English summary

The brain is a very important organ, since it directs almost all actions the body can execute. For instance, the brain regulates muscle movement, receives and processes sensory inputs, and keeps you alive by regulating your breathing, heartbeat and consciousness. In addition, the brain allows for cognitive processes like social interaction, personality, learning and memory.

The brain contains around 100 billion brain cells which are called neurons. These neurons have a specific shape, with a central soma and multiple long extensions (see Figure 1A). Neurons have to communicate with each other for the brain to function. Neurons communicate by sending messages packed in small “parcels” (vesicles) from one neuron to the next. The transfer of this message happens at the synapse. The synapse is the site where long extensions of two neurons meet, and where messenger molecules pass a small space between the two neurons. In the neuron that sends information, the pre-synaptic neuron, the messenger molecules are packed into vesicles. These vesicles need to fuse with the membrane on the outside of the neuron (the plasma membrane) to release these molecules. Subsequently, the signalling molecules can bind to receptors on the neuron which receives information, the post-synaptic neuron, who “reads” the message (see Figure 1B).

There are two types of vesicles involved in neuronal communication: synaptic vesicles (SVs) and dense core vesicles (DCVs) (see Figure 1C). Synaptic vesicles are small and contain neurotransmitters. Neurotransmitters regulate fast communication, like controlling muscle movement and sending visual information from the eyes to the brain. The way neurons communicate with synaptic vesicles is quite well known, because of extensive research over the last decades. On the other hand, dense core vesicles are relatively big compared to synaptic vesicles and contain neuromodulators (which include neuropeptides and neurotrophic factors). These neuromodulators are involved in processes which are relatively slow, for instance development, learning and behaviour. An

Figure 1. A: Two neurons communicate at the synapse. B (zoom of A): Vesicles fuse with the plasma membrane to release their content. Receptors on a post-synaptic neuron can pick up their message. C: Neurons contain two types of vesicles. D: SNARE proteins bridge the vesicle with the plasma membrane to enable fusion.
example of a neuromodulator is oxytocin, popularly known as the “cuddle hormone” for being involved in establishing social connections. Since far less research has focussed on dense core vesicles, the details of neuronal communication via dense core vesicles are not known. Therefore, this thesis aims to provide more insight in neuronal communication via dense core vesicles. The focus is to determine which proteins regulate fusion of dense core vesicles with the plasma membrane, and how these proteins regulate fusion. Also, we aim to discover how the number of dense core vesicle fusion events changes during neuronal development. In Box 1 (next page), we describe the methods to study vesicle fusion.

In Chapter 1, we introduce the topic by describing the molecular mechanisms of how vesicles fuse with the plasma membrane. Vesicles can not spontaneously fuse with the plasma membrane, but require multiple proteins to mediate this process. Essential proteins are the SNARE proteins (see Figure 1D). One SNARE protein (VAMP) is inserted into the vesicle. Two other SNARE proteins (SNAP and syntaxin) are bound to the plasma membrane. The SNARE proteins all contain a helix, a special protein structure which allows proteins to bind to each other. When the helices of the SNARE proteins (one in VAMP and syntaxin, two helices in SNAP*) bind, the SNARE complex is formed. The SNARE complex functions as a bridge between the vesicle and the plasma membrane to enable vesicle fusion. The SNARE complex is assisted by multiple other proteins, including CAPS**.

In Chapter 3 and 4, we study VAMP proteins (one of the SNARE proteins, see Figure 1D). There are seven different VAMP proteins, and we show that VAMP1, VAMP2 and VAMP3 are important for dense core vesicle fusion in neurons. The other four VAMP proteins can not support dense core vesicle fusion. Synaptic vesicle fusion only requires VAMP2. CAPS proteins interact with the SNARE proteins to help them bind each other. In Chapter 5, we study CAPS proteins. Proteins contain different functional parts, and we show that all the parts of CAPS are important for dense core vesicle fusion. We find that CAPS proteins are essential for dense core vesicles, but less important for synaptic vesicles if the intercellular calcium is high. Communication via dense core vesicles is very important during neuronal development. In Chapter 2, we study whether the dense core vesicles themselves change during development of the neuron. We show that in young neurons, only some neuromodulators are abundantly present. Also, the number of dense core vesicles increases over time in line with the increased size of the neuron. In Chapter 3, we study how many dense core vesicles fuse with the plasma membrane at different time points during the development of a neuron. We find that in young neurons only a few vesicles fuse, and that after ten days this number increases by a factor 10. Interestingly, this increase is not gradually but happens abruptly from day 5 to 6 (counted from the day the neurons were cultured).

In Chapter 6, we summarize and discuss our findings. This thesis provides more understanding of how proteins guide dense core vesicle fusion. We find subtle differences with the fusion mechanism of synaptic vesicles. Also, we show that during the development of neurons, the number of fusion events increases abruptly.

* See also the cover of this thesis, where the ballerinas represent the helices of VAMP (green), syntaxin (yellow) and SNAP (red) bridging the dense core vesicle with the plasma membrane.
** The ballet dancer at the back cover of this thesis symbolises CAPS. See also the chapter cover pages for references to the different proteins.
**Box 1: Methods. How can we study fusion of vesicles with the plasma membrane?**

Neurons are too small to detect with the naked eye (the central part of the neuron, the soma, is roughly one hundredths of a millimetre). Therefore, all experiments are performed using a microscope. In addition, to be able to see the vesicles within the neurons, we add a colour to them using **fluorescent proteins**. Fluorescent proteins emit light of one specific colour, when you activate them by illuminating them with light of another, specific, colour. The most widely used and known fluorescent protein in cell biology is the **Green Fluorescent Protein (GFP)**, which emits a green light when you shine blue light on it. We use a modified version of GFP, which is only fluorescent at neutral or high pH (the scale for acidity). This special GFP, called **pHluorin**, is dark when inside the vesicle (because of low pH), and emits green light when the vesicle fuses with the plasma membrane (because of neutral pH outside the cell). Therefore, we can detect fusion of a vesicle with the plasma membrane as a small dot of light appearing (see Figure 2).

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**Figure 2.** A-C: Cartoon of how pHluorin (pH sensitive Green Fluorescent Protein) shows vesicles fusing with the plasma membrane. D-F: Real neuron in which a vesicle fuses with the plasma membrane, which can be seen as a green dot appearing (in F).