8, 85–111.

Bell WJ (1990) Searching behavior patterns in insects. Annual Review of Entomology, 35, 447–467.

Bell WJ, Cathy T, Roggero RJ, Kipp LR and Tobin TR (1985) Sucrose-stimulated searching behaviour of Drosophila melanogaster in a uniform habitat: modulation by period of deprivation. Animal Behaviour, 33, 436–448.

Bianco IH and Engert F (2015) Visuomotor transformations underlying hunting behavior in zebrafish. *Current biology*: CB, 25, 831–846. [PubMed: 25754638]

Blanchard RJ and Blanchard DC (1969) Crouching as an index of fear. Journal of Comparative and Physiological Psychology, 67, 370–375. [PubMed: 5787388]

Blanchard DC, Blanchard RJ and Griebel G (2005) Defensive responses to predator threat in the rat and mouse Current Protocols in Neuroscience / Editorial Board, Crawley Jacqueline N. *... [*et al.*]*, **Chapter** 8, Unit 8.19.**Chapter**

Bonini L, Ferrari PF and Fogassi L (2013) Neurophysiological bases underlying the organization of intentional actions and the understanding of others' intention. Consciousness and Cognition, 22, 1095–1104. [PubMed: 23545395]

Budick S and O'Malley D (2000) Locomotor repertoire of the larval zebrafish: swimming, turning and prey capture. J Exp Biol, 203, 2565–2579. [PubMed: 10934000]

Burgess HA and Granato M (2007) Modulation of locomotor activity in larval zebrafish during light adaptation. Journal of Experimental Biology, 210, 2526–39. [PubMed: 17601957]

Burgess HA, Schoch H and Granato M (2010) Distinct retinal pathways drive spatial orientation behaviors in zebrafish navigation. Current Biology, 20, 381–386. [PubMed: 20153194]

Cahill G, Hurd M and Batchelor M (1998) Circadian rhythmicity in the locomotor activity of larval zebrafish. Neuroreport, 9, 3445–3449. [PubMed: 9855296]

Caldwell HK and Albers HE (2015) Oxytocin, Vasopressin, and the Motivational Forces that Drive Social Behaviors. Current Topics in Behavioral Neurosciences.

Carter MC and Dixon FG (1982) Habitat Quality and the Foraging Behaviour of Coccinellid Larvae. Journal of Animal Ecology, 51, 865–878.

Cheung CC, Kurrasch DM, Liang JK and Ingraham HA (2013) Genetic labeling of steroidogenic factor-1 (SF-1) neurons in mice reveals ventromedial nucleus of the hypothalamus (VMH) circuitry beginning at neurogenesis and development of a separate non-SF-1 neuronal cluster in the ventrolateral VMH. The Journal of Comparative Neurology, 521, 1268–1288. [PubMed: 22987798]

Chiu CN and Prober DA (2013) Regulation of zebrafish sleep and arousal states: current and prospective approaches. Frontiers in Neural Circuits, 7, 58. [PubMed: 23576957]

Clift D, Richendrfer H, Thorn RJ, Colwill RM and Creton R (2014) High-throughput analysis of behavior in zebrafish larvae: effects of feeding. Zebrafish, 11, 455–461. [PubMed: 25153037]

Colgan P (1993) The motivational basis of fish behaviour. Behaviour of Teleost Fishes, 31–50.

Crawley JN (2007) What's Wrong With My Mouse: Behavioral Phenotyping of Transgenic and Knockout Mice. John Wiley & Sons.

Creed RP, Jr and Miller JR (1990) Interpreting animal wall-following behavior. Experientia, 46, 758–761.

Croy MI and Hughes RN (1991) The influence of hunger on feeding behaviour and on the acquisition of learned foraging skills by the fifteen-spined stickleback, Spinachia spinachia L. Animal Behaviour, 41, 161–170.

Curtright A, Rosser M, Goh S, Keown B, Wagner E, Sharifi J, et al. (2015) Modeling nociception in zebrafish: a way forward for unbiased analgesic discovery. PloS One, 10, e0116766. [PubMed: 25587718]

Day JEL, Kyriazakis I and Lawrence AB (1995) The effect of food deprivation on the expression of foraging and exploratory behaviour in the growing pig. Applied Animal Behaviour Science, 42, 193–206.

De Marco RJ, Groneberg AH, Yeh C-M, Treviño M and Ryu S (2014) The behavior of larval zebrafish reveals stressor-mediated anorexia during early vertebrate development. Frontiers in Behavioral Neuroscience, 8, 367. [PubMed: 25368561]

Diehl F, White RS, Stein W and Nusbaum MP (2013) Motor Circuit-Specific Burst Patterns Drive Different Muscle and Behavior Patterns. The Journal of Neuroscience, 33, 12013–12029. [PubMed: 23864688]

Dreosti E, Lopes G, Kampff AR and Wilson SW (2015) Development of social behavior in young zebrafish. Frontiers in Neural Circuits, 39.

Dugué GP, Lörincz ML, Lottem E, Audero E, Matias S, Correia PA, et al. (2014) Optogenetic recruitment of dorsal raphe serotonergic neurons acutely decreases mechanosensory responsivity in behaving mice. PloS One, 9, e105941. [PubMed: 25148042]

Ebert U and Ostwald J (1992) Serotonin modulates auditory information processing in the cochlear nucleus of the rat. Neuroscience Letters, 145, 51–54. [PubMed: 1461567]

Emran F, Rihel J, Adolph AR, Wong KY, Kraves S and Dowling JE (2007) OFF ganglion cells cannot drive the optokinetic reflex in zebrafish. Proc Natl Acad Sci U S A, 104, 19126–31. [PubMed: 18025459]

Engeszer RE, Da Barbiano LA, Ryan MJ and Parichy DM (2007) Timing and plasticity of shoaling behaviour in the zebrafish, Danio rerio. Anim Behav, 74, 1269–1275. [PubMed: 18978932]

Farrell TC, Cario CL, Milanese C, Vogt A, Jeong J-H and Burton EA (2011) Evaluation of spontaneous propulsive movement as a screening tool to detect rescue of Parkinsonism phenotypes in zebrafish models. Neurobiology of Disease, 44, 9–18. [PubMed: 21669287]

Fernandes AM, Fero K, Arrenberg AB, Bergeron SA, Driever W and Burgess HA (2012) Deep Brain Photoreceptors Control Light-Seeking Behavior in Zebrafish Larvae. Current Biology, 22, 2042–7. [PubMed: 23000151]

Fero K, Yokogawa T and Burgess HA (2011) The behavioral repertoire of larval zebrafish. *Zebrafish Models in Neurobehavioral Research* (eds A. Kalueff), & J. Cachat), pp. 249–291. Springer, New York.

Flavell SW, Pokala N, Macosko EZ, Albrecht DR, Larsch J and Bargmann CI (2013) Serotonin and the neuropeptide PDF initiate and extend opposing behavioral states in C. elegans. Cell, 154, 1023–1035. [PubMed: 23972393]

Forlano PM and Cone RD (2007) Conserved neurochemical pathways involved in hypothalamic control of energy homeostasis. The Journal of Comparative Neurology, 505, 235–248. [PubMed: 17879270]

Gahtan E, Sankrithi N, Campos JB and O'Malley DM (2002) Evidence for a widespread brain stem escape network in larval zebrafish. J Neurophysiol, 87, 608–14. [PubMed: 11784774]

Ganz J, Kaslin J, Freudenreich D, Machate A, Geffarth M and Brand M (2012) Subdivisions of the adult zebrafish subpallium by molecular marker analysis. The Journal of Comparative Neurology, 520, 633–655. [PubMed: 21858823]

Garey J, Goodwillie A, Frohlich J, Morgan M, Gustafsson JA, Smithies O, et al. (2003) Genetic contributions to generalized arousal of brain and behavior. Proceedings of the National Academy of Sciences, USA, 100, 11019–22.

Gerlach G, Hodgins-Davis A, Avolio C and Schunter C (2008) Kin recognition in zebrafish: a 24-hour window for olfactory imprinting. Proc Biol Sci, 275, 2165–70. [PubMed: 18544507]

Graham CH (1967) Robert Sessions Woodworth 1869–1962: A Biographical Memoir by Clarence H. Graham. National Academy of Sciences, Washington DC.

Greenspan RJ and Ferveur JF (2000) Courtship in Drosophila. Annual Review of Genetics, 34, 205–232.

Gutierrez-Triana JA, Herget U, Lichtner P, Castillo-Ramírez LA and Ryu S (2014) A vertebrate-conserved cis-regulatory module for targeted expression in the main hypothalamic regulatory region for the stress response. BMC developmental biology, 14, 41. [PubMed: 25427861]

Herget U, Wolf A, Wullimann MF and Ryu S (2014) Molecular neuroanatomy and chemoarchitecture of the neurosecretory preoptic-hypothalamic area in zebrafish larvae. The Journal of Comparative Neurology, 522, 1542–1564. [PubMed: 24127437]

Hinz C, Gebhardt K, Hartmann AK, Sigman L and Gerlach G (2012) Influence of kinship and MHC class II genotype on visual traits in zebrafish larvae (Danio rerio). PloS One, 7, e51182. [PubMed: 23251449]

Hurd M and Cahill G (2002) Entraining Signals Initiate Behavioral Circadian Rhythmicity in Larval Zebrafish. Journal of Biological Rhythms, 17, 307–314. [PubMed: 12164247]

Hurley LM and Pollak GD (1999) Serotonin differentially modulates responses to tones and frequency-modulated sweeps in the inferior colliculus. Journal of Neuroscience, 19, 8071–8082. [PubMed: 10479707]

Irons TD, Kelly PE, Hunter DL, Macphail RC and Padilla S (2013) Acute administration of dopaminergic drugs has differential effects on locomotion in larval zebrafish. Pharmacology, Biochemistry, and Behavior, 103, 792–813.

Kapsimali M, Vidal B, Gonzalez A, Dufour S and Vernier P (2000) Distribution of the mRNA encoding the four dopamine D(1) receptor subtypes in the brain of the european eel (Anguilla anguilla): comparative approach to the function of D(1) receptors in vertebrates. The Journal of Comparative Neurology, 419, 320–343. [PubMed: 10723008]

Kaslin J and Panula P (2001) Comparative anatomy of the histaminergic and other aminergic systems in zebrafish (Danio rerio). J Comp Neurol, 440, 342–77. [PubMed: 11745628]

Kastenhuber E, Kratochwil CF, Ryu S, Schweitzer J and Driever W (2010) Genetic dissection of dopaminergic and noradrenergic contributions to catecholaminergic tracts in early larval zebrafish. The Journal of Comparative Neurology, 518, 439–458. [PubMed: 20017210]

Kohn M (1951) Satiation of hunger from food injected directly into the stomach versus food ingested by mouth. Journal of Comparative and Physiological Psychology, 44, 412–422. [PubMed: 14888734]

Kõks S (2015) Experimental Models on Effects of Psychostimulants. International Review of Neurobiology, 120, 107–129. [PubMed: 26070755]

Koubi HE, Robin JP, Dewasmes G, Le Maho Y, Frutoso J and Minaire Y (1991) Fasting-induced rise in locomotor activity in rats coincides with increased protein utilization. Physiology & Behavior, 50, 337–343. [PubMed: 1745678]

Kurrasch DM, Cheung CC, Lee FY, Tran PV, Hata K and Ingraham HA (2007) The neonatal ventromedial hypothalamus transcriptome reveals novel markers with spatially distinct patterning. *The Journal of Neuroscience*: The Official Journal of the Society for Neuroscience, 27, 13624–13634.

Kurrasch DM, Nevin LM, Wong JS, Baier H and Ingraham HA (2009) Neuroendocrine transcriptional programs adapt dynamically to the supply and demand for neuropeptides as revealed in NSF mutant zebrafish. Neural Development, 4, 22. [PubMed: 19549326]

Landis C and W.A. H (1939) The Startle Pattern. Farrar & Rinehart, New York.

Lange M, Norton W, Coolen M, Chaminade M, Merker S, Proft F, et al. (2012) The ADHD-susceptibility gene lphn3.1 modulates dopaminergic neuron formation and locomotor activity during zebrafish development. Molecular Psychiatry, 17, 946–954. [PubMed: 22508465]

Lebestky T, Chang JS, Dankert H, Zelnik L, Kim YC, Han KA, et al. (2009) Two different forms of arousal in Drosophila are oppositely regulated by the dopamine D1 receptor ortholog DopR via distinct neural circuits. Neuron, 64, 522–36. [PubMed: 19945394]

Leitner DS, Powers AS and Hoffman HS (1980) The neural substrate of the startle response. Physiol Behav, 25, 291–7. [PubMed: 7413836]

Lyon EP (1904) On rheotropism. I. - Rheotropism in fishes. Am J Physiol, 12, 149–161.

Machluf Y, Gutnick A and Levkowitz G (2011) Development of the zebrafish hypothalamus. Annals of the New York Academy of Sciences, 1220, 93–105. [PubMed: 21388407]

Mahler SV, Moorman DE, Smith RJ, James MH and Aston-Jones G (2014) Motivational activation: a unifying hypothesis of orexin/hypocretin function. Nature Neuroscience, 17, 1298–1303. [PubMed: 25254979]

Manoli M and Driever W (2014) nkx2.1 and nkx2.4 genes function partially redundant during development of the zebrafish hypothalamus, preoptic region, and pallidum. Frontiers in Neuroanatomy, 8, 145. [PubMed: 25520628]

Marder E (2012) Neuromodulation of Neuronal Circuits: Back to the Future. Neuron, 76, 1–11. [PubMed: 23040802]

Marder E and Calabrese RL (1996) Principles of rhythmic motor pattern generation. Physiological Reviews, 76, 687–717. [PubMed: 8757786]

Marder E, O'Leary T and Shruti S (2014) Neuromodulation of Circuits with Variable Parameters: Single Neurons and Small Circuits Reveal Principles of State-Dependent and Robust Neuromodulation. Annual Review of Neuroscience, 37, 329–346.

Marquart GD, Tabor KM, Brown M, Strykowski JL, Varshney GK, LaFave MC, et al. (2015) A 3D Searchable Database of Transgenic Zebrafish Gal4 and Cre Lines for Functional Neuroanatomy Studies. Frontiers in Neural Circuits, 9, 78. [PubMed: 26635538]

McElligott M and O'Malley D (2005) Prey Tracking by Larval Zebrafish: Axial Kinematics and Visual Control. Brain Behav Evol, 66, 177–96. [PubMed: 16088102]

McGinty VB, Lardeux S, Taha SA, Kim JJ and Nicola SM (2013) Invigoration of reward seeking by cue and proximity encoding in the nucleus accumbens. Neuron, 78, 910–922. [PubMed: 23764290]

McPherson AD, Barrios JP, Luks-Morgan SJ, Manfredi JP, Bonkowsky JL, Douglass AD, et al. (2016) Motor Behavior Mediated by Continuously Generated Dopaminergic Neurons in the Zebrafish Hypothalamus Recovers after Cell Ablation.: Current biology CB, 26, 263–269. [PubMed: 26774784]

Mogenson GJ, Jones DL and Yim CY (1980) From motivation to action: Functional interface between the limbic system and the motor system. Progress in Neurobiology, 14, 69–97. [PubMed: 6999537]

Morton GJ, Meek TH and Schwartz MW (2014) Neurobiology of food intake in health and disease.. Nature Reviews Neuroscience, 15, 367–378. [PubMed: 24840801]

Moses J, Loucks JA, Watson HL, Matuszewich L and Hull EM (1995) Dopaminergic drugs in the medial preoptic area and nucleus accumbens: Effects on motor activity, sexual motivation, and sexual performance. Pharmacology Biochemistry and Behavior, 51, 681–686.

Mueller T and Guo S (2009) The distribution of GAD67-mRNA in the adult zebrafish (teleost) forebrain reveals a prosomeric pattern and suggests previously unidentified homologies to tetrapods. The Journal of Comparative Neurology, 516, 553–568. [PubMed: 19673006]

Mueller T and Wullimann MF (2002) BrdU-, neuroD (nrd)- and Hu-studies reveal unusual non-ventricular neurogenesis in the postembryonic zebrafish forebrain. Mechanisms of Development, 117, 123–135. [PubMed: 12204253]

Mueller T and Wullimann MF (2009) An evolutionary interpretation of teleostean forebrain anatomy. Brain, Behavior and Evolution, 74, 30–42.

Mueller T, Wullimann MF and Guo S (2008) Early teleostean basal ganglia development visualized by zebrafish Dlx2a, Lhx6, Lhx7, Tbr2 (eomesa), and GAD67 gene expression. The Journal of Comparative Neurology, 507, 1245–1257. [PubMed: 18181142]

Munk P (1995) Foraging behaviour of larval cod (Gadus morhua) influenced by prey density and hunger. Marine Biology, 122, 205–212.

Murray EJ (1964) Motivation and Emotion. Prentice-Hall Englewood Cliffs, NJ.

Nusbaum MP, Blitz DM, Swensen AM, Wood D and Marder E (2001) The roles of co-transmission in neural network modulation. Trends in Neurosciences, 24, 146–154. [PubMed: 11182454]

de Oliveira Crisanto K, de Andrade WMG, de Azevedo Silva KD, Lima RH, de Oliveira Costa MSM, de Souza Cavalcante J, et al. (2015) The differential mice response to cat and snake odor. Physiology & Behavior, 152, 272–279. [PubMed: 26471840]

Paredes R and Agmo A (1989) Stereospecific actions of baclofen on sociosexual behavior, locomotor activity and motor execution. Psychopharmacology, 97, 358–364. [PubMed: 2497485]

Paredes RG and Agmo A (2004) Has dopamine a physiological role in the control of sexual behavior? A critical review of the evidence. Progress in Neurobiology, 73, 179–226. [PubMed: 15236835]

Perrot-Sinal TS, Ossenkopp KP and Kavaliers M (1999) Brief predator odour exposure activates the HPA axis independent of locomotor changes. Neuroreport, 10, 775–780. [PubMed: 10208547]

Petzold GC, Hagiwara A and Murthy VN (2009) Serotonergic modulation of odor input to the mammalian olfactory bulb. Nat Neurosci, 12, 784–91. [PubMed: 19430472]

Pfaff DW, Martin EM and Faber D (2012) Origins of arousal: roles for medullary reticular neurons. Trends in Neurosciences, 35, 468–476. [PubMed: 22626543]

Pfaff D, Ribeiro A, Matthews J and Kow LM (2008) Concepts and mechanisms of generalized central nervous system arousal. Annals of the New York Academy of Sciences, 1129, 11–25. [PubMed: 18591465]

Phelps EA and LeDoux JE (2005) Contributions of the amygdala to emotion processing: from animal models to human behavior. Neuron, 48, 175–187. [PubMed: 16242399]

Portugues R and Engert F (2011) Adaptive locomotor behavior in larval zebrafish. Frontiers in Systems Neuroscience, 5, 72. [PubMed: 21909325]

Prober DA, Rihel J, Onah AA, Sung R-J and Schier AF (2006) Hypocretin/Orexin Overexpression Induces An Insomnia-Like Phenotype in Zebrafish. J. Neurosci, 26, 13400–13410. [PubMed: 17182791]

Prober DA, Zimmerman S, Myers BR, McDermott BM, Jr., Kim SH, Caron S, et al. (2008) Zebrafish TRPA1 channels are required for chemosensation but not for thermosensation or mechanosensory hair cell function. J Neurosci, 28, 10102–10. [PubMed: 18829968]

Puelles L and Rubenstein JLR (2015) A new scenario of hypothalamic organization: rationale of new hypotheses introduced in the updated prosomeric model. Frontiers in Neuroanatomy, 9, 27. [PubMed: 25852489]

Ramaekers MG, Verhoef A, Gort G, Luning PA and Boesveldt S (2016) Metabolic and Sensory Influences on Odor Sensitivity in Humans. Chemical Senses, 41, 163–168. [PubMed: 26567260]

Randlett O, Wee CL, Naumann EA, Nnaemeka O, Schoppik D, Fitzgerald JE, et al. (2015) Whole-brain activity mapping onto a zebrafish brain atlas. Nature Methods.

Rink E and Wullimann M (2001) The teleostean (zebrafish) dopaminergic system ascending to the subpallium (striatum) is located in the basal diencephalon (posterior tuberculum). Brain Res, 889, 316–30. [PubMed: 11166725]

Rink E and Wullimann MF (2002) Connections of the ventral telencephalon and tyrosine hydroxylase distribution in the zebrafish brain (Danio rerio) lead to identification of an ascending dopaminergic system in a teleost. Brain Res Bull, 57, 385–7. [PubMed: 11922994]

Robbins TW (1997) Arousal systems and attentional processes. Biol Psychol, 45, 57–71. [PubMed: 9083644]

Ronneberger O, Liu K, Rath M, Ruebeta D, Mueller T, Skibbe H, et al. (2012) ViBE-Z: a framework for 3D virtual colocalization analysis in zebrafish larval brains. Nat Methods, 9, 735–42. [PubMed: 22706672]

Rupp B, Wullimann MF and Reichert H (1996) The zebrafish brain: a neuroanatomical comparison with the goldfish. Anatomy and Embryology, 194, 187–203. [PubMed: 8827327]

Ryu S, Mahler J, Acampora D, Holzschuh J, Erhardt S, Omodei D, et al. (2007) Orthopedia homeodomain protein is essential for diencephalic dopaminergic neuron development. Curr Biol, 17, 873–80. [PubMed: 17481897]

de Sá DSF, Plein DE, Schulz A, Oitzl MS, Blumenthal TD and Schächinger H (2014) Acoustic startle reactivity while processing reward-related food cues during food deprivation: Evidence from women in different menstrual cycle phases and men. Psychophysiology, 51, 159–167. [PubMed: 24354658]

Salamone JD (1988) Dopaminergic involvement in activational aspects of motivation: Effects of haloperidol on schedule-induced activity, feeding, and foraging in rats. Psychobiology, 16, 196–206.

Salamone JD (1992) Complex motor and sensorimotor functions of striatal and accumbens dopamine: involvement in instrumental behavior processes. Psychopharmacology, 107, 160–174. [PubMed: 1615120]

Salamone JD, Correa M, Farrar A and Mingote SM (2007) Effort-related functions of nucleus accumbens dopamine and associated forebrain circuits. Psychopharmacology, 191, 461–482. [PubMed: 17225164]

Salamone JD, Pardo M, Yohn SE, López-Cruz L, SanMiguel N and Correa M (2015) Mesolimbic Dopamine and the Regulation of Motivated Behavior. Current Topics in Behavioral Neurosciences.

Schneirla T (1959) An evolutionary and developmental theory of biphasic processes underlying approach and withdrawal. Nebraska Symposium on Motivation, 7, 1–42.

Sharma SC, Berthoud VM and Breckwoldt R (1989) Distribution of substance P-like immunoreactivity in the goldfish brain. The Journal of Comparative Neurology, 279, 104–116. [PubMed: 2464008]

Sheibani V and Farazifard R (2006) Dorsal raphe nucleus stimulation modulates the response of layers IV and V barrel cortical neurons in rat. Brain Res Bull, 68, 430–5. [PubMed: 16459198]

Sogard SM and Olla BL (1997) The influence of hunger and predation risk on group cohesion in a pelagic fish, walleye pollock Theragra chalcogramma. Environmental Biology of Fishes, 50, 405–413.

Souza BR, Romano-Silva MA and Tropepe V (2011) Dopamine D2 receptor activity modulates Akt signaling and alters GABAergic neuron development and motor behavior in zebrafish larvae. *The Journal of Neuroscience*: The Official Journal of the Society for Neuroscience, 31, 5512–5525.

Stein W (2009) Modulation of stomatogastric rhythms. Journal of Comparative Physiology A, 195, 989–1009.

Stoop R, Hegoburu C and van den Burg E (2015) New opportunities in vasopressin and oxytocin research: a perspective from the amygdala. Annual Review of Neuroscience, 38, 369–388.

Striedter GF (2005) Principles of Brain Evolution. Sinauer Associates, Sunderland, Mass.

Swanson LW and Petrovich GD (1998) What is the amygdala? Trends in Neurosciences, 21, 323–331. [PubMed: 9720596]

Tay TL, Ronneberger O, Ryu S, Nitschke R and Driever W (2011) Comprehensive catecholaminergic projectome analysis reveals single-neuron integration of zebrafish ascending and descending dopaminergic systems. Nat Commun, 2, 171. [PubMed: 21266970]

von Trotha JW, Vernier P and Bally-Cuif L (2014) Emotions and motivated behavior converge on an amygdala-like structure in the zebrafish. The European Journal of Neuroscience, 40, 3302–3315. [PubMed: 25145867]

Ware DM (1972) Predation by Rainbow Trout (Salmo gairdneri): the Influence of Hunger, Prey Density, and Prey Size. Journal of the Fisheries Research Board of Canada, 29, 1193–1201.

Weed JL, Lane MA, Roth GS, Speer DL and Ingram DK (1997) Activity measures in rhesus monkeys on long-term calorie restriction. Physiology & Behavior, 62, 97–103. [PubMed: 9226348]

Weil ZM, Zhang Q, Hornung A, Blizard D and Pfaff DW (2010) Impact of generalized brain arousal on sexual behavior. Proc Natl Acad Sci U S A, 107, 2265–70. [PubMed: 20080607]

Wester JC and McBain CJ (2014) Behavioral state-dependent modulation of distinct interneuron subtypes and consequences for circuit function. Current Opinion in Neurobiology, 29, 118–125. [PubMed: 25058112]

Wise RA and Bozarth MA (1987) A psychomotor stimulant theory of addiction. Psychological Review, 94, 469–492. [PubMed: 3317472]

Wolman MA, Jain RA, Liss L and Granato M (2011) Chemical modulation of memory formation in larval zebrafish. Proceedings of the National Academy of Sciences, USA, 108, 15468–73.

Woods IG, Schoppik D, Shi VJ, Zimmerman S, Coleman HA, Greenwood J, et al. (2014) Neuropeptidergic Signaling Partitions Arousal Behaviors in Zebrafish. The Journal of Neuroscience, 34, 3142–3160. [PubMed: 24573274]

Woodworth RS (1918) Dynamic Psychology. Columbia University Press.

Wullimann MF and Mueller T (2004) Teleostean and mammalian forebrains contrasted: Evidence from genes to behavior. The Journal of Comparative Neurology, 475, 143–162. [PubMed: 15211457]

Wullimann MF and Puelles L (1999) Postembryonic neural proliferation in the zebrafish forebrain and its relationship to prosomeric domains. Anatomy and Embryology, 199, 329–348. [PubMed: 10195307]

Yeh C-M, Glöck M and Ryu S (2013) An Optimized Whole-Body Cortisol Quantification Method for Assessing Stress Levels in Larval Zebrafish. PLoS ONE, 8, e79406. [PubMed: 24223943]

Yokogawa T, Hannan MC and Burgess HA (2012) The dorsal raphe modulates sensory responsiveness during arousal in zebrafish. The Journal of Neuroscience, 32, 15205–15215. [PubMed: 23100441]

Yokogawa T, Marin W, Faraco J, Pezeron G, Appelbaum L, Zhang J, et al. (2007) Characterization of sleep in zebrafish and insomnia in hypocretin receptor mutants. PLoS Biol, 5, 2379–2397.

Zahm DS (2006) The evolving theory of basal forebrain functional-anatomical “macrosystems.” Neuroscience and Biobehavioral Reviews, 30, 148–172. [PubMed: 16125239]

Zahm DS, Parsley KP, Schwartz ZM and Cheng AY (2013) On lateral septum-like characteristics of outputs from the accumbal hedonic “hotspot” of Peciña and Berridge with commentary on the transitional nature of basal forebrain “boundaries.” The Journal of Comparative Neurology, 521, 50–68. [PubMed: 22628122]

Zhdanova IV, Wang SY, Leclair OU and Danilova NP (2001) Melatonin promotes sleep-like state in zebrafish. Brain Res, 903, 263–8. [PubMed: 11382414]

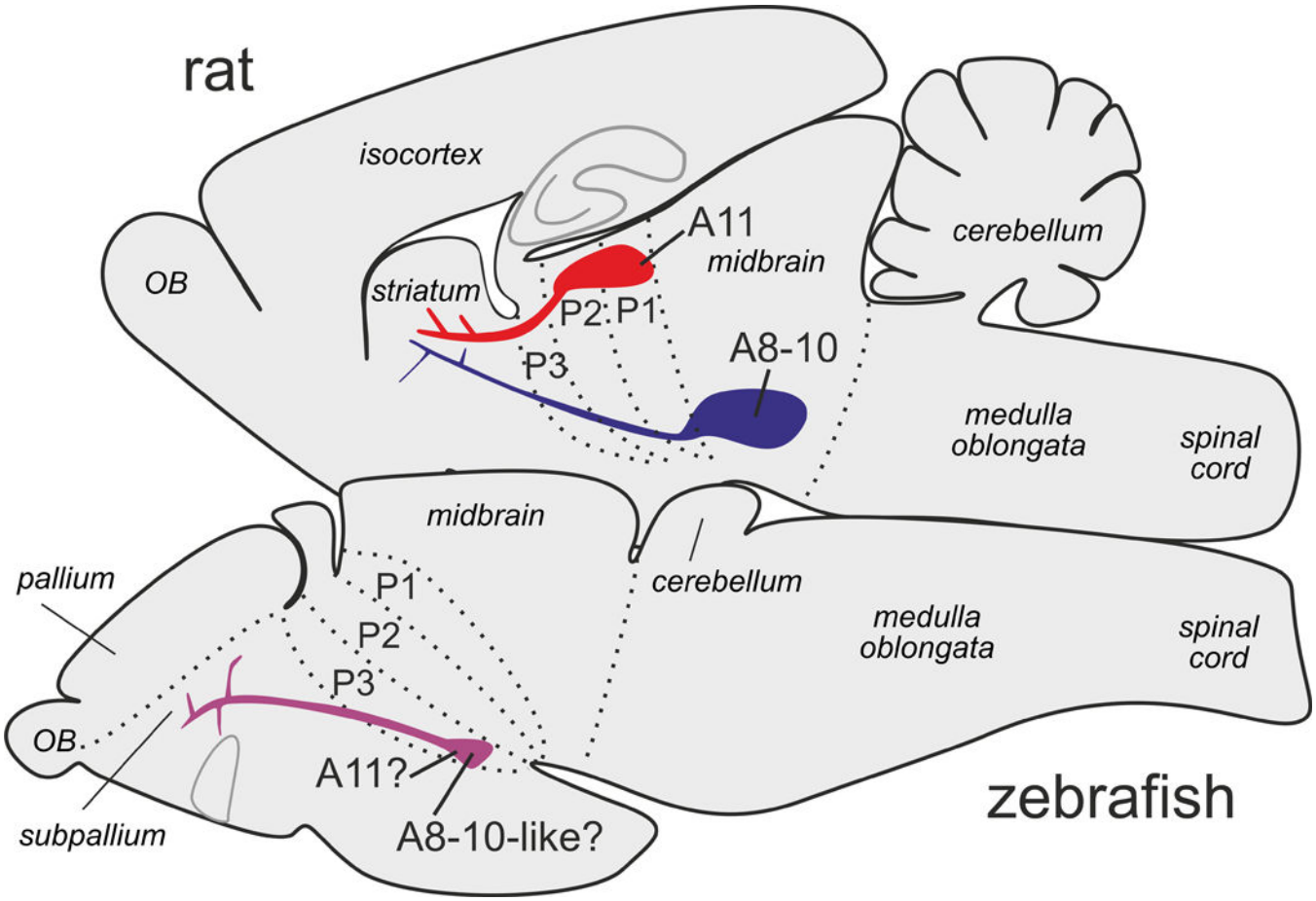

Figure 1:
Schematic comparison of the zebrafish (teleostean) and the rat (mammalian) ascending dopaminergic systems.