Functional Analysis of the Escape Response of Zebrafish

Herbert Weidner
email: herbertweidner@gmx.de
Am Stutz 3, 63864 Glattbach, Germany

The brains of fish and tadpoles show that surprisingly few neurons are sufficient to trigger reactions vital to survival. The escape reaction functions without memory and without adaptation of synaptic transmission; it is a reproducible connection of several neurons. Despite decades of research, we are still a long way from understanding the entire circuit. Comments and suggestions on brain research from a physicist's perspective.

1 Introduction

Plants can survive without a brain, but animals cannot. A brain enables them to search for food, recognize certain dangers in time, and choose a favorable escape route. How can a combination of a few neurons perform these tasks reliably? It seems unlikely that we can find the answers in the brains of highly evolved animals with billions of neurons and good memories. We will not be able to understand the function of these complex connections if we do not even understand how the brain of simple animals works, which often consists of only a few hundred neurons. The tasks of small brains are straightforward and can easily be described in words: searching for food and escaping danger. It always involves the movement of the animal's body. These actuators provide responses, how neurons connect incoming information and control the muscles to produce sensitive behavior. Sensitive in the sense that the animals survive for a longer period of time.

What do we know so far? 234 years ago, Galvani described [1] that the connection between brain and muscles is accompanied by electrical impulses. Even 73 years after the presentation of the first model of nerve conduction [2], there is still controversy about whether the action potentials in axons are electrical or mechanical in nature [3]. Is that important? Analyses of how an action potential gets from A to B are of little help if you want to understand the information processing of a network of neurons. With ever better research methods, it has recently been possible to create complete connectomes of flies and fish. It remains to be seen how much these extensive maps will help in understanding signal processing.

Figure 1 shows a tiny connectome from the field of electronics. Is this the circuit of a motor control or a motion detector? Where are the connections for the inputs and outputs? To understand how this circuit works, you have to activate it and measure signals at strategically favorable points. From the measurement data, one can determine the technical properties of individual components and recognize the function of assemblies. The process is called *reverse engineering* and is used in electronics to analyze the function and purpose of unknown devices. If you give small brains sufficiently simple tasks, *reverse engineering* could help to understand how neurons work. The escape reaction of zebrafish is simple enough and clearly structured.

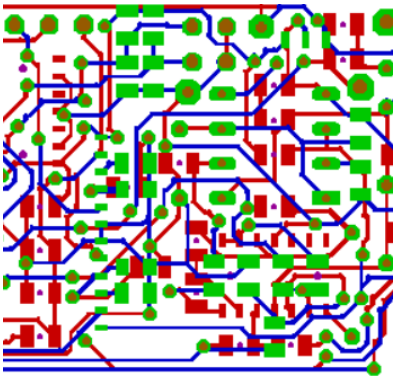

Figure 1): *Double layer of a 'printed circuit board' on which components such as transistors, capacitors and resistors are mounted and 'wired'. The brain of a fly contains a hundred times more layers.*

2 The Escape-Function

Animals have to react to external stimuli in order to find food; however, they also have to recognise dangers and move quickly to avoid being eaten. Fish and similar animals have little time to think; a genetically determined program controls the rapid escape: the body bends in a characteristic manner, followed by swimming through periodic tail movements. In the animals' brains, two conspicuous Mauthner neurons are involved in this control, which react to certain stimuli faster than other neurons [4]. Together with a few other neurons, they form a hard-wired 'black box' that contains only a few neurons with unknown internal circuitry.

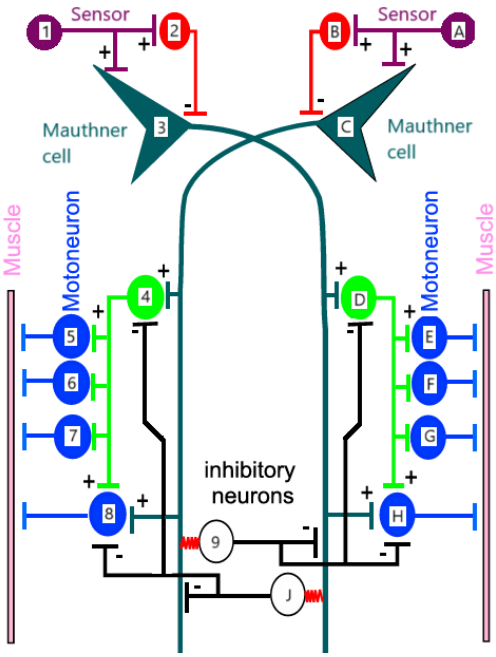

Figure 2): *A variant of the presumed basic circuit of the nerve connections that generates an escape reflex (dorsal view). Sensors 1 and A generate alarm signals that are passed on to both Mauthner cells 3 and C. Their axons cross and carry the signal via a few intermediary neurons to the muscles on the other side of the body. When a sensor on the left side fires, only muscles on the right side of the body contract so that the head and tail bend away from the source of the alarm. Inhibitory synapses 2 and B as well as 9 and J are intended to prevent all muscles from contracting at the same time. The motor control for the subsequent escape is missing in this image.*

In the literature, you often find suggestions similar to those in Figure 2. If you program this circuit on a computer using If–Then–commands, it becomes clear that this basic

circuit does not even generate the start phase of the well-known escape reflex. The most important defects or errors:

- Probably, the sensors fire repeatedly. It is not ensured that exactly *one* of the two Mauthner cells generates exactly *one* action potential (AP).
- Experimentally, it has been established that an AP starts a stereotypical behavior pattern (*C-start*) after a noticeably short delay, which after a short time changes into a swimming movement. Before the muscles on one side of the body contract, the muscles on the opposite side must relax so that the body can bend into a C-shaped curve. The transmission path for the corresponding commands is missing.
- The one-sided muscle contraction must end after a few milliseconds so that the behavior pattern *swimming* can be started. The timer ($t \approx 10$ ms) is missing.
- It is not ensured that *neither* of the two Mauthner cells generates an AP during the escape movement (duration $>$ 100 ms), because that would cause a renewed and braking C-curvature.

The basic circuit in Figure 2 is incomplete because it is not clear what causes the one-sided muscle contraction to end, when the body is roughly straight again and why the animal then swims in a straight line. After each start stimulus, these processes occur always in the same order. The fish performs these tasks like an automaton.

3 The logic of the sequence control

From a technical point of view, the escape reflex is a sequential control: a start impulse triggers a fixed sequence of processes. As soon as one process ends, the next one starts. A process ends as soon as a defined time has elapsed or a defined goal has been reached. The process does not require recourse to previous experience (no memory) and no adjustment of synaptic transmission. Actually a very simple control.

The aim of this study is to model a sequential control as a computer program that generates the observed movements of a fish in the correct order. In other words: If the program controlled each muscle of a fish separately via a wire connection, the fish should execute a C-start on command, which is no different from natural behavior. The possibility of measuring time is initially chosen as the central control element.

It is predictable that the control may require components that are known in technology but have not (yet) been discovered in previous biological studies. For example, the resolution of electron microscopes is barely sufficient to reliably identify electrical *gap junctions*. How do you know if it is a symmetrical or a rectifying gap junction [8–10]? Chemical synapses are easier to see, but the postsynaptic effect is not recognized: do they increase or reduce the probability that the following neuron will generate an AP?

The prerequisite for a process control is the creation of a functional specification. Here we describe the functional process and the chronological sequence of individual steps in a way that has been proven millions of times. The logic is defined, not the details of the

technical implementation. Sometimes it is necessary to program how the machine should react in the event of errors. Prerequisites for this process: A clear left-right blueprint applies to sensors, nerves, muscles and axons.

1. Every sensor that reports an alarm → Create an AP of the connected Mauthner cell. (Purpose: The more sensors are involved and the faster an AP is created, the clearer the best escape direction is chosen.)

2. Wait until $U_{AP} > 0$ → Send a blocking signal to the axon hillock of the *other* Mauthner cell (Reciprocal Inhibition). Save this command-1. (Purpose: Suppress all AP for a certain period of time)

3. Stop all running programs such as eating, swimming... Relax all muscles, block all sensors. Save all this as command-2.

4. If the AP was created in the left Mauthner cell → contract all muscles on the right side. If the AP was created in the right Mauthner cell → contract all muscles on the left side. (The animal's body forms a C)

5. Save this command-3. Start timer-1 with $t_1 = 10$ ms. (All timers run backwards 6, 5, 4, 3,.. and report reaching the value zero.)

6. Wait until $t_1 = 0$, then delete command-3 (C-shape is formed). Relax all muscles. Start timer-2 with $t_2 = 3$ ms. (Purpose: Wait until body is almost straight again)

7. If $t_2 = 0$ *and* the body was curved to the left → start swimming program-a. If curved to the right → swimming program-b. Start timer-3 with $t_3 = 60$ ms. (Purpose: Swim straight)

8. Delete command-1. (C-starts may be triggered again. In case of alarm: → delete command-2, continue with item-1, restart the escape program)

9. Wait until $t_3 = 0$, then delete command-2 and end the swimming program. This means that the C-start is finished after $t_{total} \approx 80$ ms.

There is probably a single swimming program in the brain with different entry points to enable a smooth transition from the C-curve to swimming. Details of the swimming program are not discussed here.

The program described is based on three timers with different durations, each longer than the typical duration of an AP. When precision is low, technical devices usually use monostable flip-flops in which a capacitor is slowly discharged. Whether and how this is done in the brain can be clarified experimentally through ablation experiments. In this way, we would learn how a population of neurons must be wired in order to perform a specific function when activated.

The control sequence outlined above shows the most important steps for controlling a complete C-start. In some animals, such as the zebrafish, the signal chain also runs via the *bilaterally-clustered midbrain nucleus of the medial longitudinal fasciculus (nMLF)*,

which does not exist in other animals. If this detour is interrupted, only the first part of the C-start is observed [5].

The flowchart contains three 1-bit memories (command-1, -2 and -3) with overlapping scopes. Presumably these are not organized as random access memories, but as simple set-reset latches. (Circuit proposals for neural latches and timers are discussed in a subsequent paper).

Nobody planned the escape program in animals. In the race against predators, it has been gradually perfected through mutations and natural selection to such an extent that the animals' probability of survival is sufficiently high.

Programming on a computer confirms that the sequence control shown in section 3 produces the familiar movement of the fish's body during an escape reaction. This can also be achieved by simulating the brain in the form of an electronic circuit with components such as transistors and resistors. These projects are easy to carry out, but do not explain how real neurons solve this task.

4 The search for the timer

The working speed of neurons ($\sim$ 1 ms) is shorter than the contraction speed of muscles ($\sim$ 10 ms) at the beginning of the escape. Therefore, the brain has to delay further control signals in order not to disturb the C-curvature. The following steps also occur sequentially. In technology, the problem is solved using timers. How do you build a biological timer? Can this be achieved by combining already known neurons or does it require additional building blocks with suitable properties?

In [6] it is correctly recognized that (at least) one timer is necessary. Instead of looking for and verifying a solution with a biologically plausible neural circuit, the simulation uses an electronic circuit (NbO2 memristor). This solution does not solve any question of brain research.

The *CoLo neuron* appears to play an important role in the escape response of the zebrafish [7]. During the escapes, the CoLo neuron fires three successive spikes. During the swimming phase, the cell receives inhibition. Could this be a part of the timer circuit we are looking for? Where does the start signal come from? Who counts the spikes? Which cells are affected?

The flow chart (section 3) can be modified so that no timer is needed. Each step requires a start signal and an end criterion to start the next step. The exact type of end criterion is often unknown, but can be determined experimentally. In technology, limit switches are often used as the end criterion: when a certain degree of curvature is exceeded, a switch closes and signals *'target reached'*. So far, no switch has been discovered at the end of a fish's tail, so there must be another end criterion. If the flow chart contains a timer, the C-curvature is accomplished after a few milliseconds – regardless of whether and how much the body is curved. This can be tested experimentally: fix the fish's head and reduce the freedom of movement of the tail. If the start of the next step *cannot* be altered, either a timer is built in or there is a third – unknown – way to start the next step.

5 Discussion

The escape reaction of zebrafish is clearly structured and the control is undoubtedly a hard-wired 'black box' that contains remarkably few neurons and works very reliably. The control is of low complexity and the flow chart developed in section 3 can be easily implemented using standard technical components. The electronic circuit requires functional blocks such as timers. So far there is no experimental confirmation of the existence of biological timers and their basic circuit. This question could be clarified by the following experiments:

- Is the schedule in section 3 implemented in the brain of zebrafish?
- How to localize strategically favorable neurons? What signals do you measure there?
- How many neurons make up a biological timer?
- Are there *spiral fiber neurons*? These FET-like components would enable neural circuits that one can only dream of [11–13].
- Are *spiral fiber neurons* only found in the axon hillock of Mauthner cells?

The answers would significantly expand the pool of neuron models. **Tell me what to look for and I will find it**.

References

[1] L. Galvani, De viribus electricitatis in motu musculari. („Über Kräfte der Electricität bei Muskelbewegung“). Bologna 1791.

[2] A. Hodgkin, A. Huxley, A quantitative description of membrane current and its application to conduction and exitation in nerve, J. Physiol. (1952), 117, 500-544

[3] S. Andersen, Towards a thermodynamic theory of nerve pulse propagation, Progress in Neurobiology (2009), 88, 104-113

[4] H. Korn, D. Faber, The Mauthner Cell Half a Century Later: A Neurobiological Model for Decision-Making?, Neuron, Vol. 47, 13–28, July 7, 2005, DOI 10.1016/j.neuron.2005.05.019

[5] K. Christie, K.Severi, Motor behavior: A feedforward circuit for zebrafish escape, Current Biology 31, R948–R971, August 9, 2021, https://doi.org/10.1016/j.cub.2021.06.032

[6] Y. Yang et al., Firing feature-driven neural circuits with scalable memristive neurons for robotic obstacle avoidance, 2024, https://www.nature.com/articles/s41467-024-48399-7

[7] C. Satou et al., Functional Role of a Specialized Class of Spinal Commissural Inhibitory Neurons during Fast Escapes in Zebrafish, The Journal of Neuroscience, 2009 • 29(21):6780–6793

[8] N. Palacios-Prado, W. Huetteroth, A. Pereda, Hemichannel composition and electrical synaptic transmission: molecular diversity and its implications for electrical rectification, Front Cell Neurosci. 2014 15:8:324

[9] A. Miller, A. Pereda, The electrical synapse – molecular complexities at the gap and beyond, Dev Neurobiol. 2017 May ; 77(5): 562–574

[10] P. Phelan et al., Molecular Mechanism of Rectification at Identified Electrical Synapses in the Drosophila Giant Fiber System, Current Biology 18, 1955–1960, December 23, 2008

[11] T. Furukawa et al., A THIRD TYPE OF INHIBITION IN THE MAUTHNER CELL OF GOLDFISH, J Neurophysiol. 1963 Sep:26:759-74. doi: 10.1152/jn.1963.26.5.759

[12] V. Pachauri, S. Ingebrandt, Biologically sensitive field-effect transistors: from ISFETs to NanoFETs, Essays in Biochemistry (2016) 6081–90

[13] D. Faber, A. Pereda, Two Forms of Electrical Transmission Between Neurons, Front. Mol. Neuros., 2018, Volume 11:427