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Abstract

Injuries of the adult nervous system such as peripheral lesions or central focal strokes lead to substantial structural and functional reorganization of neural circuits and boosting these intrinsic neuroplastic processes is critical for functional recovery. Various evidence is pointing to compensatory take-over of lost functions not only by adjacent perilesional areas but also to an important role of the contralesional hemisphere in recovery, but the cellular resolution of underlying structural changes has been rather limited.

Here I am using the adult brain of *Drosophila melanogaster* as an experimental model to determine the cellular dynamics of injury induced synaptic plasticity. Previous studies have shown that peripheral lesions within the adult fly olfactory system result in rapid degeneration of sensory afferents but little changes in the overall integrity of the postsynaptic circuit. By employing a recently developed activity dependent GRASP technology, I could show that loss of sensory input results in specific structural remodeling of synaptic contacts between excitatory projection neurons (PNs) and inhibitory local interneurons (LNs). For example, unilateral lesion results in differential increase of GABAergic inhibitory synapses on two main output neurons, PNv1 and PNv5. To determine the effect of neuronal activity on synaptic remodeling I analyzed the effect of chronic sensory stimulation on the injured brain region compared to the unaffected hemisphere. Interestingly, while synaptic inhibition increases at the injured site, a novel type of excitatory interaction between specific PN classes develop at a homologue circuit of the unaffected hemisphere, indicating a compensatory mechanism of sensory perception. Finally, by analyzing changes in synaptic activity following sensory deprivation, the injury-specific responses on circuit remodeling could be determined.

In summary, in-depth analysis of lesion induced plasticity in a confined brain circuit of *Drosophila* not only revealed, that the mature nervous system is capable of synaptic remodeling in a neuron-type specific fashion but also a possible role of synaptic activity in compensatory responses. With these central features of injury-response in mammals conserved in *Drosophila*, this experimental model offers excellent conditions to identify essential genes for such plasticity events which might also help to understand possible ways of recovery in lesioned human brains.

Zusammenfassung

Verletzungen des Nervensystems, wie beispielsweise der Verlust von Gliedmaßen und Schlaganfälle, führen zu grundlegenden strukturellen und funktionellen Veränderungen von neuronalen Schaltkreisen. Diese intrinsischen Prozesse richtig zu verstärken könnte den Schlüssel darstellen um eine möglichst gute Wiederherstellung von neuronalen Funktionen zu gewährleisten. Aktuelle Forschungen konnten zeigen, dass nicht nur angrenzende Hirnareale eine unterstützende Rolle bei der Wiederherstellung von Funktionen darstellen können, sondern dass auch Hirnareale auf der kontralateralen Gehirnhälfte diese Rolle einnehmen können. Jedoch gilt es noch einige Fragen zu klären, wie diese Prozesse auf zellulärer Ebene stattfinden.

In dieser Arbeit wurde das Gehirn von *Drosophila melanogaster* als experimentelles Modell herangezogen, um der zellulären Dynamik von synaptischer Plastizität, welche in Folge von Verletzungen auftritt, auf den Grund zu gehen. Vorangegangene Arbeiten haben gezeigt, dass einseitiges Entfernen der Antennen von Fliegen zu rascher Degeneration von Nervenbahnen führt, jedoch Neurone welche als synaptischer Partner fungieren würden nicht davon beeinflusst zu werden scheinen. Durch Anwenden einer Aktivitäts-abhängigen GRASP Technik war es mir möglich deutliche Veränderungen an excitatorischen sowie inhibitorischen Synapsen aufzuzeigen, welche als Folge von Verletzungen an peripheren Sinnesorganen auftreten. Beispielsweise konnte gezeigt werden, dass eine einseitige Entfernung der Antennen eine erhöhte Aktivität von inhibitorischen Synapsen auf zwei excitatorischen Output Neurone zur Folge hat. Um den Effekt von neuronaler Aktivität auf synaptische Veränderungen zu bestimmen, habe Ich die Auswirkung von chronischer sensorischer Stimulation auf der verletzten Hemisphäre mit der unverletzten Hemisphäre verglichen. Interessanterweise, während wie erwähnt inhibitorische Synapsen erhöhte Aktivität auf der verletzten Seite vorweisen, zeigte eine excitatorische Synapse zwischen zwei Projektionsneuronen erhöhte Aktivität in der unverletzten Hemisphäre, was auf einen kompensatorischen Prozess in der Geruchswahrnehmung deutet.

Mit dieser detailreichen Analyse von Verletzungs-induzierter synaptischer Plastizität in einem festgelegten neuronalen Netzwerk in *Drosophila* konnte nicht nur gezeigt werden, dass das adulte Nervensystem fähig ist Neuronen spezifisch Synapsen zu remodellieren, sondern auch,

dass neuronale Aktivität in Folge von Verletzungen möglicherweise auf kompensatorische Antworten hindeutet. Da diese Reaktion auf Verletzungen, welche in Säugetieren mehrfach beschrieben wurde, in *Drosophila* konserviert zu sein scheint, bietet dieses experimentelle Modell exzellente Bedingungen für weitere Forschung, um Gene zu identifizieren die für solche plastischen Prozesse essentiell sind und möglicherweise zu einem besseren Verständnis von funktioneller Wiederherstellung in verletzten Gehirnen des Menschen führen.

1. Introduction

1.1 Lesion induced plasticity – A short insight into mammal research

In mammals, injuries of the adult nervous system such as peripheral lesions or central focal strokes lead to substantial structural and functional reorganization of neural circuits. For instance, it was described that peripheral lesion causes a decreased activity of neurons in the respective projection zone. Eventually, neurons of the affected area start to reorganize, forming new connections (Sammons and Keck, 2015). It was also shown, that depending on the severity of the damage inflicted on the brain, the system either tries to induce recovery by recruiting nearby surviving neurons to reestablish lost connections or, if the damage occurred in a bigger region, the contralesional hemisphere sends projections to reinnervate the homologue injured area to aid recovery (Biernaskie et al., 2005; Liu et al. 2009; see review: Jones and Adkins, 2015) (Fig.1). Shortly after such events, changes in the balanced homeostasis between excitatory and inhibitory circuit modulation were reported and thus appropriately boosting or inhibiting these intrinsic neuroplastic processes might be critical to restore function (Carmichael, 2012; Sammons and Keck, 2015).

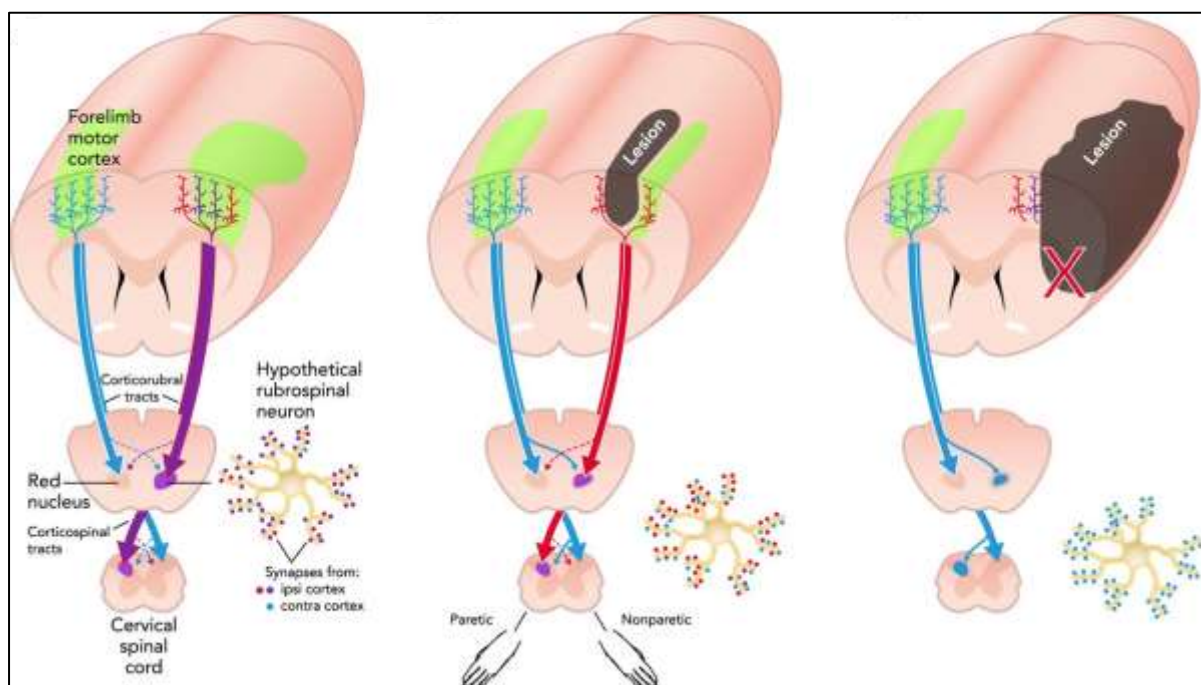


Figure 1. Schematic of a rat brain showing the forelimb representation area in green. Depending on the severity of the stroke, the system might have more than one way to reorganize itself. After smaller lesions, remaining neurons might reinnervate the injured area, while recovery after larger lesions might be dependent on innervation of neurons from the contralesional hemisphere. Adapted from Jones and Adkins (2015)

Additionally, a better understanding of the neuronal changes linked with limb loss needs to

be pursued to help and shape the development of new prosthesis (Wheaton, 2017). The important interplay between excitatory and inhibitory circuit modulation has been also reported for people who underwent procedures to emulate limb loss, such as anesthetic creams (Bjorkman et al., 2004) and ischemic nerve block (Ziemann et al., 1998). GABA, the most important inhibitory neurotransmitter in human brains, plays a crucial role in reorganization and plasticity after peripheral lesion, as it was suggested by Levy et al. (2002). Further, it was shown that peripheral injury leads to a reduced inhibition by GABA in the motor cortex which is connected to muscles proximal to the ischemia (Ziemann et al., 1998) and that there are also implications for the contralateral hemisphere. Werhahn et al. (2002a) noted, that a nerve block in one limb also induces heightened excitability in brain regions connected with the other limb. In another experiment, Werhahn et al. (2002b) could show, that the contralateral communication between the hemispheres after peripheral lesion promotes that the unaffected hand performs better in a grating orientation task which tests for tactile acuity and described this as a potential way of compensation (Fig. 2).

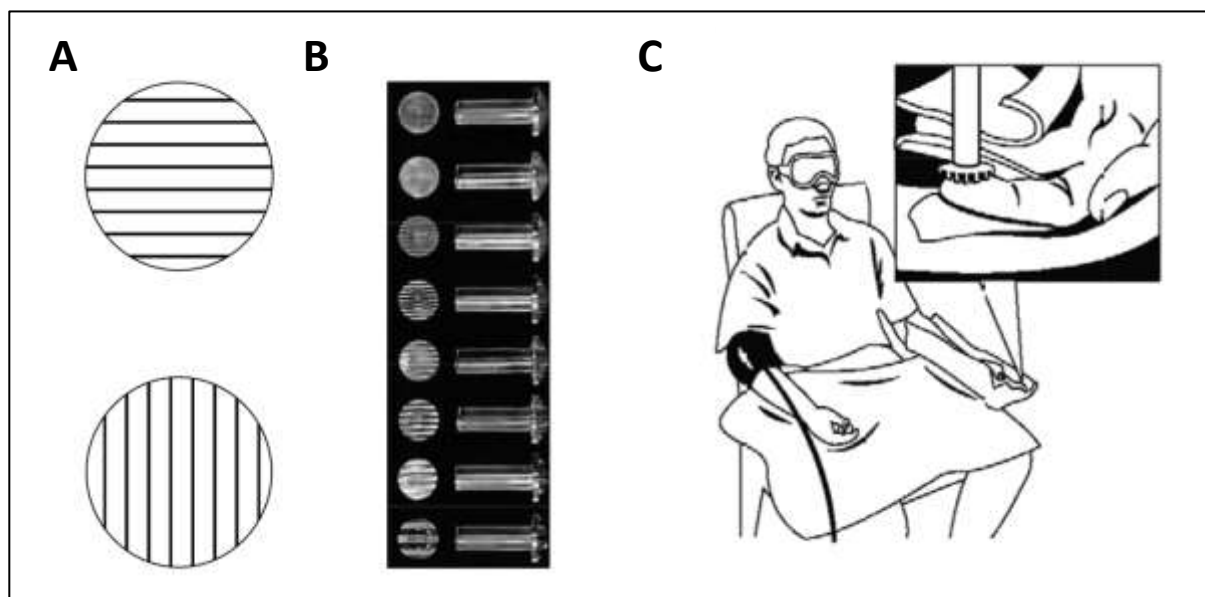


Figure 2. Grating orientation task (GOT) setup as it was used by Werhahn et al. (2002b). A: Surface of the plastic domes which were used in the task, B: Set of various plastic domes, C: Participants were seated and had to feel the orientation of the gratings with their left index finger while the right arm was anesthetized with an ischemic nerve block. Adapted from Werhahn et al. (2002b)

However, the role of GABAergic inhibition in plasticity after lesion/stroke seems to be even more complex and only in the recent years scientists made great progress to get a clearer picture on that topic. According to Carmichael (2012) it is necessary to distinguish between

phasic and tonic GABA inhibition, where phasic GABA inhibition happens directly at the synapse and tonic GABA inhibition affects the whole neuron. Most importantly, stroke leads to a decrease of phasic GABA inhibition while tonic GABA inhibition is seemingly increasing. Following that, Clarkson et al. (2010) found out that decreasing tonic inhibition after stroke is beneficial for recovery. Seemingly contrary to other findings, Hiu et al. (2016) showed, that after stroke GABAergic phasic inhibition is increased specifically in pyramidal neurons of layer 5 in mice and that enhancing this inhibition is also beneficial in recovery following stroke. In sum, previous research shows a strong indication that any form of lesion to the brain or peripheral neurons can greatly impact synaptic plasticity raising the question about the underlying mechanism that regulates the specific changes in the balance of excitation and inhibition to modify circuit function.

Interestingly, there is also evidence that several genes have different expression levels post injury and that those expression levels recapitulate early development with high neuronal plasticity (Zeiler and Krakauer, 2013). Li et al. (2011) identified various genes being expressed in neurons of the peri-infarct area after stroke, which are involved in axonal sprouting and proliferation and suggested a connection to recovery. Zeiler and Krakauer (2013) concluded, that some genes (e.g. BDNF – brain derived neurotrophic factor) which show a higher expression after stroke can also be detected with similar expression levels in motor learning tasks, suggesting that there is a specific timeframe after a stroke in which the damaged area is much more prone to plasticity.

Following that, recent research could show that there is a critical period after lesion in which all those mechanisms associated with functional reorganization are most prominent, indicating that the adult brain may utilize plasticity mechanisms following injury which are very well established in early brain development and were originally described by Hubel and Wiesel in 1962 (as cited in Espinosa and Stryker, 2012) as the critical period in juvenile animals (Carmichael, 2006; see review: Zeiler and Krakauer, 2013). Reportedly, the best timeframe to start rehabilitation programs in stroke patients is the first few weeks after stroke occurred (Dewey et al. 2007)

According to Levin et al. (2009), it is necessary to distinguish between real recovery and compensation. Recovery means, that brain regions related to a specific task regain their initial function after the area has been damaged, while compensation indicates that neurons, which had a distinct function and connectivity before, now adapt and occupy the role of neurons which were damaged. This distinction is important, since rehabilitation programs should be directed to support the recovery of damaged brain regions or, if it would be more helpful, to enhance the compensation of other neurons fulfilling the role of injured neurons (e.g. motoric tasks).

Generally, the common notion among scientists working in this field is, that an enriched environment for individuals who suffered from strokes is beneficial for the recovery of the patient (Johansson, 2004; Risedal et al., 2002). However, the severity of the stroke should be kept in mind (Dobkin, 2005) and therefore the correct rehabilitation program should be made for each patient individually (Brewer et al., 2013). During the last decade, various approaches were suggested to help patients who suffered from stroke, ranging from motor training with application of the CIMT method (Constraint – induced movement therapy), to electrical stimulation as well as stem cell therapy and robotic therapy (Wolf et al., 2006; Brown et al., 2006; Lo et al., 2010; Abe et al., 2012).

Regeneration research in mammals, including humans, was performed largely at the systems level using techniques such as functional MRI and PET and only in the past decade new approaches to study the structural changes at the cellular level were introduced, such as two photon imaging and calcium imaging (Dijkhuizen et al., 2001; Winship and Murphy, 2009; Sammons and Keck, 2015). Genetic methodology to analyze neural circuit organization and function at cellular resolution has been advanced substantially in the *Drosophila* brain but there is so far little knowledge about the structural plasticity in the adult fly following traumatic injury, so the aim of my thesis was to determine the level of structural plasticity. For this I exploited the detailed knowledge about the cellular organization of the adult olfactory system combined with the novel GRASP technology (*GFP reconstitution across synaptic partners*) to visualize synaptic contacts within neural circuits.

1.2 The olfactory system of *Drosophila melanogaster*

The olfactory circuitry of *Drosophila* displays several features which support the identification of conserved mechanisms underlying brain plasticity. First of all, the structural organization of the olfactory system is strikingly similar between insects and mammals, but the number of neurons involved is much smaller which makes it much more feasible for experimental approaches (Jefferis et al., 2002). In addition, the synaptic glomeruli in the *Drosophila* olfactory system are easily distinguishable by positions and sizes (Laissue et al., 1999) and a large set of cell type specific expression lines allow to visualize and manipulate each neuronal element within glomerular microcircuits. Another advantage of using the *Drosophila* olfactory system for exploring the dynamics of injury-induced adult plasticity is the fact, that neurogenesis is completed at adult eclosion, in contrary to the situation in adult mammals in which substantial amounts of neurons are also adult-born (Berdnik et al., 2006).

A lot of research has been done to characterize and understand the olfactory system of *Drosophila melanogaster*. The fly has two olfactory sensory organs, the antenna and maxillary palps, which contain the ORNs. There are approximately 1200 ORNs located on each antenna, while there are around 120 ORNs on each maxillary palp (Stocker, 1994 as cited in de Bruyne et al., 2001). Up to 4 of those neurons are housing in sensory hair, called sensilla, which can be found on the surface on either of the 2 sensory organs. Furthermore, these sensilla can also be subdivided into 3 different types: basiconic, trichoid and coeloconic (de Bruyne et al., 2001). While on the antennae all sensilla types can be found, only a single class of basiconic sensilla can be located on the surface of maxillary palps (de Bruyne et al., 1999). The dendrites of these ORNs recognize different odorants, depending on their expressed odorant receptors (ORs) (Vosshall and Stocker, 2007). Altogether *Drosophila m.* has around 62 ORs (Robertson et al., 2003). Couto et al. (2005) mentioned that a mouse has up to 2 million ORNs and roughly 1000 ORs. Not all ORNs express OR genes, as there also GR genes (gustatory receptor) and IR genes (ionotropic receptor) expressed (Clyne et al., 2000; Rytz et al., 2013). Especially the gustatory receptor genes GR21a and GR63a, which are expressed in basiconic sensilla c, are important for this thesis since they are only responsive to CO₂, which was an important reagent in the experiments shown later, and projecting to V-glomerulus. They don't rely on another co-receptor and it was shown that when both genes are expressed in ORNs, usually expressing

an OR gene, CO₂ detection is possible. Flies show a strong avoidance behavior when confronted with CO₂ (Jones et al., 2007).

1.3 The microcircuit of V-glomerulus

To understand the microcircuit of V-glomerulus and how injury might induce synaptic plasticity at the various synapses of this glomerulus, the pathway of odorant detection needs to be addressed, starting with the stimulation at the dendrites of ORNs, up to memorization and learning behavior in higher brain centers.

Odors get detected by odorant receptors located in the dendrites of ORNs which in turn results in the generation of an action potential. These action potentials travel along the axons to the first olfactory processing centers of *Drosophila m.* called the antennal lobes (AL). The AL consist of ~43 densely packed neuropils (Laissue et al, 1999), the glomeruli, where the axons of ORNs form synapses with dendrites of the second order neurons, namely projection neurons (PNs) and local interneurons (LNs) (Hallem and Carlson, 2004). PNs convey the incoming signal to higher brain centers like the Mushroom body (MB) or the lateral horn (LH) where the information is further processed. LNs don't have axons, which means that their purpose is to modulate the intraglomerular and interglomerular communication between neurons, mostly in an inhibitory way (Wilson and Laurent, 2005) but an excitatory type was also described by Shang et al. in 2007. Interestingly, the ORNs generally follow the one neuron – one receptor principle, with a few exceptions as mentioned by Couto et al. (2005) (Fig.3). In addition to that, ORNs which express the same receptor converge onto a single glomerulus, which allows to map the different projections to distinct locations within the AL (Vosshall et al., 2000). Furthermore, most the ORNs send their axons ipsilateral as well as to the contralateral AL, while a small portion only innervates on the ipsilateral side (Stocker et al., 1990 as cited in Gao et al., 2000), which includes the V-glomerulus. On the contrary, the majority of PNs have unilateral projections, but PNs with bilateral projections have also been described for V-glomerulus (Lin et al., 2013). In sum, the fact that in most of the cases ORNs express just one OR gene and these neurons are converging onto one glomerulus results in that odorants, to which ORNs react with differently strong responses depending on their receptor's response profile, leads to an odorant specific activation of multiple glomeruli. These different activation patterns, are the underlying necessity for odor discrimination (Hallem and Carlson, 2004).

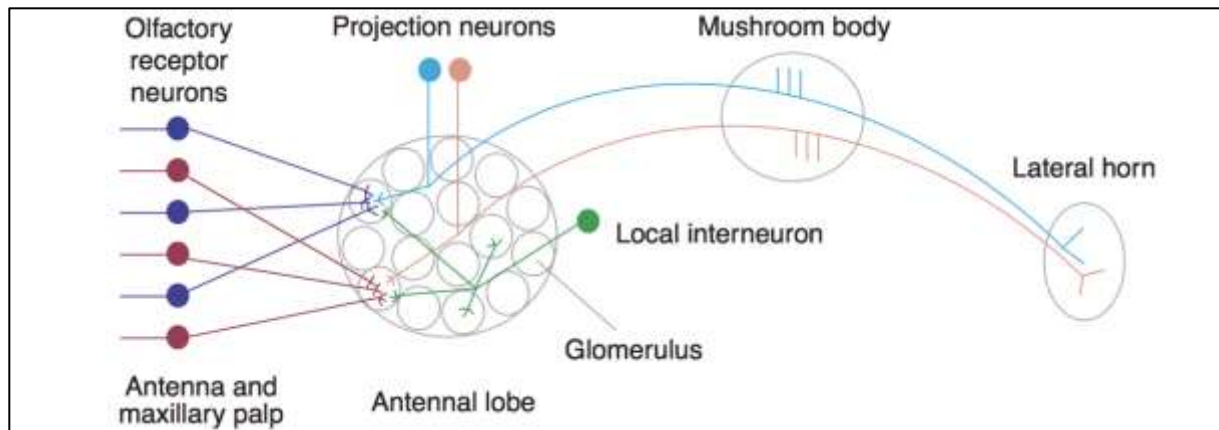


Figure 3. Schematic of *Drosophila melanogaster*'s olfactory system. Adapted from Hallem and Carlson (2004)

Focusing on V-glomerulus leads to the realization that the intra-glomerular circuitry is much more complex (Fig.4). As noted previously, ORNs expressing GR21a and GR63a project their axons to the V-glomeruli in each hemisphere. Other than most ORNs, these neurons innervate only the ipsilateral antennal lobe, which means that there is no direct sensory commissure between the antennal lobes connecting both V-glomeruli (Rajashekhar and Shamprasad, 2004). The axons of these ORNs form synapses with different classes of projection neurons which all have their own identity and innervation patterns (Lin et al., 2013; Tanaka et al., 2012). For the experiments conducted in this thesis the focus lies on three classes of projection neurons, PNv1, PNv3 and PNv5, each with different structural and functional properties. PNv5 is a unilaterally projecting excitatory cholinergic neuron and receives major synaptic input from ORNs and therefore most likely defines the main channel for CO₂ detection (Batawi, unpublished). PNv1 is also excitatory in nature, but in contrast to PNv5 receives bilateral sensory input via an additional contralateral projection to the V-glomerulus in the other hemisphere and then sends the axons to higher brain centers such as the lateral horn and the calyx of the mushroom bodies (Lin et al., 2013). PNv3 on the other hand is a GABAergic inhibitory neuron (Lin et al., 2013) with an innervation pattern resembling the one of local interneurons. One class of local interneuron, LNv2, was also a part of the analysis in this thesis. This ipsilateral neuron is also GABAergic, therefore inhibitory and densely projecting its dendrites in each glomerulus of the antennal lobes, thereby broadly modulating olfactory perception (Sachse et al., 2007).

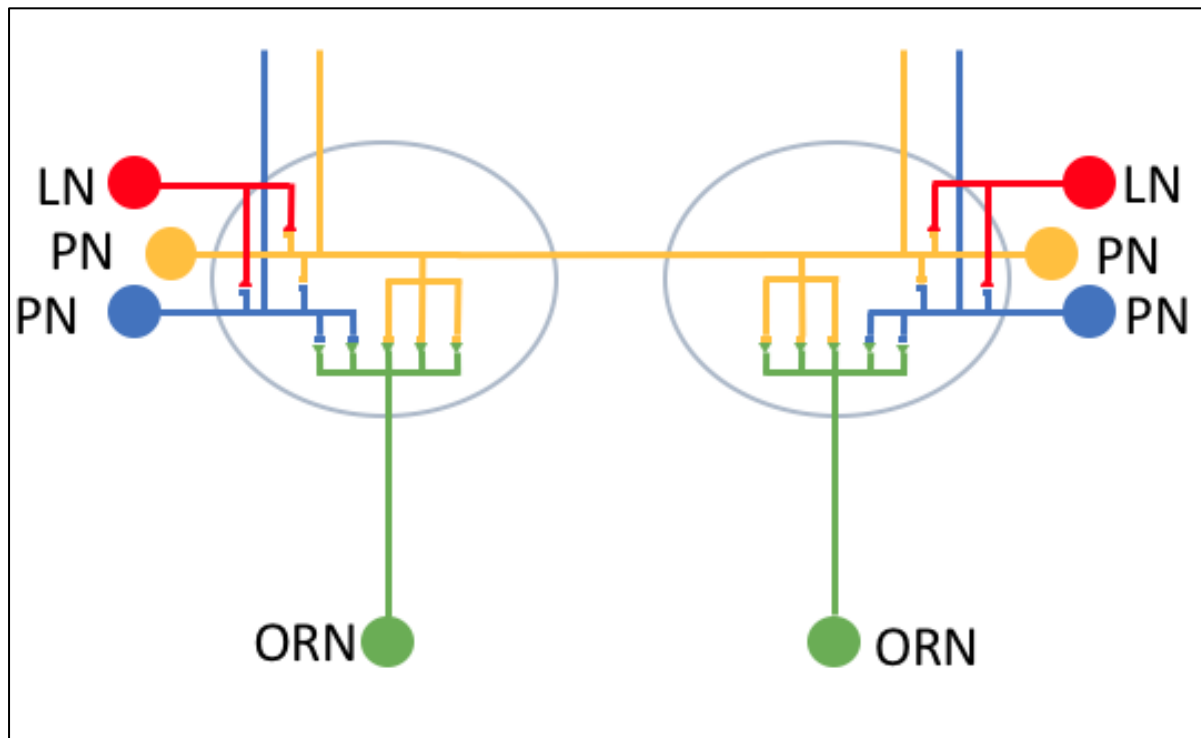


Figure 4. Schematic of V-glomerulus' microcircuit. Shown are ORNs (olfactory receptor neuron) unilateral and bilateral PNs (projection neurons) and inhibitory LNs (local interneurons)

1.4 Adult plasticity in the *Drosophila* olfactory system

Adapting to its environment is a key element for all organisms. Since odors are an important cue in nature, adaptations to a constant abundance or concentration could lead to an increased fitness level of the respective organism if habituation occurs and the animal can direct its cognitive abilities towards other sensations (Das et al., 2011). After being chronically stimulated with a given odorant, animals either respond less or show enhanced behavioral attraction (Devaud et al., 2001; Kidd et al., 2015). These changes are not only shown in behavior but also anatomically by Sachse et al. (2007). Their study revealed a reversible volume increase in the V glomerulus after exposure to CO₂. The plastic changes also led to altered neuronal activity of innervating LNs and decreased output of PNs in the lateral horn.

Plasticity is not only activity dependent, as Sachse et al. (2007) could show in their study, but has also been reported in response to injury. What is known so far about how the olfactory system responds to injury? To answer this question, it is necessary to address what happens at axons themselves if they get severed from their cell bodies and what ablation of ORNs and PNs causes in the antennal lobes, regarding the synaptic circuitry.

MacDonald et al. (2006) could show that severed axons undergo a degeneration process which is comparable to WD (Wallerian degeneration) found in mammals. Furthermore, they also argued that WD might lead to the release of attracting signals, which are recognized by the glia engulfment receptor *drpr* (*Draper*). *Drpr* was first described by Freeman et al. (2003), and is now known to be essential for glia cells to detect neuronal debris and initiate clearing it from the system which takes approximately 5 days (MacDonald et al., 2006).

Berdnik et al. (2006) showed that neurons within a single glomerulus can go through plastic changes, if either ORNs or PNs are ablated with toxins, but the wiring specificity is still highly stable (Fig.5). Neither ORNs form synapses with other PN classes outside of their respective glomerular border after their PN synaptic partner is ablated, nor PNs project their dendrites across other glomeruli to connect with other ORNs. However, they showed that intraglomerular plasticity can be observed after a unilateral antenna cut, in which ORNs contralateral to the lesion increase the density of their axon terminals in the injured hemisphere.

Knowing that the olfactory system is very robust, in terms of overall microcircuit stability following peripheral lesions and the fact that local remodeling can be observed upon injury-induced neuron loss, the main goal of my thesis described here was to determine the characteristics of central plasticity after sensory neuron degeneration.

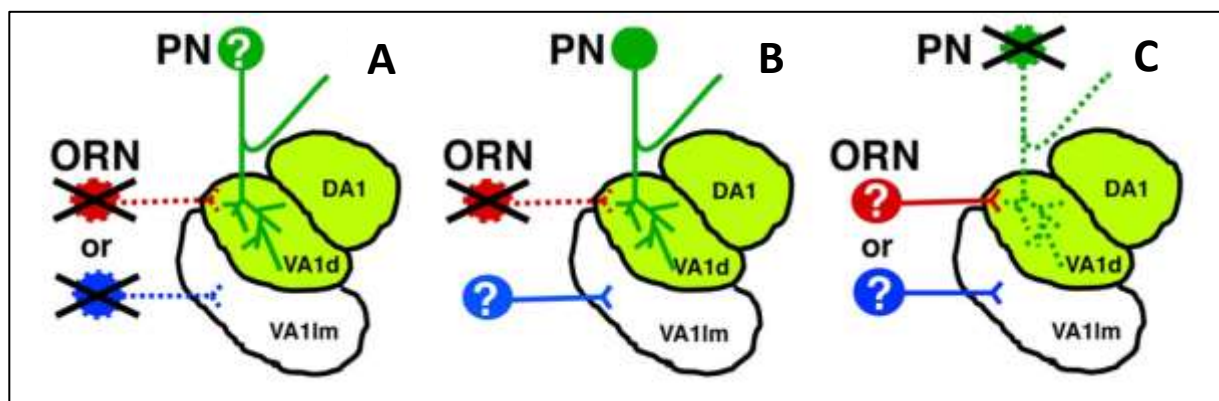


Figure 5. Cell ablation experiments done by Berdnik et al. (2006). A: Elimination of ORNs and the effect on PNs, B: Elimination of ORNs and the effect on neighboring ORNs, C: Elimination of PNs and the effect on ORNs; adapted from Berdnik et al. (2006)

Recent research has been rather scarce on that topic and the novel activity dependent GRASP technique was never used before to address such questions. Kazama et al. (2011) could show, that excitatory LNs increase their input on deafferented PNs and they argued that this event

might be a compensation for sensory input loss. I wanted to determine if and to what extent lesion influences the activity of a whole circuit of both excitatory and inhibitory neurons.

Is it possible to track plasticity by using GRASP? Is altered activity an indicator for compensation or is it just an effect of the preceding lesion? Does compensation rely on the increased activity of excitatory system modulating neurons, as it was shown by Kazama et al. (2011) or do inhibitory synapses contribute to that? If the microcircuit tries to compensate for the sensory input loss, does it rely on ipsilateral changes at the synapses or is the contralesional hemisphere (think of the bilateral projection of PNv1) also involved in recovery processes as it was shown in mammal research? Understanding the impact of lesion on such a small scale such as the microcircuit of V-glomerulus might help us to understand what happens at a synapse level after lesion on a much bigger scale, in much more complex organisms like mammals and eventually humans. The fly brain and the human brain, despite the vast difference in complexity and simply the number of neurons, share a lot of similarities and the idea to use *Drosophila* for these kind of experiments is not far off. A wide range of tools and the comparably easy cultivating conditions allow for precise manipulations of the fly genome to specifically answer the questions above.

2. Material & Methods

2.1 Fly culturing

Flies in our lab were cultured and raised in disposable plastic vials which are filled with self-cooked food which is made on a regular basis by people of our department (For the recipe refer to the Fly food section). Crosses were usually set up in small vials by putting male and female virgin flies inside until eggs were visible. Then the parent flies were transferred into a new vial to prevent mixing with the offspring and thus making it impossible to keep track of their genotype. Stocks on the other hand were usually kept in big vials, which can hold much more flies until the food spoils, to ensure a large enough quantity of flies to pick from. Flies in vials were kept at around 25°C on shelves in our laboratory with the option of putting them in various incubators which are set to different temperatures (18°C / 25°C) needed for the individual experiments conducted by people in our workgroup. Depending on the experiment

done in this thesis, flies were also put in a 5% CO₂ incubator (Tritech Research; DigiTherm®) at 25°C to explore the effect on the microcircuit of the CO₂ sensitive V-glomerulus.

2.2 Fly food

The recipe is the one used by TAs (technical assistant) in our laboratory. Slight variations were possible depending on the consistency on the food, but the changes are very minor so pre-requisites for all conducted experiments could be considered as equal (Table 1)

20 l water	1400 g corn flour	170 g agar
200 g soy flour	350 g dry yeast	1400 g malt extract
700 g sugar-beet syrup	80 ml proprionic acid	300ml Nipagin solution

Table 1. Ingredients of the fly food cooked by TAs in our lab

2.3 Fly stocks

The stocks which were used for all the experiments are all very well established and explored in our lab. Lines starting with BC (BCxxx) are our lab internal brain circuit numbers which refer to specific Rubin numbers (Rxxx), which can be ordered from Bloomington *Drosophila* Stock center, Indiana, USA. VT lines were sourced from Vienna *Drosophila* RNAi Center, Vienna, Austria. NP lines (NPxxx) were ordered from Kyoto Stock center, Kyoto, Japan (Table 2). These lines were needed to visualize the different synapses which can be found in V-glomerulus' microcircuit.

Transgene	Genotype	Stock number	Source
<i>PNv1 Gal4</i>	<i>PNv1 Gal4 / III</i>	200516VT	Vienna <i>Drosophila</i> RNAi center
<i>PNv3 Gal4</i>	<i>PNv3 Gal4 / III</i>	012760VT	Vienna <i>Drosophila</i> RNAi center

<i>PNv5 Gal4</i>	<i>BC0554 / III</i>	R44A02	Bloomington <i>Drosophila</i> Stock center
<i>LNv2 Gal4</i>	<i>LNv2 Gal4 / I</i>	NP2426-P	Kyoto Stock center
<i>PNv1 lexA</i>	<i>PNv1 lexA / CyO; TM2/TM6</i>	200516VT	Vienna <i>Drosophila</i> RNAi center
<i>PNv5 lexA</i>	<i>PNv5 lexA / CyO; TM2/TM6</i>	R44A02	Bloomington <i>Drosophila</i> Stock center
"GRASP"	<i>Sp / CyO; UAS-syb::spGFP¹⁻¹⁰ lexAopCD4::spGFP¹¹ / TM2</i>	-	Chi-Hon Lee

Table 2. Stocks used for all experiments. BCxxx lines are the lab internal brain circuit numbers which can be also found and ordered by their Rubin numbers Rxxx from Bloomington *Drosophila* stock center in the US. VTxxx lines are from the Vienna *Drosophila* RNAi center in Vienna. NP lines were ordered from the Kyoto Stock center in Japan

2.4 Crosses

The following crosses were used for all experiments featured in this thesis. In the first step the lexA lines were combined with the GRASP stock (Fig. 6).

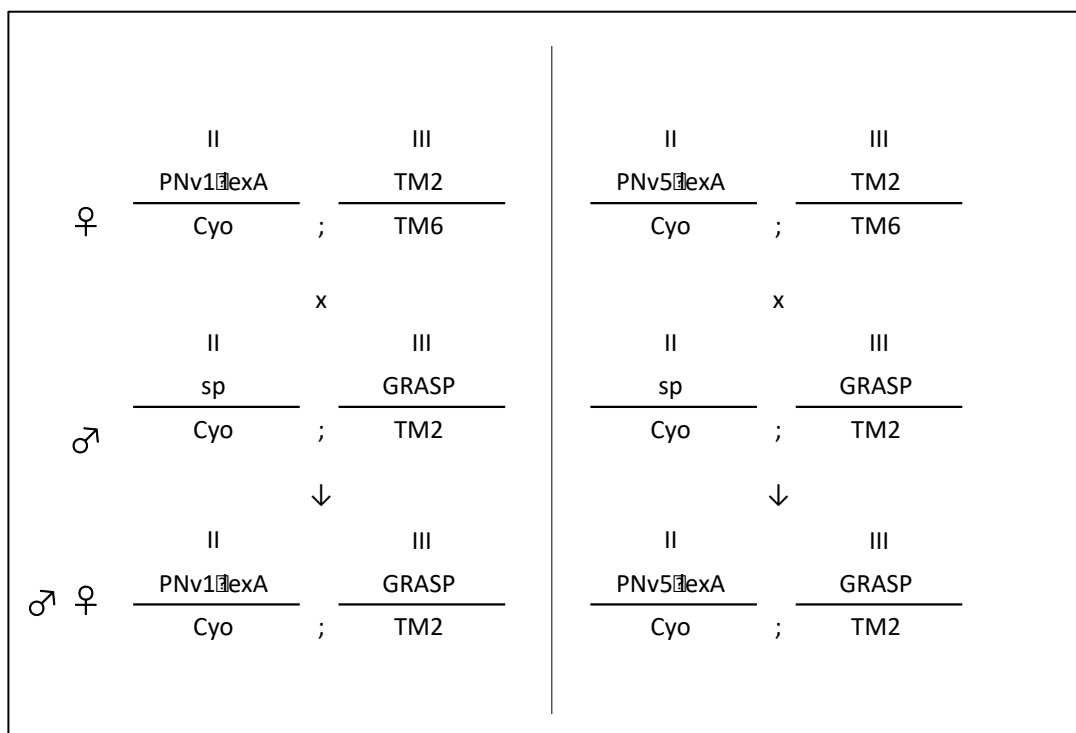


Figure 6. The *lexA* lines are crossed with the GRASP stock to allow further experimenting

The newly established stocks could then be further crossed with the available Gal4 lines (Gal4 on third chromosome) to allow GRASP to work and visualize the different synapses of V-glomerulus' microcircuit (Fig. 7).

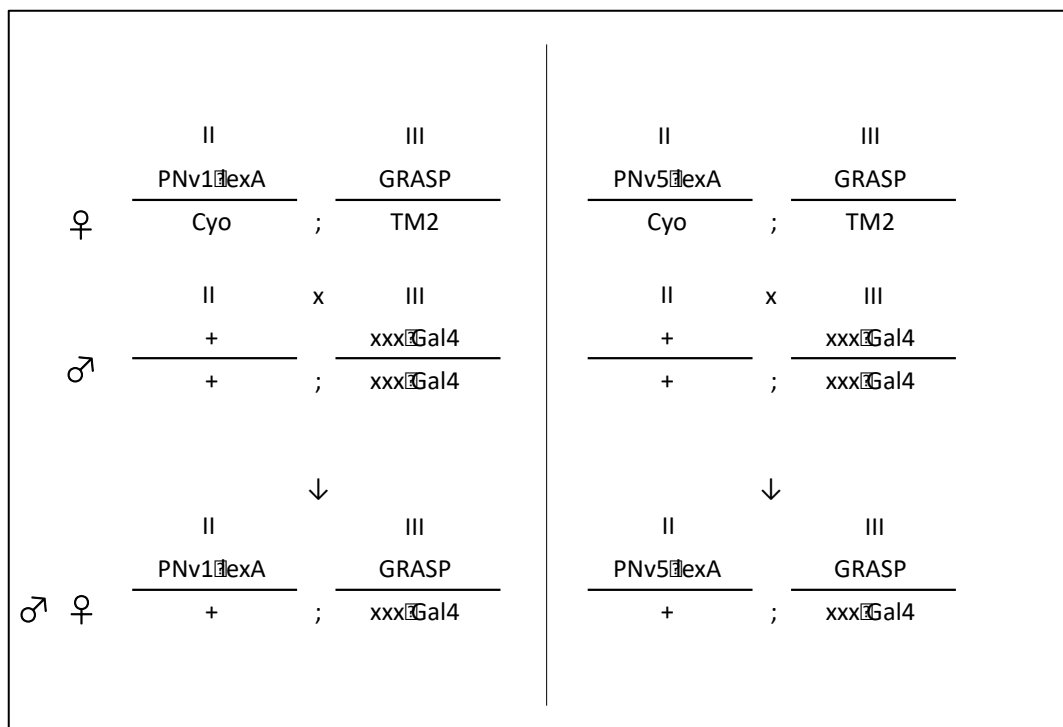


Figure 7. New stocks need to be crossed to Gal4 lines to drive the expression on both presynaptic and postsynaptic neurons

Since the LNv2 Gal4 driver was located on the first chromosome, the genotype of the cross was different (Fig. 8).

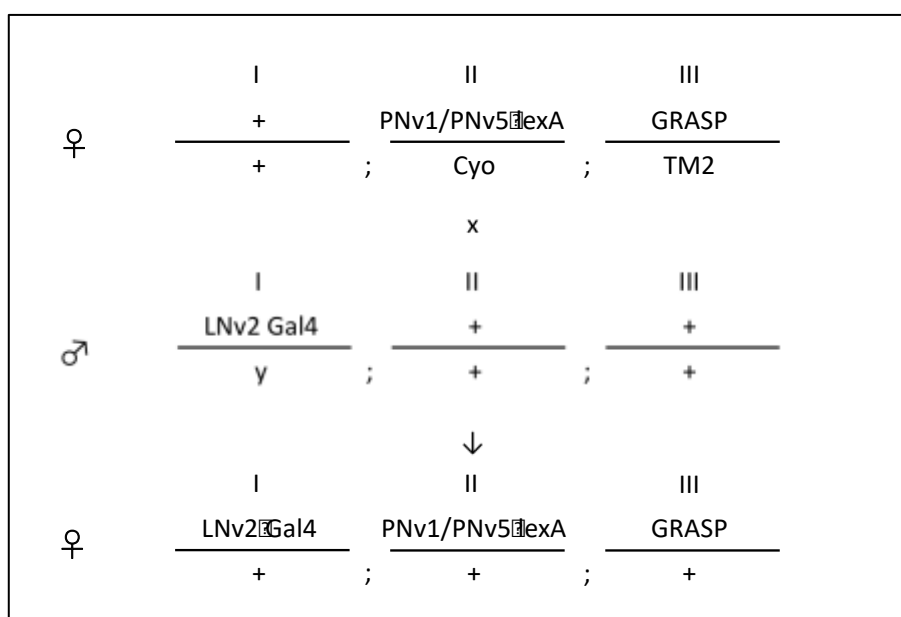


Figure 8. Crossing scheme for the new stocks with LNv2 Gal4

Male flies of the offspring didn't have the Gal4 driver and were kept as an internal control to check if GRASP is working as intended.

2.5 Genetic tools

2.5.1 Binary expression systems

As a necessity to apply the GRASP technique featured in the experiments of this thesis, it was important to understand the properties of the available binary expression systems, namely the Gal4 / UAS system and the *lexA* / *lexAop* system. Since 1988, when Fischer et al. showed that Gal4 drives the expression of a reporter gene which was downstream of the UAS sequence, this binary system, which originates from yeast (*saccharomyces cerevisiae*) (Laughon et al., 1984), quickly became one, if not the most popular genetic tool when working with *Drosophila melanogaster*. As of today, there are specific Gal4 drivers for most neurons or tissues in the fly and the applications of this binary expression system are varied (Duffy, 2002). For the experiments, which are shown in this thesis, male and female flies were crossed with each other to make use of the binary expression system. One sex had to carry the tissue specific Gal4 driver and the other sex the gene of interest or reporter which gets transcribed after Gal4 binds to the UAS (Fischer, 1988). The other binary expression system, *lexA* / *lexAop*, can be used parallel to the Gal4 / UAS system which obviously allows for more freedom in activating genes of interest in specific neurons and follows the same principles as the Gal4 / UAS system (Lai et al., 2006).

2.5.2 GRASP technique

GRASP is the abbreviation for GFP reconstitution across synaptic partners and it is based on two separate non-fluorescent split GFP fragments which are located on the presynapse and postsynapse respectively. The larger fragment, GFP¹⁻¹⁰, is fused with neuronal-synaptobrevin, a protein of the synaptic vesicle membrane. If an action potential reaches the presynaptic terminal, vesicles fuse with the synaptic membrane and therefore syb:spGFP¹⁻¹⁰ gets exposed to the synaptic cleft. That's why the GRASP used in this thesis is also referred to as an activity dependent GRASP, because the more APs reaching the synapse, the more vesicles fuse with the synaptic membrane, thus leading to more syb:spGFP¹⁻¹⁰ available in the synaptic cleft, ready to reconstitute with the smaller GFP11 counterpart which is already exposed to the

cleft, fused to the transmembrane protein CD4. If the two fragments now reconstitute a fully functional fluorescent protein is formed, which allows a visualization of the chosen synapse given that the drivers in the pre-, - and postsynapse are in fact driving the expression in neurons which are connected with each other (Macpherson et al. 2015) (Fig. 9).

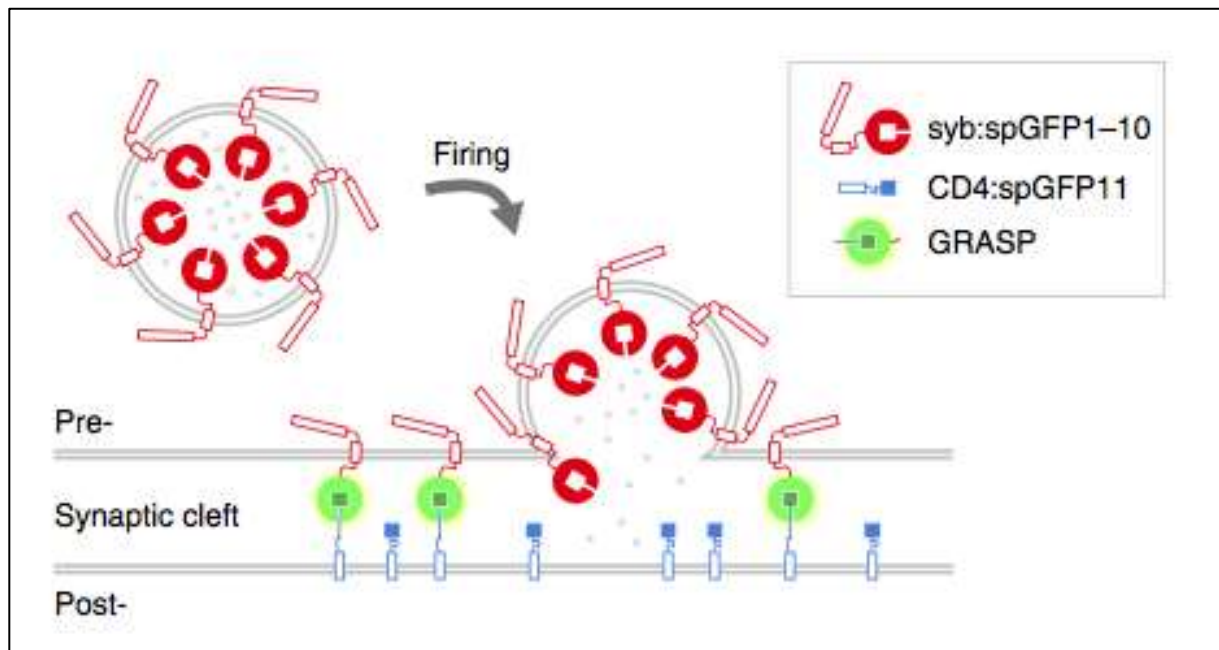


Figure 9. N-synaptobrevin bound GFP1-10 gets exposed to the synaptic cleft upon stimulation by an incoming action potential. There it can reconstitute with a CD4 bound GFP11 fragment to form a functional fluorescent protein; Adapted from Macpherson et al. (2015)

2.6 Inducing Injury

2.6.1 Time table

Since the major interest in this thesis was to explore if and to what extent injury induces synaptic plasticity, the process to apply lesion to the flies was vital. It was shown, that as of day 1 after injury like antennal ablation, the severed axons start to disintegrate due to Wallerian degeneration. 3 days after injury, a GFP expression driven by an ORN driver is barely visible anymore and after 5 days the GFP signal is gone, indicating that the severed axonal debris is completely cleared from the system (MacDonald et al. 2006). Based on these findings, a time table was constructed with 3 different time points on which the effect of antennal ablation was investigated. (Fig. 10).

2.6.2 Ablation process

The antenna cut was scheduled on the first day after hatching. Usually flies are getting anesthetized with CO₂ before killing them with 96% alcohol but here the flies should be neither

killed nor would the use of CO₂ be applicable since the effect of CO₂ on synaptic plasticity in combination with injury was also considered to be explored. Therefore, the vials containing the different crosses were cooled down in a box full of ice until the flies' movement was significantly slowed down which allowed for further handling with them. The immobilized flies were then put on a plastic plate, which itself was put on a small tray filled with ice. This tray could be moved under a light microscope, where the flies could be selected for the antenna cut. Depending on the condition, the ablation was done unilaterally or bilaterally with the addition of flies which were not lesioned to have control groups at hand. For the unilateral cut condition, the right antenna was selected to be removed. High precision forceps were used as the tools of choice for this procedure. The now injured flies were separated by gender and put into small vials containing fly food. These vials then were put in 25°C either at 0,08% CO₂ (ambient air) on our lab shelves or at 5% CO₂ in the incubator for the different durations to cover all time points as previously mentioned. The procedure was the same for my UV-glue experiments. Instead of removing the antennae a drop of the glue was applied to cover the third antennal segment.

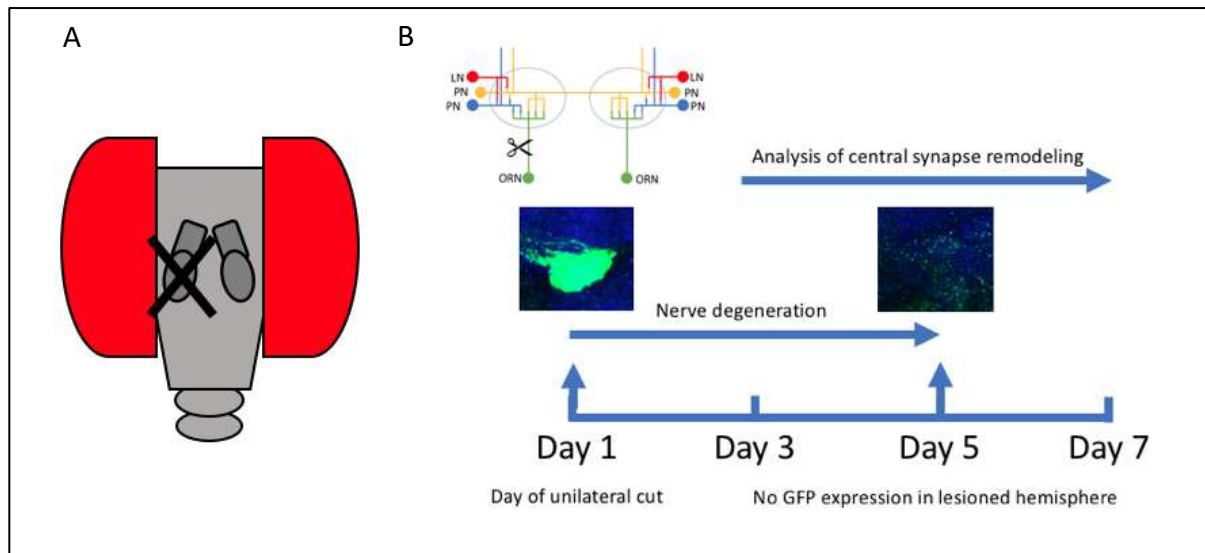


Figure 10. A. For the unilateral cut condition, the right antenna of the flies was ablated. B. A GFP expression driven by an ORN Gal4 Driver vanishes approximately 5 days after the respective antenna is cut and the axonal debris is removed from the system

2.7 Dissection, staining protocol, imaging and processing

2.7.1 Dissection

After the flies were kept at 0.08% or 5% CO₂ for the desired amount of time the vials were again put in a box full of ice to immobilize them. Since the flies were already preselected and

separated by gender, they were now killed by using 96% alcohol to allow dissection. After several washes in PBS (Phosphate-Buffer-Solution) the flies were put in a droplet of the very same liquid on a silicon plate and put under a light microscope. The dissections themselves were conducted with two high precision forceps, as they were used in the lesioning procedure.

2.7.2 Staining protocol

This staining protocol is commonly used in our lab, although slight variations are possible, depending on the experiments. The following step by step guide is the one which was used for all dissections needed in this thesis.

- Fix dissected brain in 2% PFA (Paraformaldehyde) for 1 hour
- Dispose PFA in correct container and wash the brains 3 times in PBT (PBS + TritonX-100 0,3%), 15 minutes each
- Add Goat serum to the brains and give them for at least 1 hour on a shaker
- Remove Goat serum and dispose it
- Mix Goat serum and anti-NCAD (rat) with a ration of 1:10 to make the 1st antibody
- Add antibody to fly brains and incubate overnight in 4°C on a shaker
- Remove 1st antibody (can be reused up to three times)
- Wash brains 3 times with PBT, 15 minutes each
- Prepare second antibody, mix Goat serum with A647 anti rat with a ratio of 1:500
- Remove PBT from brains and add 2nd antibody, cover the sample box with aluminum foil to reduce the risk of light damaging your antibody
- Incubate samples for 3 hours at room temperature (~ 25°C)
- Remove 2nd antibody and discard
- Wash the samples 3 times with PBT, 15 minutes each
- Mount samples with Vectashield

2.7.3 Imaging

The mounted samples were imaged with a Leica DM6000CM confocal microscope. It was important to adjust the settings beforehand and keep them the same for all samples to avoid mistakes in the GFP intensity measurements. A HD-laser was chosen to image the brains because of the higher sensitivity which was needed to detect a GRASP signal in synapses with

low activity. Following that, the gain for the green channel (GFP) was kept at the same value for the whole imaging process while the gain of the blue channel (NCAD) was adapted as needed to get the best as possible resolution for the background. The zoom factor was set to 3x to ensure that the area of interest had about the same size for all samples. The brains were scanned at rate of 200Hz and the stepsize between the individual slices was fixed to 1,5 micrometers.

2.7.4 Processing

The digital pictures were further processed with the public domain program “Fiji” which is basically the program “ImageJ” but already packaged with many useful plugins needed in various scientific fields. With this program, the blue background color (neuropil) could be further adapted to get a better contrast with the green GFP channel (V-glomerulus). The green channel was not changed to guarantee the same prerequisites when it comes to GFP intensity quantification.

2.8 GFP intensity measurements

The intensity measurements were also done with “Fiji”. The procedure followed the guide written by Christine Labno from the University of Chicago. The first step was to set a fixed circle which can be put over the region of interest. The GFP intensity measurements were done within the area of said circles. Therefore, it was important to select a diameter which was suitable to use for a wide range of brains without being too small and not give a good representation of the intensity, or not being too big to include background noise to the quantification. After testing numerous diameters in multiple brains, a diameter of 65 micrometers was chosen, which appeared to be the optimal value to ensure a good representation. For the intensity measurements itself, the built-in measurement tool of “Fiji” was used. The program uses comparisons of grey values to identify intensity, so any value above the threshold grey level is considered as a raise in intensity. Now, after the preparations were done, each GRASP signal located in V-glomerulus, for each cross in both hemispheres could be measured. To circumvent the problem of quantifying background noise as well, separate measurements from within other areas of the antennal lobes were done and subtracted from the measurements of V-glomerulus.

2.9 Statistics

Xlstat, which is a Microsoft excel add-in, was used for all comparative calculations because of its simple user interface and good accessibility. For comparisons between the hemispheres, for example if one side shows higher GFP intensity after lesion than the other, Mann-Whitney's U-test was considered to be the best test available, since the prerequisites for a parametric test were not fulfilled. To compare more than two independent samples the Kruskal-Wallis test for non-parametric data was chosen, followed by Dunn's test.

3. Results

3.1 Analyses of interhemispheric GRASP intensities

3.1.1 GRASP intensity measurements

The first step was to apply the GRASP technique in flies without any previously induced neuronal lesions to determine possible interhemispheric differences in the GRASP intensity of the glomerular connections. As mentioned in the Methods section, the GRASP used in this thesis is an adapted form constructed by Macpherson et al. (2015) which works in an activity dependent manner. Therefore, any differences in GFP intensity which were quantified in the following GRASP experiments reflect differences of synaptic activity. In total, 6 different types of synaptic connections within the V-glomerulus were selected and can be divided into two main classes, the GABAergic input of PNv3 and LNV2 onto the bilateral projection neuron PNv1, the unilateral projection neuron PNv5 and the reciprocal connection between PNv1 and PNv5 (Fig. 11).

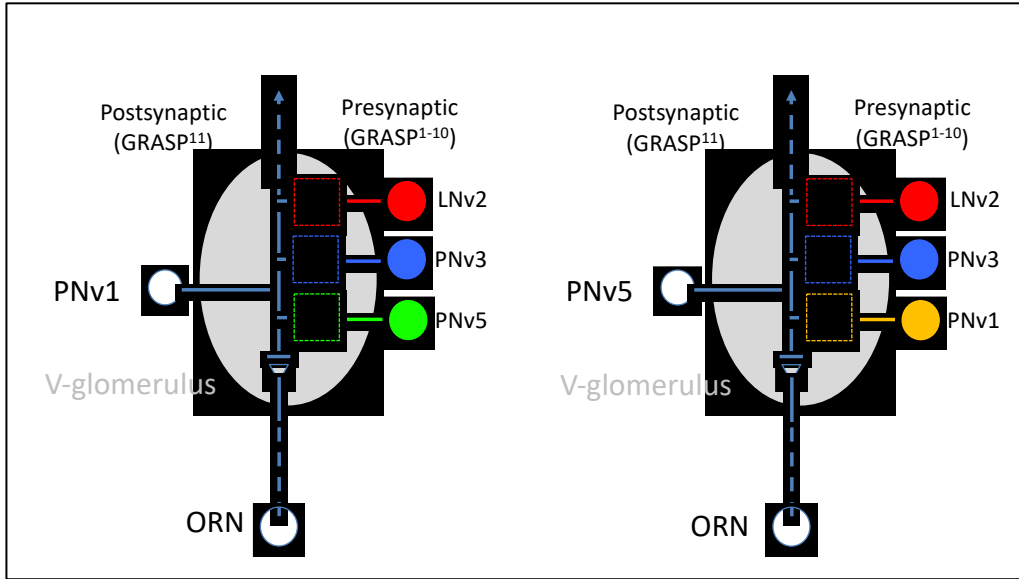


Figure 11. Schematic of synapses which were visualized with GRASP and were used for GFP intensity quantifications

The following experiments will show the GFP intensity measurements of the GRASP signal between two connected neurons. As mentioned in the Material & Methods section, it was necessary to create a predefined circle which marks the ROI (region of interest) (Fig. 12). Within this ROI the GFP intensity measurements were done with a built-in tool of “Fiji”. The program

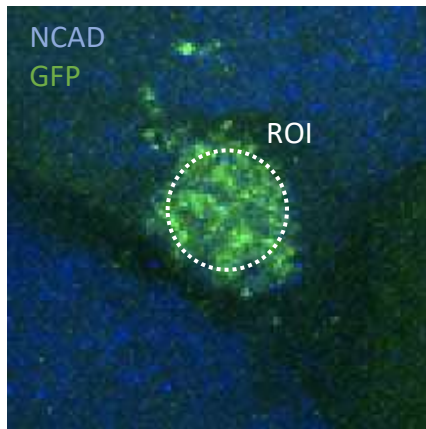


Figure 12. The GFP intensity of V-glomerulus was measured within the border of a predefined circle which surrounds the ROI (region of interest)

quantifies grey levels within this ROI and calculates any deviation from a base grey value as a change of intensity, from which I additionally subtracted the background intensity to get a proper representation of the real intensity value within the border of said circle. Therefore, if I refer to values when describing the GFP intensity measurements, it is important to know that they are in relation to this base grey value and represent the positive increase above threshold. These measurements were done in both hemispheres for all brains available in each scenario. Then,

for each synapse, the measurements of one hemisphere were grouped together and compared with the measurements of the other hemisphere and possible significant differences between both sides were calculated with the U-test. For the following experiments, the p-values of those statistical test are shown for each day. Results for LNV2 synapses in male flies are not shown since they were used as an internal control to validate the functionality of GRASP. Since my crossing method didn't allow for a functional GRASP construct in male flies no endogenous GFP signal was visible.

3.1.1.1 Synaptic input onto PNv1

The GRASP connection of PNv3 onto PNv1 (PNv3 → PNv1) of male flies revealed no statistical difference between the two hemispheres throughout all days (3 days $p=0.134$; 5 days $p=0.829$; 7 days $p=0.468$) and none of the calculated mean GFP intensities reached a value above 20 (Fig. 13). Similarly, in female flies the measurements revealed intensities in the same range and no significant differences between the hemispheres were detected either (3 days $p=0.694$; 5 days $p=0.937$; 7 days $p=0.246$) (Fig. 13)

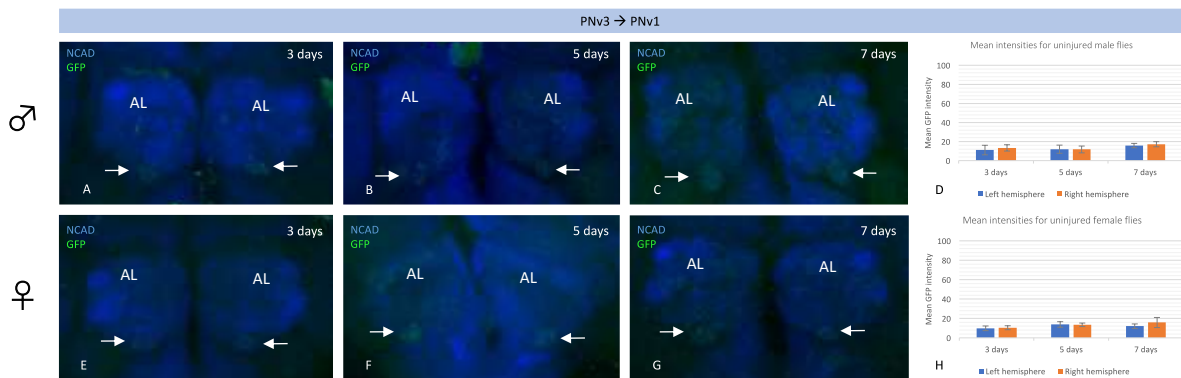


Figure 13. GRASP-GFP intensity measurements of the PNv3 → PNv1 synapse: (A-C) Antennal lobes (ALs) of male flies stained with anti-NCAD (blue, neuropil marker) and visible endogenous GFP (green) in the V-glomerulus (white arrows), (E-G) ALs of female flies stained with anti-NCAD and visible endogenous GFP in the V-glomerulus, (D) GFP intensity measurements in male flies, (H) GFP intensity measurements in female flies (* $p<0.05$, $n=4-8$)

The PNv5 → PNv1 synapse didn't differ much from the PNv3 → PNv1 synapse. Neither male flies (3 days $p=0.635$; 5 days $p=0.800$; 7 days $p=0.690$) nor female flies (3 days $p=0.746$; 5 days $p=0.651$; 7 days $p=0.690$) showed significant GFP differences between the hemispheres (Fig. 14). For both types of synaptic connections, the GFP intensities are increasing within the first week. Furthermore, overall females have slightly higher GFP intensities indicating a sex-specific activity of the PNv5 → PNv1 synapse (Fig. 14)

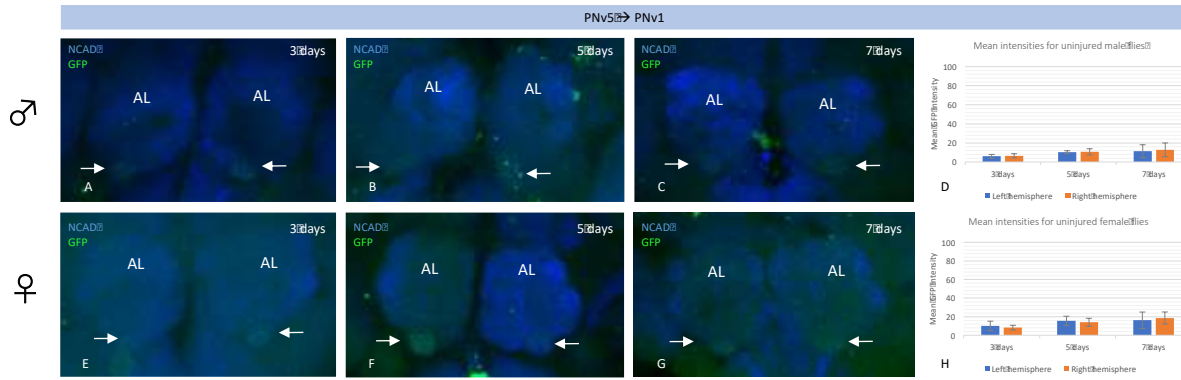


Figure 14. GRASP-GFP intensity measurements of the PNv5 → PNv1 synapse: (A-C) Antennal lobes (ALs) of male flies stained with anti-NCAD (blue, neuropil marker) and visible endogenous GFP (green) in the V-glomerulus (white arrows), (E-G) ALs of female flies stained with anti-NCAD and visible endogenous GFP in the V-glomerulus, (D) GFP intensity measurements in male flies, (H) GFP intensity measurements in female flies (* $p < 0.05$, $n = 3-5$)

Analyzing the GFP expression for the LNV2 → PNv1 synapse revealed a dramatic increase in the GRASP intensity between day 5 and day 7. The measurements were peaking at a value of 87 above threshold while the intensities for 3 and 5 days were only around a value of 20 above threshold (Fig. 15). Compared with the previous two synapses this increase of intensity on day 7 seems to be specific for the GABAergic LNV2 → PNv1 synapse. A comparison of GFP intensities between the two hemispheres revealed no significant differences (3 days $p = 0.833$; 5 days $p = 0.802$; 7 days $p = 0.971$) (Fig. 15).

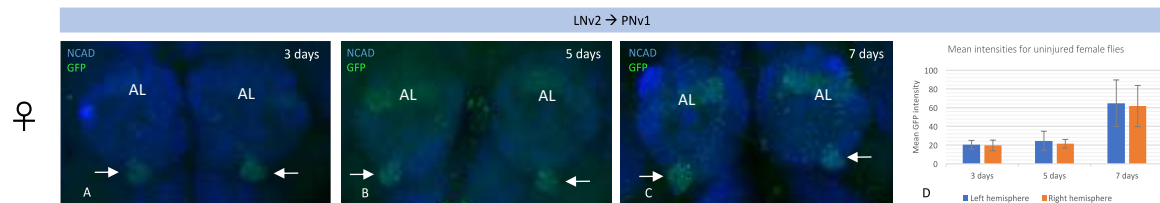


Figure 15. GRASP-GFP intensity measurements of the LNV2 → PNv1 synapse: (A-C) Antennal lobes (ALs) of female flies stained with anti-NCAD (blue, neuropil marker) and visible endogenous GFP (green) in the V-glomerulus (white arrows), (D) GFP intensity measurements in female flies (* $p < 0.05$, $n = 4-5$)

In summary, within the V-glomerulus, PNv1 receives rather low synaptic input from neighboring excitatory and inhibitory neurons and no significant differences in interglomerular GFP intensities could be observed. However, it was shown that the inhibitory input from LNV2 on PNv1 was extensively increased after 7 days.

Next I determined the average GRASP intensities of the synaptic input onto PNv5 to spot possible differences with the previously analyzed synaptic input onto PNv1.

3.1.1.2 Synaptic input onto PNv5

The synaptic input of PNv3 on PNv5 was low according to the GFP intensity measurements (Fig. 16). Surprisingly, the U-test found a statistical significant difference on day 3 in male flies (3 days $p=0.027$; 5 days $p=0.578$; 7 days $p=0.400$) suggesting increased activity of this synapse in the left hemisphere (Fig. 16). Female flies differed from this result since no significant difference was found on any day (3 days $p=0.413$; 5 days $p=0.310$; 7 days $p=0.540$) (Fig. 16).

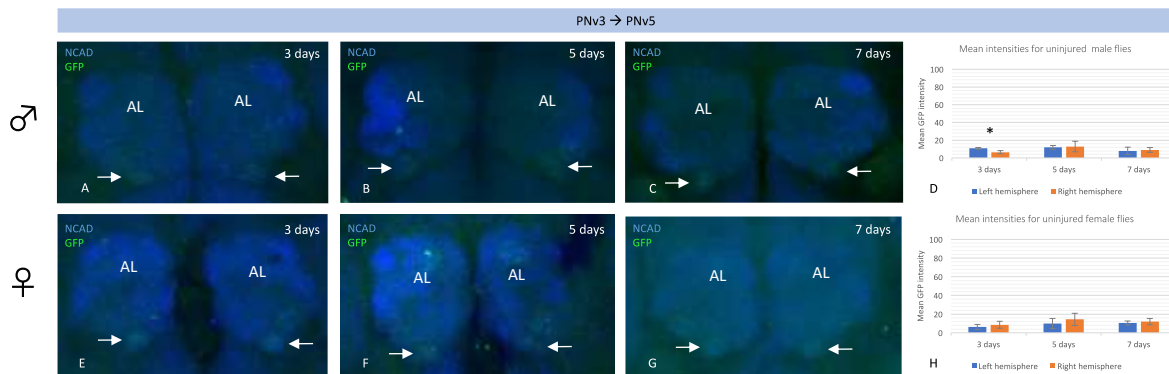


Figure 16. GRASP- GFP intensity measurements of the PNv3 → PNv5 synapse: (A-C) Antennal lobes (ALs) of male flies stained with anti-NCAD (blue, neuropil marker) and visible endogenous GFP (green) in the V-glomerulus (white arrows), (E-G) ALs of female flies stained with anti-NCAD and visible endogenous GFP in the V-glomerulus, (D) GFP intensity measurements in male flies, (H) GFP intensity measurements in female flies (* $p<0.05$, $n=4-7$)

Similarly to the PNv5 → PNv1 synapse, analysis of the PNv1 → PNv5 synapse indicates a sex-specific activity. Measurements in male flies showed low GFP intensity values on day 3 and day 7, while on day 5 both hemispheres displayed higher activity (Fig. 17). A comparison of the hemispheres resulted in no significant differences (3 days $p=0.397$; 5 days $p=0.468$; 7 days $p=0.600$) (Fig. 17). In female flies the measured GFP intensities stayed in a similar range on all days and the hemispheres didn't significantly differ from each other (3 days $p=0.078$; 5 days $p=0.635$; 7 days $p=0.971$) (Fig. 17).

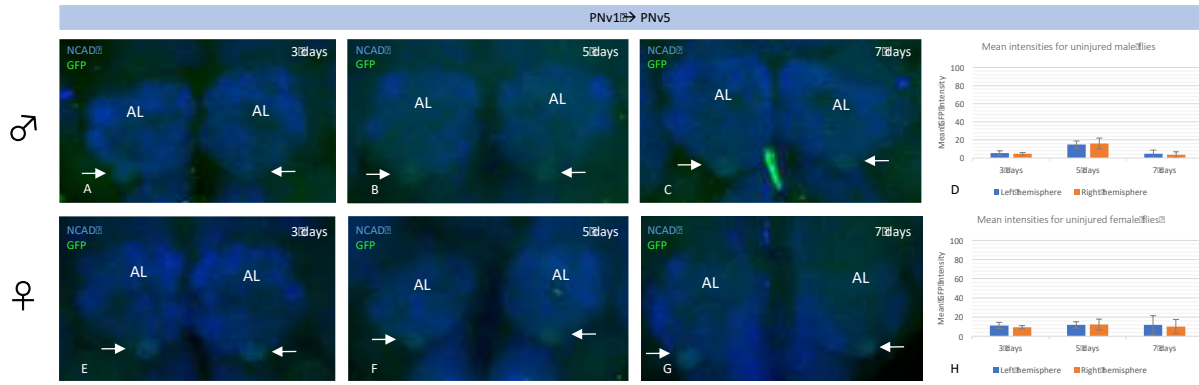


Figure 17. GRASP-GFP intensity measurements of the PNv1 → PNv5 synapse: (A-C) Antennal lobes (ALs) of male flies stained with anti-NCAD (blue, neuropil marker) and visible endogenous GFP (green) in the V-glomerulus (white arrows), (E-G) ALs of female flies stained with anti-NCAD and visible endogenous GFP in the V-glomerulus, (D) GFP intensity measurements in male flies, (H) GFP intensity measurements in female flies (* $p < 0.05$, $n = 3-7$)

In comparison with the previous two synapses, the LNV2 → PNv5 synapse showed overall higher GFP intensity with values of 20 above threshold (Fig. 18). No significant differences between the hemispheres were calculated by the U-test (3 days $p = 0.873$; 5 days $p = 0.743$; 7 days $p = 0.400$) (Fig. 18). Interestingly the GABAergic input from LNV2 onto PNv5 didn't increase between day 5 and day 7 as it was shown for the LNV2 → PNv1 synapse (Fig. 15).

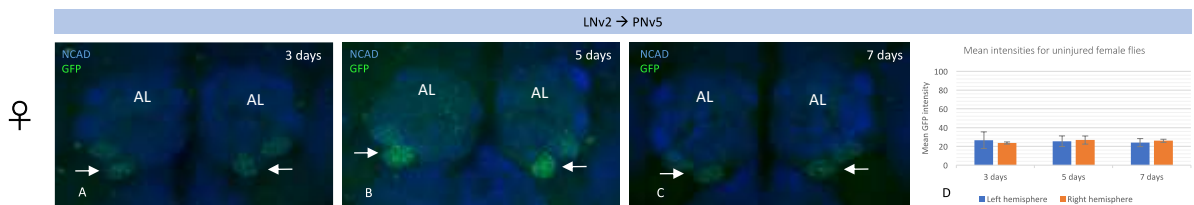


Figure 18. GRASP-GFP intensity measurements of the LNV2 → PNv5 synapse: (A-C) Antennal lobes (ALs) of female flies stained with anti-NCAD (blue, neuropil marker) and visible endogenous GFP (green) in the V-glomerulus (white arrows), (D) GFP intensity measurements in female flies (* $p < 0.05$, $n = 4-5$)

Comparing the results of synaptic input onto PNv1 and PNv5 shows that there are no interhemispheric differences of measured GFP intensities apart from the PNv3 → PNv5 synapse, where a significant difference was shown on day 3 in male flies. The GABAergic input from LNV2 onto PNv1 and PNv5 was different from each other, because a drastic increase of GFP intensity was observed for the LNV2 → PNv1 synapse on day 7 which was absent for the synapse with PNv5.

3.1.2 GRASP in unilaterally lesioned flies

After analyzing the GFP expression levels in uninjured control flies, I explored how and to what extent unilateral lesion affects measured GFP intensities. It was shown that the GFP intensities in unharmed flies didn't differ between the hemispheres besides on one day for the PNv3 →

PNv5 synapse. For this reason, an increase or decrease of measured GFP intensities after unilateral ablation would indicate injury-induced changes of synaptic activity. For all the following antennal ablation experiments the right hemisphere represents the injured side.

3.1.2.1 Changes of synaptic input onto PNv1

The overall measured GFP intensities of the PNv3 → PNv1 synapse were low, but unilateral lesion in male flies led to an increased GFP intensity on the injured side reaching the highest intensity on day 5 (Fig. 19). This differential GFP intensity measurements indicate a higher GABAergic input from PNv3 onto PNv1 after ablation of one antenna. Yet, male flies which underwent unilateral lesion showed no statistical difference between the two hemispheres on day 3 ($p=0.682$) and day 5 ($p=0.090$), but showed a significant difference on day 7 ($p=0.024$) (Fig. 19). For female flies a similar distribution of GFP intensity values can be observed. In the unilateral cut condition, the injured side had generally a higher GFP intensity compared to the unharmed side where it peaked again after 5 days (Fig. 19). A significant statistical difference between the two hemispheres could be observed on day 5 (3 days $p=0.639$; 5 days $p=0.024$; 7 days $p=0.199$) (Fig. 19).

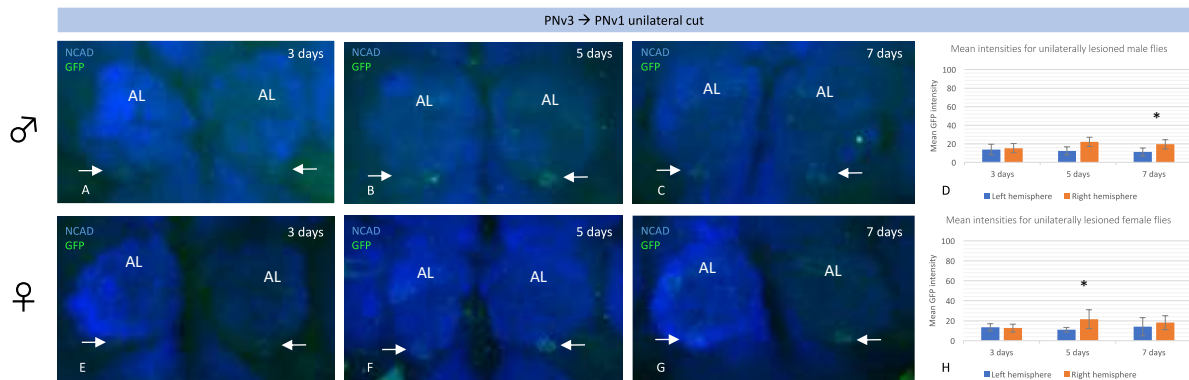


Figure 19. GRASP-GFP intensity measurements of the PNv3 → PNv1 synapse after unilateral lesion: (A-C) Antennal lobes (ALs) of male flies stained with anti-NCAD (blue, neuropil marker) and visible endogenous GFP (green) in the V-glomerulus (white arrows), **(E-G)** ALs of female flies stained with anti-NCAD and visible endogenous GFP in the V-glomerulus, **(D)** GFP intensity measurements in male flies, **(H)** GFP intensity measurements in female flies (* $p<0.05$, $n=5-7$)

In contrast to the PNv3 → PNv1 synapse, no significant GFP intensity differences could be found for unilaterally lesioned male flies at the PNv5 → PNv1 synapse (3 days $p=0.769$; 5 days $p=0.333$; 7 days $p=0.548$) (Fig. 20). Like in male flies no significant GFP intensity differences after unilateral ablation could be found in female flies within one week (3 days $p=0.697$; 5

days=0.800; 7 days p=0.617) (Fig. 20). Interestingly the two sexes differed in their GFP expression levels most noticeable on day 7 (Fig. 20).

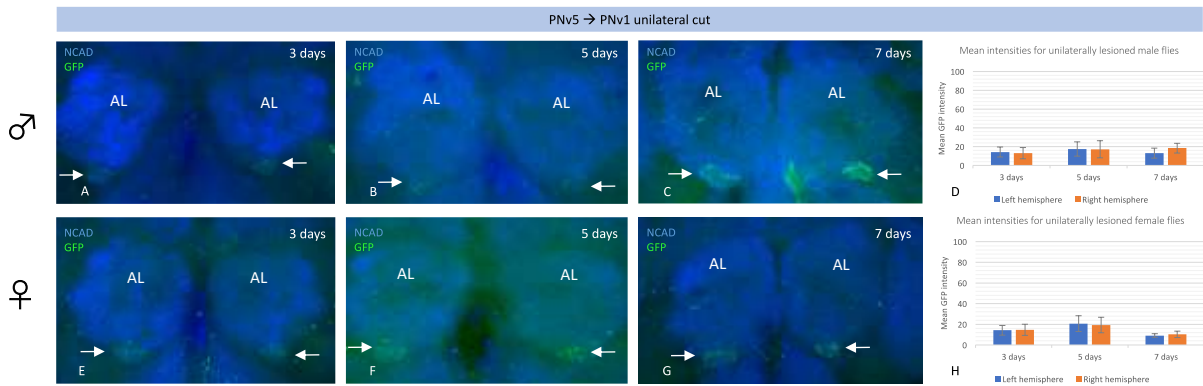


Figure 20. GRASP-GFP intensity measurements of the PNv5 → PNv1 synapse after unilateral lesion: (A-C) Antennal lobes (ALs) of male flies stained with anti-NCAD (blue, neuropil marker) and visible endogenous GFP (green) in the V-glomerulus (white arrows), (E-G) ALs of female flies stained with anti-NCAD and visible endogenous GFP in the V-glomerulus, (D) GFP intensity measurements in male flies, (H) GFP intensity measurements in female flies (*p<0.05, n=6-7)

The LNV2 → PNv1 synapse displayed the greatest response to the unilateral cut. The injured hemisphere showed greater GFP intensity values on all days, while the overall GFP intensity increased up until day 7 (Fig. 21). Due to the high variance within one sample no statistically significant GFP intensity differences could be found for any day (3 days p=0.090; 5 days p=0.625; 7 days p=0.156) (Fig. 21).

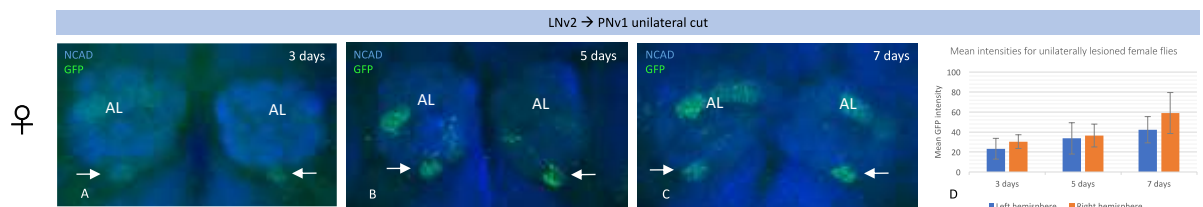


Figure 21. GRASP-GFP intensity measurements of the LNV2 → PNv1 synapse after unilateral lesion: (A-C) Antennal lobes (ALs) of female flies stained with anti-NCAD (blue, neuropil marker) and visible endogenous GFP (green) in the V-glomerulus (white arrows), (D) GFP intensity measurements in female flies (*p<0.05, n=7-8)

In summary, I found that the PNv3 → PNv1 and the LNV2 → PNv1 synapses had the strongest response to unilateral lesion. There was a trend that the synapses in the injured hemisphere responded with heightened activity which suggests that this change was injury-induced. Most interestingly, both PNv3 and LNV2 are GABAergic inhibitory neurons, so that this differential activity caused by lesion might be dependent on the nature of the involved neurons.

Now, after the observation that a unilateral lesion can cause injury-induced plasticity, I wanted to explore if the same applies for synaptic connections onto PNv5. It was already shown in

uninjured flies, that connections onto PNv1 and PNv5 differ in their activity and therefore it is not unlikely that a lesion affects synapses with PNv5 differently.

3.1.2.2 Changes of synaptic input onto PNv5

The PNv3 → PNv5 synapse showed vastly different results (Fig. 22) if compared with the PNv3 → PNv1 synapse (Fig. 19). In males, while the latter was more active in the injured hemisphere (Fig. 19), this couldn't be shown for the PNv3 → PNv5 synapse (Fig. 22). Furthermore, no significant GFP intensity differences between the two hemispheres could be calculated (3 days $p=0.123$; 5 days $p=0.897$; 7 days $p=0.984$) (Fig. 22). Female flies showed similar results, although with slightly lower mean GFP intensity values. No significant intensity differences between the injured and uninjured hemisphere could be observed (3 days $p=0.937$; 5 days $p=0.914$; 7 days $p=0.800$) (Fig. 22)

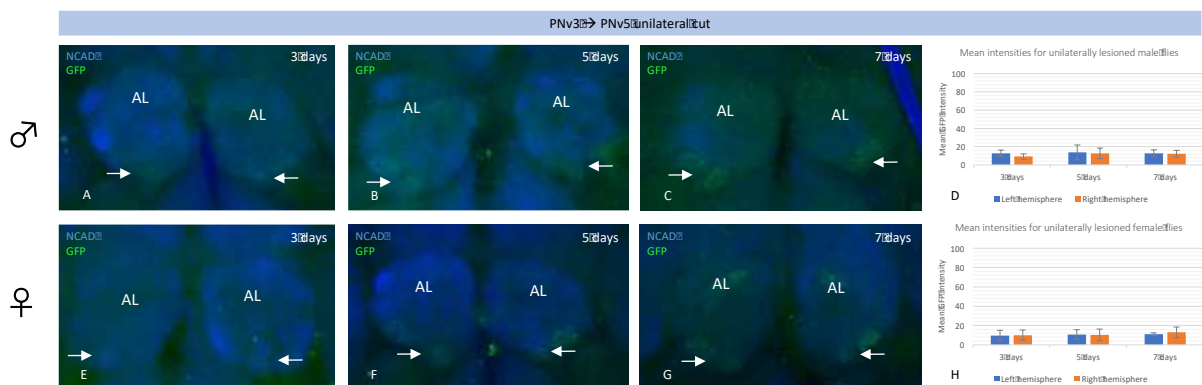


Figure 22. GRASP-GFP intensity measurements of the PNv3 → PNv5 synapse after unilateral lesion: (A-C) Antennal lobes (ALs) of male flies stained with anti-NCAD (blue, neuropil marker) and visible endogenous GFP (green) in the V-glomerulus (white arrows), (E-G) ALs of female flies stained with anti-NCAD and visible endogenous GFP in the V-glomerulus, (D) GFP intensity measurements in male flies, (H) GFP intensity measurements in female flies (* $p<0.05$, $n=4-7$)

GFP intensity measurements of the PNv1 → PNv5 synapse revealed a sex specific activity like it was already observed in uninjured flies (Fig. 17). Males showed fluctuations if the GFP intensities were compared within one week, while in female flies no such observation was possible (Fig. 23). Despite this sex-specific difference of activity neither male flies (3 days $p=0.532$; 5 days $p=0.333$; 7 days $p=0.548$) nor female flies (3 days $p=0.416$; 5 days $p=0.162$; 7 days $p=0.333$) showed interhemispheric GFP intensity differences after unilateral lesion (Fig. 23).

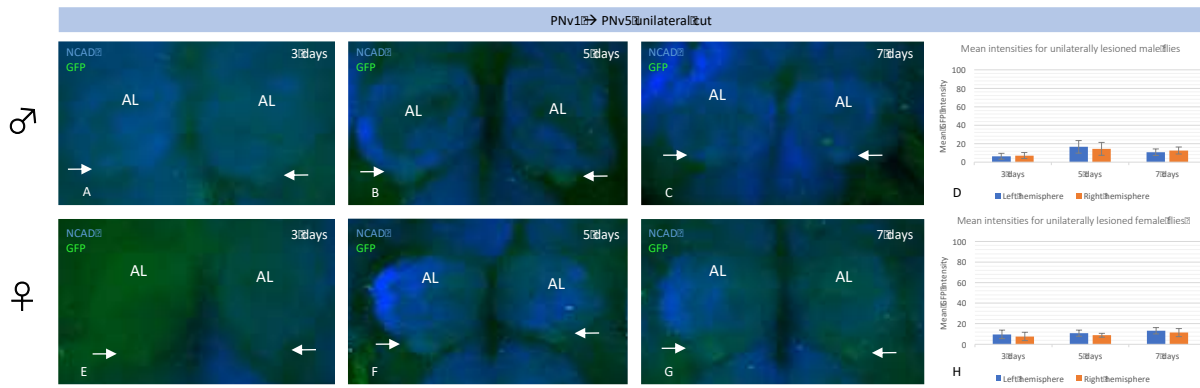


Figure 23. GRASP-GFP intensity measurements of the PNv1 → PNv5 synapse after unilateral lesion: (A-C) Antennal lobes (ALs) of male flies stained with anti-NCAD (blue, neuropil marker) and visible endogenous GFP (green) in the V-glomerulus (white arrows), (E-G) ALs of female flies stained with anti-NCAD and visible endogenous GFP in the V-glomerulus, (D) GFP intensity measurements in male flies, (H) GFP intensity measurements in female flies (* $p < 0.05$, $n = 5-7$)

The LNV2 → PNv5 synapse displayed high GFP intensities throughout all conditions (Fig. 24). Interestingly, following a unilateral ablation of one antenna, this synapse showed a distinct activity pattern within 1 week (Fig. 24). While on day 3 and day 5 the uninjured hemispheres clearly displayed a higher synaptic activity, this was changed on day 7 with higher activity in the injured hemispheres (Fig. 24). However, the difference on day 7 was not drastic enough to be significant but the hemispheres' GFP intensity differed significantly from each other on day 3 (3 days $p = 0.030$; 5 days $p = 0.190$; 7 days $p = 0.579$) (Fig. 24).

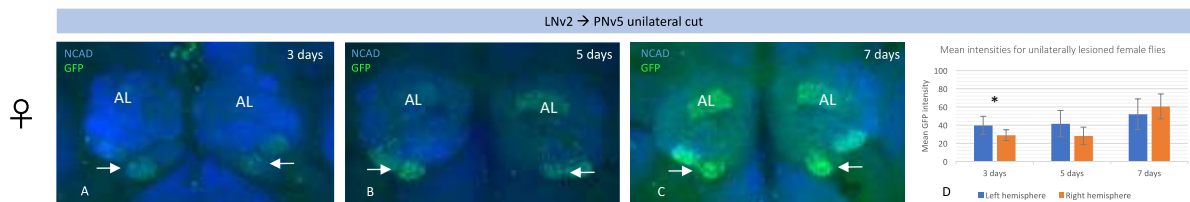


Figure 24. GRASP-GFP intensity measurements of the LNV2 → PNv5 synapse after unilateral lesion: (A-C) Antennal lobes (ALs) of female flies stained with anti-NCAD (blue, neuropil marker) and visible endogenous GFP (green) in the V-glomerulus (white arrows), (D) GFP intensity measurements in female flies (* $p < 0.05$, $n = 5-8$)

In summary, it was shown that connections onto PNv5 indeed responded differently to unilateral lesion compared to synaptic connections onto PNv1. This is especially true if the GABAergic input from PNv3 and LNV2 onto PNv1 and PNv5 are compared. These inhibitory synapses increase their activity onto PNv1 in the injured hemisphere, while the synapse between LNV2 and PNv5 initially shows increased activity in the unharmed hemisphere and the synapse between PNv3 and PNv5 doesn't seem to show any specific response to lesion at all.

3.1.3 GRASP in bilaterally lesioned flies

After the results of uninjured and unilaterally lesioned flies were obtained, I next determined the changes in GFP intensity following the removal of both antennae. Due to bilateral lesion, flies should have no ability to sense odors anymore, which are usually perceived with the antennae, thus it might be possible that the olfactory system responds with injury-induced plasticity resulting in a differential activity pattern compared to unilateral lesion and unharmed flies.

3.1.3.1 Changes of synaptic input onto PNv1

A bilateral lesion resulted in GFP intensity measurements more resembling those of uninjured flies (Fig. 13) than unilaterally lesioned flies (Fig. 19) for PNv3 → PNv1 synapses. In male flies, a bilateral cut led to no visible or statistically significant GFP intensity differences (3 days $p=0.819$; 5 days $p=0.671$; 7 days $p=0.079$) (Fig.25). No obvious sex-specific activity differences were observed and there was no significant GFP intensity difference between the two hemispheres in female flies (3 days $p=0.165$; 5 days $p=0.629$; 7 days $p=0.770$) (Fig. 25).

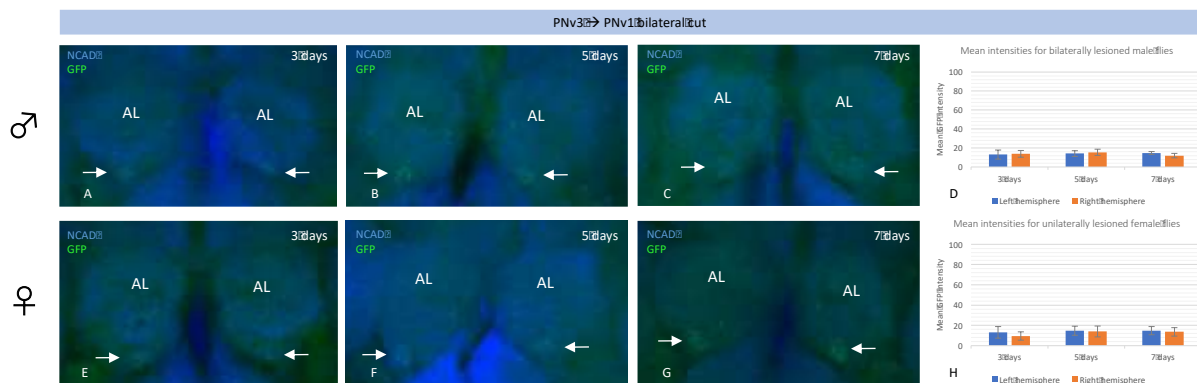


Figure 25. GRASP-GFP intensity measurements of the PNv3 → PNv1 synapse after bilateral lesion: (A-C) Antennal lobes (ALs) of male flies stained with anti-NCAD (blue, neuropil marker) and visible endogenous GFP (green) in the V-glomerulus (white arrows), (E-G) ALs of female flies stained with anti-NCAD and visible endogenous GFP in the V-glomerulus, (D) GFP intensity measurements in male flies, (H) GFP intensity measurements in female flies (* $p<0.05$, $n=4-8$)

The activity pattern of the PNv5 → PNv1 synapse after bilateral lesion has resemblance with the activity of the same synapse in the unilateral cut condition (Fig. 20). Measurements in male flies revealed no significant GFP intensity differences within one week (3 days $p=0.945$; 5 days $p=0.872$; 7 days $p=0.457$) (Fig 26). Following the same trend as males, females didn't show a significant differential activity in both hemispheres (3 days $p=0.730$; 5 days $p=1.000$; 7 days $p=0.825$) (Fig. 26). Interestingly, male flies displayed a lower activity in both hemispheres

on day 3 while the values in female flies were almost doubled on this day. This might be another case of sex-specific activity of the connection from PNv5 onto PNv1 (Fig. 26).

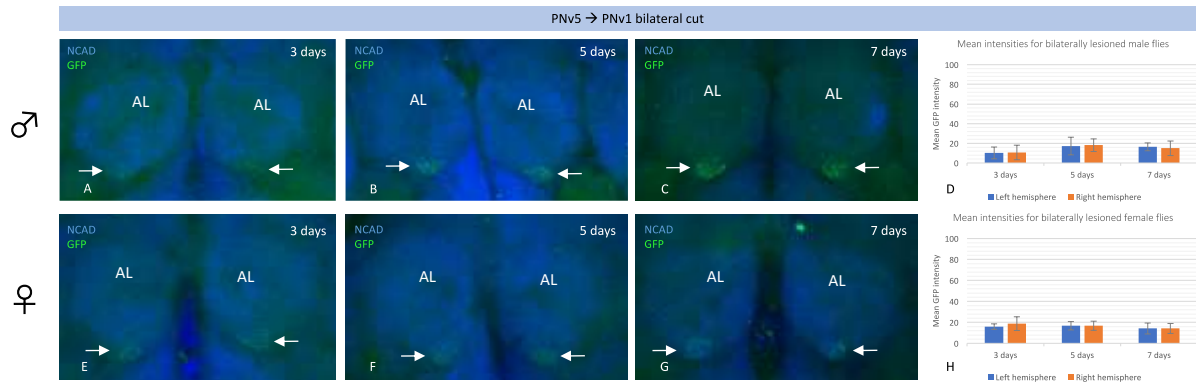


Figure 26. GRASP-GFP intensity measurements of the PNv5 → PNv1 synapse after bilateral lesion: (A-C) Antennal lobes (ALs) of male flies stained with anti-NCAD (blue, neuropil marker) and visible endogenous GFP (green) in the V-glomerulus (white arrows), (E-G) ALs of female flies stained with anti-NCAD and visible endogenous GFP in the V-glomerulus, (D) GFP intensity measurements in male flies, (H) GFP intensity measurements in female flies (* $p < 0.05$, $n = 5-7$)

Compared with the unilateral cut (Fig. 21), the bilateral cut didn't reveal such a prominent increase in GFP intensities over the course of one week if the LNV2 → PNv1 synapse was examined. This difference is most obvious on day 7, because after unilateral lesion the GFP intensities reached their highest values and after a bilateral lesion the measurements showed the lowest GFP intensities (Fig. 27). No significant differences between the hemispheres were found (3 days $p = 0.942$; 5 days $p = 1.000$; 7 days $p = 0.659$) (Fig. 27).

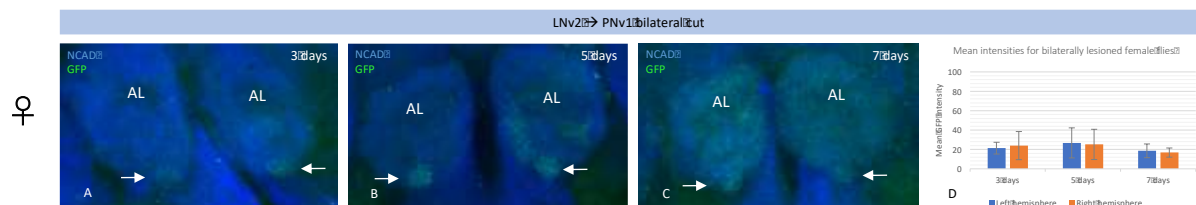


Figure 27. GRASP-GFP intensity measurements of the LNV2 → PNv1 synapse after bilateral lesion: (A-C) Antennal lobes (ALs) of female flies stained with anti-NCAD (blue, neuropil marker) and visible endogenous GFP (green) in the V-glomerulus (white arrows), (D) GFP intensity measurements in female flies (* $p < 0.05$, $n = 5-6$)

Analyses of the various synaptic connections onto PNv1 after a bilateral lesion showed that the GFP intensity differences between the hemispheres, which were observed in the unilateral cut condition for the connections with PNv3 and LNV2, were not visible when both antennae are ablated, indicating that if one odor perception organ is still functional it impacts the synaptic activity/plasticity differently.

In the last step of this part of my analyses I wanted to determine again how a bilateral lesion is effecting the connections onto PNv5. The previous data of uninjured flies and unilaterally lesioned flies showed differential activity patterns of neurons connected onto PNv1 and PNv5, so if this tendency remains it might be possible that a bilateral lesion has a different effect on the following synapses.

3.1.3.2 Changes of synaptic input onto PNv5

The GFP intensity measurements of the PNv3 → PNv5 synapse revealed low intensity values (Fig 28) which are similar to the measurements of the PNv3 → PNv1 synapse after bilateral ablation (Fig. 25). A comparison between the two injured hemispheres could show that there are no interhemispheric GFP intensity differences in male (3 days $p=0.595$; 5 days $p=0.817$; 7 days $p=0.413$) and female flies (3 days $p=0.487$; 5 days $p=0.246$; 7 days $p=0.797$) (Fig. 28).

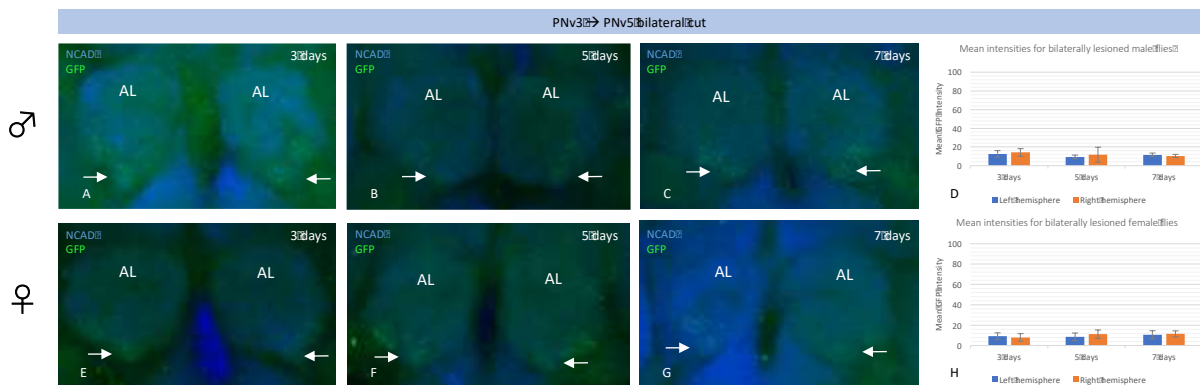


Figure 28. GRASP-GFP intensity measurements of the PNv3 → PNv5 synapse after bilateral lesion: (A-C) Antennal lobes (ALs) of male flies stained with anti-NCAD (blue, neuropil marker) and visible endogenous GFP (green) in the V-glomerulus (white arrows), (E-G) ALs of female flies stained with anti-NCAD and visible endogenous GFP in the V-glomerulus, (D) GFP intensity measurements in male flies, (H) GFP intensity measurements in female flies (* $p<0.05$, $n=5-8$)

The bilateral lesion had a drastic effect on the activity of the synapses from PNv1 onto PNv5. The GFP intensities lowered to a range which was barely quantifiable (Fig. 29). In fact, out of the brains which have been looked at, a few didn't show a GFP signal at all. Following this observation, no significant differences between the hemispheres were found in males (3 days $p=1.000$; 5 days $p=0.460$; 7 days $p=0.349$) as well as female flies (3 days $p=1.000$; 5 days $p=1.000$; 7 days $p=1.000$) (Fig. 29).

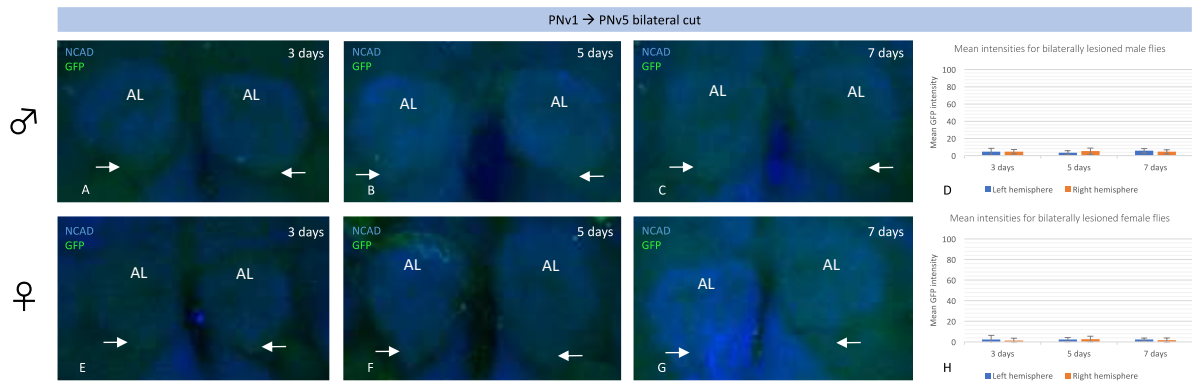


Figure 29. GRASP-GFP intensity measurements of the PNv1 → PNv5 synapse after bilateral lesion: (A-C) Antennal lobes (ALs) of male flies stained with anti-NCAD (blue, neuropil marker) and visible endogenous GFP (green) in the V-glomerulus (white arrows), (E-G) ALs of female flies stained with anti-NCAD and visible endogenous GFP in the V-glomerulus, (D) GFP intensity measurements in male flies, (H) GFP intensity measurements in female flies (* $p < 0.05$, $n = 2-5$; low n result from not quantifiable GFP intensities)

For the LNV2 → PNv5 synapse, the GFP intensity measurements remained stable and at a similar level at day 3 and day 5 with an increase on day 7 (Fig. 30). No significant differences between the hemispheres were found (3 days $p = 0.952$; 5 days $p = 0.970$; 7 days $p = 0.151$) (Fig. 30). In comparison, the LNV2 → PNv1 synapse displayed a lower activity in both hemispheres on day 7 (Fig. 27).

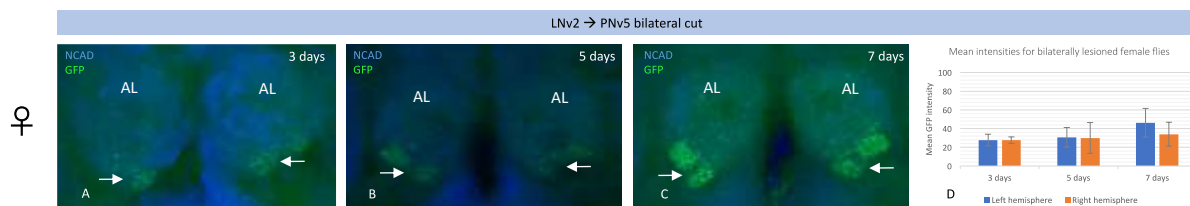


Figure 30. GRASP-GFP intensity measurements of the LNV2 → PNv5 synapse after bilateral lesion: (A-C) Antennal lobes (ALs) of female flies stained with anti-NCAD (blue, neuropil marker) and visible endogenous GFP (green) in the V-glomerulus (white arrows), (D) GFP intensity measurements in female flies (* $p < 0.05$, $n = 5-6$)

In summary, the bilateral ablation of antennae had also a differential effect on connections onto PNv5 compared to PNv1. Most interestingly, PNv1 showed a drastically decreased excitatory input on PNv5, while the reciprocal connection displayed a rather stable activity within one week. Taken together, I could observe that every inspected synapse reacted with unique activity levels following lesion. Unilateral lesion and bilateral lesion have a different effect on the plasticity of each synapse. Ablating only one antenna resulted in increased activity of LNV2 and PNv3 in the injured hemisphere, suggesting heightened inhibitory modulation.

3.2 Synaptic plasticity following chronic sensory stimulation

Since I was working with V-glomerulus, which is sensitive to CO₂, I wanted to explore if exposure to elevated levels of the odor will lead to a different pattern of synaptic activity. Sachse

et al. (2007) found out that V-glomerulus undergoes structural reorganization after exposure to CO₂ which might be also true if the circuit is analyzed at the synapse level. Therefore, the experiments from the previous section were repeated, but this time the flies were exposed to 5% CO₂ for 3, 5 or 7 days (the previous data showed results at ambient air which has about 0.08% CO₂).

3.2.1 The effect of chronic sensory activity on microcircuit organization

First, I wanted to focus on the GFP intensity differences between the hemispheres in uninjured flies to see whether CO₂ exposure might lead to an increased activity in one side, which would indicate laterality for odor perception.

3.2.1.1 Synaptic input onto PNv1 after chronic exposure to CO₂

GFP intensity measurements at the PNv3 → PNv1 synapse in male flies showed that the intensities for both sides increased the longer the flies were exposed to elevated CO₂ levels (Fig. 31). The differences between the hemispheres were not statistically significant (3 days $p=0.561$; 5 days $p=0.460$; 7 days $p=0.063$). In female flies, the inhibitory input from PNv3 onto PNv1 was the highest on day 5 and even decreased on day 7 (Fig. 31). However, no significant GFP intensity differences between the hemispheres (3 days $p=0.310$; 5 days $p=1.000$; 7 days $p=0.603$) could be observed (Fig. 31)

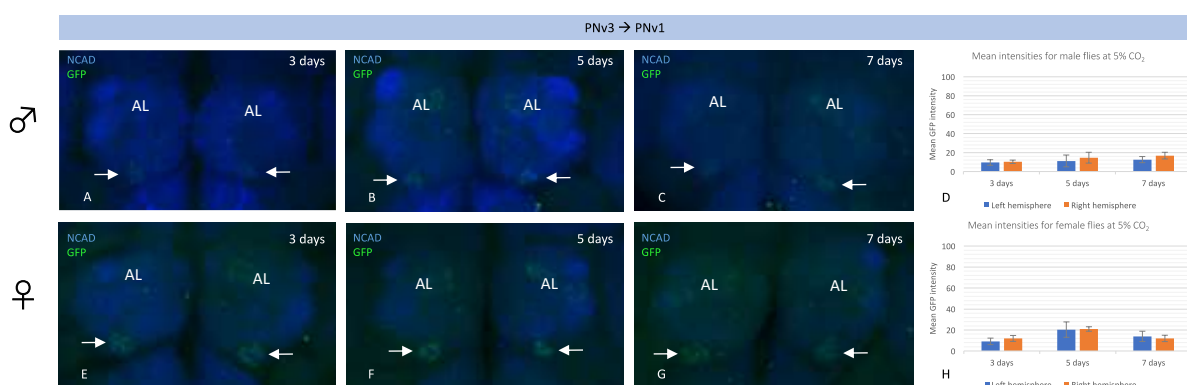


Figure 31. GRASP-GFP intensity measurements of the PNv3 → PNv1 synapse at 5% CO₂: (A-C) Antennal lobes (ALs) of male flies stained with anti-NCAD (blue, neuropil marker) and visible endogenous GFP (green) in the V-glomerulus (white arrows), (E-G) ALs of female flies stained with anti-NCAD and visible endogenous GFP in the V-glomerulus, (D) GFP intensity measurements in male flies, (H) GFP intensity measurements in female flies (* $p<0.05$, $n=5-7$)

Interestingly, the exposure to 5% CO₂ led to a shift of GFP intensity levels within one week in flies for measurements at the PNv5 → PNv1 synapse (Fig. 32). While being exposed to ambient

air, male flies reached their highest GFP intensity on day 7 (Fig. 14) but after exposure to elevated CO₂ levels the highest GFP intensity could be found on day 3 getting weaker on day 5 and day 7. Despite that, no significant difference could be found between the hemispheres in male flies (3 days $p=0.455$; 5 days $p=0.314$; 7 days $p=0.357$). The same observation was made for female flies, that on day 3 the excitatory input from PNv5 onto PNv1 was increased (Fig. 32). As in male flies, no significant GFP intensity difference (3 days $p=0.500$; 5 days $p=0.603$; 7 days $p=0.865$) between the hemispheres was detected (Fig. 32).

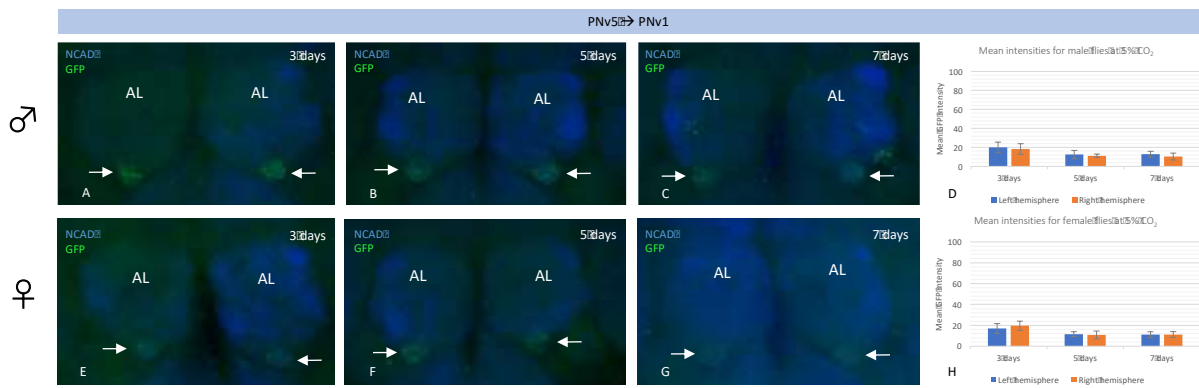


Figure 32. GRASP-GFP intensity measurements of the PNv5 → PNv1 synapse at 5% CO₂: (A-C) Antennal lobes (ALs) of male flies stained with anti-NCAD (blue, neuropil marker) and visible endogenous GFP (green) in the V-glomerulus (white arrows), (E-G) ALs of female flies stained with anti-NCAD and visible endogenous GFP in the V-glomerulus, (D) GFP intensity measurements in male flies, (H) GFP intensity measurements in female flies ($*p<0.05$, $n=4-6$)

Surprisingly, the GFP intensities on day 7 were lower at 5% CO₂ (Fig. 33) than at 0.08% (Fig. 15) in uninjured flies for the LNv2 → PNv1 synapse. Here, the activity peaked on day 5 and slightly decreased by day 7 (Fig. 33). Like at 0.08% CO₂, no significant GFP intensity differences (3 days $p=0.500$; 5 days $p=0.558$; 7 days $p=0.351$) between the hemispheres were detected (Fig. 33).

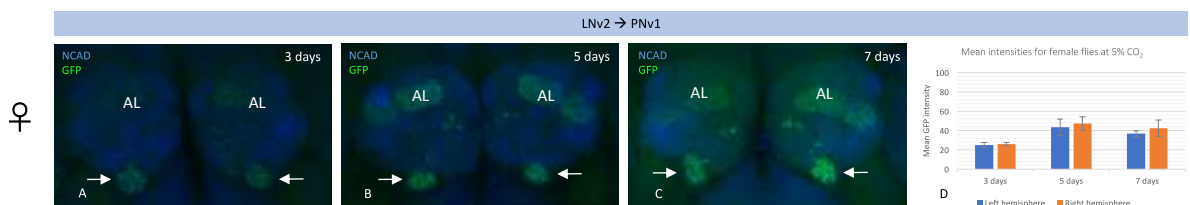


Figure 33. GRASP-GFP intensity measurements of the LNv2 → PNv1 synapse at 5% CO₂: (A-C) Antennal lobes (ALs) of female flies stained with anti-NCAD (blue, neuropil marker) and visible endogenous GFP (green) in the V-glomerulus (white arrows), (D) GFP intensity measurements in female flies ($*p<0.05$, $n=3-6$)

In summary, I could show that the exposure to elevated CO₂ concentrations leads to differential GFP intensities within one week of exposure to 5% CO₂, but it didn't result in significant differences between the hemispheres for connections onto PNv1. Furthermore, I observed

that chronic exposure to 5% CO₂, does influence the plasticity of the investigated synaptic connections as there were noticeable differences if compared with the results of these synapses at 0.08% CO₂. Next, I wanted to determine if chronic sensory stimulation affects synaptic activity onto PNv5 differently.

3.2.1.2 Synaptic input onto PNv5 after chronic exposure to CO₂

The GABAergic input from PNv3 onto PNv5 was not much affected by chronic exposure to 5% CO₂. GFP intensity measurements of both, male and female flies, revealed low intensity values around 10 above threshold (Fig. 34). Neither male (3 days p=0.078; 5 days p=0.647; 7 days p=0.974) nor female flies (3 days p=0.933; 5 days p=0.613; 7 days p=0.476) showed significant differences of GFP intensity if the hemispheres were compared (Fig. 34). If comparing this synapse at 5% CO₂ (Fig. 34) and 0.08% CO₂ (Fig. 16), it is worth mentioning that for elevated CO₂ levels the mean GFP intensity values were generally lower than the GFP intensity values at 0.08% CO₂.

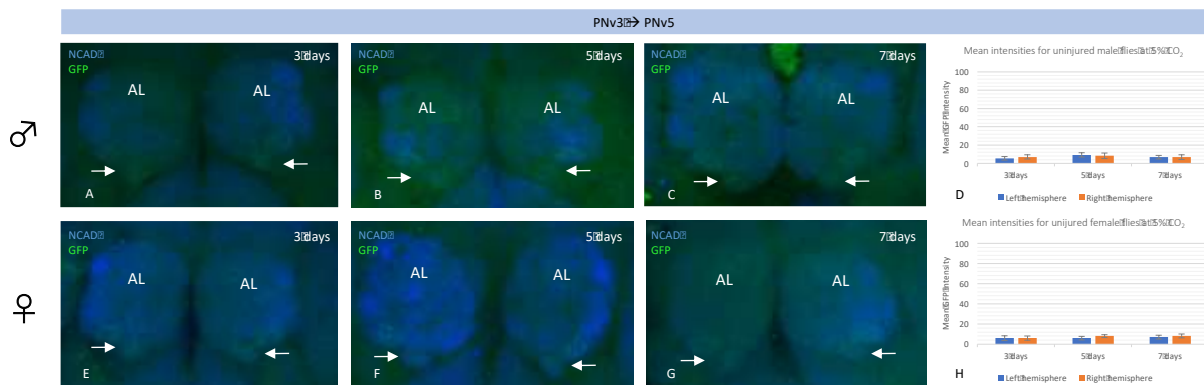


Figure 34. GRASP-GFP intensity measurements of the PNv3 → PNv5 synapse at 5% CO₂. (A-C) Antennal lobes (ALs) of male flies stained with anti-NCAD (blue, neuropil marker) and visible endogenous GFP (green) in the V-glomerulus (white arrows), (E-G) ALs of female flies stained with anti-NCAD and visible endogenous GFP in the V-glomerulus, (D) GFP intensity measurements in male flies, (H) GFP intensity measurements in female flies (*p<0.05, n=5-9)

In contrast to measurements done at ambient air (Fig. 17), here, for the PNv1 → PNv5 synapse, the male flies show a relatively stable GFP intensity level for both hemispheres up until day 5, with an evident rise of GFP intensities at day 7, which indicates a large increase of excitatory input from PNv1 onto PNv5 (Fig. 35). As it was observed in male flies, GFP intensity measurements in female flies also revealed an increase of activity on day 7, although not reaching the intensity values of male flies (Fig. 35). However, a comparison between the hemispheres showed no significant GFP intensity differences in male flies (3 days p=0.421; 5 days

p=0.159; 7 days p=0.690) and female flies (3 days p=0.645; 5 days p=0.613; 7 days p=0.937) (Fig. 35). With these results, the PNv1 → PNv5 synapse showed the strongest response to the CO₂ exposure.

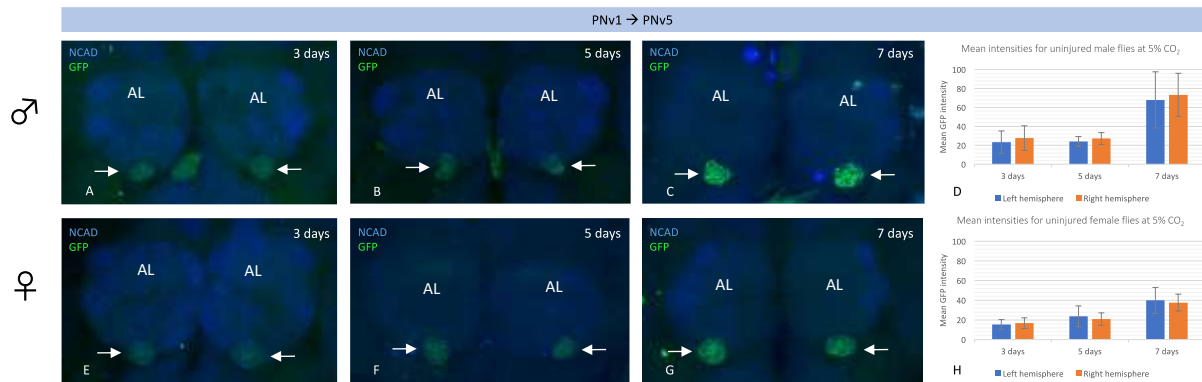


Figure 35. GRASP-GFP intensity measurements of the PNv1 → PNv5 synapse at 5% CO₂: (A-C) Antennal lobes (ALs) of male flies stained with anti-NCAD (blue, neuropil marker) and visible endogenous GFP (green) in the V-glomerulus (white arrows), (E-G) ALs of female flies stained with anti-NCAD and visible endogenous GFP in the V-glomerulus, (D) GFP intensity measurements in male flies, (H) GFP intensity measurements in female flies (*p<0.05, n=5-7)

GFP intensity quantifications of the LNV2 → PNv5 showed that the GFP intensities were decreasing the longer flies were kept at 5% CO₂ (Fig. 36), indicating a decrease of inhibitory input of LNV2 onto PNv5. This synapse didn't show this trend at 0.08% CO₂ (Fig. 18), because there the GFP intensity values stayed around 20 above threshold. The U-test revealed no significant GFP intensity differences between the hemispheres (3 days p=0.582; 5 days p=0.681; 7 days p=0.365) (Fig. 36).

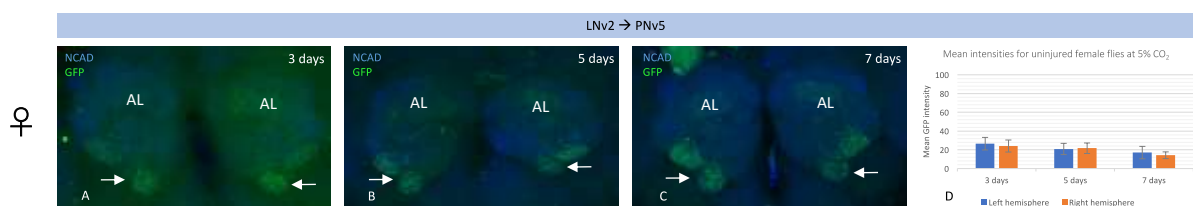


Figure 36. GRASP-GFP intensity measurements of the LNV2 → PNv5 synapse at 5% CO₂: (A-C) Antennal lobes (ALs) of female flies stained with anti-NCAD (blue, neuropil marker) and visible endogenous GFP (green) in the V-glomerulus (white arrows), (D) GFP intensity measurements in female flies (*p<0.05, n=5-9)

In summary, I could show that chronic exposure to 5% CO₂ does influence synaptic activity. Not all connections respond with the same change of activity, because the LNV2 → PNv5 synapse showed decreased activity at the end days of the week, PNv3 → PNv5 barely showed any response to chronic exposure while the excitatory input from PNv1 onto PNv5 was drastically increased on day 7. Compared with connections onto PNv1, synapses with PNv5 clearly showed a more varied response to chronic exposure to CO₂.

3.2.2 Unilateral lesion-induced plasticity after chronic exposure to CO₂

Now that I know how chronic exposure to CO₂ affects synaptic activity in uninjured flies, I wanted to address how exposure to 5% CO₂ affects unilaterally lesioned flies. I could already show that the inhibitory input from PNv3 and LNV2 onto PNv1 was increased in the injured hemisphere at 0.08% CO₂ and therefore it will be interesting to investigate if that changes at higher CO₂ concentrations.

3.2.2.1 Changes of synaptic input onto PNv1 after chronic exposure to CO₂

Analyzing the inhibitory input from PNv3 onto PNv1 after unilaterally lesioning the flies showed, that in both, male and females, the activity was higher in the injured hemisphere (Fig. 37). However, if compared with the GFP intensity measurements at 0.08% CO₂ (Fig. 19), the overall GFP intensity was higher if the flies were chronically exposed to 5% CO₂ (Fig. 37). By day 7 the difference between the hemispheres was larger, suggesting an activity boosting effect by exposure to 5% CO₂ (Fig. 37). The U-test revealed a significant difference in males on day 7 (3 days $p=0.433$; 5 days $p=0.686$; 7 days $p=0.029$) and in females after 3 and 7 days of CO₂ exposure (3 days $p=0.016$; 5 days $p=0.135$; 7 days $p=0.011$) (Fig. 37).

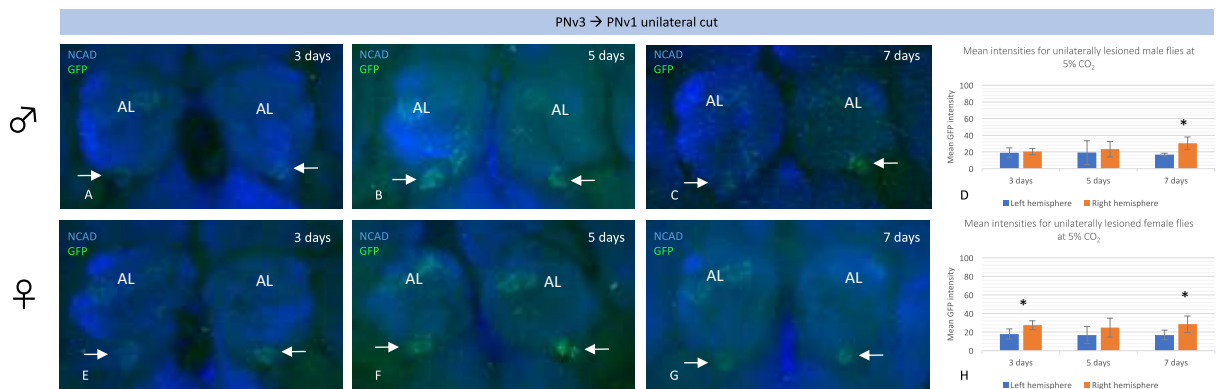


Figure 37. GRASP-GFP intensity measurements of the PNv3 → PNv1 synapse at 5% CO₂ after unilateral lesion: (A-C) Antennal lobes (ALs) of male flies stained with anti-NCAD (blue, neuropil marker) and visible endogenous GFP (green) in the V-glomerulus (white arrows), (E-G) ALs of female flies stained with anti-NCAD and visible endogenous GFP in the V-glomerulus, (D) GFP intensity measurements in male flies, (H) GFP intensity measurements in female flies (* $p<0.05$, $n=4-8$)

The exposure to 5% CO₂ didn't have much of an impact on the excitatory input from PNv5 onto PNv1 since none of the mean intensity values reaches above a value of 20 (Fig. 38). This also applies for this synapse at 0.08% CO₂ (Fig. 20). Like at 0.08% CO₂, no significant GFP intensity difference was found for the unilateral cut condition in male flies (3 days $p=0.500$; 5 days

p=0.314; 7 days p=0.617). Female flies also didn't show a significant GFP intensity difference between the hemispheres (3 days p=0.905; 5 days p=0.683; 7 days p=0.929) (Fig. 38).

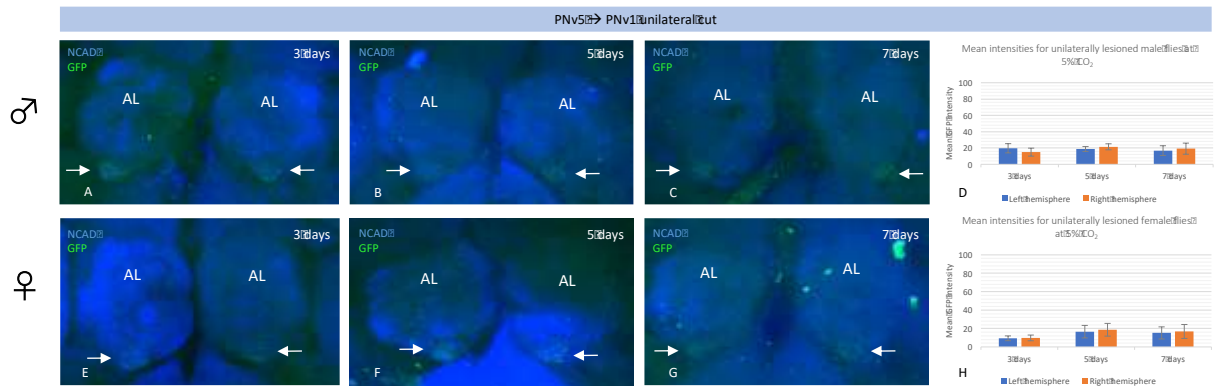


Figure 38. GRASP-GFP intensity measurements of the PNv5 → PNv1 synapse at 5% CO₂ after unilateral lesion: (A-C) Antennal lobes (ALs) of male flies stained with anti-NCAD (blue, neuropil marker) and visible endogenous GFP (green) in the V-glomerulus (white arrows), (E-G) ALs of female flies stained with anti-NCAD and visible endogenous GFP in the V-glomerulus, (D) GFP intensity measurements in male flies, (H) GFP intensity measurements in female flies (*p<0.05, n=3-6)

The inhibitory input from LNV2 onto PNv1 was increased in the injured hemisphere (Fig. 39). Therefore, the unilaterally lesioned flies exposed to 5% CO₂ showed similar responses as it has been shown at 0.08% CO₂ (Fig 21). This differential increase was potentiated until day 7 and due to the chronic exposure (Fig. 39), the difference between the hemispheres was much more evident compared with measurements at 0.08% CO₂. Despite the clear difference, statistical significance could only be shown on day 3 (3 days p=0.002; 5 days p=0.121; 7 days p=0.095) (Fig. 39).

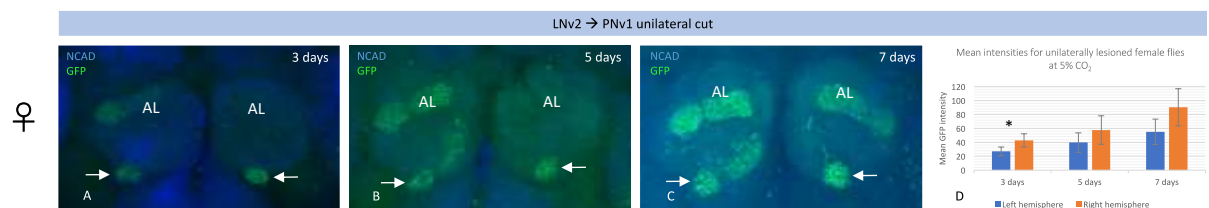


Figure 39. GRASP-GFP intensity measurements of the LNV2 → PNv1 synapse at 5% CO₂ after unilateral lesion: (A-C) Antennal lobes (ALs) of female flies stained with anti-NCAD (blue, neuropil marker) and visible endogenous GFP (green) in the V-glomerulus (white arrows), (D) GFP intensity measurements in female flies (*p<0.05, n=5-6)

Analyses of connections onto PNv1 showed, that the trend, which was already observable after unilateral lesion at 0.08% CO₂ is still true for these connections at 5% CO₂. The inhibitory neurons PNv3 and LNV2 still show higher activity in the injured hemisphere. However, chronic stimulation with 5% CO₂ seems to boost overall GFP intensity which can result in a larger GFP intensity difference as it was shown on day 7 for the PNv3 → PNv1 synapse and on all three days for the LNV2 → PNv1 synapse.

Connections of PNv1 and PNv3 onto PNv5 didn't respond strongly to unilateral lesion at 0.08% CO₂ and the synapse between LNV2 and PNv5 initially showed higher activity in the uninjured hemisphere while on day 7 it was the opposite. In the next section I wanted to determine if these observations are still true when those connections are investigated after chronic exposure to 5% CO₂.

3.2.2.2 Changes of synaptic input onto PNv5 after chronic exposure to CO₂

The exposure to higher CO₂ levels didn't push the PNv3 → PNv5 synapse to show significant differences in GFP intensity between the injured and uninjured side in male flies (3 days $p=0.513$; 5 days $p=0.076$; 7 days $p=0.897$) (Fig. 40). Noticeably, males had their highest mean GFP intensity values on day 3 after unilateral lesion (Fig. 40), while showing more stable values throughout all days when exposed to ambient air (Fig. 22). In female flies, the observation that on day 3 the mean GFP intensity values are the highest compared to day 5 and day 7 was not as evident as in male flies (Fig. 40). Nevertheless, opposing to males, females did show a significant GFP intensity difference between the two hemispheres on day 5 (3 days $p=0.270$; 5 days $p=0.008$; 7 days $p=0.167$) (Fig. 40).

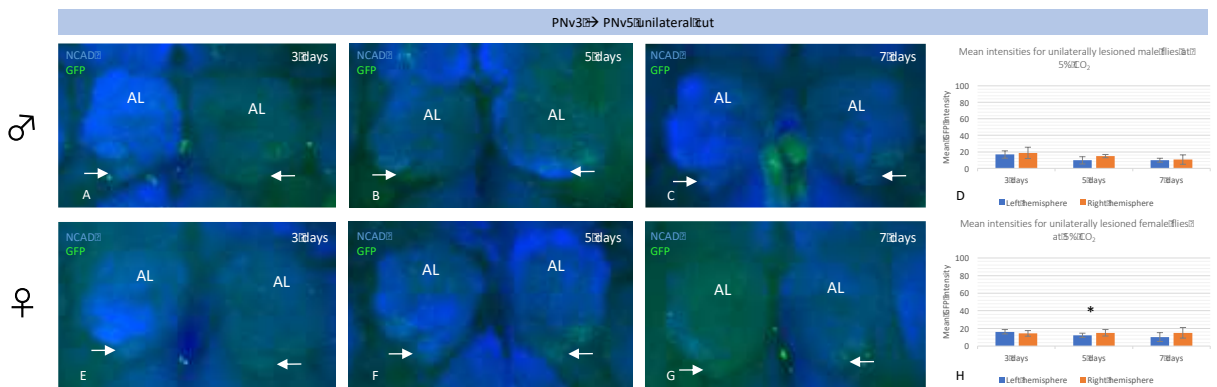


Figure 40. GRASP-GFP intensity measurements of the PNv3 → PNv5 synapse at 5% CO₂ after unilateral lesion: (A-C) Antennal lobes (ALs) of male flies stained with anti-NCAD (blue, neuropil marker) and visible endogenous GFP (green) in the V-glomerulus (white arrows), **(E-G)** ALs of female flies stained with anti-NCAD and visible endogenous GFP in the V-glomerulus, **(D)** GFP intensity measurements in male flies, **(H)** GFP intensity measurements in female flies (* $p<0.05$, $n=5-8$)

Surprisingly, upon chronic exposure to 5% CO₂, the GFP intensity values showed a distinct pattern for the PNv1 → PNv5 synapse. On all 3 days, the uninjured side had a higher GFP intensity (Fig. 41). This was not the case if this synapse was investigated at 0.08% CO₂ (Fig. 23). This resulted in a significant GFP intensity difference between the hemispheres on day 7 in

male flies (3 days $p=0.069$; 5 days $p=0.111$; 7 days $p=0.035$) (Fig. 41). Although the GFP expression was similar in female flies, no significant difference between the injured and uninjured side could be detected (3 days $p=0.054$; 5 days $p=0.082$; 7 days $p=0.087$) (Fig. 41).

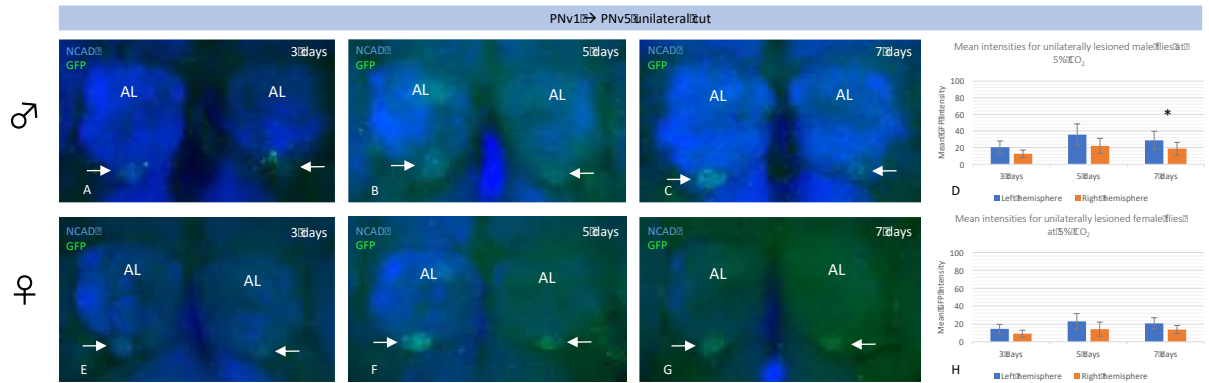


Figure 41. GRASP-GFP intensity measurements of the PNv1 → PNv5 synapse at 5% CO₂ after unilateral lesion: (A-C) Antennal lobes (ALs) of male flies stained with anti-NCAD (blue, neuropil marker) and visible endogenous GFP (green) in the V-glomerulus (white arrows), (E-G) ALs of female flies stained with anti-NCAD and visible endogenous GFP in the V-glomerulus, (D) GFP intensity measurements in male flies, (H) GFP intensity measurements in female flies (* $p<0.05$, $n=5-11$)

The inhibitory synaptic connection between LNv2 and PNv5 also showed a differential activity pattern after chronic exposure to 5% CO₂. The GFP intensity values stayed in a more similar range on day 3 and day 5 (Fig. 42) in comparison with measurements at 0.08% CO₂ (Fig. 24). However, at 0.08% CO₂ as well as at 5% CO₂ the injured side is showing higher intensities on day 7 (Fig. 42). Here at 5% CO₂, no significant GFP intensity differences between the hemispheres were observed (3 days $p=0.379$; 5 days $p=0.841$; 7 days $p=0.074$) (Fig. 42) as it was the case at 0.08% CO₂.

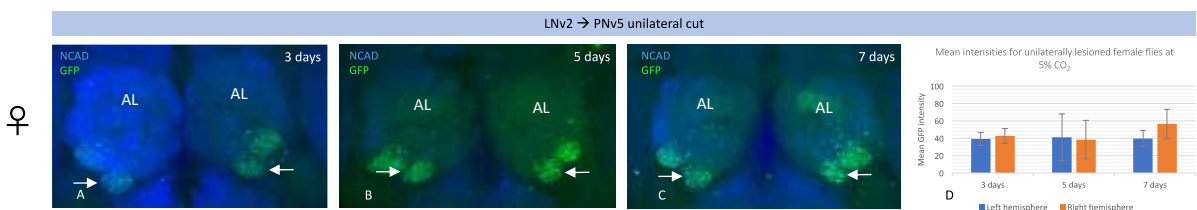


Figure 42. GRASP-GFP intensity measurements of the LNv2 → PNv5 synapse at 5% CO₂ after unilateral lesion: (A-C) Antennal lobes (ALs) of female flies stained with anti-NCAD (blue, neuropil marker) and visible endogenous GFP (green) in the V-glomerulus (white arrows), (D) GFP intensity measurements in female flies (* $p<0.05$, $n=5-10$)

The most interesting result from the GFP intensity quantification of neurons forming synapses with PNv5 was, that the excitatory input from PNv1 onto PNv5 was increased in the uninjured hemisphere, but only after chronic exposure to 5% CO₂. This suggests that not only lesion induces plasticity, but that injury in combination with chronic exposure to CO₂ can unmask new activity patterns.

3.2.3 Bilateral lesion-induced plasticity after chronic exposure to CO₂

Flies, which were bilaterally lesioned should have no possibility to sense CO₂, since all ORNs which express the CO₂ responsive receptor genes GR21a and GR63a are located on the antennae, which are both ablated (Jones et al., 2007). Therefore, no influence from CO₂ on activity in either hemisphere was expected and the GFP intensity measurements should resemble the ones which were done for bilaterally lesioned flies at 0.08% CO₂.

3.2.3.1 Changes of synaptic input onto PNv1 after chronic exposure to CO₂

The PNv3 → PNv1 synapse didn't show a changed activity pattern after chronic exposure (Fig. 43). In male flies, the bilateral lesion led to negligible GFP intensity differences between both hemispheres and no significance was found (3 days=0.734; 5 days p=0.500; 7 days p=0.690). Measurements in female flies resulted in very similar values and the hemispheres didn't differ from each other (3 days p=0.629; 5 days p=0.381; 7 days p=0.358) (Fig. 43).

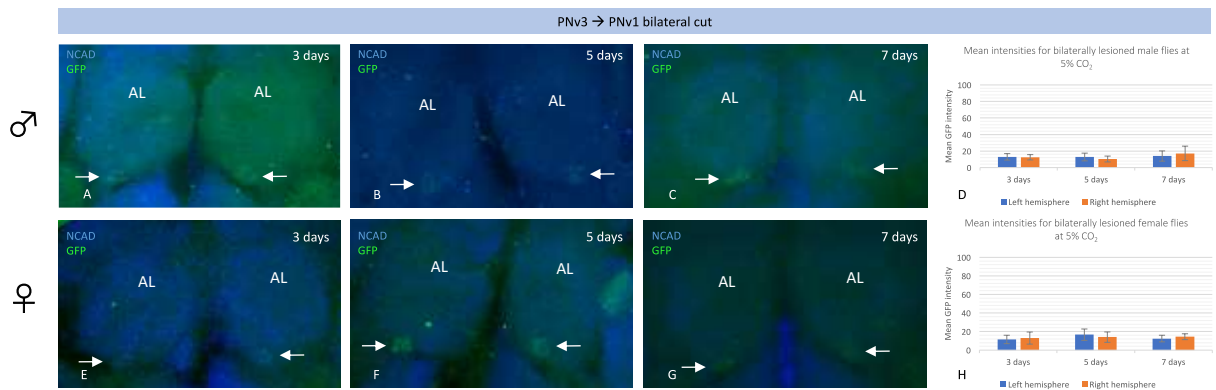


Figure 43. GRASP-GFP intensity measurements of the PNv3 → PNv1 synapse at 5% CO₂ after bilateral lesion: (A-C) Antennal lobes (ALs) of male flies stained with anti-NCAD (blue, neuropil marker) and visible endogenous GFP (green) in the V-glomerulus (white arrows), (E-G) ALs of female flies stained with anti-NCAD and visible endogenous GFP in the V-glomerulus, (D) GFP intensity measurements in male flies, (H) GFP intensity measurements in female flies (*p<0.05, n=4-7)

As it was observed at the synaptic connection between PNv3 and PNv1, the PNv5 → PNv1 synapse didn't show a strong response to chronic exposure after bilateral lesion (Fig. 44). No significant interhemispheric differences of GFP intensity could be observed in male (3 days p=0.587; 5 days p=0.722; 7 days p=0.873) and female flies (3 days p=0.086; 5 days p=0.548; 7 days p=0.768) (Fig. 44).

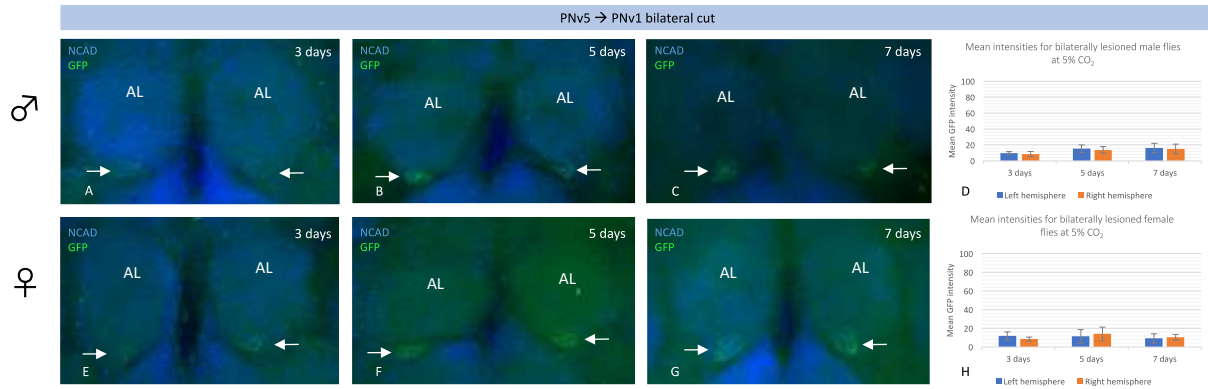


Figure 44. GRASP-GFP intensity measurements of the PNv5 → PNv1 synapse at 5% CO₂ after bilateral lesion: (A-C) Antennal lobes (ALs) of male flies stained with anti-NCAD (blue, neuropil marker) and visible endogenous GFP (green) in the V-glomerulus (white arrows), (E-G) ALs of female flies stained with anti-NCAD and visible endogenous GFP in the V-glomerulus, (D) GFP intensity measurements in male flies, (H) GFP intensity measurements in female flies (* $p < 0.05$, $n = 4-5$)

The U-test found no significant difference between the hemispheres (3 days $p = 0.286$; 5 days $p = 0.516$; 7 days $p = 0.889$) for GFP intensity measurements done at the LNv2 → PNv1 synapse (Fig. 45). Interestingly I could see that the GFP intensities were higher on day 7 (Fig. 45) if the flies were exposed to the high CO₂ compared with flies exposed to ambient air (Fig. 27).

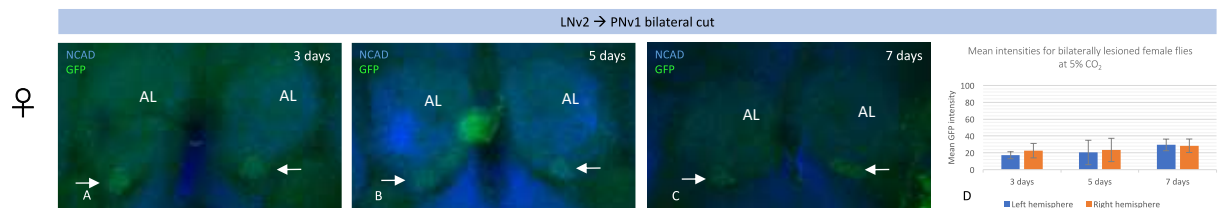


Figure 45. GRASP-GFP intensity measurements of the LNv2 → PNv1 synapse at 5% CO₂ after bilateral lesion: (A-C) Antennal lobes (ALs) of female flies stained with anti-NCAD (blue, neuropil marker) and visible endogenous GFP (green) in the V-glomerulus (white arrows), (D) GFP intensity measurements in female flies (* $p < 0.05$, $n = 5$)

A bilateral ablation combined with chronic exposure to CO₂ didn't increase the activity in left or right hemispheres significantly, if the connections onto PNv1 were investigated. However, it wasn't always clear if CO₂ influences the absolute activity of those synapses or not. This was addressed later in the results section. Next, I wanted to determine if synapses with PNv5 respond differently to bilateral ablation and chronic exposure to 5% CO₂.

3.2.3.2 Changes of synaptic input onto PNv5 after chronic exposure to CO₂

At the PNv3 → PNv5 synapse in males, the mean GFP intensity values for either side are increasing until day 7, indicating a stronger inhibition of PNv5 (Fig. 46). A similar trend was observed in female flies. The GFP intensity values steadily increased towards day 7 with the right hemisphere reaching a plateau on day 5 (Fig. 46). Neither hemisphere showed a significantly

higher activity in male (3 days $p=0.059$; 5 days $p=0.565$; 7 days $p=0.548$) and female flies (3 days $p=0.714$; 5 days $p=0.857$; 7 days $p=0.103$) (Fig.46).

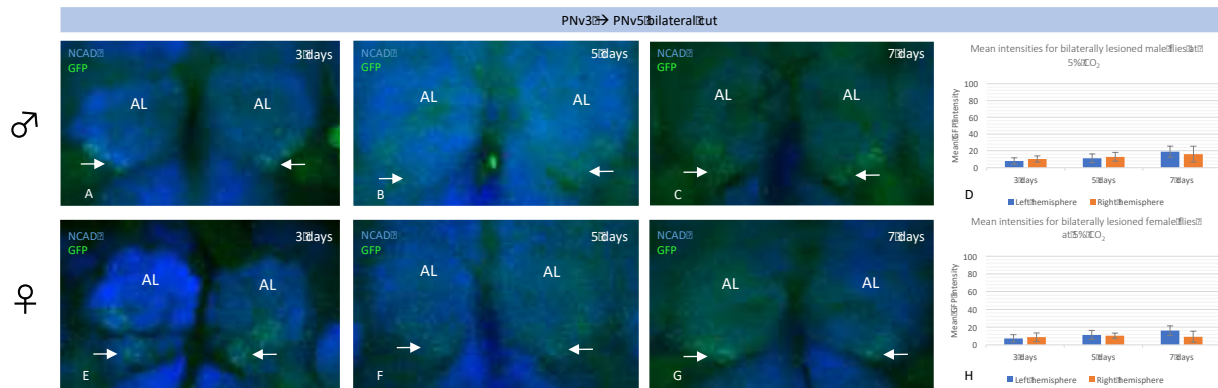


Figure 46. GRASP-GFP intensity measurements of the PNv3 → PNv5 synapse at 5% CO₂ after bilateral lesion: (A-C) Antennal lobes (ALs) of male flies stained with anti-NCAD (blue, neuropil marker) and visible endogenous GFP (green) in the V-glomerulus (white arrows), **(E-G)** ALs of female flies stained with anti-NCAD and visible endogenous GFP in the V-glomerulus, **(D)** GFP intensity measurements in male flies, **(H)** GFP intensity measurements in female flies (* $p<0.05$, $n=5-8$)

The bilateral cut led to a similar result at the PNv1 → PNv5 synapse as it was already shown at 0.08% CO₂ (Fig. 29). The GFP signals were barely measurable and in fact, it was impossible to quantify GFP intensities on day 3 in male flies (Fig. 47). No significant GFP intensity differences between the hemispheres were found in males (5 days $p=0.500$; 7 days $p=0.642$) and females (3 days $p=0.751$; 5 days $p=0.771$; 7 days $p=0.500$) (Fig. 47).

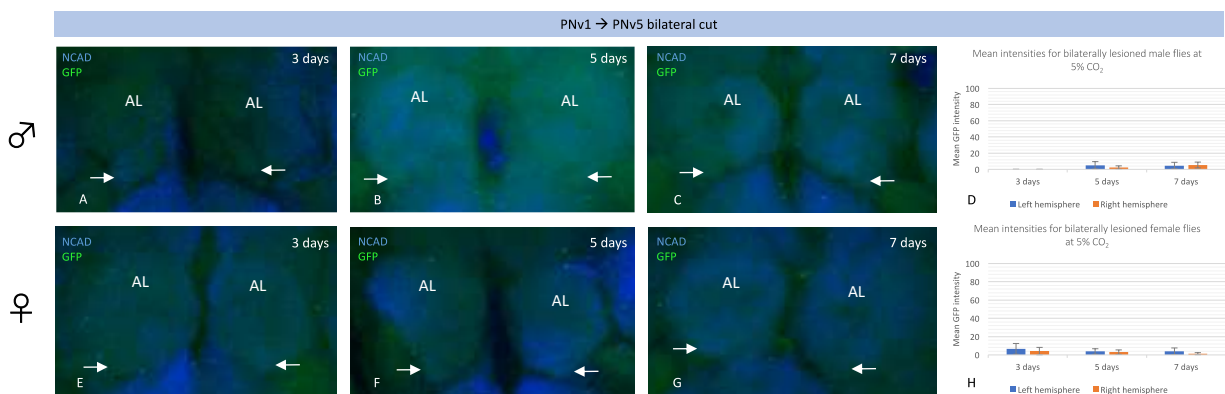


Figure 47. GRASP-GFP intensity measurements of the PNv1 → PNv5 synapse at 5% CO₂ after bilateral lesion: (A-C) Antennal lobes (ALs) of male flies stained with anti-NCAD (blue, neuropil marker) and visible endogenous GFP (green) in the V-glomerulus (white arrows), **(E-G)** ALs of female flies stained with anti-NCAD and visible endogenous GFP in the V-glomerulus, **(D)** GFP intensity measurements in male flies, **(H)** GFP intensity measurements in female flies (* $p<0.05$, $n=0-6$)

The GFP intensity measurements of the LNV2 → PNv5 synapse displayed no clear trend. The activity of this synapse seemed to fluctuate as none of the days showed similar results (Fig. 48). The GFP intensity was much more stable between the two hemispheres if compared at 0.08% CO₂ (Fig. 30). Still, as it was observed at 0.08% CO₂ (Fig. 30), the GFP intensities were

higher on day 7 when exposed to 5% CO₂, but here the right hemispheres reached higher intensity values above threshold. None of the differences were big enough to result in a statistically significant difference (3 days $p=0.157$; 5 days $p=0.532$; 7 days $p=0.286$) (Fig. 48).

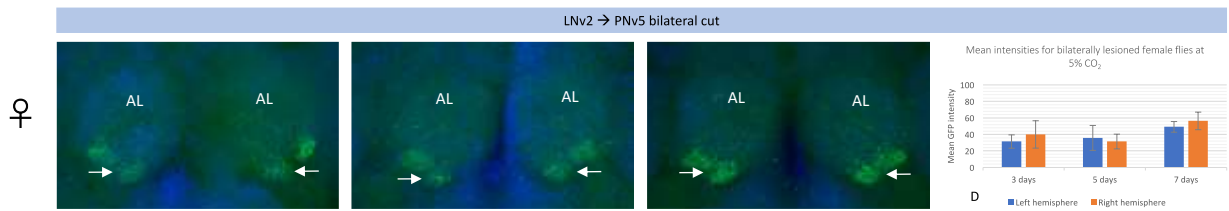


Figure 48. GRASP-GFP intensity measurements of the LNV2 → PNv5 synapse at 5% CO₂ after bilateral lesion: (A-C) Antennal lobes (ALs) of female flies stained with anti-NCAD (blue, neuropil marker) and visible endogenous GFP (green) in the V-glomerulus (white arrows), (D) GFP intensity measurements in female flies (* $p<0.05$, $n=5-7$)

Results for synaptic connections onto PNv5 after bilateral lesion and exposure to 5% CO₂ could show, that the PNv1 → PNv5 synapse was most affected (Fig. 47). This is a strong indication that the activity of this synapse is heavily influenced by CO₂. Comparing synaptic inputs on PNv1 and PNv5 after bilateral lesion shows that the activity is not significantly higher in one hemisphere, but that there are still activity fluctuations which need to be further addressed.

3.3 Comparative analyses of the applied lesions and control flies

In the previous chapter, I focused on interhemispheric differences following lesion and chronic CO₂ exposure. In this section, the goal will be to determine the influence of injury-induced plasticity in contrast to uninjured flies in more detail. I could already show that different lesions lead to different GFP intensity distributions but a direct comparison will highlight activity changes and how they differ from each other. The statistical test of choice was the Kruskal-Wallis test (including Dunn's test as a post-hoc test) with Bonferroni correction to compare multiple nondependent samples. In the unilateral cut condition, the right antenna was ablated and therefore the right hemisphere represents the injured hemisphere. Abbreviations (uni= unilateral cut, bila= bilateral cut and unc= uncut control condition) were used to describe the calculated significant p-values of the statistical tests.

3.3.1 PNv3 → PNv1

Comparing lesioned and uninjured flies revealed that the activity was the highest in unilaterally lesioned flies, especially in the injured right hemisphere. In addition, all significant differences which were found between the lesioning conditions and uninjured flies were observed if the unilateral cut condition was compared with any of the other two conditions (Fig. 49-51).

Day 3

Exposure to 0.08% CO₂ didn't reveal differences between the three conditions in neither male nor female flies (Fig. 49). However, at 5% CO₂ a significant difference was found in the left and right hemispheres between the unilateral and uncut control condition of male flies (left hemisphere uni - unc $p=0.003$; right hemisphere uni - unc $p=0.0001$) (Fig. 49). In females, the GFP intensities of the right hemispheres differed significantly between the unilateral cut condition and the other two conditions (uni - bila $p=0.016$; uni - unc $p=0.006$) (Fig. 49).

Day 5

On day 5, no significant differences between the lesioned and unharmed flies were found (Fig. 50). The Kruskal-Wallis showed a significant difference between the right hemispheres of the unilateral condition and the bilateral cut condition after chronic exposure to 5% CO₂ ($p=0.027$), this result was not significant after the Bonferroni correction (Fig. 50).

Day 7

At day 7, a significant difference was found between the unilateral cut condition and the bilateral cut condition, if the right hemispheres of male flies at atmospheric air were compared (uni - bila $p=0.013$) (Fig. 51). In female flies the unilateral cut condition differed from the bilateral and uncut condition if the right hemispheres were compared after chronic exposure to CO₂ (uni - bila $p=0.011$; uni - unc $p=0.0001$) (Fig. 51).

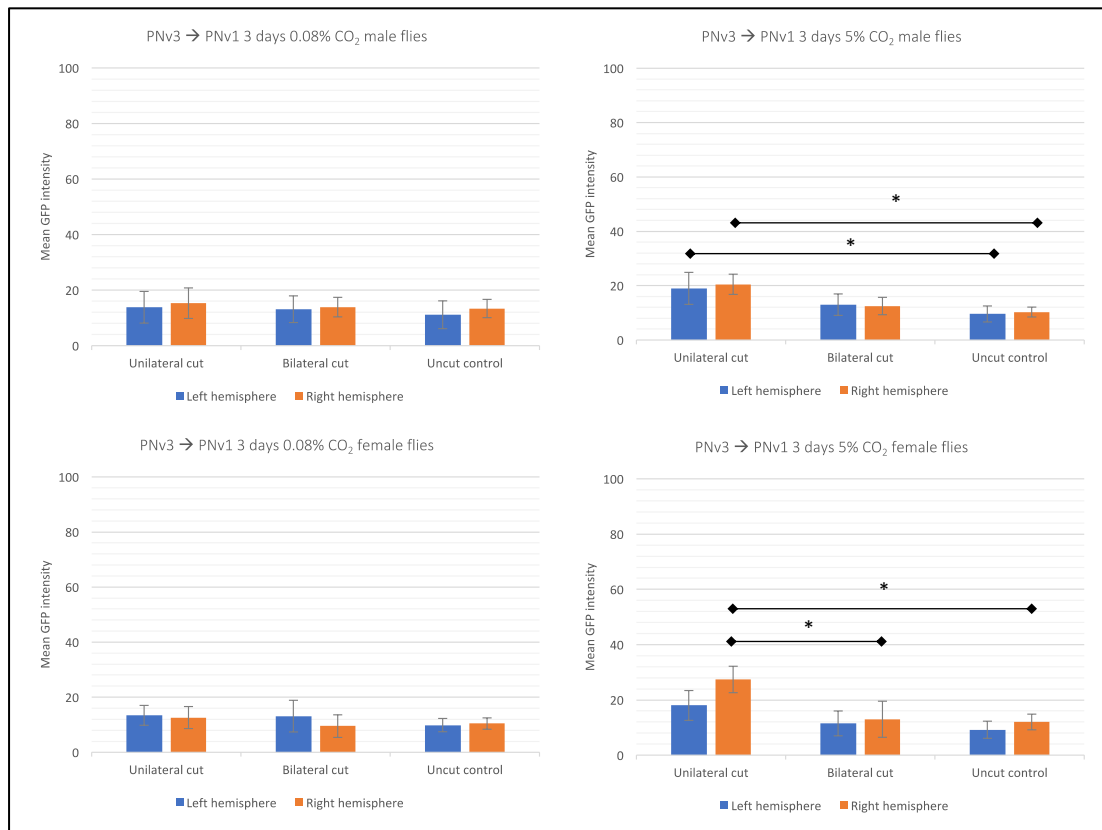


Figure 49. Comparing the GFP intensities between the different lesioning conditions in male and female flies with 0.08% and 5% CO₂ treatment. The bars represent the two hemispheres and horizontal lines are highlighting possible significant differences between the lesioning conditions (* $p < 0.0167$ after Bonferroni correction; $n = 4-7$)

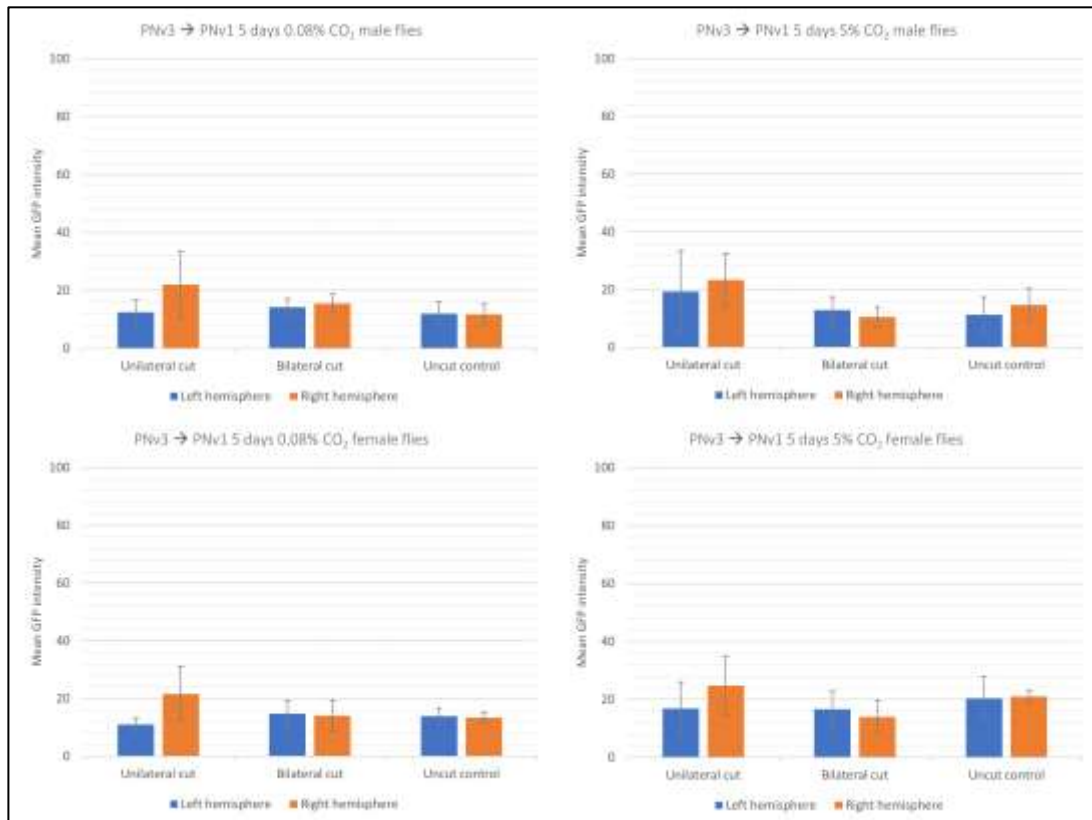


Figure 50. Comparing the GFP intensities between the different lesioning conditions in male and female flies with 0.08% and 5% CO₂ treatment. The bars represent the two hemispheres and horizontal lines are highlighting possible significant differences between the lesioning conditions (* $p < 0.0167$ after Bonferroni correction; $n = 4-7$)

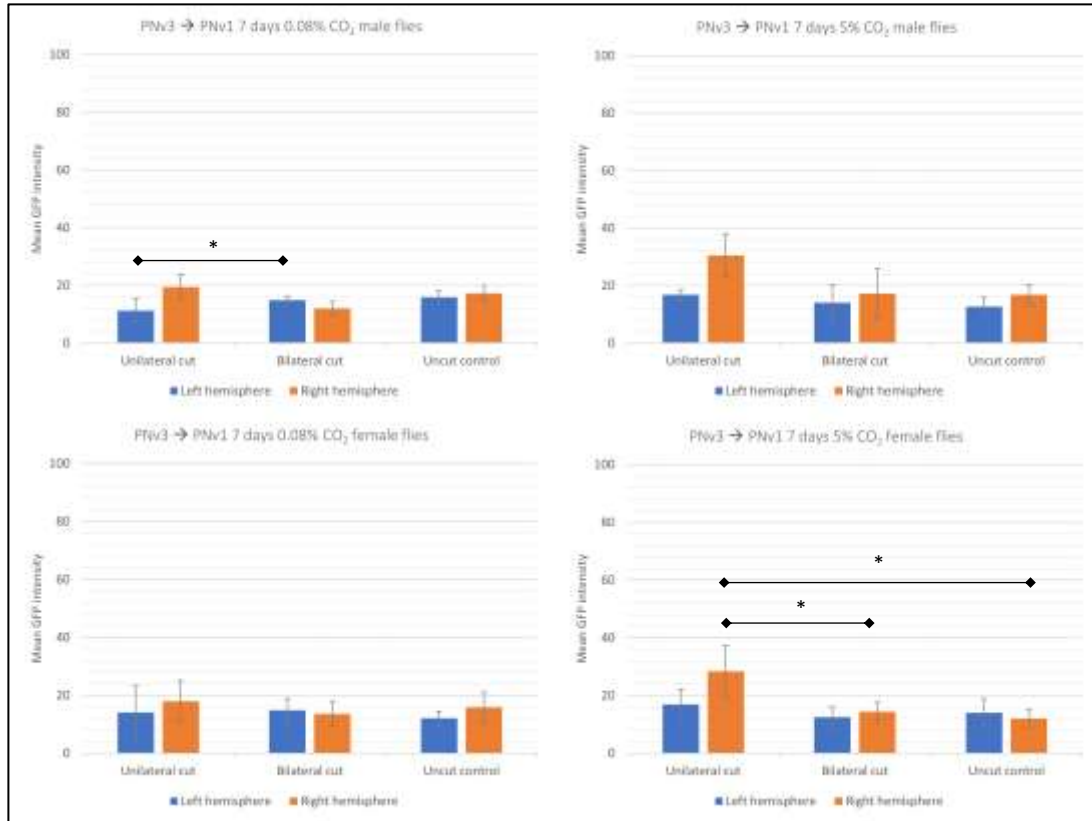


Figure 51. Comparing the GFP intensities between the different lesioning conditions in male and female flies with 0.08% and 5% CO₂ treatment. The bars represent the two hemispheres and horizontal lines are highlighting possible significant differences between the lesioning conditions (* $p < 0.0167$ after Bonferroni correction; $n = 4-8$)

3.3.2 PNv5 → PNv1

The comparison between lesioned and uninjured flies revealed several significant differences. While on day 3, it was impossible to see a clear trend which lesioning condition caused the highest synaptic activity (Fig. 52), results of day 5 and day 7 allowed for a more insightful interpretation (Fig. 53 and 54). Apart from day 7, when female flies were exposed to ambient air, the GFP intensities reached their highest values if flies were unilaterally lesioned.

Day 3

On this day, numerous significant differences were detected (Fig. 52). In male flies, the measurements in left hemispheres following a unilateral cut differed significantly from the left hemispheres of uninjured flies if previously exposed to ambient air (uni – unc $p = 0.008$). A comparison between the applied lesions and control in male flies exposed to 5% CO₂ revealed, that the measurements of the bilateral cut condition are significantly different than the measurements of the uncut control condition, which is true for both hemispheres (left hemisphere bila – unc $p = 0.005$; right hemisphere bila – unc $p = 0.007$) (Fig. 52). In females, if exposed to ambient

air, a significant difference was calculated between bilaterally lesioned flies and uninjured flies for the right hemispheres (bila – unc $p=0.001$) (Fig. 52). If exposed to 5% CO₂, a significant difference could be observed between unilaterally lesioned flies and control flies for left hemispheres (uni – unc $p=0.006$) and between bilaterally lesioned flies and unharmed flies for right hemispheres (bila – unc $p=0.008$) (Fig. 52)

Day 5

After 5 days, the GFP intensities between the different conditions stayed in a more equal range although the unilaterally lesioned flies displayed the highest GFP intensities (Fig. 53). There was only one finding of a statistically significant difference between the unilaterally lesioned flies and the uninjured flies at 5% CO₂ for the males' right hemispheres (uni – unc $p=0.006$) (Fig. 53).

Day 7

Similar to day 5, day 7 was lacking the numerous significant differences (Fig. 54) between lesioned flies and unharmed flies like they were shown on day 3 (Fig. 52). Still, a significant difference was found between unilaterally lesioned and uninjured female flies' right hemispheres if the 0.08% CO₂ treatment groups were compared (uni – unc $p=0.016$) (Fig. 54). Despite one case of higher GFP intensities in uninjured female flies at 0.08% CO₂, the uninjured flies were usually showing the lowest activity at this synapse according to the measured GFP intensities (Fig. 54).

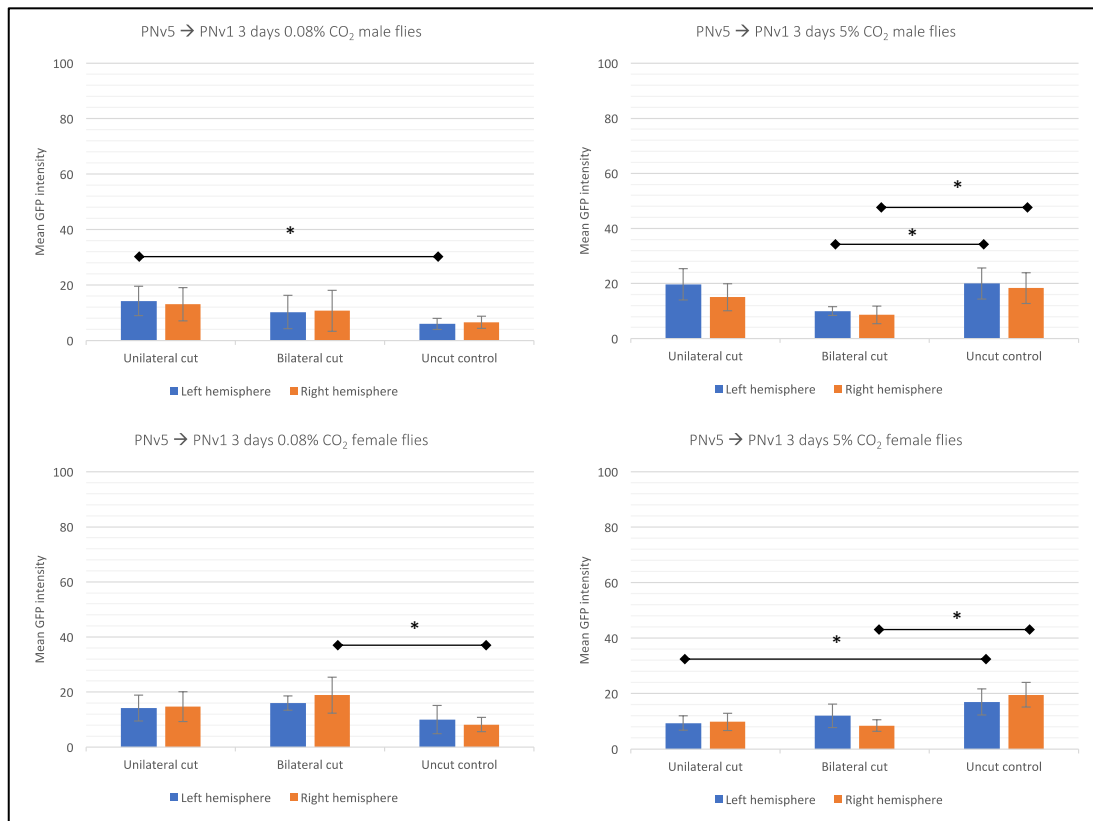


Figure 52. Comparing the GFP intensities between the different lesioning conditions in male and female flies with 0.08% and 5% CO₂ treatment. The bars represent the two hemispheres and horizontal lines are highlighting possible significant differences between the lesioning conditions (* $p < 0.0167$ after Bonferroni correction; $n=3-7$)

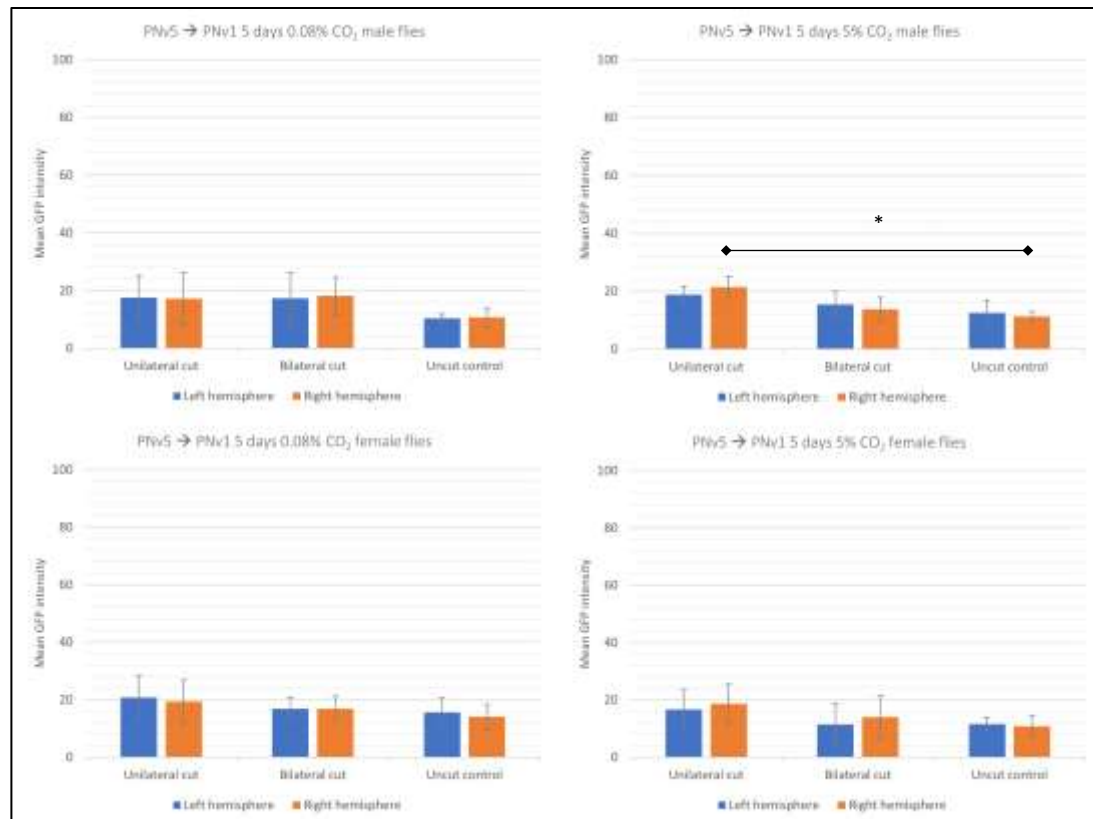


Figure 53. Comparing the GFP intensities between the different lesioning conditions in male and female flies with 0.08% and 5% CO₂ treatment. The bars represent the two hemispheres and horizontal lines are highlighting possible significant differences between the lesioning conditions (* $p < 0.0167$ after Bonferroni correction; $n=3-7$)

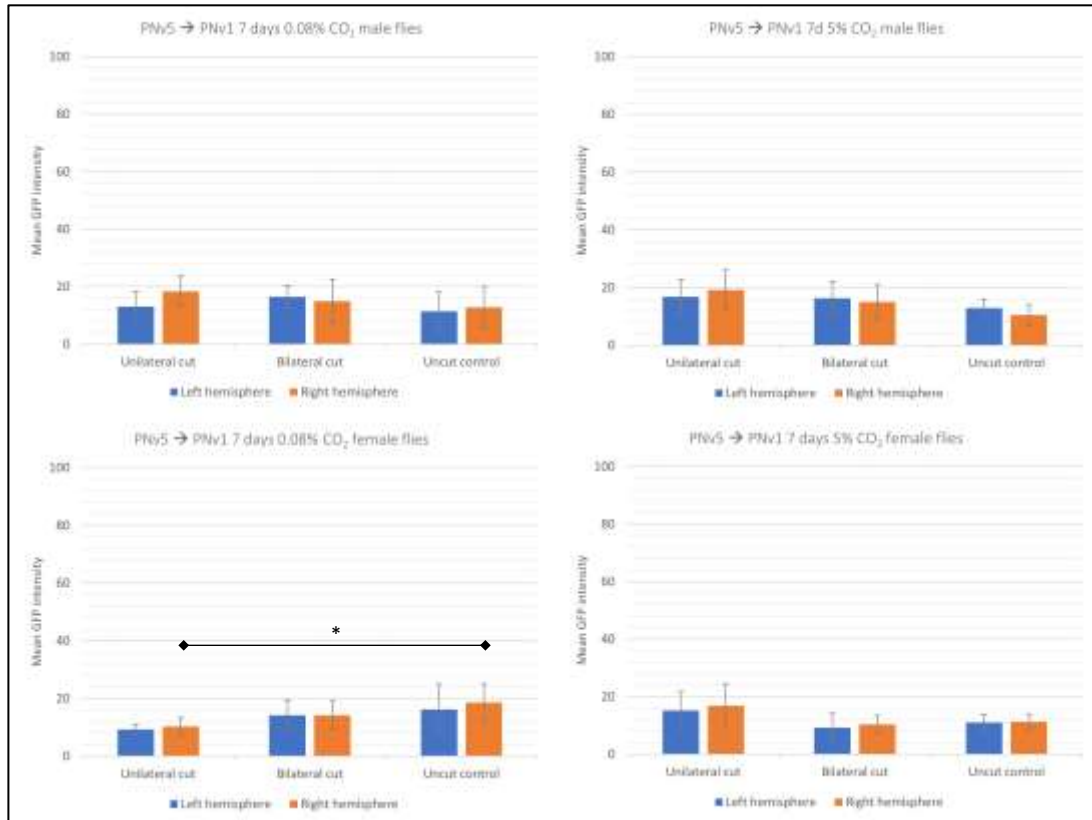


Figure 54. Comparing the GFP intensities between the different lesioning conditions in male and female flies with 0.08% and 5% CO₂ treatment. The bars represent the two hemispheres and horizontal lines are highlighting possible significant differences between the lesioning conditions (* $p < 0.0167$ after Bonferroni correction; $n=5-7$)

3.3.3 LNV2 → PNv1

Following the same trend as the previously discussed synapses, the unilateral ablation resulted in the highest GFP intensities on average, here with the injured right hemisphere clearly displaying the higher values, thus resembling higher synaptic activity and showing multiple significant differences if compared with the other conditions. Interestingly, GFP intensity measurements in uninjured flies also revealed some noteworthy findings. After 3 days, the activity is increasing in both hemispheres not necessarily tied to exposure to higher levels of CO₂ as it can be seen on day 7 for flies in ambient air (Fig. 55-57).

Day 3

At ambient air, no significant differences between the lesioned and uninjured flies were found. However, at 5% CO₂, significance was detected if the unilateral and bilateral cut condition were compared. The differences could be observed in the left and right hemispheres (left hemisphere uni – bila $p=0.006$; right hemisphere uni – bila $p=0.004$) (Fig. 55).

Day 5

After 5 days, there was also no statistical difference between the conditions at 0.08% CO₂. After chronic stimulation with CO₂, a significant difference was found between the unilaterally and bilaterally lesioned flies, but here only if the right hemispheres were compared (p=0.003) (Fig. 56).

Day 7

At day 7, more significant differences were found (Fig. 57). Within flies at atmospheric air, bilaterally lesioned flies differed significantly from the uninjured flies if the left hemispheres were compared (bila – unc p=0.004) (Fig. 57). For comparisons of the right hemispheres, the GFP measurements of flies of the unilateral cut condition were significantly different than measurements of flies of the bilateral cut condition, and the latter were again significantly different than flies of the uncut control condition (uni – bila p=0.004; bila – unc p=0.011) (Fig. 57). In flies exposed to 5% CO₂, the GFP intensity measurements after a unilateral cut differed significantly from the bilateral cut in both hemispheres (left hemisphere uni – bila p=0.016; right hemisphere uni – bila p=0.001) (Fig. 57).

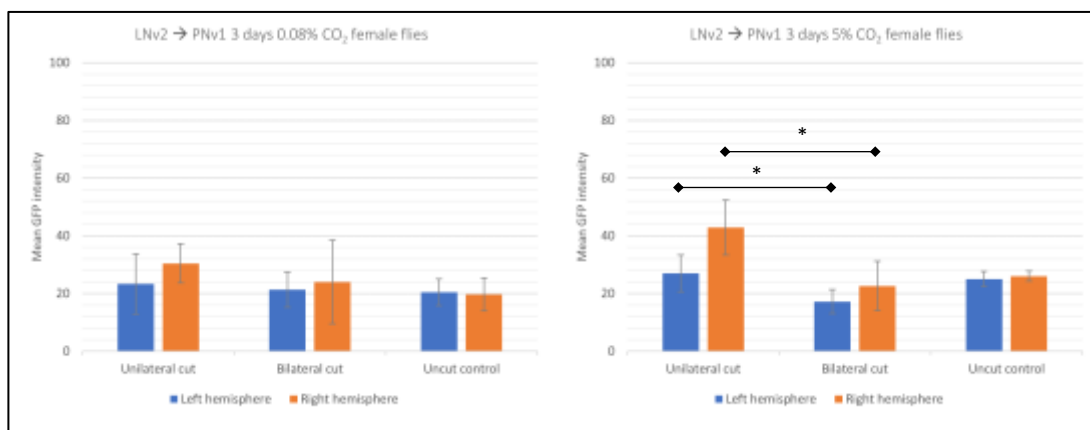


Figure 55. Comparing the GFP intensities between the different lesioning conditions in female flies with 0.08% and 5% CO₂ treatment. The bars represent the two hemispheres and horizontal lines are highlighting possible significant differences between the lesioning conditions (*p< 0.0167 after Bonferroni correction; n=5-7)

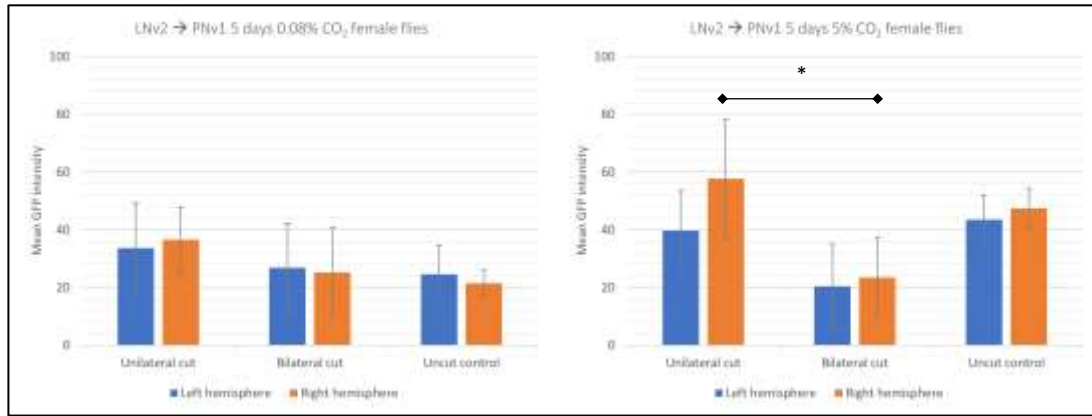


Figure 56. Comparing the GFP intensities between the different lesioning conditions in female flies with 0.08% and 5% CO₂ treatment. The bars represent the two hemispheres and horizontal lines are highlighting possible significant differences between the lesioning conditions (* $p < 0.0167$ after Bonferroni correction; $n = 5-8$)

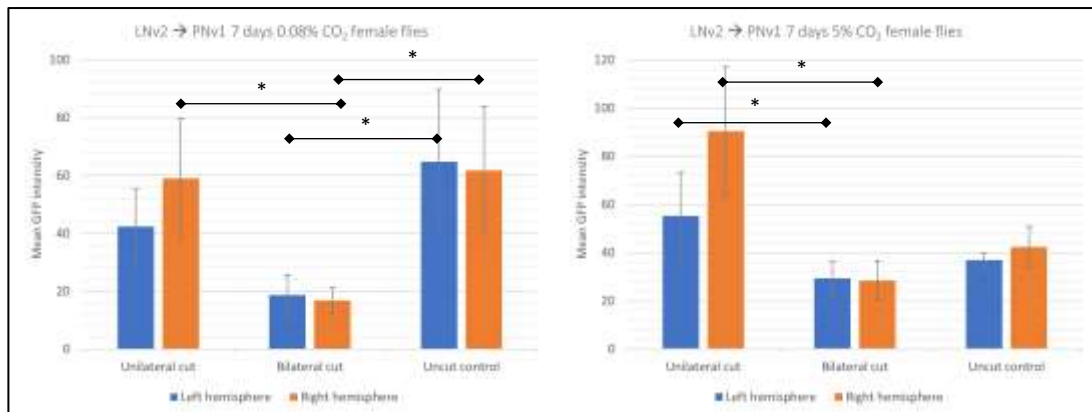


Figure 57. Comparing the GFP intensities between the different lesioning conditions in female flies with 0.08% and 5% CO₂ treatment. The bars represent the two hemispheres and horizontal lines are highlighting possible significant differences between the lesioning conditions (* $p < 0.0167$ after Bonferroni correction; $n = 4-7$)

3.3.4 PNv3 → PNv5

A comparison between the lesioning conditions and uninjured flies revealed that upon exposure to 5% CO₂, the uninjured flies displayed the lowest activity at this synapse if compared to the unilateral and bilateral ablation resulting in multiple significant differences, while at 0.08% CO₂ the measured GFP intensities were in a more equal range across all lesioning conditions (Fig. 58-60).

Day 3

After 3 days, a significant difference between the bilateral cut condition and the uncut control condition could be observed for measurements in right hemispheres of male flies at 0.08% CO₂ (bila – unc $p = 0.003$) (Fig. 58). At 5% CO₂, the unilaterally lesioned flies showed to be different than the bilateral and uninjured flies, if the left hemispheres were compared (uni – bila $p = 0.009$; uni – unc $p = 0.0001$) (Fig. 58). The right hemispheres were significantly different between the unilateral and uncut control condition (uni – unc $p = 0.0001$) (Fig. 58). In female flies,

the GFP intensity measurements didn't reveal any significant differences between lesioned flies and uninjured flies at 0.08% CO₂ (Fig. 58). If the conditions were compared after chronic exposure to 5% CO₂ a significant distinction between the unilateral cut condition and the uncut control condition was calculated with the Kruskal-Wallis test. This was true for both hemispheres (left hemisphere uni – unc p=0.004; right hemisphere uni – unc p=0.006) (Fig. 58)

Day 5

On day 5, no significant differences could be observed in male flies of the 0.08% CO₂ groups (Fig. 59). When the male flies were exposed to 5% CO₂ a significant difference was seen between unilaterally lesioned flies and control flies in a comparison of the right hemispheres (uni – unc p=0.006) (Fig. 59). Like in male flies, there were no significant differences in females when exposed to low levels of CO₂ (Fig. 59). Being exposed to high concentrations of CO₂ led to statistically relevant differences between the unilateral cut condition and the uncut control condition. However, in female flies this significance could be observed for both hemispheres (left hemisphere uni – unc p=0.006; right hemisphere uni – unc p=0.004) (Fig. 59).

Day 7

Like on day 5 (Fig. 59), there were no significant differences between the conditions at 0.08% CO₂ in male flies (Fig. 60). If they were exposed to 5% CO₂ for the same amount of time it resulted in a statistically relevant distinction between the left hemispheres following the bilateral cut and the control flies (bila – unc p=0.002) (Fig. 60). Significant differences were also absent in female flies when comparing the lesioned flies and unharmed flies at 0.08% CO₂ (Fig. 60). At high CO₂ concentrations, the Kruskal-Wallis test revealed that the same conditions as in male flies were significantly different from each other in female flies if the left hemispheres were compared (bila – unc p=0.010) (Fig. 60).

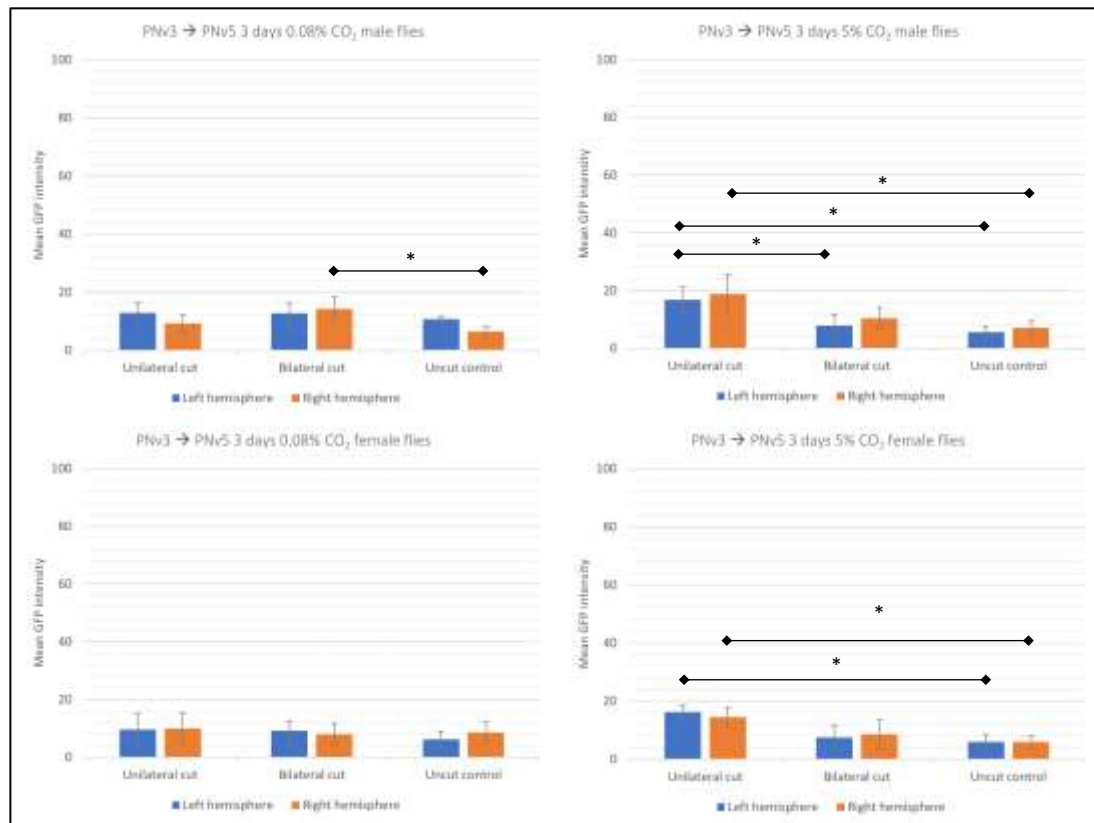


Figure 58. Comparing the GFP intensities between the different lesioning conditions in male and female flies with 0.08% and 5% CO₂ treatment. The bars represent the two hemispheres and horizontal lines are highlighting possible significant differences between the lesioning conditions (* $p < 0.0167$ after Bonferroni correction; $n=4-8$)

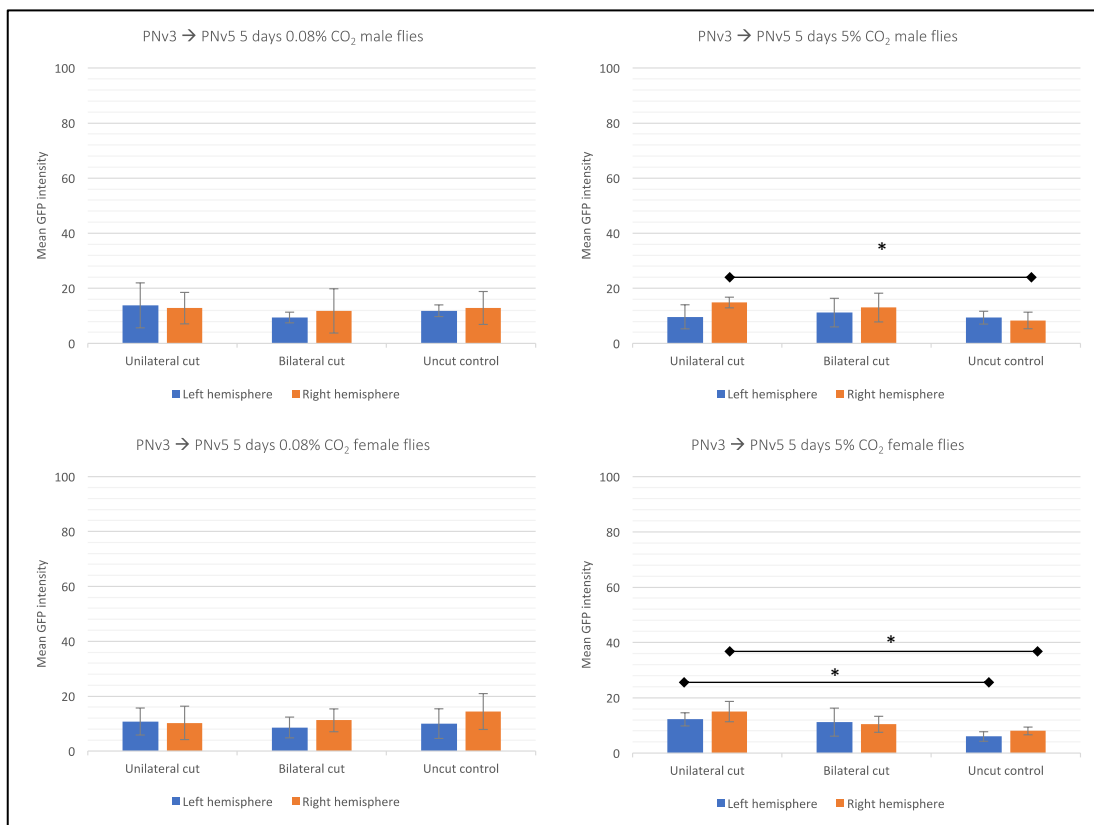


Figure 59. Comparing the GFP intensities between the different lesioning conditions in male and female flies with 0.08% and 5% CO₂ treatment. The bars represent the two hemispheres and horizontal lines are highlighting possible significant differences between the lesioning conditions (* $p < 0.0167$ after Bonferroni correction; $n=4-8$)

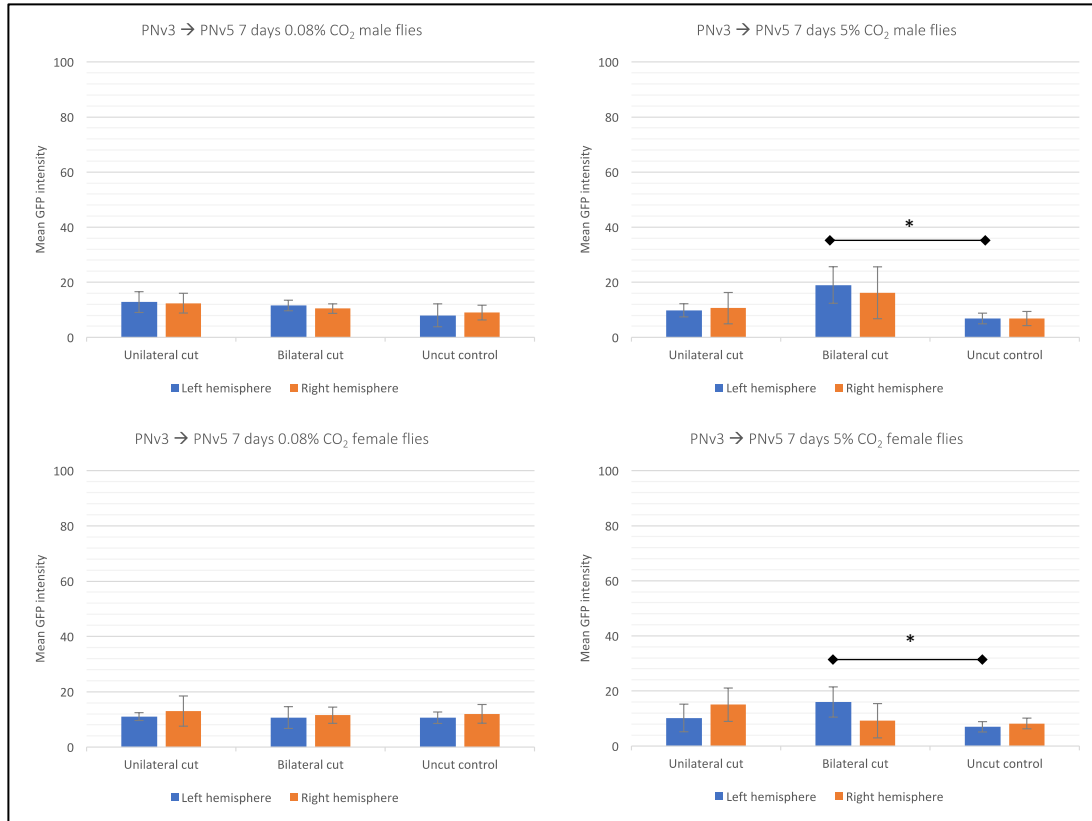


Figure 60. Comparing the GFP intensities between the different lesioning conditions in male and female flies with 0.08% and 5% CO₂ treatment. The bars represent the two hemispheres and horizontal lines are highlighting possible significant differences between the lesioning conditions (* $p < 0.0167$ after Bonferroni correction; $n=4-7$)

3.3.5 PNv1 → PNv5

This synapse showed a great response to 5% CO₂ which is especially evident in uninjured flies after 7 days of chronic exposure (Fig. 63). Interestingly, after bilateral ablation the activity in this synapse was vastly reduced (Fig. 61-63), further suggesting that the activity of this synapse is highly connected with the ability to sense the odor. Furthermore, it is very evident, that a unilateral lesion in addition to an exposure to 5% CO₂ caused heightened activity in the uninjured hemispheres on all days (Fig. 61-63). Multiple significant differences between the lesioning conditions could be shown. Another interesting observation was, that comparing the different conditions at this synapse didn't show a higher synaptic activity in lesioned flies compared to the uninjured as it was the case for all the other synapses. Here the measured intensities of unharmed flies were in the same range or higher if compared with the values of unilaterally lesioned flies (Fig. 61-63).

Day 3

Male flies displayed low GFP intensity levels across all three conditions at 0.08% CO₂ (Fig. 61) and no significant differences between them were found. When exposed to 5% CO₂, it was

possible to observe a noticeable response for flies in the unilateral cut condition and the uncut control condition, while it was not possible to measure GFP intensity above threshold for flies of the bilateral cut condition (Fig. 61). The differences between the two quantifiable conditions were not drastic enough to show significance (Fig. 61). In females, the picture was different. In ambient air, GFP intensity measurements from both hemispheres of bilaterally lesioned flies were significantly different than the measurements done in both hemispheres of uninjured flies (left hemisphere bila – unc $p=0.016$; right hemisphere bila – unc $p=0.007$) (Fig. 61). Exposure to high CO₂ levels led to an increase in the measured GFP intensities for all lesioning conditions, resulting in a significant intensity difference for the right hemispheres between the bilaterally lesioned flies and control flies (bila – unc $p=0.001$) (Fig. 61).

Day 5

According to the GFP intensity measurements and the assumption that changes are going along with synaptic activity, this synapse underwent major adaptations. While the values stayed low following a bilateral cut, the measurements for the unilaterally lesioned flies and uninjured flies were higher than on day 3 (compare Fig. 62 and 61). Therefore, in male flies at 0.08% CO₂, the left hemispheres following a bilateral lesion differed significantly from the left hemispheres of the other two conditions (uni – bila $p=0.005$; bila – unc $p=0.013$). For the right hemispheres, the significance was still given if the bilaterally lesioned and the uninjured flies were compared (bila – unc $p=0.009$) (Fig. 62). At 5% CO₂, a significant difference was calculated between the unilateral cut condition and the bilateral cut condition for the left hemispheres (uni – bila $p=0.004$) and between the bilateral cut condition and the uncut control for the right hemispheres (bila – unc $p=0.008$) (Fig. 62). Analyses of female flies ended up with similar results. However, at 0.08% CO₂, the bilateral cut condition resulted in significantly different GFP intensity values than those of the unilateral cut condition and the uncut control condition for both hemispheres (left hemisphere uni – bila $p=0.011$; bila – unc $p=0.007$; right hemisphere uni – bila $p=0.016$; bila – unc $p=0.006$) (Fig. 62). If the conditions were compared after chronic exposure to 5% CO₂, the left hemispheres of female flies after bilateral lesion showed significantly different GFP intensities than the left hemispheres of other conditions (uni – bila $p=0.011$; bila – unc $p=0.007$) (Fig. 62). A comparison of right hemispheres showed significance only between bilaterally lesioned female flies and female control flies (bila – unc $p=0.003$) (Fig. 62).

Day 7

The results achieved at day 7 proved once more that this synapse shows the greatest response to CO₂ exposure. While at 0.08% CO₂, male flies didn't show significant differences between the conditions for left hemispheres, a significant difference was found for right hemispheres. Unilaterally lesioned flies had significantly different GFP intensity values than bilaterally lesioned flies and uninjured flies (uni – bila p= 0.011; uni – unc p= 0.013) (Fig. 63). Exposure to 5% CO₂ led to a huge increase of measured GFP intensities. Like on the previous days, the values for the bilateral cut condition stayed very low, but the unilateral cut condition resulted once more in an increased intensity for the uninjured left hemisphere and the intensity measurements for uninjured flies reached high values (Fig. 63). Following that, both hemispheres of the bilateral cut condition showed significantly different GFP intensity values than those of the other conditions (left hemisphere uni – bila p=0.005; bila – unc p=<0.0001; right hemisphere uni – bila p=0.007; bila – unc p=<0.0001) (Fig. 63). Again, in females a similar picture could be observed. At 0.08% CO₂ however, no significant differences between the conditions were found for either hemisphere (Fig. 63). A significant difference was expected, since the GFP intensities were very low for the bilateral cut condition compared to the unilateral cut condition and the control condition but the amount of brains which had a measurable intensity was so low that they could not get picked up by the Kruskal-Wallis test. A comparison between lesioned and unharmed female flies at 5% CO₂ showed, that the GFP intensities of left hemispheres in bilaterally lesioned flies differed significantly from the measurements of left hemispheres in uninjured flies (bila – unc p=0.002). The same could be shown for the right hemispheres of bilaterally lesioned female flies and control flies (bila – unc p=0.001) (Fig. 63).

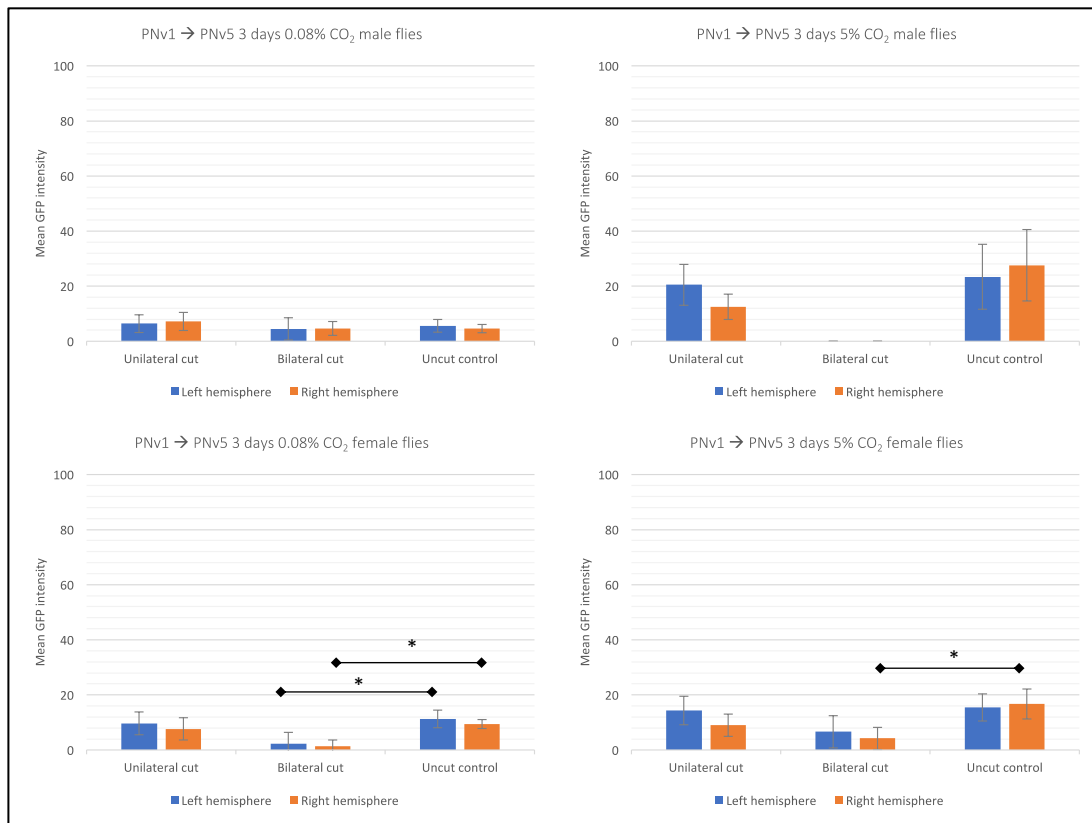


Figure 61. Comparing the GFP intensities between the different lesioning conditions in male and female flies with 0.08% and 5% CO₂ treatment. The bars represent the two hemispheres and horizontal lines are highlighting possible significant differences between the lesioning conditions (* $p < 0.0167$ after Bonferroni correction; $n=0-7$).

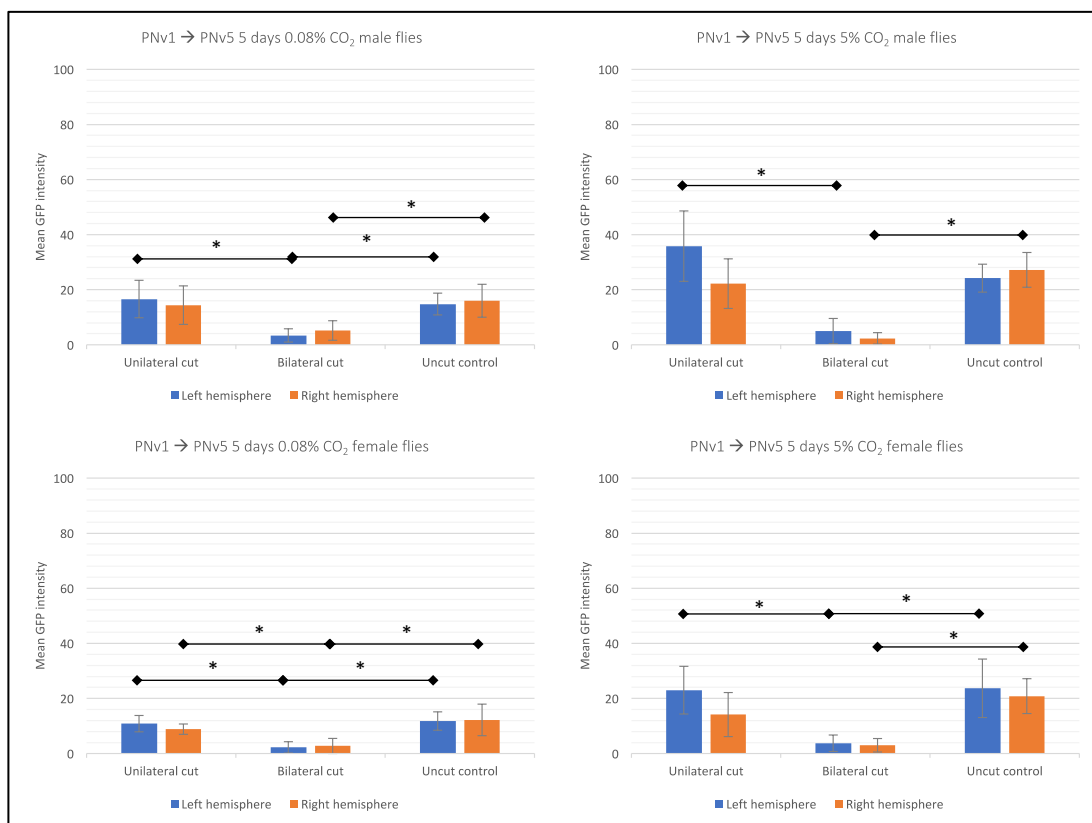


Figure 62. Comparing the GFP intensities between the different lesioning conditions in male and female flies with 0.08% and 5% CO₂ treatment. The bars represent the two hemispheres and horizontal lines are highlighting possible significant differences between the lesioning conditions (* $p < 0.0167$ after Bonferroni correction; $n=2-7$).

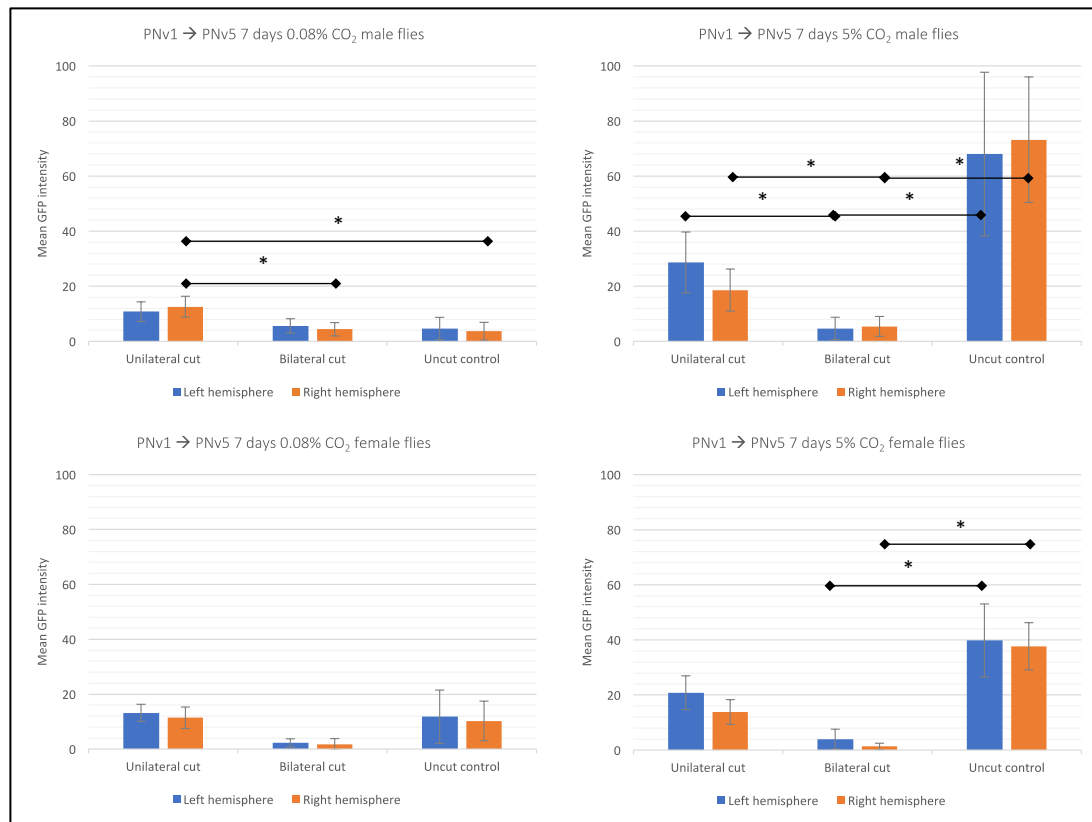


Figure 63. Comparing the GFP intensities between the different lesioning conditions in male and female flies with 0.08% and 5% CO₂ treatment. The bars represent the two hemispheres and horizontal lines are highlighting possible significant differences between the lesioning conditions (* $p < 0.0167$ after Bonferroni correction; $n = 2-11$)

3.3.6 LNV2 → PNv5

This synapse showed, that the activity in uninjured flies is not much affected by exposure to 5% CO₂, but a preceding lesion in addition to CO₂ exposure induces plasticity. Uninjured flies displayed the lowest GFP intensity values which resulted in multiple significant differences (Fig. 64-66)

Day 3

Comparing the conditions with a focus on the left hemispheres didn't result in significant differences at 0.08% CO₂. For the right hemispheres, the unilateral cut caused a significantly different activity level compared with uninjured flies (uni – unc $p = 0.011$) (Fig. 64). Looking at the different groups which were exposed to 5% CO₂ a significant difference between the unilateral cut condition and the uncut control condition was found for both hemispheres (left hemisphere uni – unc $p = 0.004$; right hemisphere uni – unc $p = 0.010$) (Fig. 64).

Day 5

There were no significant GFP intensity differences if the conditions were compared in ambient air, although the uninjured hemisphere after unilateral lesion clearly showed heightened activity (Fig. 65). At 5% CO₂, a significant distinction between the unilaterally lesioned flies and the uncut control flies was calculated with the Kruskal-Wallis test for both hemispheres (left hemisphere uni – unc p=0.004; right hemisphere uni – unc p=0.010) (Fig. 65).

Day 7

After 7 days of exposure to 0.08% CO₂, there were no significant differences between the left hemispheres, but the GFP intensity of right hemispheres of the unilaterally lesioned flies varied significantly from right hemispheres of control flies (uni – unc p=0.003) (Fig. 66). Flies which were exposed to 5% CO₂ for 7 days showed more significant GFP intensity differences. The measurements of the bilateral cut condition and the uncut control condition diverged significantly from each other if the GFP intensities of left hemispheres were compared (bila – unc p=0.001). For the right hemispheres, the GFP intensities of uninjured flies differed significantly from those of both the other conditions. (uni – unc p=0.005; bila – unc p=0.010) (Fig. 66).

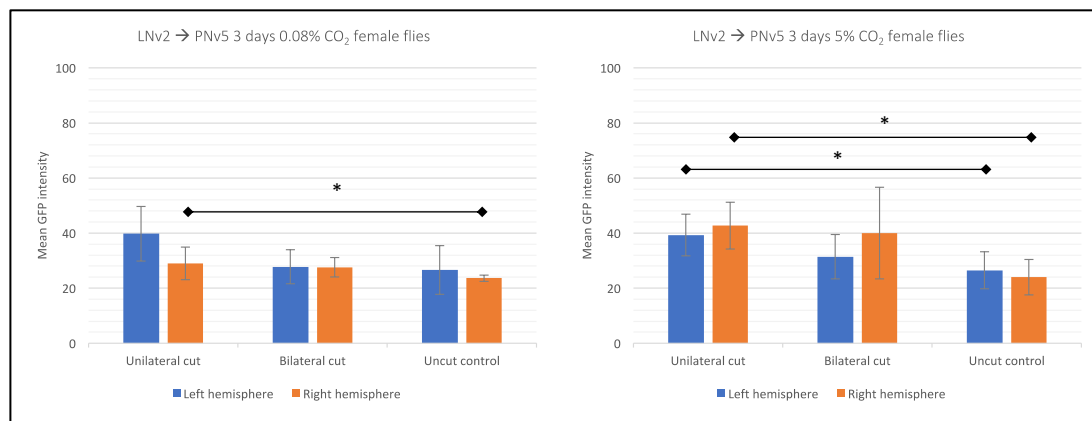


Figure 64. Comparing the GFP intensities between the different lesioning conditions in female flies with 0.08% and 5% CO₂ treatment. The bars represent the two hemispheres and horizontal lines are highlighting possible significant differences between the lesioning conditions (*p< 0.0167 after Bonferroni correction; n=5-10).

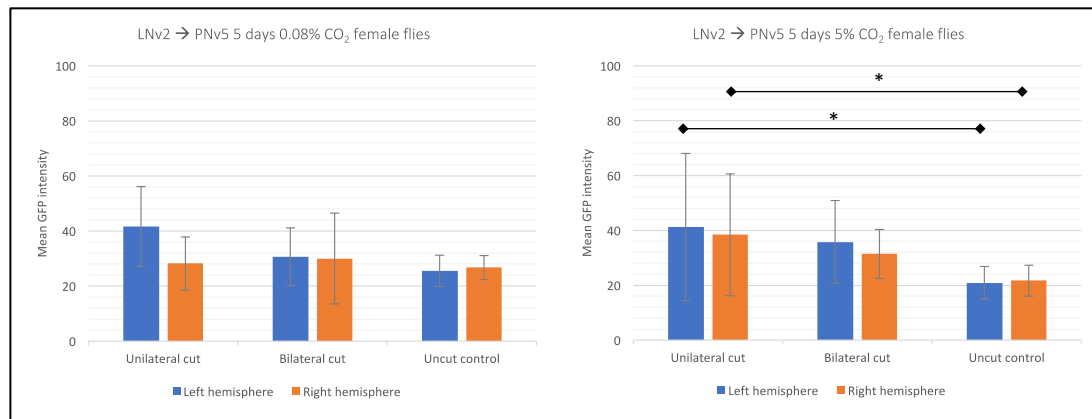


Figure 65. Comparing the GFP intensities between the different lesioning conditions in female flies with 0.08% and 5% CO₂ treatment. The bars represent the two hemispheres and horizontal lines are highlighting possible significant differences between the lesioning conditions (* $p < 0.0167$ after Bonferroni correction; $n = 4-9$).

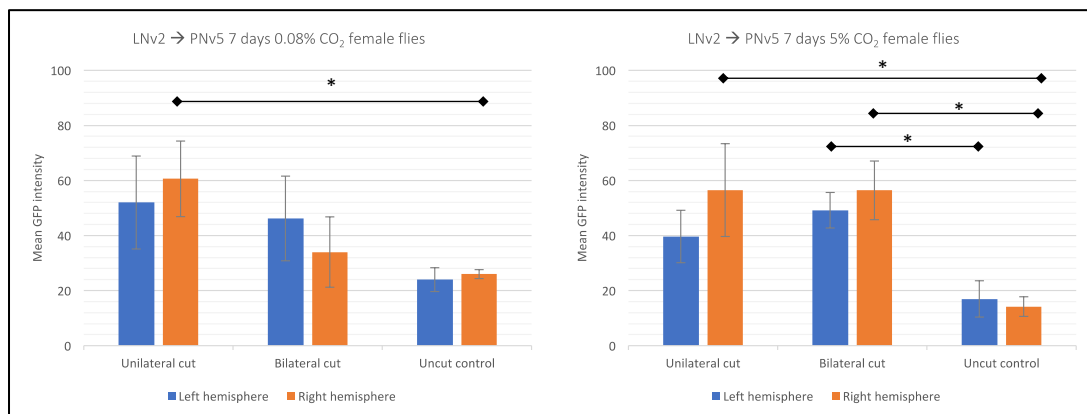


Figure 66. Comparing the GFP intensities between the different lesioning conditions in female flies with 0.08% and 5% CO₂ treatment. The bars represent the two hemispheres and horizontal lines are highlighting possible significant differences between the lesioning conditions (* $p < 0.0167$ after Bonferroni correction; $n = 4-6$).

3.4 Analyzing the impact of CO₂ on synaptic plasticity

Now, after I explored the possible GFP intensity differences between the hemispheres depending on the lesioning condition in 0.08% CO₂ and 5% CO₂ and comparing the differential effect of the different ablation procedures on synaptic activity, it was necessary to explore the impact of elevated CO₂ exposure for each hemisphere after lesion. The following diagrams will illustrate a side by side comparison of the same hemispheres where one bar will show the hemisphere when exposed to 0.08% CO₂ and the other bar represents the hemisphere at 5% CO₂. Significant results may give more information about the characteristics of each synapse since I could already tell, with the knowledge of the previous sections, that the synapses do respond very differently to the various lesioning conditions and both CO₂ treatments, but here

the focus is not on interhemispheric differences but on intrahemispheric differences. Significant p-values are shown for comparisons on all days between ambient air and chronic exposure to 5% CO₂.

3.4.1 Uninjured flies

Comparisons of uninjured flies show that both hemispheres respond very similar to exposure of 5% CO₂, but also that the various synapses have a distinct response profile, ranging from decreased activity after exposure to increased activity.

3.4.1.1 PNv3 → PNv1

In male flies, 5% CO₂ exposure didn't boost the activity to make it a significant difference (Fig. 67). In females, there was a noticeable increase at day 5 for both hemispheres at 5% CO₂, which resulted in a significant difference for the right hemispheres (p=0.012). Otherwise the GFP intensities kept rather stable between the different CO₂ treatments (Fig. 67).

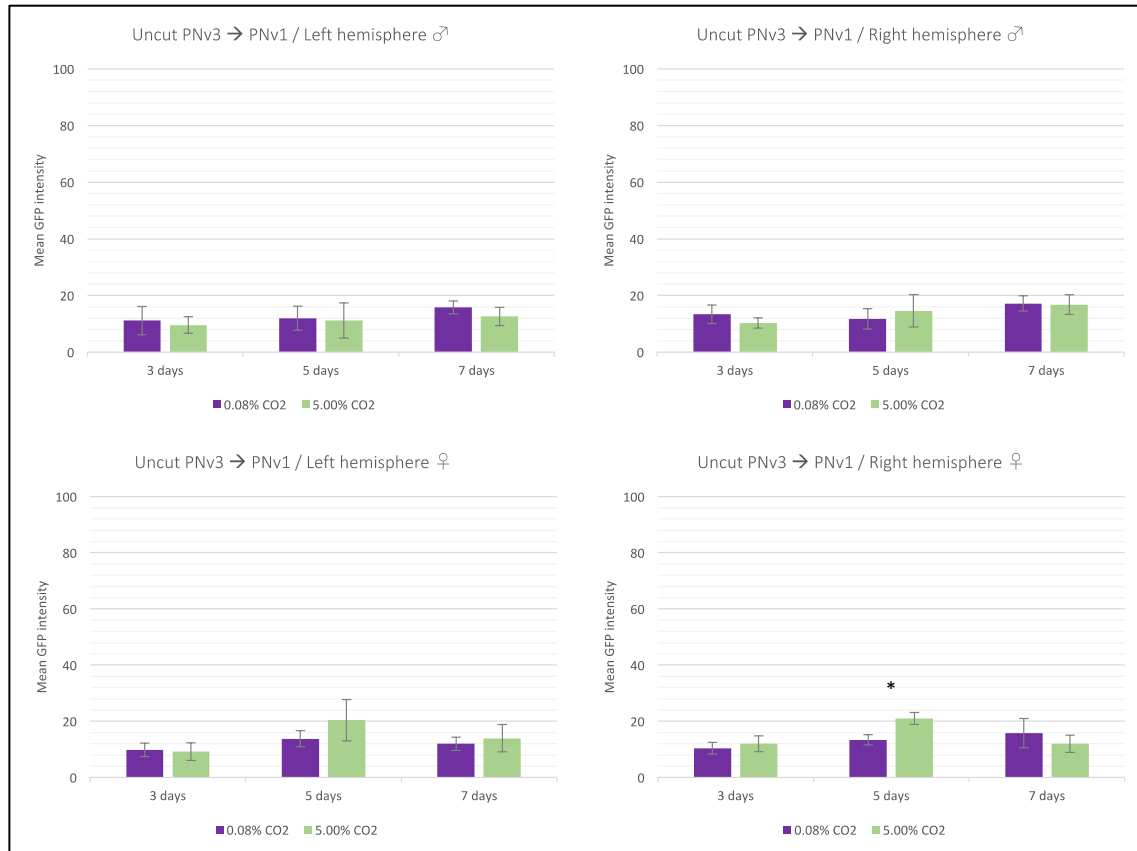


Figure 67. Comparison of GFP intensities between the 0.08% CO₂ and the 5% CO₂ treatment for the PNv3 → PNv1 synapse. Shown are both hemispheres and sexes compared to the same hemisphere and sex at the selected time points with the respective preceding lesioning condition (*p<0.05; n=4-8)

3.4.1.2 PNv5 → PNv1

Analysis of the PNv5 → PNv1 synapse revealed interesting observations if the influence of CO₂ was investigated. If the focus lies on the hemispheres exposed to 0.08% CO₂ it was shown that the GFP intensities are rising in both hemispheres, in both sexes up to day 7 (Fig. 68). If the hemispheres were exposed to 5% CO₂ the opposite was true. On day 3 both hemispheres displayed their highest activity at this synapse, progressively getting weaker over time (Fig. 68). In male flies this resulted in a significant difference at day 3 in both hemispheres (left hemisphere $p=0.008$; right hemisphere $p=0.008$) and in females on day 3 and 7 for the right hemispheres (3 days $p=0.012$; 7 days $p=0.048$) (Fig. 68).

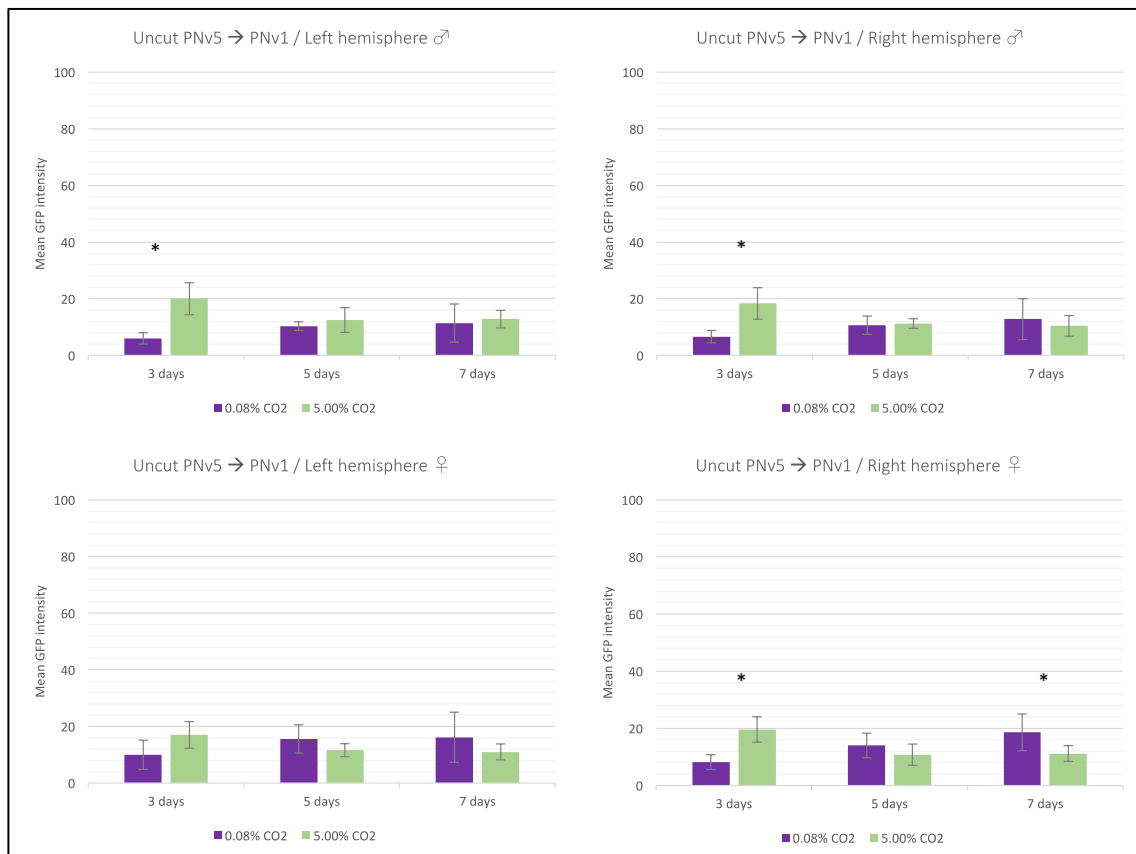


Figure 68. Comparison of GFP intensities between the 0.08% CO₂ and the 5% CO₂ treatment for the PNv5 → PNv1 synapse. Shown are both hemispheres and sexes compared to the same hemisphere and sex at the selected time points with the respective preceding lesioning condition (* $p<0.05$; $n=3-6$)

3.4.1.3 LNv2 → PNv1

Here, GABAergic synapses in both hemispheres increase their activity over time, but the increase is different if the 0.08% CO₂ and 5% CO₂ treatment were compared, since the values

kept on being more stable for flies exposed to 5% CO₂ after 5 days (Fig. 69). A significant difference between the treatments was found on day 5 for both hemispheres (left hemisphere $p=0.017$; right hemisphere $p=0.008$) (Fig. 69).

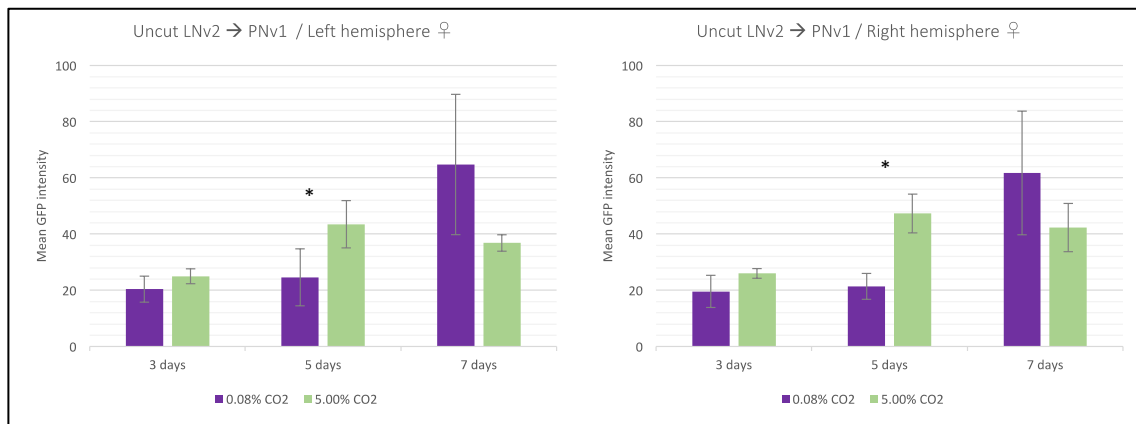


Figure 69. Comparison of GFP intensities between the 0.08% CO₂ and the 5% CO₂ treatment for the LNV2 → PNv1 synapse. Shown are both hemispheres of female flies compared with the same hemisphere at the selected time points with the respective preceding lesioning condition (* $p<0.05$; $n=3-6$)

3.4.1.4 PNv3 → PNv5

Overall, The PNv3 → PNv5 synapse didn't show much response following chronic stimulation with CO₂. In most of the cases the GFP intensity was even lower when exposed to higher CO₂ levels. Still, a significant difference was found at day 3 in the left hemispheres of male flies ($p=0.006$) (Fig. 70).

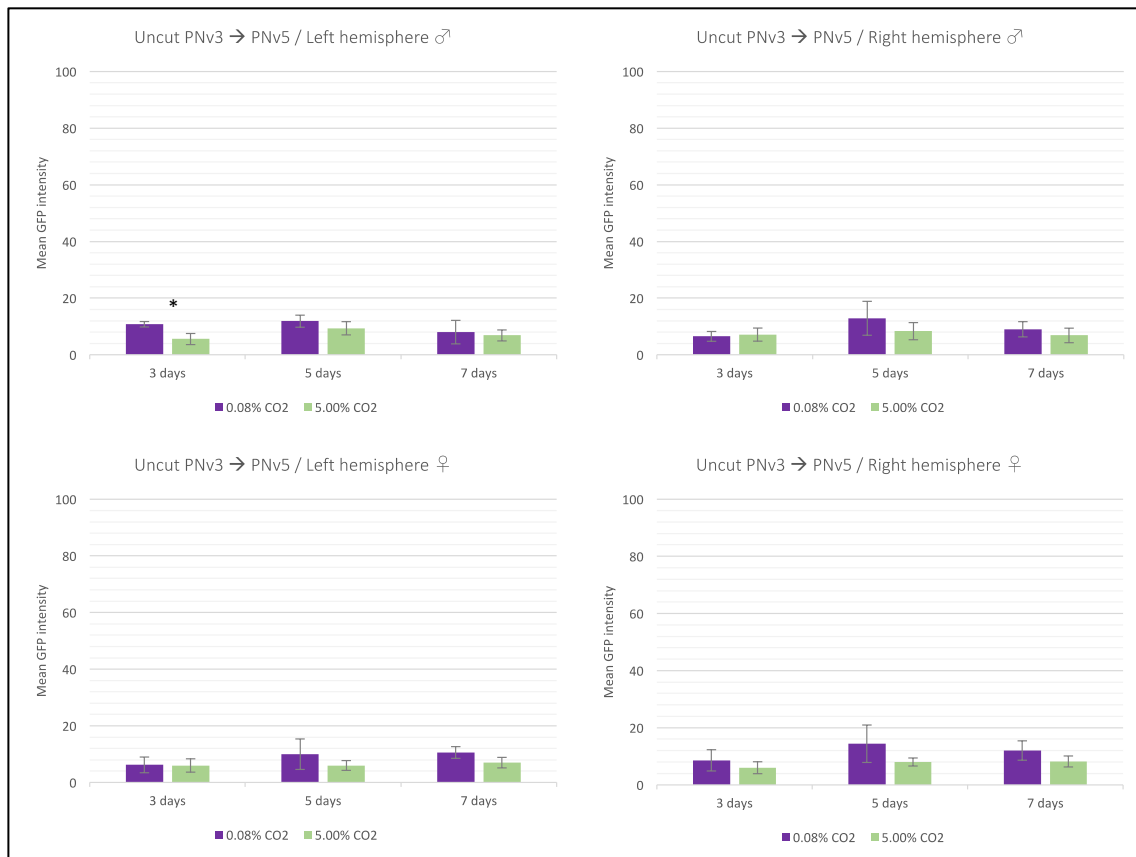


Figure 70. Comparison of GFP intensities between the 0.08% CO₂ and the 5% CO₂ treatment for the PNv3 → PNv5 synapse. Shown are both hemispheres and sexes compared to the same hemisphere and sex at the selected time points with the respective preceding lesioning condition (* $p < 0.05$; $n = 4-9$)

3.4.1.5 PNv1 → PNv5

This synapse showed the greatest response following chronic stimulation with 5% CO₂. In male flies, a significant GFP intensity difference between high and low CO₂ levels could be found at all time points in the left hemispheres and on day 3 and day 7 for the right hemispheres (left hemisphere: 3 days $p = 0.008$; 5 days $p = 0.024$; 7 days $p = 0.036$; right hemisphere: 3 days $p = 0.011$; 7 days $p = 0.036$) (Fig. 71). In females, the difference wasn't that drastic, but significance was revealed on day 7 for the left hemispheres ($p = 0.010$) as well as day 3 and day 7 for the right hemispheres (3 days $p = 0.001$; 5 days $p = 0.014$) (Fig. 71).

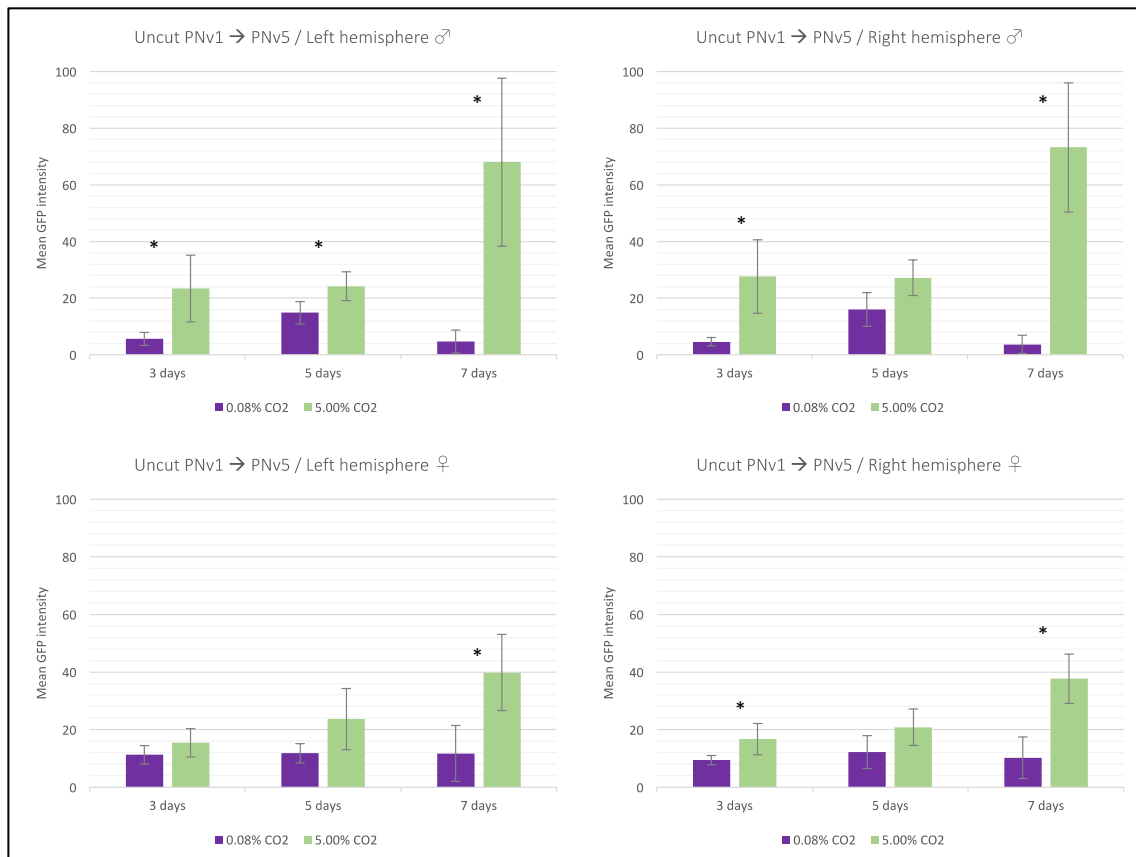


Figure 71. Comparison of GFP intensities between the 0.08% CO₂ and the 5% CO₂ treatment for the PNv1 → PNv5 synapse. Shown are both hemispheres and sexes compared to the same hemisphere and sex at the selected time points with the respective preceding lesioning condition (*p<0.05; n=3-7)

3.4.1.6 LNv2 → PNv5

Here, lower GFP intensities could be observed in hemispheres if exposed to 5% CO₂. A significant difference could be calculated at day 7 in the right hemispheres (p=0.019). The GFP intensity decreased steadily in both hemispheres at high CO₂ concentrations, with being the highest on day 3 and the lowest on day 7 (Fig. 72).

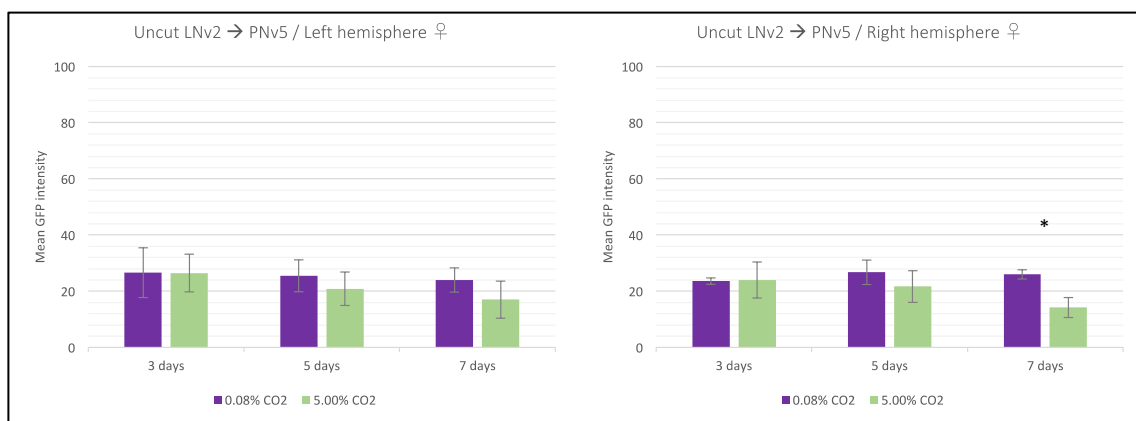


Figure 72. Comparison of GFP intensities between the 0.08% CO₂ and the 5% CO₂ treatment for the LNv2 → PNv5 synapse. Shown are both hemispheres in female flies compared with the same hemisphere at the selected time points with the respective preceding lesioning condition (*p<0.05; n=4-9)

3.4.2 Unilateral cut

Comparisons of unilaterally lesioned flies revealed that inhibitory synapses on PNv1 responded with more activity in the injured hemisphere, while this wasn't as evident at inhibitory synapses on PNv5. This differential activity was enhanced by chronic exposure to CO₂. The excitatory synapse PNv1 → PNv5 responded with higher activity in the uninjured hemisphere upon exposure to 5% CO₂. The right hemisphere represents the injured side for the following diagrams.

3.4.2.1 PNv3 → PNv1

As already observed, the PNv3 → PNv1 synapse showed higher GFP intensities in the injured hemispheres after a unilateral cut (Fig. 73). In male flies, a direct comparison of the intensity values from the 0.08 and 5% CO₂ treatment resulted in a significant difference on day 7 in the left hemispheres ($p=0.048$) and a significant difference on day 7 in the right hemispheres ($p=0.048$) (Fig. 73). In female flies a significant difference was found at day 3 ($p= 0.005$) and day 7 ($p= 0.026$) for the right hemispheres (Fig. 73).

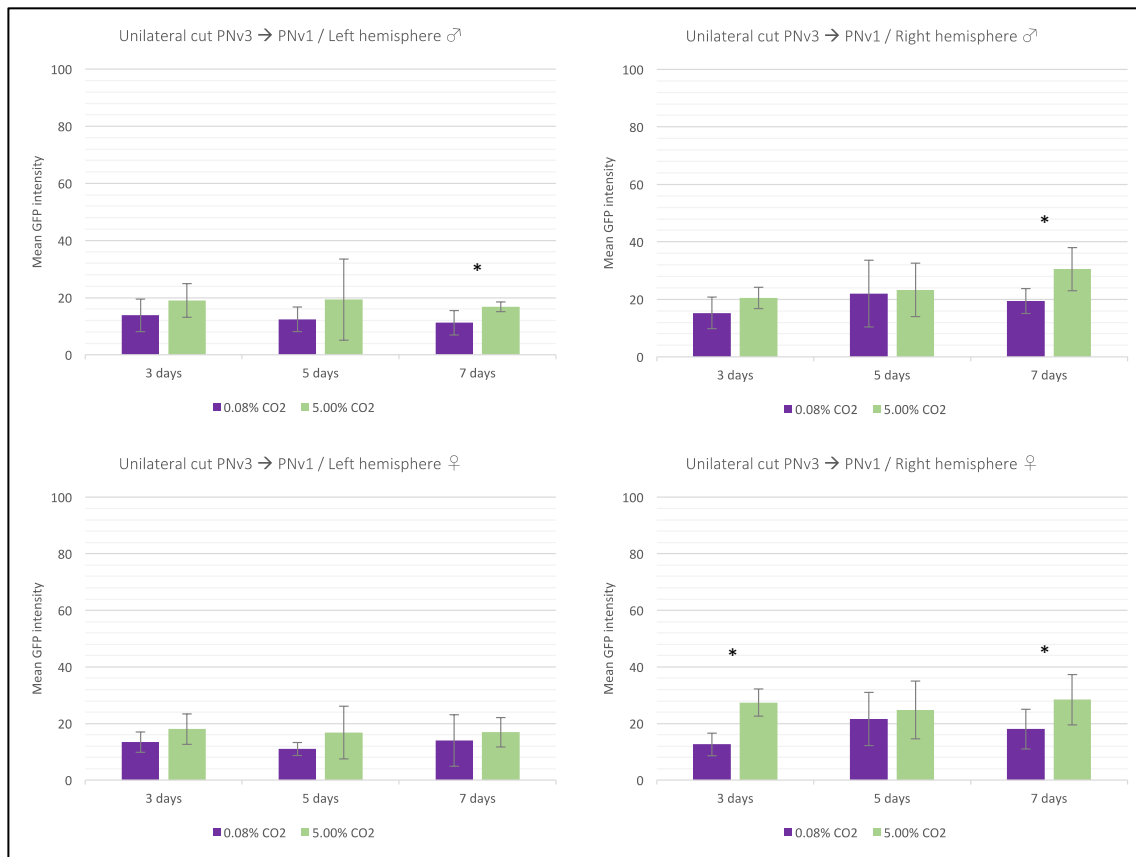


Figure 73. Comparison of GFP intensities between the 0.08% CO₂ and the 5% CO₂ treatment for the PNv3 → PNv1 synapse. Shown are both hemispheres and sexes compared to the same hemisphere and sex at the selected time points with the respective preceding lesioning condition (*p<0.05; n=4-8)

3.4.2.2 PNv5 → PNv1

No significant differences were found in male flies, but the hemispheres exposed to 5% CO₂ showed slightly higher GFP intensities. Females showed greater deviations when exposed to 5% CO₂ which resulted in a significant difference on day 7 in the left hemispheres (p=0.013) (Fig. 74). Generally, females seem to respond differently to CO₂ exposure than males when compared after a unilateral cut. On day 3 and day 5 both hemispheres displayed the higher intensities when at 0.08% CO₂, but on day 7 the hemispheres exposed to 5% CO₂ showed higher synaptic activity, indicating a sex specific response to CO₂ (Fig. 74).

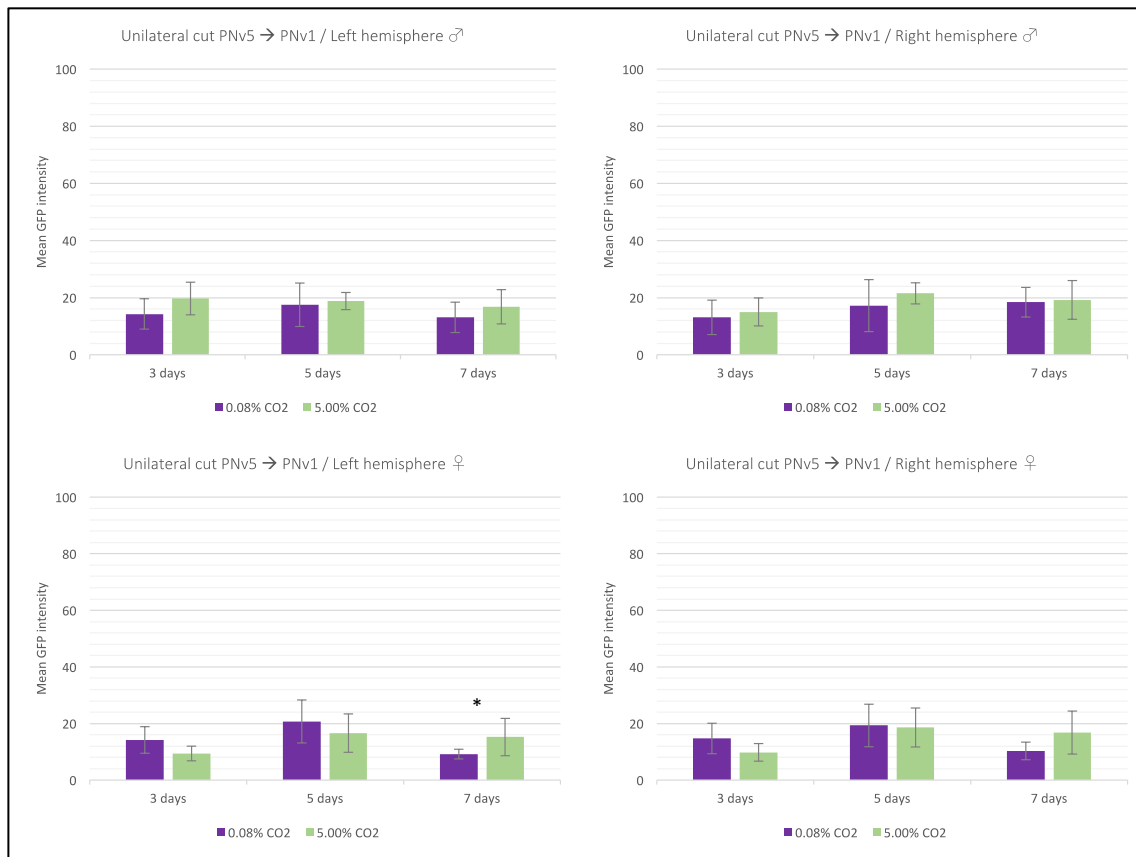


Figure 74. Comparison of GFP intensities between the 0.08% CO₂ and the 5% CO₂ treatment for the PNv5 → PNv1 synapse. Shown are both hemispheres and sexes compared to the same hemisphere and sex at the selected time points with the respective preceding lesioning condition (* $p < 0.05$; $n = 3-7$)

3.4.2.3 LNV2 → PNv1

For this synapse, exposure to a higher CO₂ concentrations caused an increase of activity for both hemispheres within one week (Fig. 75). Significant differences were found on day 3 and day 5 for the right hemispheres (3 days $p = 0.017$; 5 days $p = 0.041$). The injured right hemispheres responded with higher activity upon stimulation with 5% CO₂ (Fig. 75).

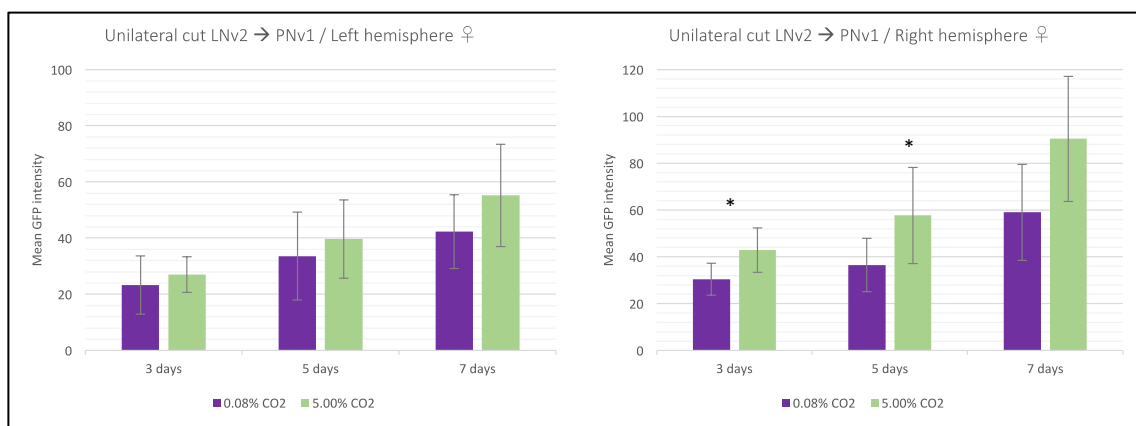


Figure 75. Comparison of GFP intensities between the 0.08% CO₂ and the 5% CO₂ treatment for the LNV2 → PNv1 synapse. Shown are both hemispheres in female flies compared with the same hemisphere and at the selected time points with the respective preceding lesioning condition (* $p < 0.05$; $n = 5-8$)

3.4.2.4 PNv3 → PNv5

The increased CO₂ levels didn't affect the activity at this synapse as strong as it did for others. Still, a significant difference was found on day 3 in female flies for the left hemispheres ($p=0.048$) (Fig. 76). On day 5 and day 7, exposure to ambient aid led to higher activity in left hemispheres of males while in females the left hemisphere showed slightly higher activity on day 7, according to the marginally higher mean GFP intensity (Fig. 76). As for the right hemispheres, exposure to 5% CO₂ increased intensity values on all days except for day 7 in male flies. The only significant difference was found on day 3 in male flies ($p=0.003$). In sum, the injured right hemisphere responded with slightly more activity upon exposure to 5% CO₂ (Fig. 76).

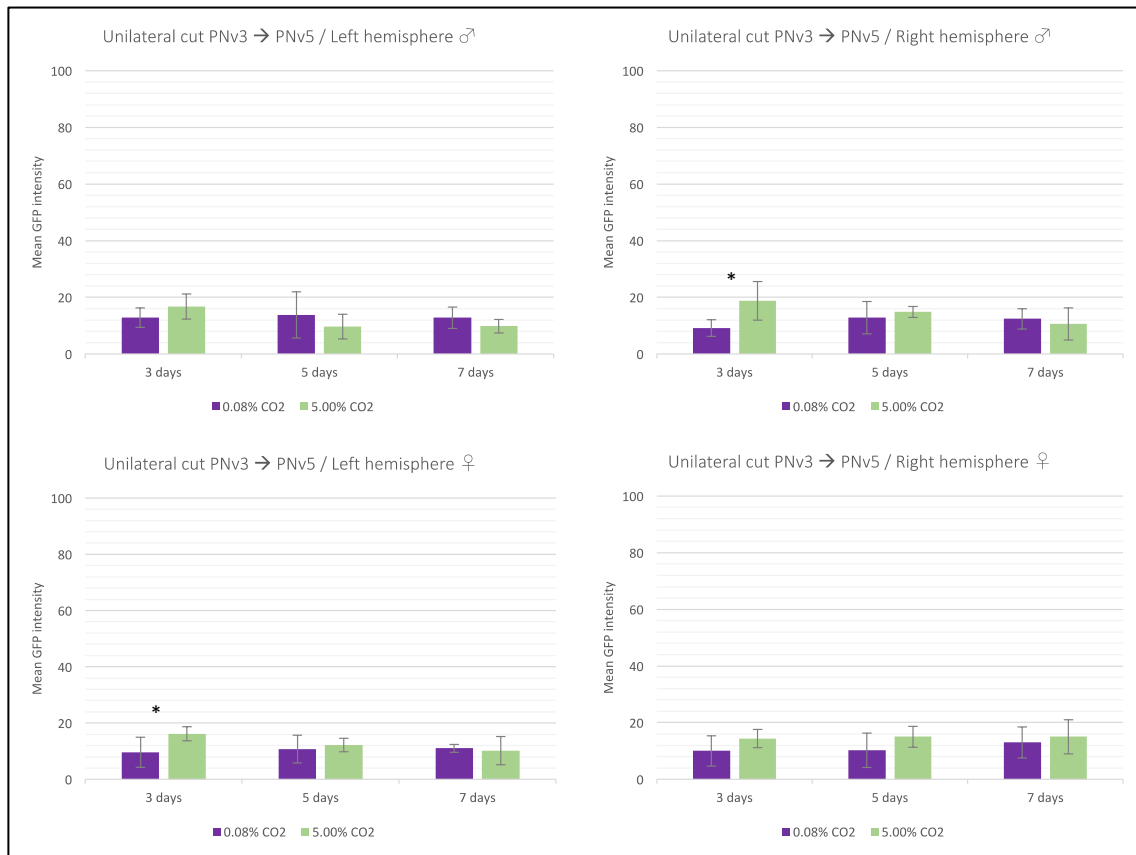


Figure 76. Comparison of GFP intensities between the 0.08% CO₂ and the 5% CO₂ treatment for the PNv3 → PNv5 synapse. Shown are both hemispheres and sexes compared to the same hemisphere and sex at the selected time points with the respective preceding lesioning condition (* $p<0.05$; $n=4-8$)

3.4.2.5 PNv1 → PNv5

This synapse stood out as the one, where upon stimulation with high CO₂ concentrations the left uninjured hemispheres responded with higher activity compared to the injured right hemispheres (Fig. 77). This was substantiated with significant differences on all days in the left uninjured hemispheres in male flies (3 days p=0.009; 5 days p=0.016; 7 days p=0.002) and only on day 3 for the right hemispheres (p=0.001) (Fig. 77). In female flies the GFP intensity difference was significant on day 5 for left hemispheres (p=0.008) (Fig. 77).

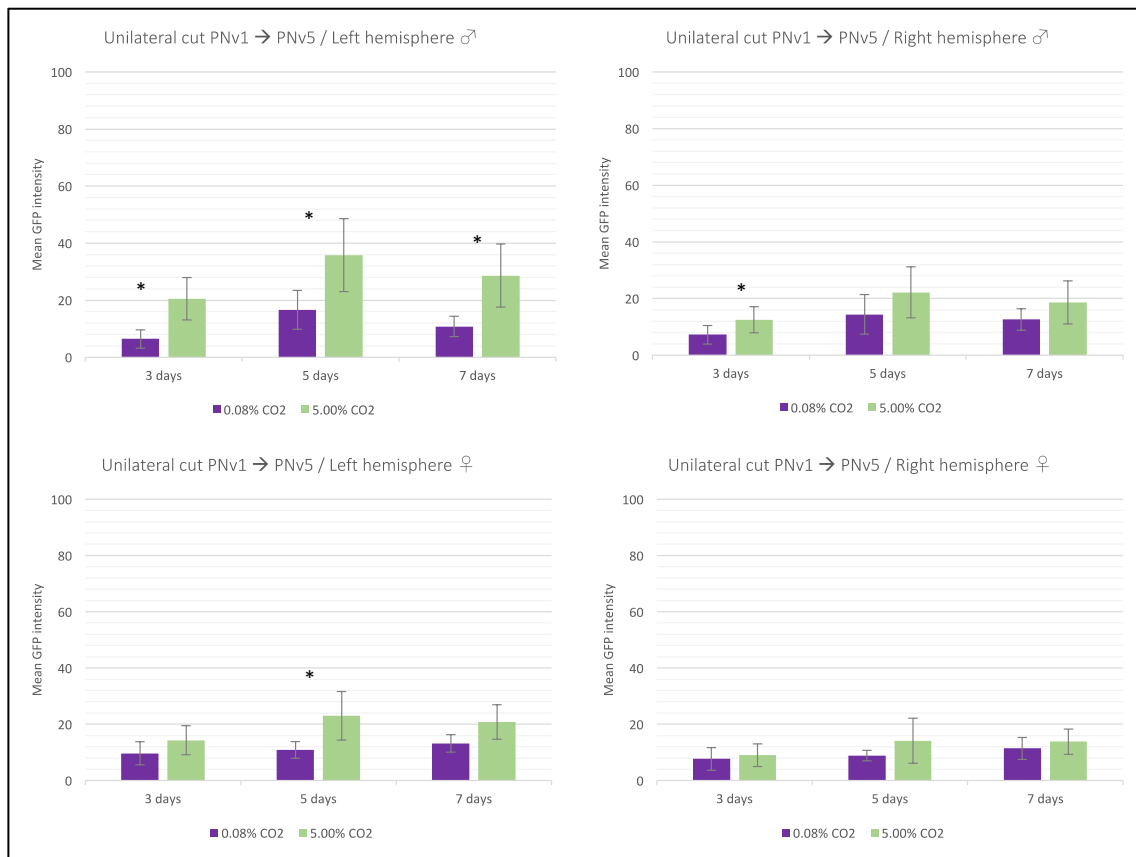


Figure 77. Comparison of GFP intensities between the 0.08% CO₂ and the 5% CO₂ treatment for the PNv3 → PNv5 synapse. Shown are both hemispheres and sexes compared to the same hemisphere and sex at the selected time points with the respective preceding lesioning condition (*p<0.05; n=5-10)

3.4.2.6 LNv2 → PNv5

Higher CO₂ levels didn't affect the uninjured left hemisphere much. However, after 7 days the GFP intensity was higher in left hemispheres at 0.08% CO₂ compared to left hemispheres at 5% but the difference wasn't significant (Fig. 78). If the injured right hemispheres were compared at low and high CO₂ levels a significant difference was calculated at day 3 (p=0.002) (Fig. 78).

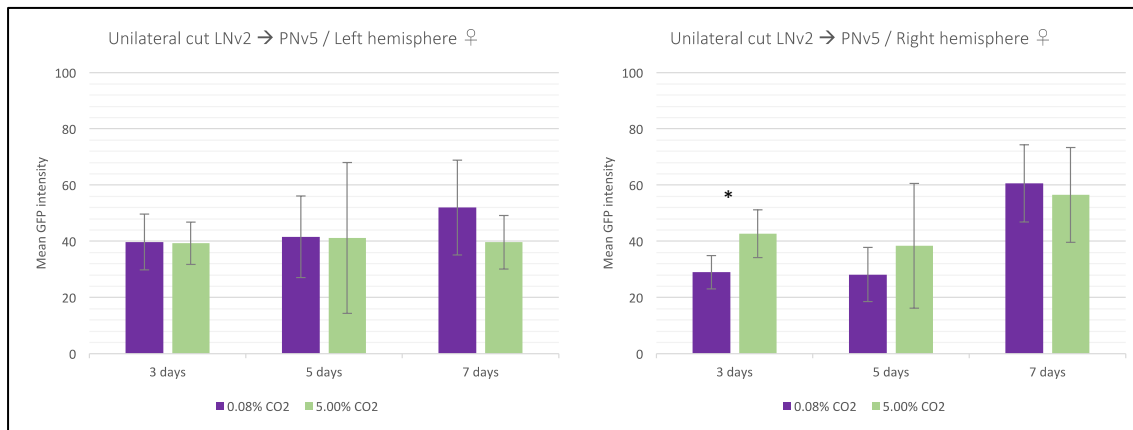


Figure 78. Comparison of GFP intensities between the 0.08% CO₂ and the 5% CO₂ treatment for the LNV2 → PNv5 synapse. Shown are both hemispheres in female flies compared with the same hemisphere at the selected time points with the respective preceding lesioning condition (* $p < 0.05$; $n = 5-10$)

3.4.3 Bilateral cut

Comparisons of flies which underwent bilateral ablation of the antennae followed by exposure to atmospheric air or chronic stimulation with CO₂ resulted in interesting findings. Although the flies should have no more capabilities to sense CO₂, multiple observations suggest otherwise. The excitatory PNv1 → PNv5 stood out as the synapse most affected by bilateral lesion, indicating a strong affiliation to CO₂ perception.

3.4.3.1 PNv3 → PNv1

High CO₂ exposure had almost no impact on the activity pattern of this synapses since the GFP intensities stayed in a similar range for both hemispheres. Therefore, no significant difference between CO₂ treatments was found in both sexes for either hemisphere (Fig. 79).

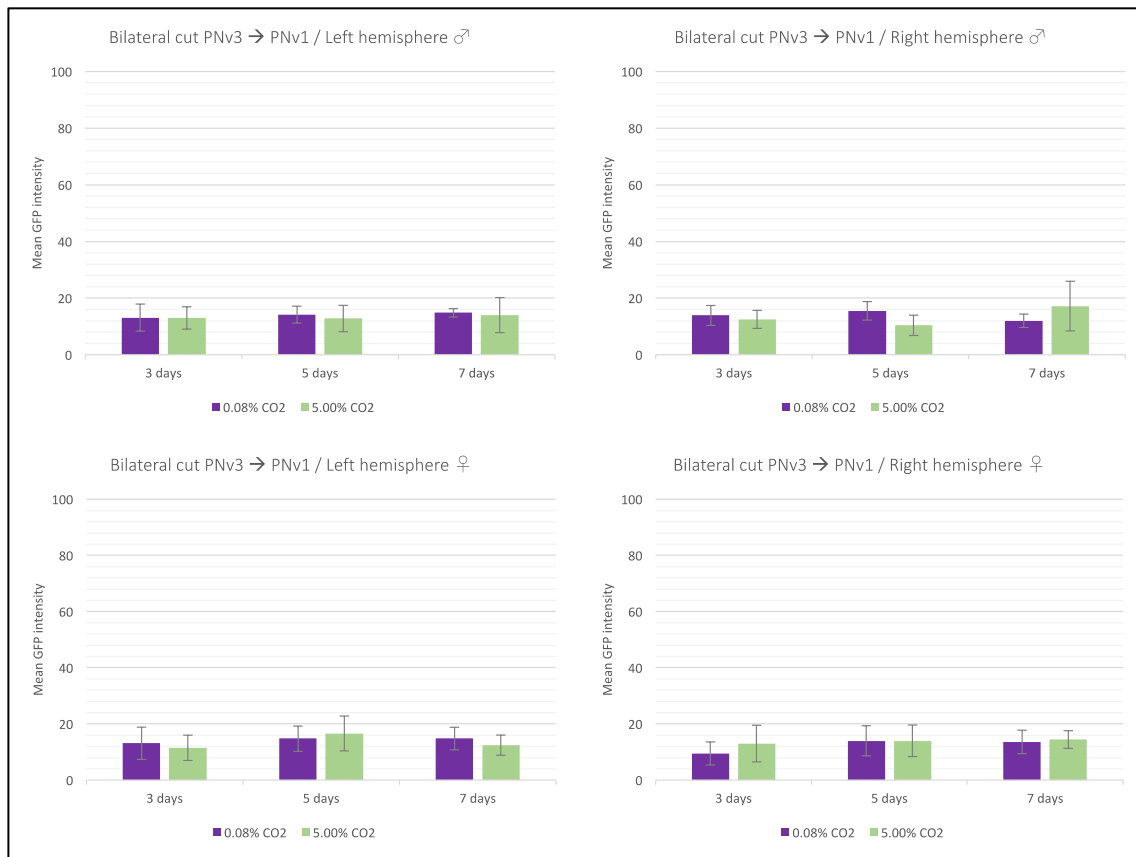


Figure 79. Comparison of GFP intensities between the 0.08% CO₂ and the 5% CO₂ treatment for the PNv3 → PNv1 synapse. Shown are both hemispheres and sexes compared to the same hemisphere and sex at the selected time points with the respective preceding lesioning condition (* $p < 0.05$; $n = 4-8$)

3.4.3.2 PNv5 → PNv1

The analysis on this synapse showed that in most cases, flies, both male and female, exposed to 0.08% CO₂ displayed a higher GFP intensity in both hemispheres than flies with the high CO₂ treatment. The only detected significant difference was found in female flies, on day 3 for right hemispheres ($p = 0.006$) (Fig. 80).

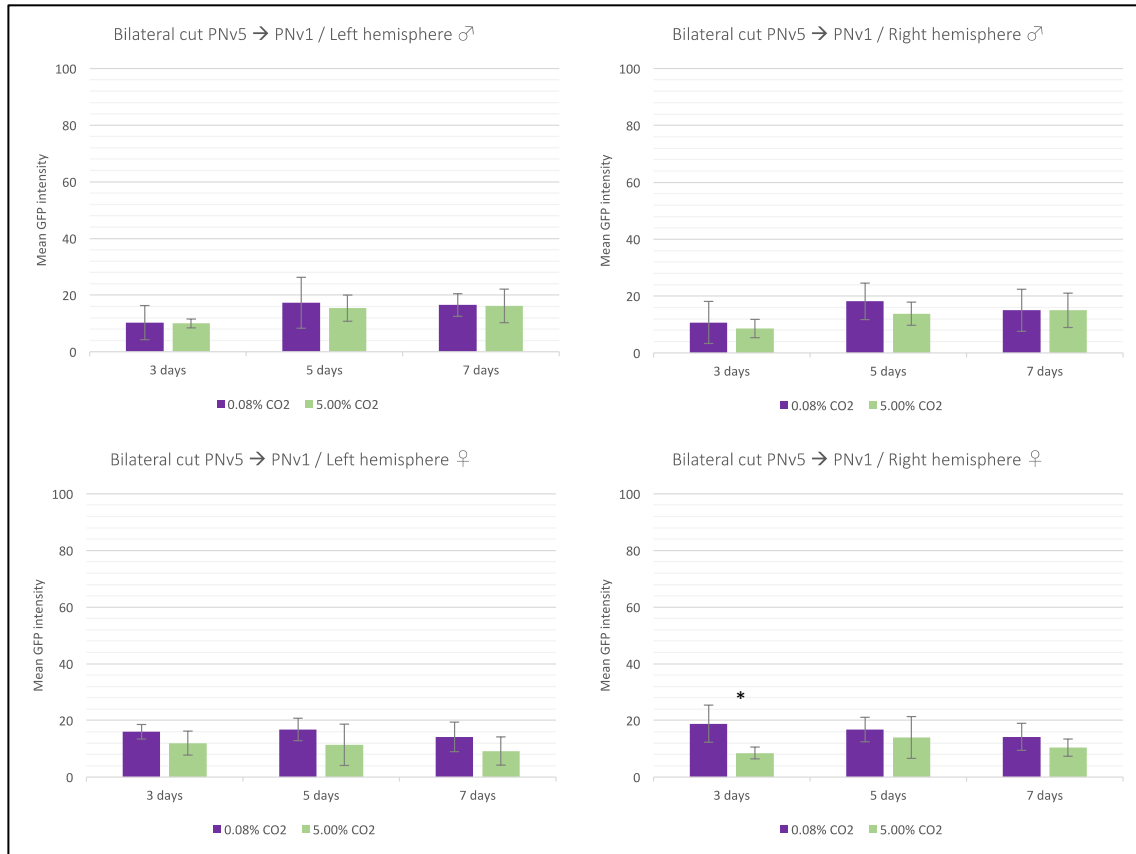


Figure 80. Comparison of GFP intensities between the 0.08% CO₂ and the 5% CO₂ treatment for the PNv5 → PNv1 synapse. Shown are both hemispheres and sexes compared to the same hemisphere and sex at the selected time points with the respective preceding lesioning condition (* $p < 0.05$; $n = 4-7$)

3.4.3.3 LNv2 → PNv1

For this synapse, the difference of being exposed to 0.08% CO₂ and 5% CO₂ was, that with exposure to ambient the GFP intensities were the lowest on day 7 for both hemispheres, while being the highest with a 5% CO₂ treatment (Fig. 81). Accordingly, this led to a significant difference at day 7 for both hemispheres (left hemisphere $p = 0.048$; right hemisphere $p = 0.040$) (Fig. 81). Such a response to 5% CO₂ was not expected because both antennae were ablated.

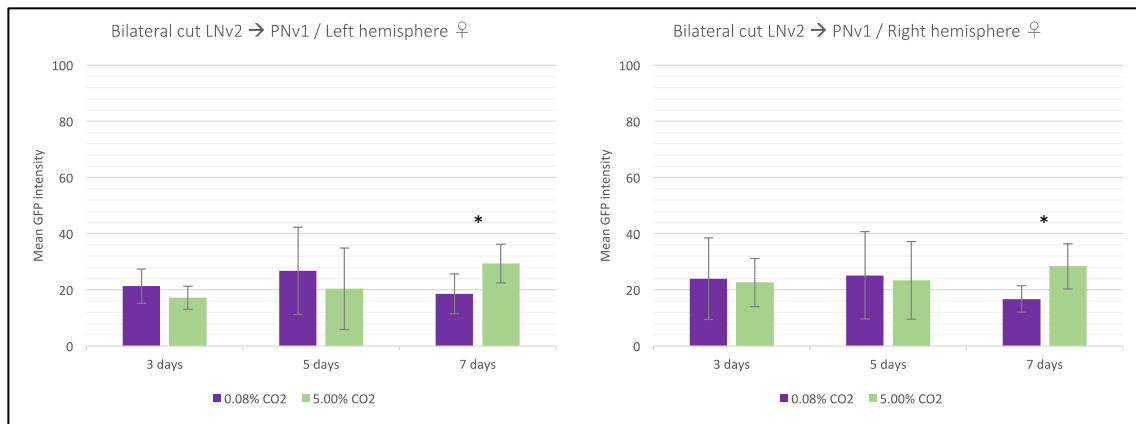


Figure 81. Comparison of GFP intensities between the 0.08% CO₂ and the 5% CO₂ treatment for the LNV2 → PNv1 synapse. Shown are both hemispheres in female flies compared with the same hemisphere at the selected time points with the respective preceding lesioning condition (* $p < 0.05$; $n = 5-6$)

3.4.3.4 PNv3 → PNv5

In male flies, a significant GFP difference between 0.08% CO₂ and 5% CO₂ treatment could be shown for both the left and right hemispheres on day 3 (left hemisphere $p = 0.009$; right hemisphere $p = 0.028$) (Fig. 82). In females, no significant differences between the two treatments were found (Fig. 82). It is also evident that synaptic activity is rising at 5% CO₂ within one week.

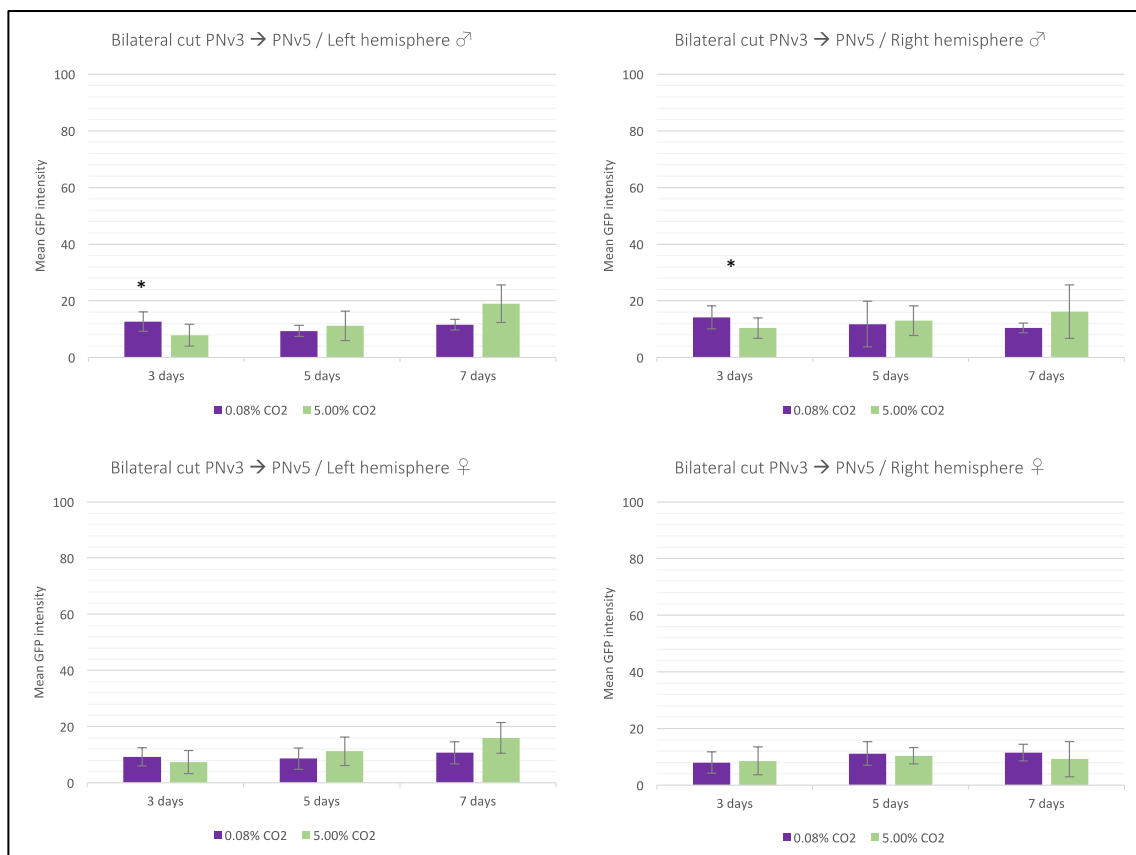


Figure 82. Comparison of GFP intensities between the 0.08% CO₂ and the 5% CO₂ treatment for the PNv3 → PNv5 synapse. Shown are both hemispheres and sexes compared to the same hemisphere and sex at the selected time points with the respective preceding lesioning condition (* $p < 0.05$; $n = 5-8$)

3.4.3.5 PNv1 → PNv5

A bilateral cut drastically lowered the GFP intensities which can be measured at this synapse, not depending on whether the flies were exposed to atmospheric air or chronically stimulated with 5% CO₂. In some brains, it was impossible to get measurements above threshold. No significant difference between the 0.08% CO₂ and the 5% CO₂ treatment could be calculated by the U-test (Fig. 83).

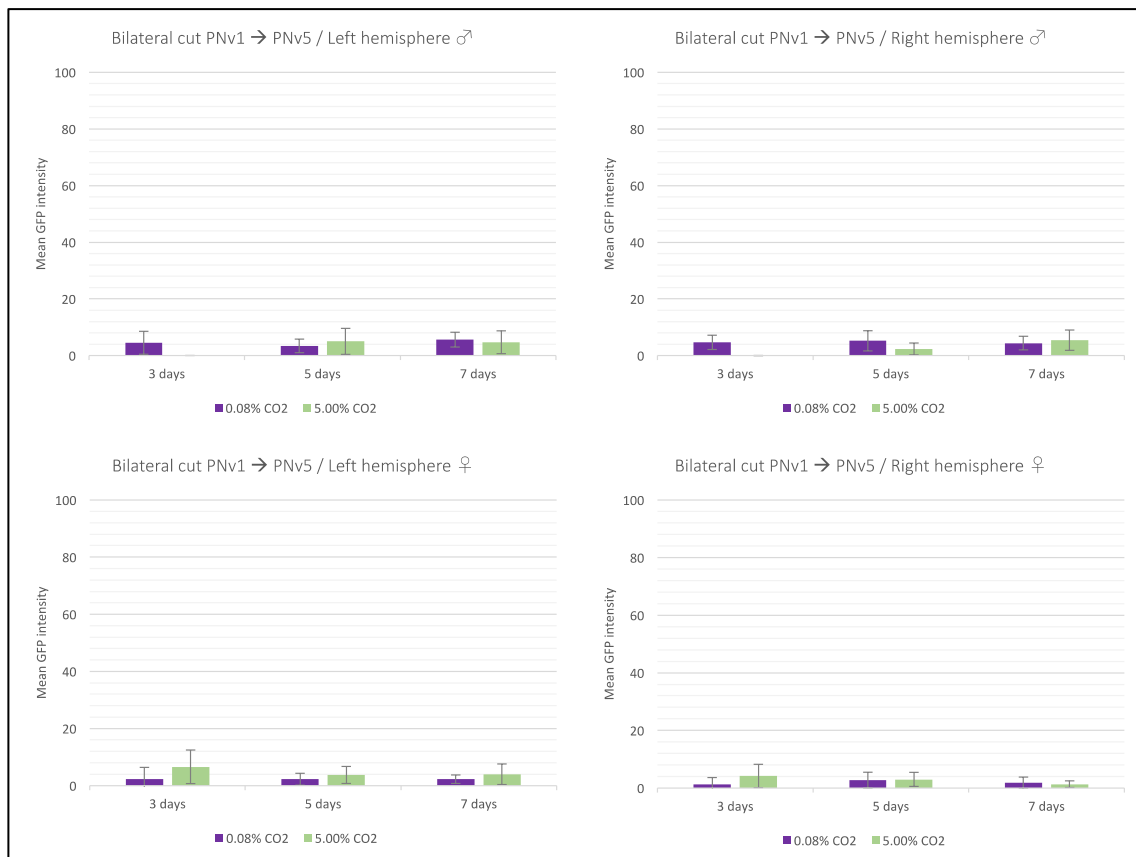


Figure 83. Comparison of GFP intensities between the 0.08% CO₂ and the 5% CO₂ treatment for the PNv1 → PNv5 synapse. Shown are both hemispheres and sexes compared to the same hemisphere and sex at the selected time points with the respective preceding lesioning condition (* $p < 0.05$; $n = 2-6$)

3.4.3.6 LNv2 → PNv5

Although the antennae have been bilaterally ablated, suggesting that the flies can't perceive CO₂ anymore, the analysis on this synaptic connection showed that flies displayed higher GFP intensities in hemispheres after chronic exposure to CO₂, indicating higher synaptic activity. However, a significant difference was only detected on day 7 for the right hemispheres ($p = 0.032$) (Fig. 84).

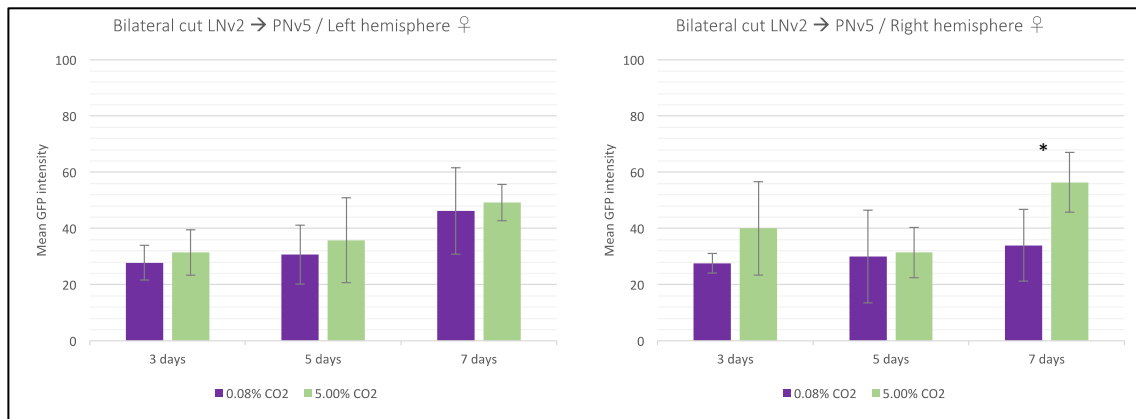


Figure 84. Comparison of GFP intensities between the 0.08% CO₂ and the 5% CO₂ treatment for the LNV2 → PNv5 synapse. Shown are both hemispheres in female flies compared with the same hemisphere at the selected time points with the respective preceding lesioning condition (* $p < 0.05$; $n = 5-7$)

3.5 Reverse unilateral cut and sensory deprivation

Now, after I explored the differences between the hemispheres of the individual conditions, the impact of CO₂ on synaptic plasticity and how the different lesions affect synaptic activity, it was to address if a reverted unilateral cut, injuring the left hemisphere instead of the right, will lead to the same GFP intensity differences but in a reverse manner. Furthermore, it was important to figure out if the plasticity occurred because of the injury itself or if sensory deprivation is enough to trigger a similar or the same response.

3.5.1 Reverse unilateral cut for selected synapses

For this experiment the LNV2 → PNv1 and the PNv3 → PNv1 synapse were chosen, because for the regular unilateral cut I could observe that the injured hemisphere showed a higher GFP intensity after 7 days at both CO₂ concentrations. The reverse unilateral cut for the LNV2 → PNv1 synapse showed, that GFP intensity differences between the hemispheres are in fact turned around, although with a lower overall GFP intensity (Fig. 85). This could be observed both at 0.08% and 5% CO₂. Same as with the regular unilateral cut no significant differences between the hemispheres were found. For the PNv3 → PNv1 synapse I could show a similar picture, but here the reverse unilateral cut didn't lead to lower GFP intensities. Additionally, while no significant difference between the hemispheres were found for the regular unilateral cut, the difference at 0.08% CO₂ was significant for the reverse one ($p = 0.016$) (Fig. 85).

3.5.2 The effect of sensory deprivation

To explore the effect of sensory deprivation, UV-glue was used to cover the antennae to hinder the flies to perceive CO₂. For the LNV2 → PNv1 synapse the sensory deprivation led to much lower measurable GFP intensity values, but the affected hemisphere (right hemisphere) was still the one which had the higher intensity (Fig. 86). Same as in the unilateral cut condition, no significant difference between the hemispheres was found. The GFP intensities which were measured for the PNv3 → PNv1 synapse were almost identical if UV-glue was applied instead of causing injury. Furthermore, when using UV-glue the intensity difference between the hemispheres was significant ($p=0,024$) (Fig. 86).

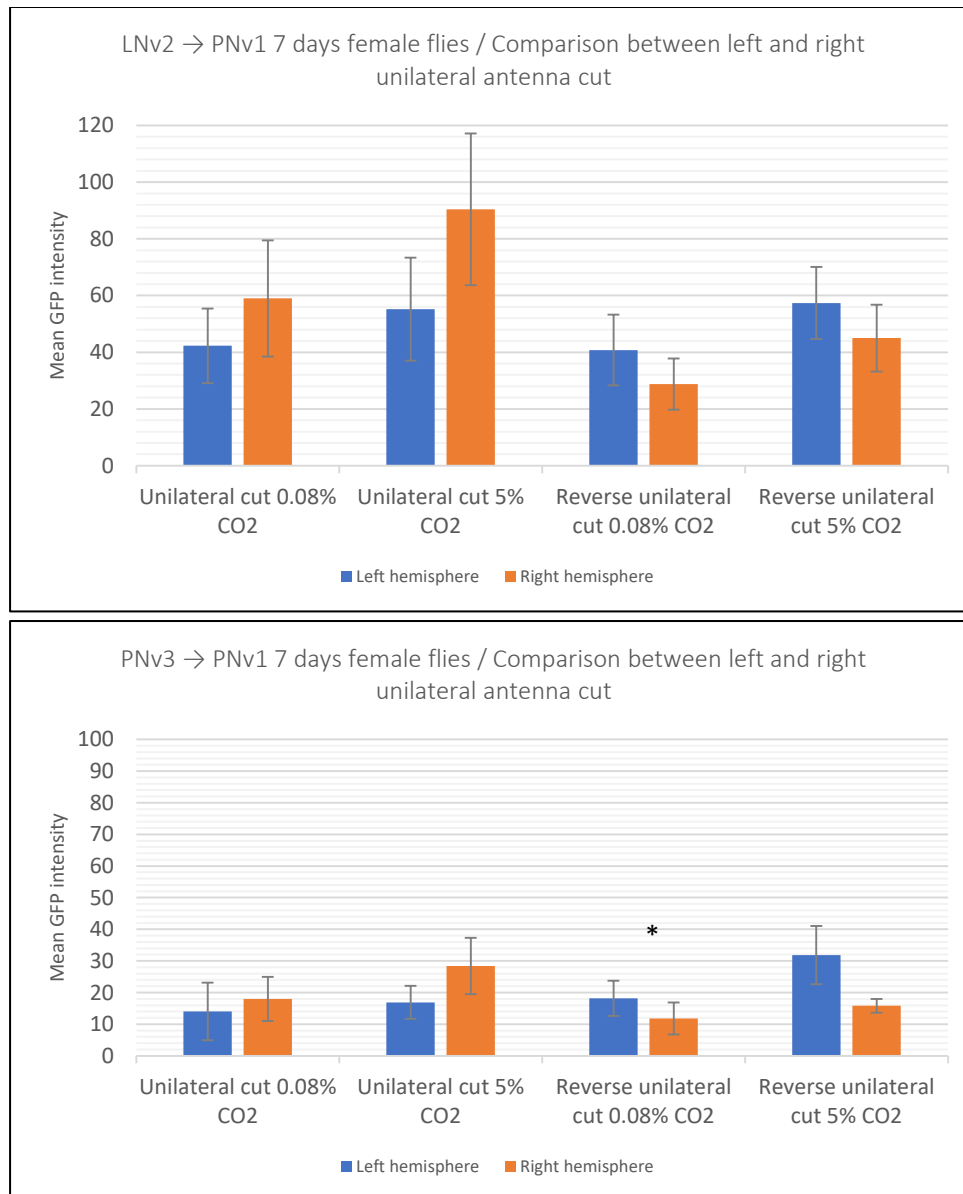


Figure 85. Exploring the difference of an injury to the left or right hemisphere for female flies after 7 days in 0.08% and 5% CO₂. The bars show the mean GFP intensity measurements for both hemispheres with their respective standard deviations; (* $p < 0.05$; $n = 4-7$)

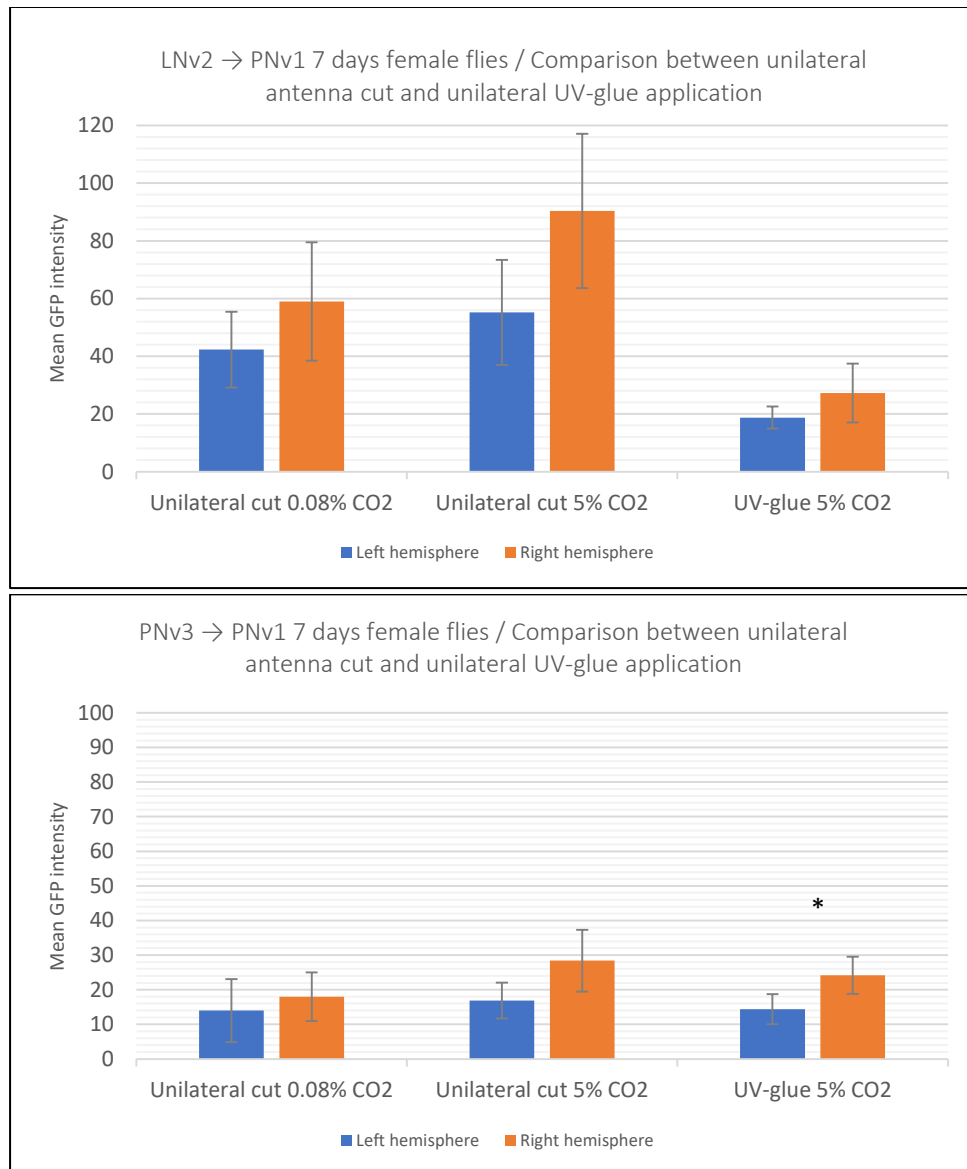


Figure 86. Comparing unilateral lesion with unilateral sensory deprivation in female flies after 7 days in 0.08% and 5% CO₂. The bars show the mean GFP intensity measurements for both hemispheres with their respective standard deviations; (* $p < 0.05$; $n = 4-8$)

4. Discussion

The analysis of olfactory circuits in the adult *Drosophila* brain described here revealed differences in central synaptic activity following peripheral lesions and that chronic stimulation of the unaffected hemisphere influences the activity level of the lesioned microcircuit. Most importantly, results described here showed that each type of central synaptic connection responds in a specific fashion to peripheral lesions and increased sensory stimulation, indicating a critical level of injury-induced synaptic plasticity in *Drosophila* central brain circuits.

Peripheral lesion and strokes in mammals result in altered excitatory and inhibitory circuit modulation (Carmichael, 2012; Sammons and Keck, 2015) and there is also evidence that not only the perilesional area, but also the contralesional hemisphere is involved in neuronal recovery (Jones and Adkins, 2015). I could show that the adult nervous system of *Drosophila* shares similarities with these structural changes in mammalian brain circuits. My study reveals, that after unilateral lesion a complex interaction between both, unilateral and bilateral, excitatory and inhibitory neurons are initiated and I propose a compensatory mechanism to enhance signal transduction for the remaining functional odor perception pathway.

4.1 Wild type circuit organization

GRASP intensity measurements in control flies at ambient air revealed that both projection neurons, PNv1 and PNv5, receive overall low synaptic input with the exception of GABAergic LNV2 (Fig. 87). Furthermore, the GABAergic input of LNV2 onto the two types of projection neurons shows a different temporal dynamic, in which the LNV2→PNv5 synapse displays a steady moderate activity level within the first week after eclosion whereas the LNV2→PNv1 synapses show a significant increase in activity after a few days (Fig. 87). This indicates a selective inhibition of PNv1 but not PNv5, thereby a modification of the two main output channels of the CO₂ pathway. In contrast, no significant differences in the synaptic activity of the main excitatory and inhibitory connections between the two hemispheres could be detected in control flies, providing an excellent experimental model to determine the specific changes in synaptic activity following unilateral peripheral lesions.

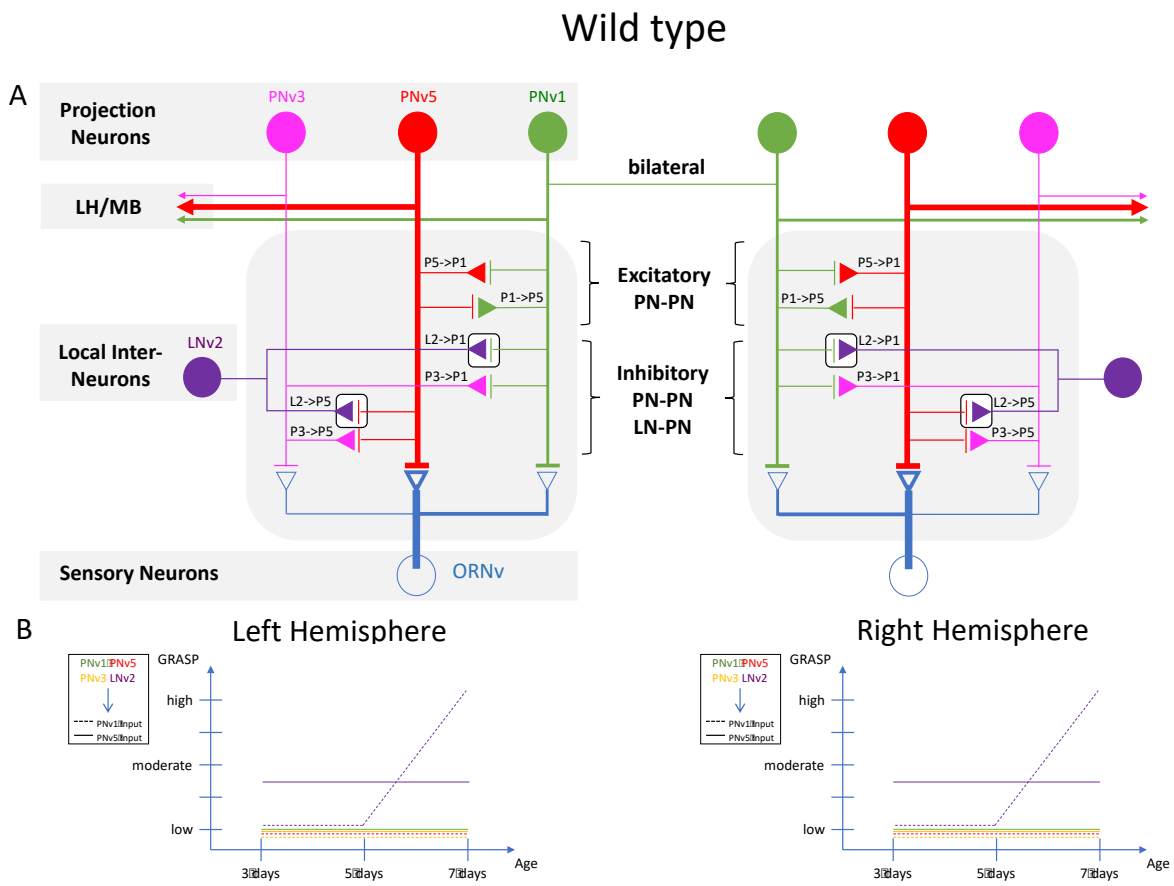


Figure 87. (A) Microcircuit of interconnected V-glomeruli. Synapses which show a distinct activity compared to other synapses are highlighted. **(B)** Diagrams are showing relative activity changes within one week for all synapses in each hemisphere. Lnv2 synapses (solid and dashed purple line) show increased activity in comparison with the other synaptic connections. The LNV2 → PNv5 synapse (solid purple line) shows steady activity while the LNV2 → PNv1 synapse (dashed purple line) shows very high activity after 5 days

4.1.1 Changes of central synaptic activity following bilateral sensory deafferentation

A bilateral ablation of the antennae almost silenced synaptic connections of the excitatory PNv1 onto PNv5 (Fig. 88). Furthermore, the synaptic activity of the LNV2 → PNv1 slightly increased until day 5, but by day 7 it decreased to lower levels again (Fig. 88). The synaptic activity of the LNV2 → PNv5 synapse was increased on day 7 in the left hemisphere, while the right hemisphere showed steady activity on all days (Fig. 88).

Interestingly, if the activity of GABAergic LNV2 synapses of bilaterally lesioned flies are compared with the activity of LNV2 synapses of uninjured flies, they show a differential activity pattern. The sudden increase of activity for the LNV2 → PNv1 synapse after 5 days in uninjured flies is absent following bilateral lesion. In fact, bilateral ablation of the antennae results in the

lowest activity on day 7. The LNv2 → PNv5 synapse shows steady activity within one week in uninjured flies while the left hemisphere displays increased activity on day 7 after bilateral lesion.

By removing both antennae, all sensory input from ORNs, involving ORNs expressing the CO₂ sensitive receptors GR21a and GR63a, should be gone (Kwon et al., 2007). However, since the antennal palps also project ORNs into the antennal lobes, a small subset of glomeruli should still be active which might influence the activity pattern of the GABAergic neurons PNv3 and LNv2, because they broadly innervate multiple glomeruli. According to this, a certain baseline activity of the investigated inhibitory neurons was expected after bilateral lesion.

However, the reciprocal excitatory connection between PNv5 and PNv1 needs to be addressed separately, because both projection neurons receive direct input from ORNs in uninjured flies and their differential activity pattern following bilateral lesion might be attributed to their different structural properties. According to previous research which also applied the activity dependent GRASP technique, it was shown that the ORN → PNv5 synapse was more active than ORN connections with other PNs, indicating that PNv5 has the highest output in higher brain centers, such as the Mushroom Bodies (Batawi, unpublished). Therefore, it was expected that the activity of PNv5 should be significantly affected following bilateral removal of the antennae. In wildtype flies, the role of PNv1, due to its bilateral nature, might be to match incoming odor signals and communicate this information to the contralateral hemisphere, to upregulate PNv5s activity via their excitatory synaptic connection if it is necessary. Deafferented PNv1s possibly detect that there are no active afferents after bilateral ablation, resulting in the very low synaptic input onto PNv5 which I could show in my results. The remaining very low activity could stem from spontaneous activity of PNv1.

PNv5 on the other hand, can't match incoming odor input from both hemispheres by itself, which might explain why it has a higher excitatory output on PNv1. However, I could show in my results, that if I compare the unilaterally and bilaterally lesioned flies with control flies, that injured flies generally showed higher synaptic activity than unharmed flies, so I suppose that injury generally interferes with the balanced homeostasis of excitatory and inhibitory circuit modulating neurons.

Bilaterally lesioned flies

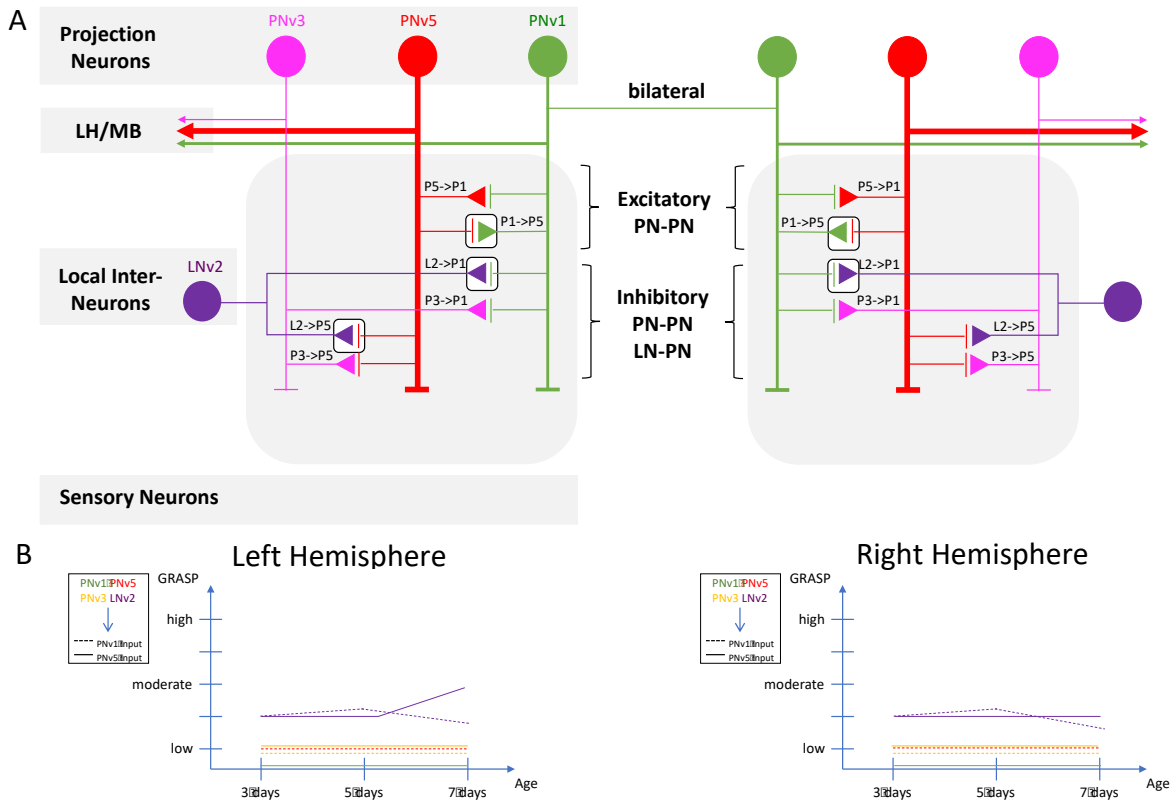


Figure 88. (A) Microcircuit of interconnected V-glomeruli. Synapses which show a distinct activity compared to other synapses are highlighted. **(B)** Diagrams are showing relative activity changes within one week for all synapses in each hemisphere. The excitatory PNv1 → PNv5 synapse displayed low synaptic activity (solid green line). LNV2 synapses showed fluctuating activity levels within one week (solid and dashed purple line)

4.1.2 Changes of central synaptic activity following unilateral sensory deafferentation

Following unilateral peripheral lesion, the reciprocal excitatory connection between PNv1 and PNv5 showed low activity in both directions, but no interhemispheric differences were observed (Fig. 89). However, the GABAergic neurons LNV2 and PNv3 displayed a differential output onto PNv1 and PNv5 following the removal of one antenna. Both inhibitory neurons showed an increased activity in the injured hemisphere for synaptic connections onto PNv1 in comparison with the uninjured hemisphere (Fig. 89). On the contrary, the synaptic input from LNV2 onto PNv5 was increased in the uninjured hemisphere, 3 and 5 days after lesion (Fig. 89). On day 7, this observation changed to the opposite, with the injured hemisphere displaying higher activity (Fig. 89). The connection between PNv3 and PNv5 didn't show any specific changes following injury (Fig. 89).

Comparing the results of the unilaterally lesioned flies with uninjured flies reveals, that the activity of the PNv5 → PNv1 synapse is slightly increased after unilateral ablation. Further, the synaptic activity of the PNv3 → PNv1 synapse in the left uninjured hemisphere is similar to the activity level of this synapse in uninjured flies, indicating that the lesion only affects the injured right hemisphere. Synapses of LNV2 onto PNv1 show steadily increasing activity in both hemispheres, keeping the differential activity between the injured and uninjured hemisphere, which is different than the sudden increase of activity on day 7 in uninjured flies and the decreasing activity in bilaterally lesioned flies.

A comparison of the synaptic activity of LNV2 → PNv5 synapses in unilaterally lesioned flies with uninjured and bilaterally lesioned flies shows that the injured hemisphere doesn't show a change of activity until day 5. This synapse starts to show a differential activity between lesioned and uninjured flies between day 5 and day 7. Furthermore, the measured GFP intensities are higher in both hemispheres after lesion, indicating a generally stronger inhibition by GABA.

These results suggest, that unilateral lesion induces synaptic changes, but that those changes seem to be synapse specific since each connection showed a distinct answer following injury. Most noticeably, unilateral lesion leads to changes in the homeostasis of excitatory and inhibitory input in each hemisphere. Moreover, as my results indicate, unilateral injury seems to have more influence on the activity of inhibitory synapses than on excitatory synapses.

Both excitatory projection neurons, PNv1 and PNv5, which I have analyzed in this thesis form synapses with ORNs which are sensitive to CO₂ (Kwon et al., 2007) and both have different functional properties, as noted previously (Batawi, unpublished; Lin et al., 2013), so this might explain the differential inhibitory input from GABAergic neurons onto these two projection neurons. I suppose, that PNv1 has capacities to detect the unilateral loss of ORN input, and due to its bilateral nature, this information gets conveyed to the contralesional hemisphere. My interpretation is, that PNv1, ipsilateral to the lesion, gets actively downregulated by the GABAergic neurons LNV2 and PNv3, to further suppress firing of a deafferented PN.

A study conducted by Kazama et al. (2011) could show that the removal of all ORN input to a specific subset of glomeruli leads to an increased activity of excitatory LNs and following that, deafferented PNs lose their glomerulus specific activity and get globally more active which in turn potentiates the signal transduction of intact maxillary palp ORNs via PNs up to higher brain centers. However, V-glomerulus only receives ipsilateral ORN input and Kazama et al. (2011) showed that unilateral antenna ablation didn't change the electrical signalling of eLNs, although all ORNs innervating the V-glomerulus in one hemisphere are removed that way, and they concluded that the compensation mechanism from eLNs gets only triggered if multiple glomeruli are completely deafferented. For my results, this might indicate that the heightened inhibitory input from LNv2 and PNv3 onto PNv1 in the injured hemisphere is V-glomerulus specific response which might not be observable for glomeruli which are bilaterally innervated by ORNs.

PNv5 on the other hand receives unilateral ORN input and no direct information about the contralateral sensory loss and therefore the change of GABAergic input onto PNv5 after unilateral lesion is most likely directed by PNv1 and therefore the adaption might be delayed. My results suggest, that initially the inhibitory input from LNv2 onto PNv5 is stronger in the uninjured hemisphere and I assume this happens because the system tries to balance the incoming odor stimuli, which results in downregulating the more active side trying to reach a similar activity level like the injured hemisphere. Unpublished data from Batawi shows, that PNv5 defines the main pathway for CO₂ detection and from studies (e.g. Kwon et al. (2007)) it is known that *Drosophila* shows a strong aversive behavior when exposed to CO₂, so even the low amount of CO₂ in atmospheric air might be enough to trigger such events.

Why the LNv2 → PNv5 synapse in the injured hemisphere is more strongly activated on day 7, so the opposite of day 3 and day 5, is not clear. It could be a direct effect caused by the lesion itself which is observable after 5 days. This is reportedly the time it needs for the glia cells to remove the severed ORNs from the system (MacDonald et al., 2006). The neurons of the microcircuit might need a specific timespan to adapt to the lesion and in the case of the LNv2 → PNv5 synapse this results in heightened GABAergic inhibition of the deafferented PN on day 7.

Unilaterally lesioned flies

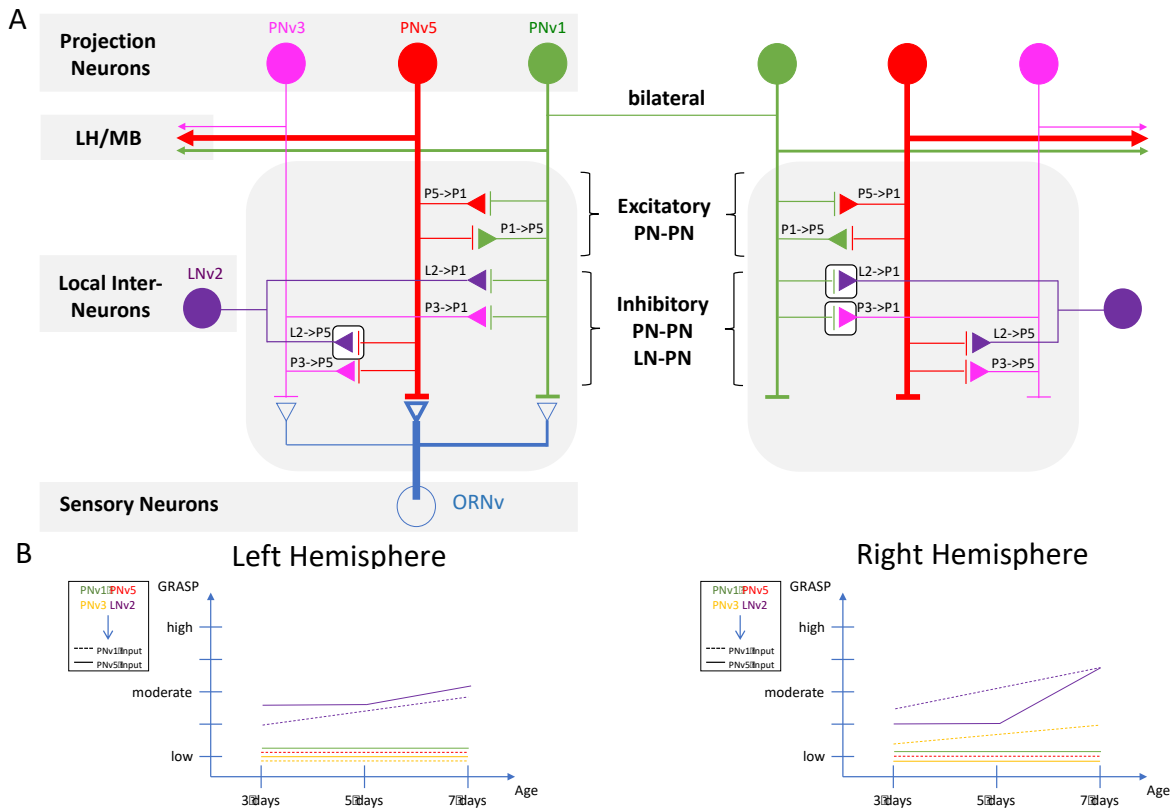


Figure 89. (A) Microcircuit of interconnected V-glomeruli. Synapses which show a distinct activity compared to other synapses are highlighted. **(B)** Diagrams are showing relative activity changes within one week for all synapses in each hemisphere. PNv3 and LNV2 synapses show increased activity in the right injured hemisphere in comparison with the left uninjured hemisphere for connections onto PNv1 (purple and yellow dashed line). The synaptic connection of LNV2 onto PNv5 was more active on day 3 and day 5 in the uninjured left hemisphere which was the opposite on day 7 (solid purple line)

In summary, compared to control flies, lesion induces plasticity, which involves changes of the complex interaction of excitatory and inhibitory neurons, which build the microcircuit of V-glomerulus. Those injury-induced changes of activity seem to be highly synapse specific. Furthermore, lesion induces higher synaptic activity for most synapses if compared to control flies, indicating that injury doesn't alter the activity of one synapse but has global effect on all synapses if the scope is V-glomerulus' microcircuit. I assume that PNv1 is matching incoming odor information, which results in a differential GABAergic input in both hemispheres, which in turn should have implications on excitatory synapses from PNv1 onto PNv5.

4.2 GRASP levels in wildtype flies following chronic stimulation with CO₂

Compared to control flies at atmospheric air, flies which were chronically stimulated with 5% CO₂ displayed changes of synaptic activity. This alteration was most noticeable for the PNv1 → PNv5 synapse, since after one week of chronic exposure the measured GFP intensities were substantially higher, indicating high excitatory input from PNv1 onto PNv5 (Fig. 90). Interestingly, when exposed to 5% CO₂, the sudden increase in synaptic activity of LNV2 → PNv1 is induced significantly earlier, indicating a critical threshold in sensory activity, while the inhibitory input from LNV2 onto PNv5 is steadily decreasing within one week (Fig. 90). Exposure to 5% CO₂ didn't lead to a differential activity pattern in either hemisphere, indicating that in uninjured wildtype flies both hemispheres show the same response, which should result in the natural avoidance behavior of *Drosophila* if confronted with CO₂ (Fig. 90).

Synaptic activity following chronic exposure to 5% CO₂ in uninjured flies can be separated in 3 groups. There are synapses which show increased activity after chronic exposure, synapses which show decreased activity after chronic exposure and synapses which showed a steadier activity within the timeframe of the conducted experiments. The excitatory connection from PNv1 onto PNv5 showed the greatest response following the exposure to 5% CO₂. This observation is supporting the notion, that the synaptic connection between the CO₂ sensitive ORN and PNv5 defines that main channel for CO₂ (Batawi, unpublished) and is compliant with my theory that PNv1 increases its excitatory input onto PNv5 to increase its activity.

To address the changes of the inhibitory synapses of LNV2 onto PNv1 and PNv5 I need to recapitulate what is known about LN function if flies are chronically exposed to odorants. Studies from Das et al. (2011) and Sachse et al. (2007) could show that if *Drosophila* gets chronically stimulated by odors, LN activity gets upregulated resulting in greater inhibition of PNs. Das et al. (2011) argued that LN activity is linked to a habituation process in the fly brain, which suggests in the case of CO₂, that flies show weaker avoidance behavior over time. Sachse et al. (2007) found that PNs have dampened output in higher brain centers after chronic stimulation which is compliant with the proposed habituation mechanism.

The results of the GABAergic input onto PNv1 and PNv5 after chronic stimulation presented in my thesis might unmask a novel mechanism of how habituation occurs in wildtype flies.

Upon chronic stimulation with 5% CO₂ it was shown that the inhibitory input from LNV2 onto PNv5 is decreasing within one week. This contradicts the theory of increased LN activity due to the onset of habituation (Das et al., 2011; Sachse et al., 2007). Furthermore, I assume, based on previous work (Batawi, unpublished), that the activity of PNv5 would be the first neuron to be attenuated if the fly gets habituated to CO₂. However, contrary to the observation for the LNV2 → PNv5 synapse, I could show that chronic exposure to CO₂ increases inhibition on PNv1. LNV2 might inhibit PNv1 more strongly during habituation, so that PNv1 on the other hand has dampened excitatory input on PNv5. I propose an indirect way of habituation, which is controlled by excitatory connection from PNv1 onto PNv5 and I assume, that the activity of PNv5 might be even higher after 7 days if this indirect effect wouldn't be present. This assumption gets further substantiated by the results, which I observed for the PNv3 → PNv5 and the PNv5 → PNv1 synapse. Chronic stimulation with CO₂ lowered the activity of PNv3 onto PNv5, indicating weaker inhibition while the excitatory input from PNv5 onto PNv1 was initially increased, but got lower within one week. On a side note, my results didn't confirm the observation from Lin et al. (2013) that PNv3 inhibits PNv1 at higher CO₂ concentrations since the measured activity levels in my thesis were similar between both CO₂ treatments and not increased at 5% CO₂.

Chronically stimulated wild type flies

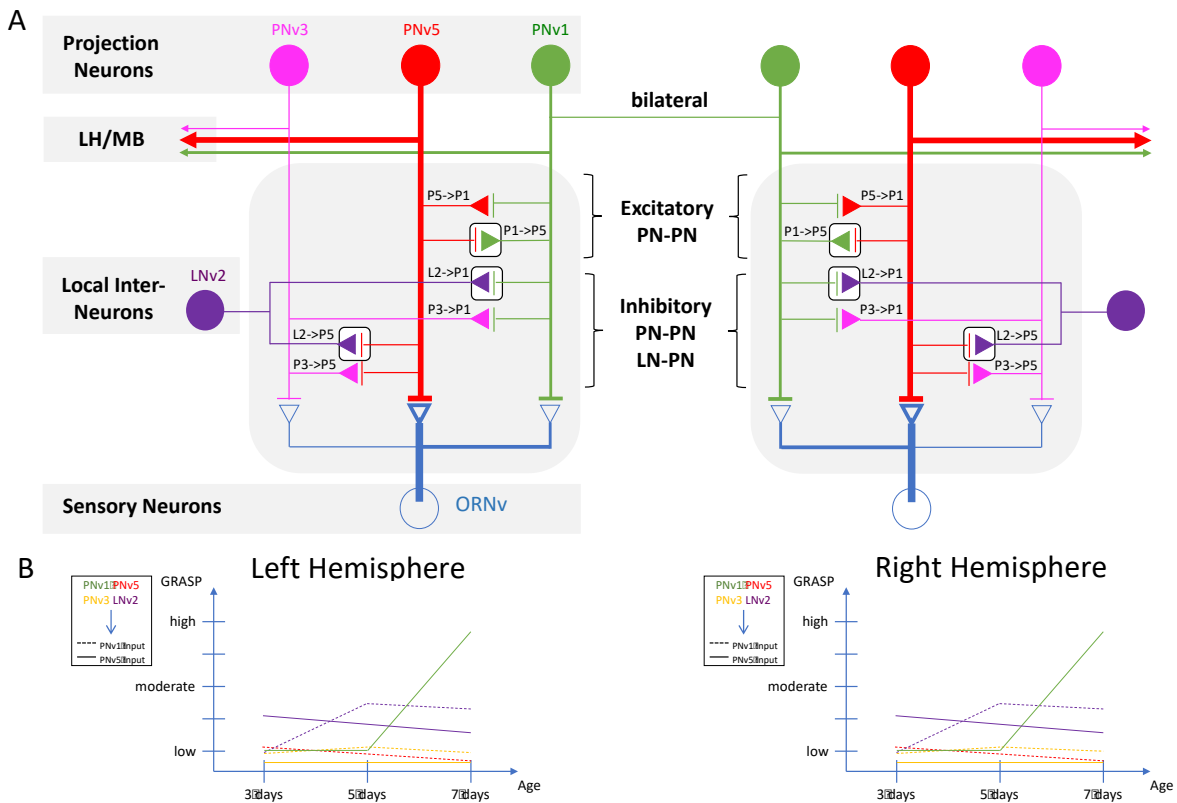


Figure 90. (A) Microcircuit of interconnected V-glomeruli. Synapses which show a distinct activity compared to other synapses are highlighted. **(B)** Diagrams are showing relative activity changes within one week for all synapses in each hemisphere. The LNV2 → PNv1 synapse shows increased activity as of day 5 (dashed purple line) while the activity of the LNV2 → PNv5 decreases within one week (solid purple line). The PNv1 → PNv5 synapse showed a substantially increased activity on day 7 following exposure to CO₂ (solid green line).

4.2.1 Changes of GRASP levels in bilaterally lesioned flies following chronic stimulation with CO₂

Bilateral ablation of the antennae with additional chronic sensory stimulation resulted in rather low activities of the synapses (Fig. 91). I could not show significant differences between the hemispheres, indicating that the bilateral lesion is affecting both hemispheres similarly. The PNv1 → PNv5 synapse was almost silent as it was already shown for bilaterally lesioned flies at atmospheric CO₂ conditions (Fig. 91). Interestingly, the GABAergic connections from LNV2 onto PNv1 and PNv5 as well as the connection from PNv3 onto PNv5 showed increasing activity when exposed to 5% CO₂, which was not observable at ambient air (Fig. 91).

The influence of CO₂ on a bilaterally lesioned system was expected to be rather low, since with the removal of the antennae, the fly's ability to sense CO₂ should be gone according to Jones

et al. (2007). Further, if the fly can't sense CO₂ anymore its response should be indifferent to exposure to CO₂, yet it is possible to see changes in synaptic activity. Initially both hemispheres respond differently to 5% CO₂, as the synaptic activity of connections from LNV2 onto PNv1 suggest, but after one week of chronic stimulation the activity is clearly higher in both hemispheres. The PNv3 → PNv5 and the LNV2 → PNv5 synapses also reveal increasing activity in both hemispheres following bilateral lesion at 5% CO₂, which is also substantially higher than in uninjured flies for the latter connection. If the fly doesn't have organs to sense CO₂, why is it possible to see these differences? This suggests, that *Drosophila melanogaster* might have another pathway to detect CO₂ besides the ORNs which are responsive to that odor. It would be interesting to do further experiments with the removal of maxillary palps because they were not included in my ablation experiments. The possibility stands, that they have properties to sense CO₂, which were not considered in this thesis.

Chronically stimulated bilaterally lesioned flies

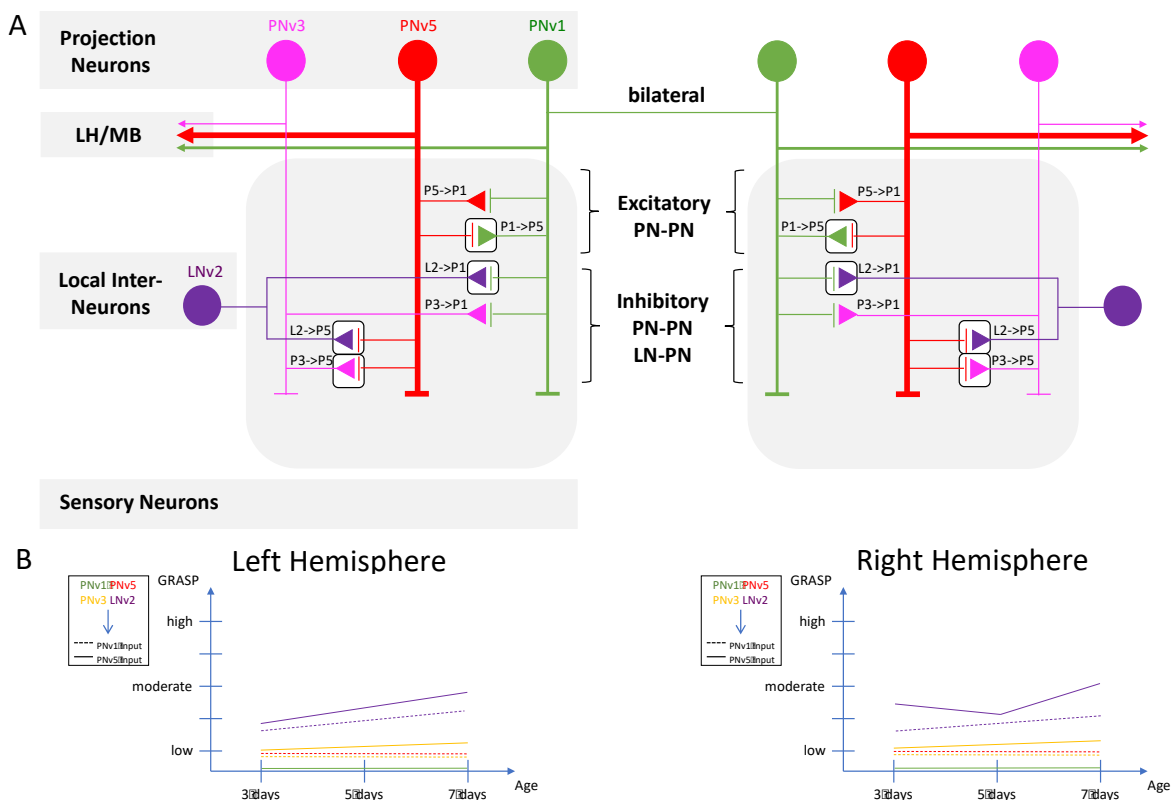


Figure 91. (A) Microcircuit of interconnected V-glomeruli. Synapses which show a distinct activity compared to other synapses are highlighted. **(B)** Diagrams are showing relative activity changes within one week for all synapses in each hemisphere. The activity of LNV2 synapses is increasing in both hemispheres within one week (solid and dashed purple line) and so was the synapse of PNv3 onto PNv5 (solid yellow line). The excitatory synapse of PNv1 onto PNv5 showed very low activity on all days (solid green line).

4.2.2 Changes of GRASP levels in unilaterally lesioned flies following chronic stimulation with CO₂

Unilateral lesion and chronic stimulation with 5% CO₂ influenced the activity of most synapses. The synapses from PNv3 and LNV2 onto PNv1 showed that the activity was increased in the injured hemisphere (Fig. 92). The inhibitory input from PNv3 onto PNv5 was also slightly increased in the injured hemisphere, although not to the same extent as it was shown for the PNv3 → PNv1 synapse (Fig. 92). The synaptic activity of connections from LNV2 onto PNv5 was also increased in the injured hemisphere, most evidently on day 7 (Fig. 92). Upon chronic stimulation with elevated CO₂ levels I could show that the excitatory cholinergic PNv1 → PNv5 synapse was clearly more active on the uninjured side (Fig. 92). Furthermore, the exposure to 5% CO₂ upregulated the absolute activity of most synapses regardless which hemisphere was analyzed but the relative interhemispheric differences caused by lesion were unaffected.

It was interesting to see that the differential activity pattern between the right injured and the left uninjured hemisphere, which I could already show after unilateral lesion in atmospheric air, was still observable. Further, since exposure to CO₂ drives activity in V-glomeruli, these interhemispheric differences are even more evident. Generally, if comparing the inhibitory output from GABAergic neurons between the hemispheres, I can observe that the right injured hemisphere is more strongly inhibited than the unharmed hemisphere, which is most likely a combined effect caused by the lesion and boosted by CO₂ exposure. This indicates that the olfactory system generally reacts with stronger inhibition after injury. On the contrary, the activity of PNv5 gets upregulated by PNv1 in the uninjured hemisphere following chronic stimulation, which was not observable at atmospheric air. Considering that flies usually respond with aversive behavior if exposed to CO₂, enhancing the activity of the remaining PNv5 with a connection to an active ORN makes sense to ensure an optimal response of the fly.

Although, it could have been assumed that this rarely happens in the wild that flies lose one of their antennae and that there is no need to have capacities to induce plasticity trying to compensate for the loss of input, it was shown that flies have this property and that the circuit of the antennal lobes is responding to injury (Kazama et al., 2011). I suggest that the bilateral PNv1 plays a crucial role for this mechanism. Rybak et al. (2016) could show that LNs, PNs and ORNs are interconnected with each other, indicating that there is a constant communication

between them. Therefore, I suppose that the contralateral projection of PNv1 is communicating with LNV2 and PNv3 on the uninjured side which in turn results in lower inhibition of PNv5, which still has connections with active afferents.

Chronically stimulated unilaterally lesioned flies

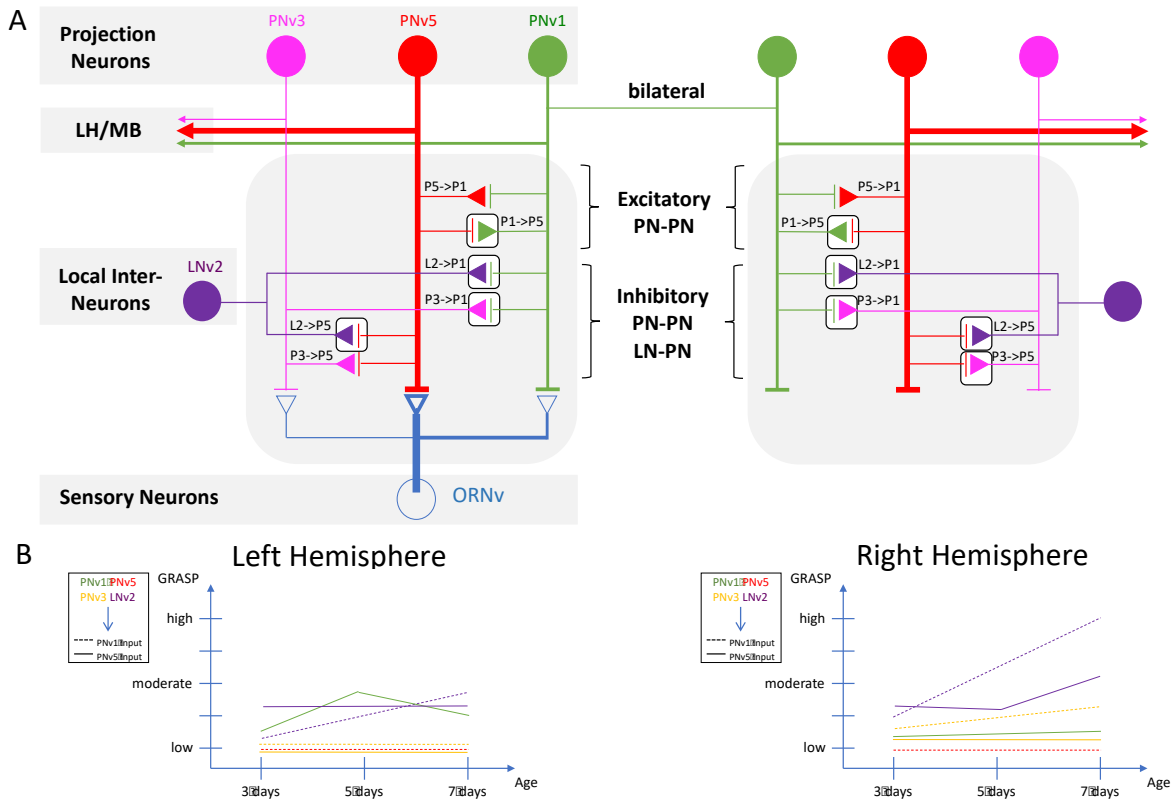


Figure 92. (A) Microcircuit of interconnected V-glomeruli. Synapses which show a distinct activity compared to other synapses are highlighted. **(B)** Diagrams are showing relative activity changes within one week for all synapses in each hemisphere. All GABAergic synapses show increased activity in the injured hemisphere (solid and dashed purple line, solid and dashed yellow line). The excitatory PNv1 → PNv5 synapses displays increased activity in the uninjured left hemisphere (solid green line).

In summary, exposure to 5% CO₂ unmasked an interaction of the PNv1 → PNv5 synapse, which is potentially crucial so that the flies remain sensitive to CO₂ and a proper aversive behavior gets elicited. Generally, lesion seems to upregulate GABAergic synapses and differential interhemispheric activity gets further influenced if the V-glomerulus is additionally stimulated by CO₂. Interestingly, bilaterally lesioned flies showed response to chronic stimulation which indicates that the increased CO₂ levels still affected the connectivity of synapses.

4.3 A reverse unilateral cut leads to a reverted GFP intensity relation between the hemispheres

Cutting the left antenna instead of the right antenna resulted in a similar but reverted GFP intensity ratio between the hemispheres. Interestingly, the synaptic input from LNV2 onto PNv1 and PNv3 onto PNv1 was differently affected. While the reverse cut didn't affect the intensity measured at the PNv3 → PNv1 synapse, it resulted in a decreased differential input from LNV2 onto PNv1. This result is surprising, since both synapses are inhibitory in nature and the innervation pattern of LNV2 and PNv3 is similar. The lower GFP intensities, which can be seen for the LNV2 → PNv1 synapse, after I made the switch and ablated the left antenna could have multiple reasons. First, unilateral lesion might affect the hemispheres differently so that the olfactory system has laterality for inducing plasticity. However, this requires further experiments to figure out if the LNV2 populations on either side possibly differ in their expression of genes which might be related to injury-induced plasticity. Second, as Chou et al. (2010) noted, the variability of LN innervation pattern can vary between animals which leads to a third possibility that the results obtained are stochastic. This differential activity pattern has to be kept in mind for further research.

4.4 Sensory deprivation is sufficient to trigger synaptic plasticity

In contrast to the results of Kazama et al. (2011), that silencing the ORNs is not sufficient to trigger plasticity I could show that the application of UV-glue on the antenna is sufficient. Interestingly, similar to the reverted unilateral cut, the GFP intensities decreased for the LNV2 → PNv1 synapse after sensory deprivation. The possibility stands, that UV-glue is not as effective as the mutation used by Kazama et al. (2011) or that the glue is corrosive so that the caused injury resembles a cut antenna, just to a lesser degree.

5. Conclusion & Future directions

As my results suggest, the olfactory system of *Drosophila melanogaster* is capable to undergo synaptic plasticity to enhance remaining connections after lesion occurs, which can be compared to results presented for research done in mammals. I could show that the interaction of inhibitory and excitatory synapses is crucial for this process and that the activity dependent

GRASP technique is capable of showing occurring changes. Still, some questions are left open, which need to be addressed in the future.

Interhemispheric communication: It remains unclear, to what degree the bilateral nature of PNv1 aids the recovery of the system and how bilateral populations of LNs respond to lesion and chronic exposure to CO₂.

Non-neuronal components: The function of glia cells is crucial for clearing the system of neuronal debris after lesion (Macdonald et al., 2006) and there are strong arguments that they are heavily involved in synapse formation and synaptic plasticity (see review: Stogsdill and Eroglu, 2017). The possibility stands that they are also involved in compensation mechanisms.

Genetic regulation: A forward genetic RNAi screen might be useful to gather more information about genes which might be crucial for synaptic plasticity and recovery after lesion. As initially mentioned, lesion leads to an upregulation of several genes which seem to be necessary for plasticity after stroke. If orthologue genes could be found in *Drosophila melanogaster*, which can be targeted with RNAi lines further insight into functional recovery after lesion could be achieved and it would strengthen the position of *Drosophila melanogaster* as an organism for such experimental approaches.

Excitatory LNs: The role of excitatory LNs was not considered in this thesis and Das et al. (2011) already noted, based on the work of Yaksi and Wilson (2010), that they might also influence habituation which cannot be neglected in further research.

Behavioral analysis for functional recovery: Another interesting aspect would be to consider a behavior assay which tests for behavioral recovery after lesion. If unilaterally lesioned flies would show an altered avoidance behavior in a T maze, similarly to the setup of Siju et al. (2014), with ambient air (0.08% CO₂) and 5% CO₂ compared to uninjured flies it would indicate an impairment in odor perception. Then, if unilaterally lesioned flies perform better after a given amount of days, more resembling the behavior of healthy flies it could be argued that flies underwent recovery following lesion. Flies might also have a specific timeframe where

the brain is more plastic and the focus in future research should be to find ways to precisely boost recovery and identify all the factors which are involved.

6. Acknowledgments

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7.1 Intensity measurement

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