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Abstract

Brain asymmetry is a widespread feature throughout the animal kingdom and has been correlated to lateralization of behavior, cognition, and neurological diseases in humans. Despite recent advances using genetically accessible model organisms like C.*elegans* or the zebrafish, the developmental basis of neural asymmetries remains largely unknown. The Asymmetric Body within the Central Complex of Drosophila *melanogaster* which has been correlated to memory function, consists of a bilateral cluster of neurons characterized by left/right differences in synaptic connectivity. Employing the powerful genetic tools available for Drosophila I could show asymmetrical connectivity to develop from a bilaterally symmetric ground state progressing through a phase of axonal remodeling. The process of remodeling is controlled by a tight spatio-temporal control of the cell adhesion molecule Fascilin II, leading to a model in which dynamic cell adhesion gates a window of heightened plasticity enabling the transition from symmetry to asymmetry. This study is the first one suggesting cell adhesion to be critical for the development of brain asymmetry, highlighting the importance of utilizing different model organisms to discover new mechanisms leading to related developmental outcomes.

Zusammenfassung

Asymmetrie im Nervensystem ist ein weitverbreitetes Phänomen im Tierreich und wurde mit der Lateralisation von Verhalten, Kognition und neurologischen Erkrankungen in Verbindung gebracht. Trotz Fortschritten in genetisch manipulierbaren Modellorganismen ist über die Entwicklung von neuronalen Asymmetrien wenig bekannt. Der „Asymmetric Body“ im „zentralen Komplex“ des Nervensystems von Drosophila *melanogaster* wurde mit Langzeitgedächtnis in Verbindung gebracht und besteht aus einer bilateralen Population von Neuronen mit Links/Rechts Unterschieden in der synaptischen Konnektivität. Mit Hilfe der

genetischen Werkzeuge für Drosophila konnte ich zeigen, dass die asymmetrische Konnektivität des „Asymmetric Body“ aus einem bilateralen Grundzustand nach einer axonalen Umbauphase entsteht. Diese Umbauphase wird durch das Zelladhäsionsmoleküls Fasciclin II reguliert. Im hier vorgestellten Modell, kontrolliert eine dynamische Zelladhäsion eine Phase erhöhter Plastizität, welche den Übergang von einer symmetrischen zu einer asymmetrischen Organisation ermöglicht. Diese Studie ist die erste, welche eine Rolle von Zelladhäsion bei der Entwicklung von neuraler Asymmetrie impliziert. Dies unterstreicht die Wichtigkeit ein Phänomen in unterschiedlichen Modellorganismen zu untersuchen, um neue Wege zu finden die zu ähnlichen Ergebnissen führen.

1. Introduction

While most living animals show a symmetrical body plan across a L/R axis (güntai, Kenny, & Shimeld, 2014) a superficial look is enough to realize that this symmetry is not perfect. In humans, for instance, the liver is positioned on the right side of the body, while the heart is located on the left. These left-right asymmetries not only show themselves in the positioning of our organs but also in our behavior, as we can experience every day on the phenomenon of handedness. Differences between the two hemispheres of our brain have been linked not only to such behavioral lateralization but also to various mental processes (Güntürkün & Ocklenburg, 2017). Associations between brain asymmetries and cognition have been found in a wide range of species including mice (Aizawa, Goto, Sato, & Okamoto, 2007), different avian species (Güntürkün & Ocklenburg, 2017), and various invertebrates (Frasnelli, 2013). These asymmetries are thought to aid cognitive function through an enhanced ability for parallel processing (Rogers, Zucca, & Vallortigara, 2004).

In humans, the first link between neural asymmetry and cognitive function was established through the discovery of lateralized areas necessary for speech production and comprehension within the left hemisphere (Berker, Berker, & Smith, 1986; Wernicke 1874). Since these early findings of language lateralization, more correlations of brain asymmetries and cognitive functions were found through the observation of brain damage and neuroimaging. Spatial attention (Li & Malhotra, 2015), as well as the processing of faces (Meng, Cherian, Singal, & Sinha, 2012; Calvo & Beltrán, 2014) and emotions (Gainotti, 2019) all show some degree of lateralization within the human brain. Perhaps reflecting the connection to cognitive function, alterations in neural asymmetries have been associated with various diseases affecting mental processing, like schizophrenia (Oertel-Knöchel & Linden, 2011), autism (Postema et al., 2019), or developmental language disorder (Herbert et al., 2005).

Despite its relevance for behavior and cognition the anatomical and developmental basis of these asymmetries remain largely unknown. However, the heritability of handedness and hemispheric speech dominance as well as findings of differential gene expression (Karlebach and Francks, 2015), between the two hemispheres suggest an at least partly genetic determination (Concha et al., 2012). In recent years genetically manipulated model organisms, most prominently C.*elegans* and the zebrafish, enabled the study of the molecular pathways underlying the development of neural asymmetries (Concha et al., 2012).

To account for the highly variable nature of neural asymmetries within and between species (Concha et al., 2012)suggested a classification into two basic classes. Class I asymmetry describes neural asymmetries with shared components occurring in different frequencies on the left and right hemispheres of the brain, while class II asymmetry occurs if a component is only present in one hemisphere. The two different types of asymmetry can manifest themselves at multiple levels of organization, including, cell bodies, neurites, synaptic connectivity, and gene

expression (Concha et al., 2012). Additionally, neural asymmetries can be classified regarding their directionality. While directional asymmetry describes the alignment of the sidedness of asymmetry within a population, anti-symmetry is characterized by a random distribution of sidedness within the population (Concha et al., 2012).

Due to its compact nervous system and its genetic accessibility, neural asymmetries have been described in most detail within the nematode C.*elegans* (Hobert, Johnston, & Chang, 2002). While AWC olfactory neurons are bilaterally symmetric regarding their morphology and position of their cell bodies, (White, Southgate, Thompson & Brenner, 1986) only one of these neurons expresses a certain olfactory receptor (Troemel, Sagasti, & Bargmann, 1999). This class II asymmetry at the level of gene expression is determined through a stochastic process depending on axonal contact of the two neurons (Troemel et al., 1999). As a result of this random process, there is no sidedness of asymmetry in the population (Graham, Freeman, & Emlen, 1993). Therefore AWC neurons can be classified as being antisymmetric.

ASE neurons, another pair of bilateral chemosensory neurons within C.*elegans* show a more complex asymmetrical organization (Hobert et al., 2014). Multiple genes are expressed either exclusively (Class II asymmetry) or predominantly (Class I asymmetry) in either the right or the left ASE neuron in a directional manner (Ortiz et al., 2006). Additionally, the same neurons display a directional class I type asymmetry at the level of cell bodies, evident through a bigger size of the right ASE cell body compared to the left one (Goldsmith, Sarin, Lockery, & Hobert, 2010). Both classes of asymmetry result from L/R differences in the early embryonic extracellular environment. Subsequent differences in chromatin configuration between the left and the right ASE neurons, then give rise to asymmetry after the last cell divisions (Goldsmith et al., 2010).

Finally, C.*elegans* displays a class II directional asymmetry at the level of whole cells. Four of its neurons are solely present at one side of the body without a homologous counterpart on the other side (Hobert et al., 2002). Although unilateral neurons have been described in other species like the hermit crab (Chapple, 1977) or the leech (Shankland & Martindale, 1989), the development of this kind of neurons in C.*elegans* is unique. While in the former two species an initially symmetric population of neurons becomes asymmetric through selective apoptosis on one hemisphere, unilateral neurons in C.*elegans* arise through asymmetric cell division producing a unilateral neuron on one side, and a different neuron type or a non-neural cell on the contralateral side (Hobert et al., 2002). Therefore unlike ASE and AWC neurons which develop asymmetry only after a prior symmetric organization, unilateral neurons in C.*elegans* never undergo a bilateral state.

The zebrafish as the second leading model for studying brain asymmetry displays directional Class I asymmetries at the levels of connectivity, (Gamse et al., 2005), gene expression (Gamse, Thisse, Thisse, & Halpern, 2003), and relative size of subnuclei (Hidenori et.al, 2005)within its diencephalon. These asymmetries were found to be at least partially dependent on a prior establishment of a Class II asymmetry in the form of the unilateral location of the parapineal organ (pp) (Gamse et al., 2003). Bilaterally symmetric precursors of the pp aggregate at the midline, followed by a migration to the left hemisphere in response to unilateral nodal signaling (Gamse et al., 2003). Therefore the zebrafish diencephalon provides an example of a hierarchy of asymmetries (Concha et al., 2012).

Despite the high diversity in class, level, and directionality in neural asymmetries, at least two principles regarding their development can be extracted from the examples given above. Firstly, asymmetry tends to develop from bilateral symmetry as seen within AWC and ASE neurons (Hobert, Johnston, & Chang, 2002) as well as the zebrafish diencephalon (Gamse et al.,

2003) and at the unilateral neurons of the hermit crab (Chapple, 1977) and the leech (Shankland & Martindale, 1989). Here unilateral C.*elegans* neurons are an exception which, unlike unilateral neurons found in the leech and the hermit crab, never show a symmetric organization. Secondly, different classes and types of asymmetry can be coupled. This can occur through a single symmetry-breaking event, leading to different asymmetries as has been observed in *C.elegans* ASE neurons, where differences in gene expression and cell size both occur due to L/R differences in the early extracellular environment (Goldsmith et al., 2010). Alternatively, the coupling of asymmetries might reflect a hierarchical organization where one type of asymmetry directly leads to further asymmetries as seen in the zebrafish diencephalon (Wilson, Bianco, Carl, Russell, & Clarke, 2008). While the zebrafish has provided valuable insights into asymmetric development of the brain, there are limitations regarding the transferability human brain development. Here nodal signaling is unlikely to be critical at least for the functional asymmetries of speech and handedness.

In 2002 Hobert et al. noted that due to its well-mapped nervous system and the availably of sophisticated genetic and molecular tools, C.*elegans* was the only invertebrate model enabling a study of neural asymmetry in great detail. This changed two years later when the discovery of the Asymmetrical Body (AB) in the Central Complex of the Drosophila brain (Pascual, Huang, Neveu, & Préat, 2004) enabled the investigation of neural asymmetry in an organism having a more complex nervous system than C.*elegans*.

The Central Complex which is compromised by a group of highly interconnected neuropiles involved in sensory integration and the generation of different locomotory behaviors (Wollf & Rubin 2018). The AB was discovered by staining the cell adhesion molecule FasII. Here a directional asymmetry at the level of protein localization was found, with only the right neuropile of the AB expressing FasII (Pascual et al., 2004). A small percentage of flies displaying a

bilaterally symmetrical expression of FasII were found to have impaired long-term memory (Pascual et al., 2004), providing a further example of a correlation between cognitive function and neural asymmetry.

Part of the anatomical basis of the AB was resolved when Jenett et al. (2012) identified a Gal4 line labeling bilateral neurons, unilaterally innervating the right hemisphere. While the axons of left neurons innervate ipsilaterally, neurons from the right hemisphere cross the midline to innervate contralaterally (Jenett et al., 2012; Markowitsch 2019). Because these neurons receive synaptic input in the superior lateral protocerebrum (SLP), Wolff and Rubin (2018) termed them AB-SLP neurons (Fig 1. A, B). A knockdown of FasII within AB-SLP neurons showed unilateral FasII expression to be a consequence of their asymmetric innervation pattern (Markowitsch, 2019). AB-SLP neurons give presynaptic input to Fan-shaped body AB neurons (AB-FB neurons) which themselves display class I asymmetry through a L/R size difference in their dendrites (Markowitsch 2019) (Fig 1. A, B).

While the adult morphology and connectivity of the AB circuit have been established (Jenett et al., 2012; Markowitsch 2019; Alsberga 2020; Wolff & Rubin 2018) (Fig 1. A, B), the developmental basis of the AB circuit is not fully resolved. Markowtisch (2019) found an early bilateral FasII expression at 30h after pupa formation (APF) (Fig. 1 C), which could indicate the establishment of asymmetry onto a symmetrical ground state of AB-SLP Neurons. Alternatively, an asymmetric pattern of axonal growth could establish asymmetry without going through a bilateral state. Regarding the development of AB-FB neurons, Alsberga (2020) found them to bilaterally segregate from FasII positive neurons dorsal to the AB until they start to innervate the AB at both hemispheres at about 35h APF (Fig 1. C-E).

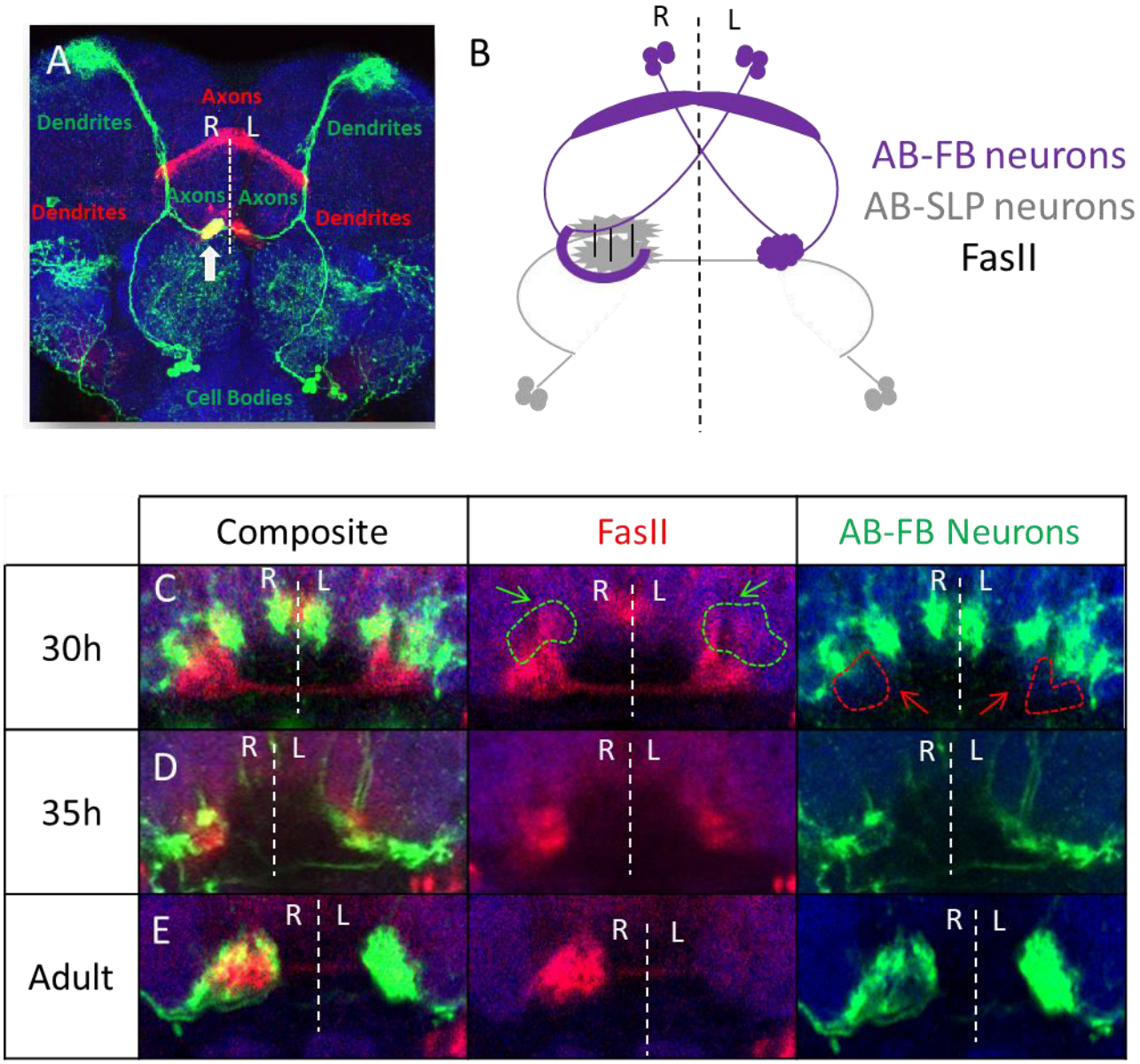


Figure 1. Organization and Development of the Asymmetric Body Circuit. **A:** Class II asymmetry of bilateral AB-SLP neurons unilaterally innervating postsynaptic FB-AB neurons, which themself display class I asymmetry showing a bigger innervation on the right than on the left hemisphere (adapted from Markowitsch (2019)). **B:** Connectivity model illustrating FasII expression, unilaterally innervating AB-SLP neurons, and AB-FB neurons. **C, D, E:** Development of postsynaptic FB-ABNs in relation to FasII. **C:** At 30h APF FasII (red) is expressed on both hemispheres. Postsynaptic FB-ABNs (green) are in close proximity *to* the FasII positive region. **D:** At 35h a fading FasII signal on the left hemisphere coincides with the movement of FB-ABNs into the AB region. **E:** In the adult, FasII expression is fully lateralized to the right side, while FB-ABNs innervation both hemispheres (adapted from Alsberga 2019).

Building on these findings the aim of this thesis was to 1. Investigate the development AB-SLP neurons, to gain insight about the developmental dynamics of these neurons in relation to AB-FB neurons 2. Investigate the role of FasII as a potential determinant of asymmetric development.

FasII, the ortholog of the mammalian NCAM (Grenningloh, Jay Rehm, & Goodman, 1991) is a homophilically binding cell adhesion molecule expressed in multiple isoforms (Grenningloh et al., 1991; Harrelson & Goodman, 1988). The isoforms have a common extracellular domain but differ in their intracellular domain (Kristiansen V., Lars, 2010) , with some variants possessing a PEST degradation sequence (Kristiansen V., Lars, 2010), leading to a higher turnover of the protein (Rechsteiner 1988). FasII was found to be involved in developmental processes like selective fasciculation during axonal outgrowth (Bastiani, Harrelson, Snow, & Goodman, 1987; Grenningloh et al., 1991), synaptic growth. (Ashley, 2005), and the pruning of neural processes (Bornstein et al., 2015).

Changing the relative levels of fasII across pre- and postsynaptic sites has been shown to lead to alterd altered patterns of synaptic connections at the neuromuscular junction (Davis, Schuster, & Goodman, 1997) as well as in the central nervous system (Baines, Seugnet, Thompson, Salvaterra, & Bate, 2002). A further level of complexity is added by findings that the relative levels of some isoforms to influence synaptic development while others do not (Ashley., 2005; Beck et al., 2012).

The sensitivity of these developmental processes to changes in FasII levels and the expression of specific isoforms is perhaps reflected by the multiple factors found to regulate FasII at multiple levels, including a micro RNA (Niu, Liu, Nian, Xu, & Zhang, 2019). and two different transcription factors. (Timmerman et al., 2013). Additionally, FasII is post-translationally regulated by multiple kinases (Bornstein et al., 2015; Hoeffer, Sanyal, &

Ramaswami, 2003; Koh, Ruiz-Canada, Gorczyca, & Budnik, 2002; Spring, Brusich, & Frank, 2016) and an endosomal protein regulating FasII membrane stability (Nahm et al., 2016).

The strong expression of FasII within the AB together with its prominent role in brain development and its dynamic expression, makes this protein a promising candidate for a determinant of AB asymmetry. Through a combination of gain and loss of function experiments and the use of Gal4 lines labeling AB-SLP neurons, AB-FB neurons as well as a transiently FasII expressing population of AB adjacent ventral Fan-shaped body neurons (vFB neurons), I could show that FasII plays a critical role in the transition from an early bilaterally symmetric organization to its asymmetric innervation pattern in the adult. These results led to a model in which asymmetric remodeling of AB-SLP neurons is controlled by FasII mediated gating of a critical window of circuit plasticity.

2. Methods

2.1. Fly stocks and genetics

Flies were raised on standard fly food at 25°C. RNAi and overexpression experiments were conducted at 29°C. For loss of function studies RNAi, well as a mutant approach, was followed. For the knockdown and overexpression experiments, the following strains were used: Fas2-Gal4 (Sulzbacher et al., 1997); RH5203-Gal4 (Jenett et al., 2012); UAS-Fas2-RNAi (Perkins et al., 2015) UAS-FasII^{PEST-} (Bornstein et al., 2015); 10x UAS-mCD8::TMT, 10x UAS-mCD8::GFP and R52H03-lexA (Jenett et al., 2012). Following hypomorphic FasII mutants were used: FasII^{EB112}, FasII^{e76} (Grenningloh et al., 1991), and FasII^{rd2} (Cheng et al., 2001). The following isoform-specific FasII mutants were provided as a gift from C. Klämbt: FasII$^{\Delta TM}$, FasII$^{\Delta PC}$; FasII$^{\Delta PB}$; FasII$^{\Delta PB\,\Delta TM}$; (Neuert, Deing, Krukkert, Naffin, & Steffes, 2019).

Table 1. List of Fly Lines

Genotype	Source
Fas2-Gal4	(Sulzbacher et al., 1997)
RH5203-Gal4	(Jenett et al., 2012)
R52H03-lexA	
UAS-Fas2-RNAi	(Perkins et al., 2015)
UAS-FasII^{PEST-}	(Bornstein et al., 2015)
10x UAS-mCD8::TMT	Bloomington Drosophila Stock Center
10x UAS-mCD8::GFP	
FasII^{EB112}	(Grenningloh et al., 1991)
FasII^{e76}	
FasII^{rd2}	(Cheng et al., 2001)
FasII$^{\Delta TM}$	(Neuert, Deing, Krukkert, Naffin, & Steffes, 2019)
FasII$^{\Delta PC}$	
FasII$^{\Delta PB}$	
FasII$^{\Delta PB\ \Delta TM}$	

2.2. Immunohistochemistry

After dissection in PBS (phosphate-buffered saline), brains were fixed in 2% PFA (paraformaldehyde) in PBS for 1h. After washing the samples (4x15m) in 0.3% PBT (PBS containing 0.3% TirtonX-100) the brains were blocked with 10% Goat Serum in PBT. After adding the primary antibodies, samples were incubated overnight at 4°C. Subsequently, the brains were washed again in PBT and the secondary antibody (diluted in Goat Serum) was added followed by overnight incubation at 4°C and washing in PBT. For mounting whole brains were placed in VECTASHIELD (Vector Laboratories).

Table 2. List of Antibodies used for Staining.

Type	Antibody	Origin	Source
Primary	α-NCadherin (Ncad)	Rat	DSHB
Primary	α-Fasciclin II (FasII)	Mouse	DSHB
Secondary	α-Rat Alexa 647	Goat	Invitrogen
Secondary	α-Mouse Alexa 568	Goat	Invitrogen
Secondary	α-Mouse Alexa 488	Goat	Invitrogen
Secondary	α-Rabbit Alexa 488	Goat	Invitrogen

2.3. Imaging and processing

Brains were imaged using a Leica Confocal Microscope TCS SP5II. Scanning was conducted with a resolution of 512 x 512 pixels at 400 Hz employing a space of 1.5µm between the single optical sections. Processing of the acquired images was done with the image processing program ImageJ®. Statistical analysis was conducted using Rstudio.

3. Results

3.1. AB-SLP Asymmetry Derives from a State of Bilaterally Symmetric Innervation.

The first aim was to determine whether the bilateral FasII expression at 30h after pupa formation (APF) observed by Markowitsch (2019), corresponds to an early bilateral organization of AB-SLP neurons. To address this, I used R52H03-lexA to drive mCD8::GFP in AB-SLP neurons at different stages of pupa development. FasII expression was determined with an antibody recognizing the transmembrane isoforms of FasII (Fig.2). FasII expression on both hemispheres overlaps with the bilateral innervation of AB-SLP neurons at 30h APF (Fig. 2 A). This suggests the bilateral FasII expression observed at 30h by Markowitsch (2019) indeed to be a consequence of an early AB-SLP neurons innervation on both hemispheres.

Left processes of AB-SLP neurons are in the process of being removed at about 35h APF In the adult, left AB-SLP neurons processes have retracted completely, resulting in a lateralized AB-SLP neuron morphology and unilateral FasII expression in the adult (Fig: 2 B, C).

At the symmetrical state of AB-SLP neuron innervation at 30h APF, a bilateral FasII expression could not only be observed within AB-SLP neurons, but also within a pair of adjacent ventral Fan-shaped Body neurons (vFB neurons) transiently expressing FasII until 35h APF (Fig.2 C). During the remodeling process between 30h and 40h APF, FasII is maintained in AB-SLP

neurons while FasII expressed by vFB neurons has been downregulated (Fig.2B.) Together these results show that AB-SLP neuron lateralization arises from the remodeling of a bilaterally symmetric ground state, following a possible FasII mediated interaction between AB-SLP neurons and vFB neurons.

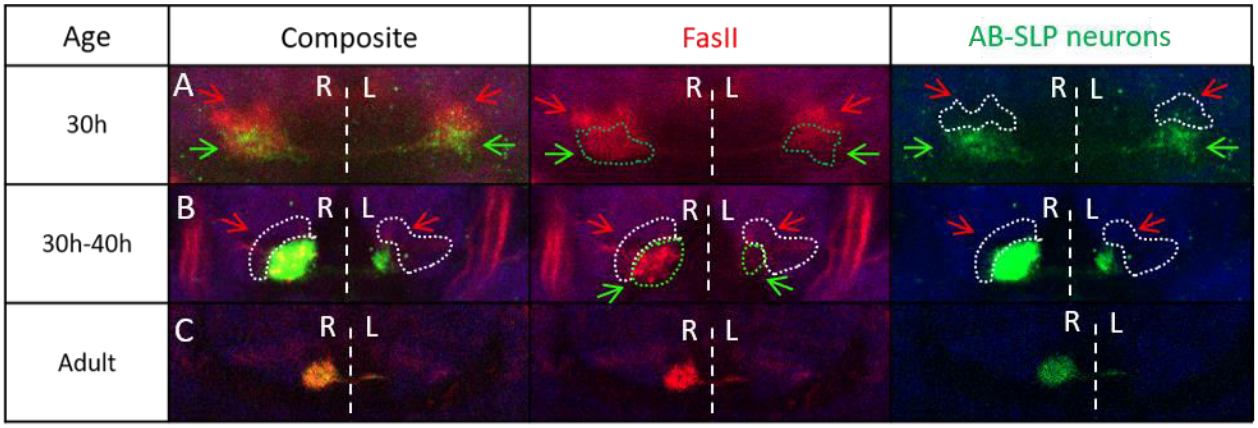


Figure 2. AB-SLP neuron asymmetry arises from a bilaterally symmetric state. A: At 30h APF AB-SLP neuron axons (green) overlap with bilateral FasII (red) expression. At this stage, FasII is also expressed by ventral Fan-shaped body neurons (vFB neurons) near the bilateral AB-SLP neurons (red arrows). **B:** Between 30h and 40h APF left processes of AB-SLP neurons have begun to retract from the left hemisphere. FasII expression is restricted to AB-SLP neurons (red and green arrows) **C:** In the adult AB-SLP neuron innervation and FasII expression is fully lateralized, Brains were stained against NCAD (blue) for reference. R52H03-lexA was used to drive mCD8::GFP in AB-SLP neurons. R: Right ,L: Left, APF: after pupa formation. Dotted Lines: AB-SLP neurons or FasII expression.

3.2. Circuit Lateralization Coincides with Downregulation of FasII in vFB Neurons.

To determine the identity of the FasII expressing vFB neurons I used the enhancer trap FasII -Gal4 to express mcd8::GFP at successive stages of AB development. FasII expression was visualized by antibody staining. Interestingly, at 30h APF FasII-Gal4 does not label the AB-SLP itself but seems to specific the distinct subset of early AB neurons (Fig. 3A). At 35h APF downregulation of FasII expression is visible in vFB neurons coinciding with the removal of left AB-SLP neurites (Fig. 3 B). In the adult, FasII expression is restricted to the right AB neuropile (Fig 3 C). The fact that FasII-Gal4 enhancer trap could not be observed within AB-SLP neurons indicates a differential regulation of FasII expression within early AB neurons (Fig.3).

Furthermore, after vFB neurons downregulate FasII, GFP expression is still visible at 35h, which could either point to a perdurance of GFP or a still active FasII enhancer Fig.3 (B).

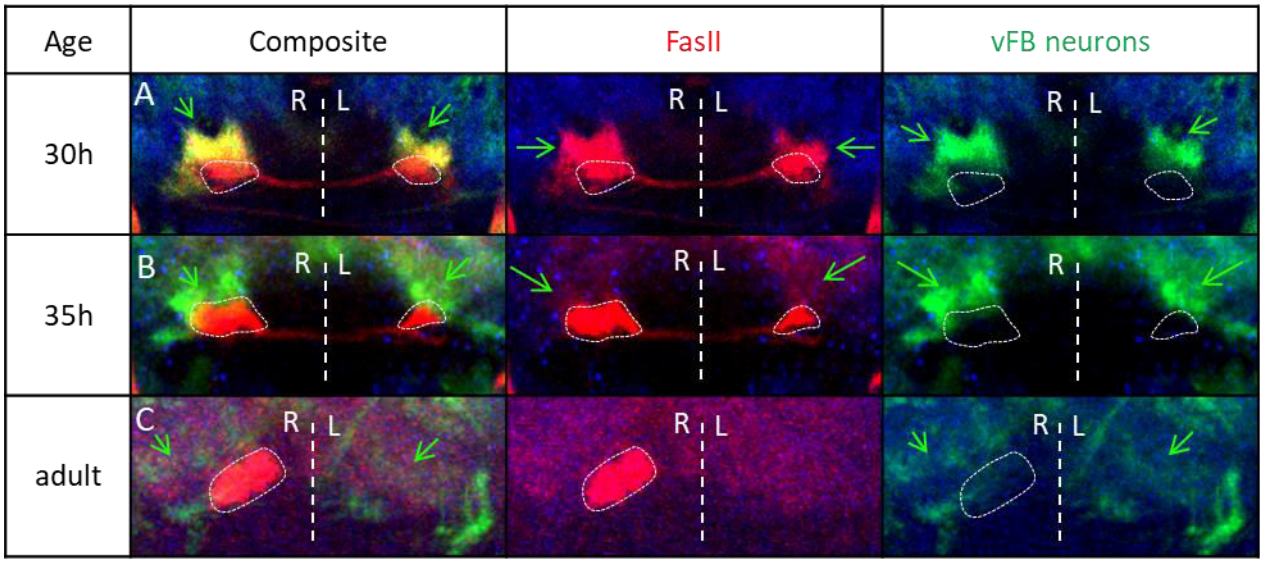


Figure 3. Downregulation of FasII within vFB neurons Coincides with Asymmetric Circuit Remodeling. **A**: At 30h APF in the bilateral stage of AB-SLP neuron Innervation, FasII is not only expressed by AB-SLP neurons (white circles) but also an adjacent population of vFB neurons (green) labeled by FasII-Gal4. **B:** At 35h hours when the left AB-SLP neuron processes begin to retract (white circles), vFB neurons downregulate FasII, while GFP expression is maintained (green arrows). **C:** In the adult, FasII expression is lateralized to the right side (white circle). Neurons labeled by FasII-Gal4 innervate the Fan-shaped Body in a layer-like fashion (green arrows). Brains were stained against NCAD (blue) for reference.

Combining these data with the previously established developmental trajectory of AB-FB neurons (Alsberga 2019) I am proposing an integrated model of AB development (Fig. 4). In the bilateral state of AB-SLP neuron innervation, transiently FasII positive vFB neurons are located between AB-SLP neurons and AB-FB neurons (Fig.4 A). After AB-SLP neuron remodeling and after vFB neurons have downregulated FasII, postsynaptic AB-FB neurons establish come into close proximity to AB-SLP neurons (Fig. 4 B). In the adult, FasII expression and AB-SLP neurons innervation are fully lateralized. Postsynaptic AB-FB neurons receive unilateral input from AB-SLP neurons and show a bigger innervation on the right hemisphere. FasII Gal4 labeled neurons can be observed in layers of the Fan-shaped Body (Fig.4 C).

In summary, the developmental analysis shows that the AB circuit progresses from a bilaterally symmetric ground state, which becomes asymmetrical through the removal of left AB-SLP neuron processes. This remodeling coincides with a downregulation of FasII in vFB neurons complex regulation of FasII expression and is followed by the AB-FB neurons coming into contact with AB-SLP neurons.

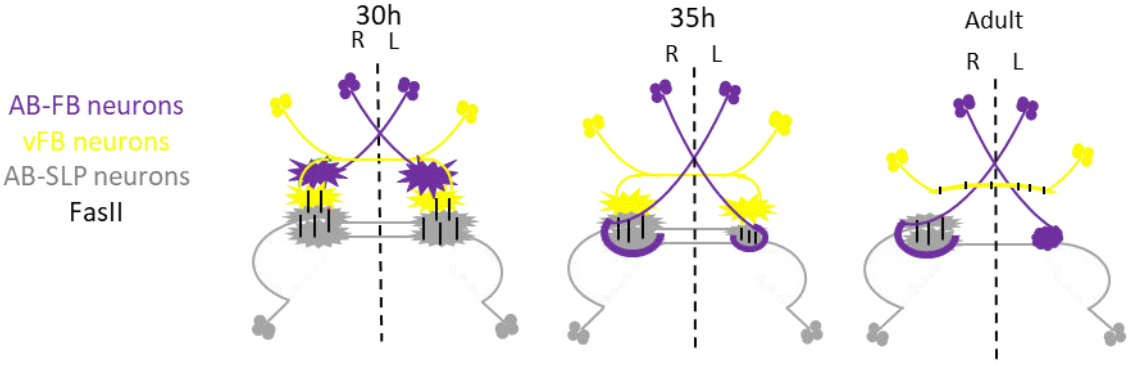
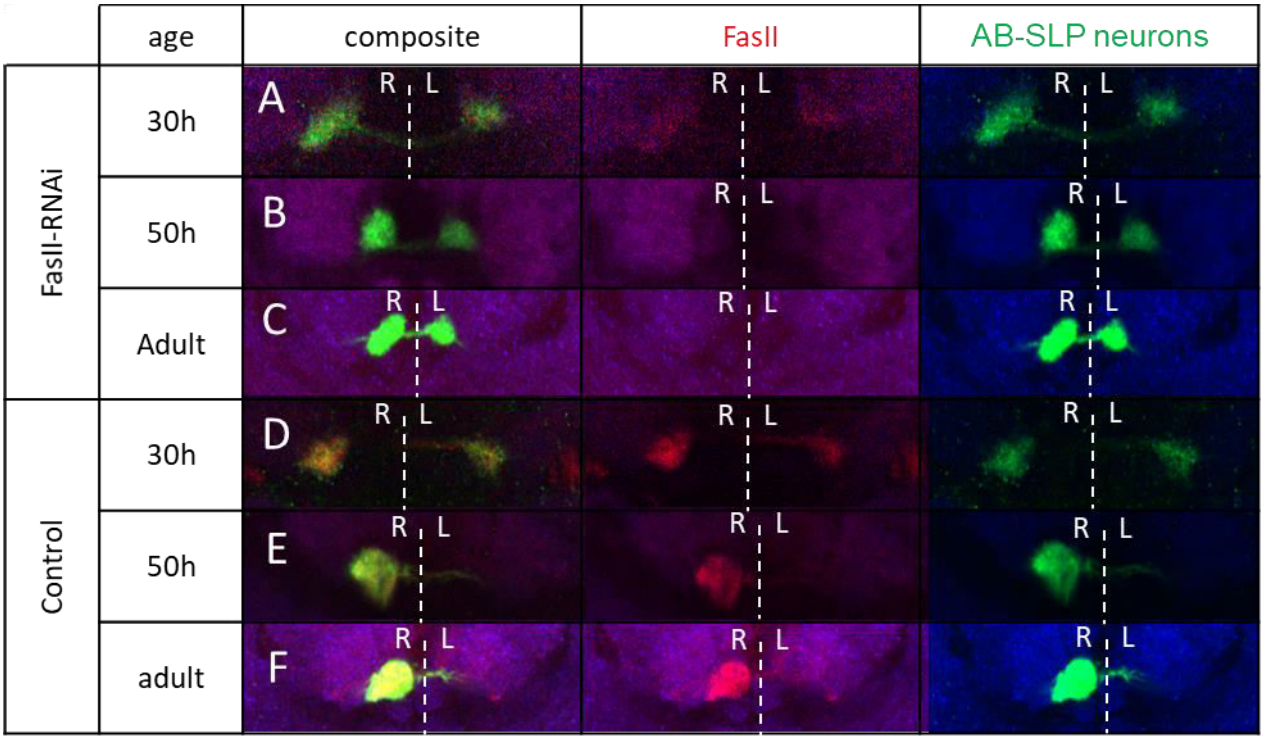


Figure 4. Model of Developmental Changes in FasII Expression and morphology within vFB neurons, AB-SLP neurons, and AB-FB neurons. A: At 30h APF, at the bilateral state of AB-SLP neuron innervation, FasII is expressed on both hemispheres by AB-SLP neurons. FasII positive vFB neurons are located between AB-SLP neurons and AB-FB neurons. **B:** At 35h APF when the left processes of AB-SLP neurons are beginning to disappear, vFB neurons downregulate FasII and AB-FB neurons integrate into the circuit. **C:** In the adult, left AB-SLP neurons processes have fully retracted, leading to a lateralized FasII expression on the right hemisphere. Bilaterally innervating AB-FB neurons show a bigger size on the right hemisphere. While FasII-Gal4 labeled neurons now innervate layers of the Fan-shaped Body. For simplicity dendrites of AB-SLP neurons and axons of AB-FB neurons are not shown.

3.3. Loss of FasII Leads to the Maintenance of the Symmetric Ground State.

To test if FasII is required to establish AB-SLP neuron asymmetry, I performed an RNAi knockdown of FasII driven by FasII-Gal4 enhancer trap Antibody staining against FasII and R52H03-lexA driven mCD8::GFP expression was used to visualize FasII expression and AB-SLP neuron morphology in experimental flies (FasII-Gal4/Y; R52H03-lexA, LexAop-mcd8::GFP/+ UAS-Fas2-RNAi/+) and controls (FasII-Gal4/Y; R52H03-lexA, LexAop-mcd8::GFP/+; TM6/+) respectively. The intensity of FasII antibody fluorescence in relation to background fluorescence intensity was measured as a proxy for FasII where a value >1 indicates

higher Intensity of FasII antibody fluorescence within the AB then the background. In the central complex of adult FasII-Gal4/UAS-FasII:RNAi flies, no anti-FasII expression could be detected (data not shown). Surprisingly, although FasII-Gal4 expression was not found to be active in AB-SLP neurons during pupal central complex circuit formation (see Fig. 3), I observed a depletion of FasII within AB-SLP neurons, indicating the notion of a non-autonomous effect of FasII expression during AB development. A significant reduction of FasII fluorescence was not only found in the adult (1.17± 0.07, n= 14; control: 2.26 ± 0.09, n=13, $p<0.001$; Mann-Whitney U test)) but already in development at 30h APF (1.21 ± 0.03 , n= 18; control: 2.2 ± 0.31; n=17, $p<0.001$; Mann-Whitney U test) (Fig 5 H). In addition, the knockdown of FasII using the FasII enhancer trap FasII Gal4 led to a significant increase in the number of brains showing symmetric AB-SLP neurons compared to controls within adult brains. (39%, n= 66; control: 9%, n=64; $p<0.001$; chi-square test) (Fig 5. G). As the bilateral AB-SLP neuron innervations following FasII-Gal4 driven FasII knock down could already be observed at 50h APF (32%, n=28; control: 0%, n=11; $p<0.05$; Fisher's exact test), FasII seems to be necessary for AB-SLP neurons to progress from an bilaterally symmetric to an asymmetric organization.



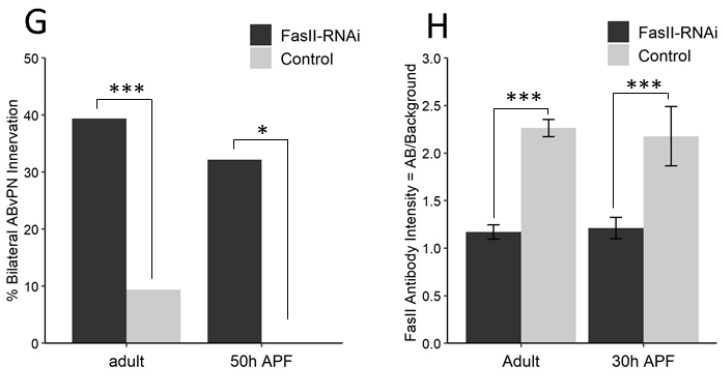


Figure 5. AB-SLP neuron Morphology and FasII Expression within a FasII-Gal4 Driven Knockdown of FasII. A-F: AB-SLP neuron morphology (green) and FasII expression (red) at successive developmental time points within knockdown flies and controls. **A, D:** At 30h APF FasII-RNAi expressing flies as well as controls show a bilateral innervation of AB-SLP neurons. **B, D:** At 50h APF AB-SLP neurons have retracted their left processes in the control while in the knockdown bilateral symmetry is maintained. **C, F:** In the adult AB-SLP neurons are lateralized in the control but not within the knockdown. Brains were stained against NCAD (blue) for reference. **G:** Percentage of Brains showing bilaterally innervating AB-SLP neurons within Fas2 Gal4 driven FasII RNAi expressing flies and controls. Chi-square Test for adults. Fisher exact test for 50h APF ; * $p<0.05$, *** $p< 0.001$. **H:** Ratio of FasII antibody fluorescence to background fluorescence within knockdown flies and controls in the adult and at 30h APF. Error bars indicate SEM. *** $p<0.001$, ns- not significant; Mann-Whitney U test.

3.4. Overexpression of FasII within vFB neurons leads to the Maintenance of Bilateral Symmetry

I next set out to determine if overexpression of FasII can inhibit asymmetric remodeling. During Drosophila metamorphosis, mushroom body (MB) remodeling requires the downregulation of FasII by a loosening of cell-cell adhesion and prolonged FasII expression result in impaired MB organization (Bornstein et al., 2015). Therefore I hypothesized that an increase in FasII mediated cell adhesion via FasII-Gal4 driven overexpression of FasII might lead to the maintenance of a bilaterally symmetric organization. Antibody staining against FasII and R52H03-lexA driven mCD8::GFP expression was used to visualize FasII expression and AB-SLP neuron morphology within overexpression(*FasII-Gal4/Y; R52H03-lexA, LexAop-mcd8::GFP/UAS-FasII*$^{PEST-)}$ and control flies respectively (FasII Gal4/Y or +; R52H03-lexA, LexAop-mcd8::GFP/CyO). To support elevated FasII protein level a *FasII* isoform without the PEST domain war used for overexpression. Intensity of FasII antibody fluorescence in relation to background fluorescence was measured as a proxy for FasII abundance at different developmental timepoints.

FasII-Gal4 driven FasII^{PEST-} overexpression led to a significantly higher percentage of bilaterally symmetric AB-SLP neuron innervations as compared to controls (40%, n= 20; control: 0%, n=19; $p<0.01$; Chi-square test), suggesting that an overabundance of FasII leads to a failure of asymmetric circuit remodeling. Interestingly, similar to the FasII-Gal4 driven knockdown, an overexpression of FasII within vFB neurons resulted in FasII-negative AB-SLP neurons in the adult central complex (0.96 ± 0.03, n= 11; control: 3.93 ± 0.42, n=12, $p<0.001$; Mann-Whitney U test) (Fig.6 A,B,G). Developmental analysis showed a loss of AB-SLP neuron intrinsic FasII from 40h APF onwards (1.5 ± 0.17, n= 10; control: 3.17 ± 0.42, n=5, $p<0.05$; t-test) (Fig.5 G). At 30h APF no difference in AB-SLP neuron FasII levels between overexpression and control

flies could be observed (3.49 ± 0.14, n= 14; control: 2.99 ± 0.23, n=18, ns; Mann-Whitney U test) (Fig.5 G). Since AB-SLP neuron remodeling is already completed at 40h within the wildtype this suggests an overabundance of FasII within vFB neurons rather than the loss of FasII in AB-SLP neurons to be the cause of the maintenance of the symmetrical state.

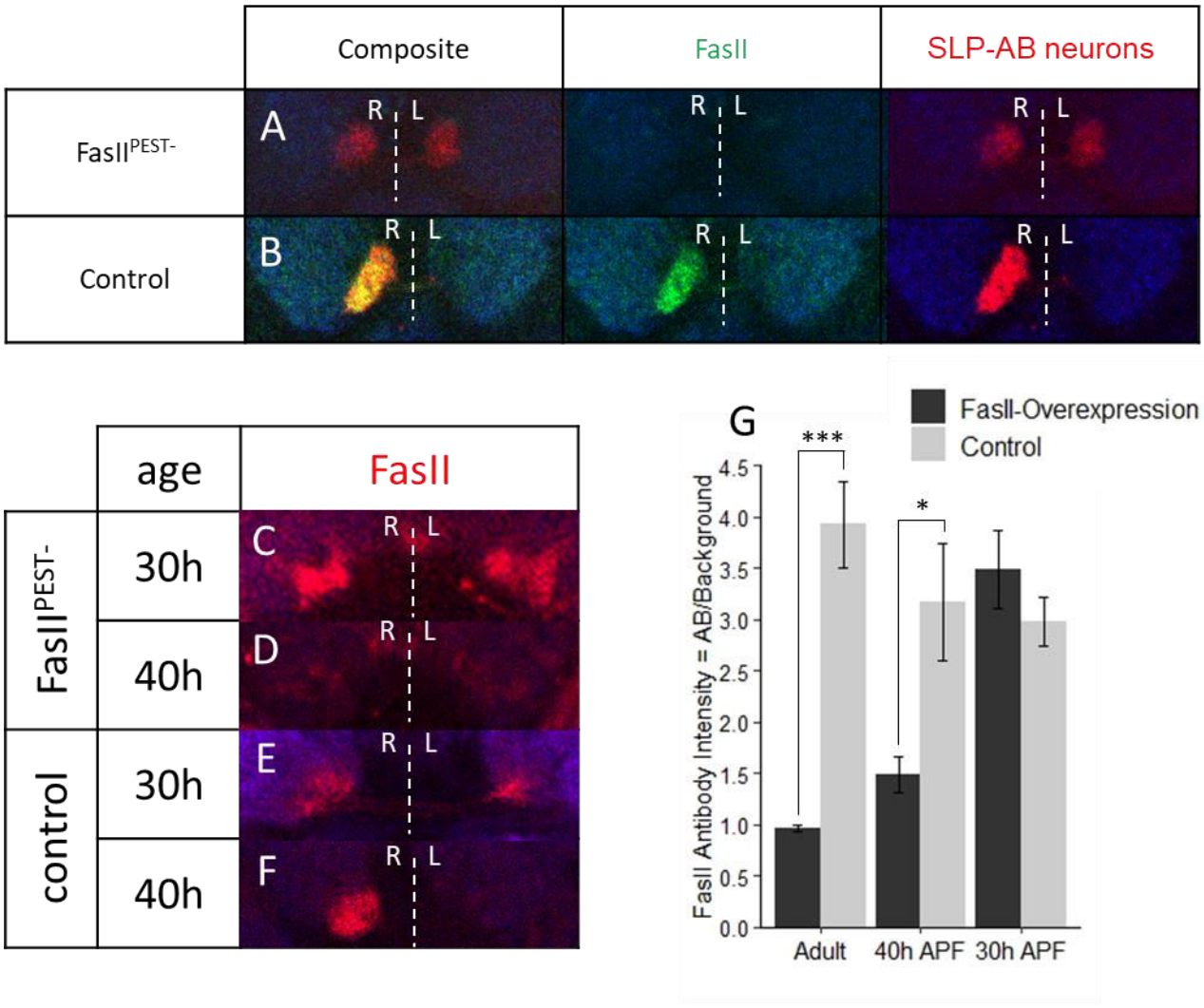


Figure 6. FasII-Gal4 Driven Overexpression of FasII^{PEST-} perturbs asymmetric remodeling of AB-SLP neurons A, B: Overexpression of FasII^{PEST-} leads to an asymmetric AB-SLP neuron Innervation pattern (red) and a loss of FasII (green). In controls, AB-SLP neurons express FasII (green) and show a lateralized innervation pattern (red). **C-F:** Developmental FasII expression (red) in overexpression flies and controls. **A, C:** At 30h APF there is no difference in FasII expression between overexpression flies and controls. **B, D:** At 40h APF FasII is significantly reduced in overexpression flies as compared to controls. Brains were stained against NCAD (blue) for reference. R52H03-lexA was used to drive mCD8::TMT in AB-SLP neurons. **G:** Intensity of FasII antibody fluorescence in relation to background fluorescence as a proxy for

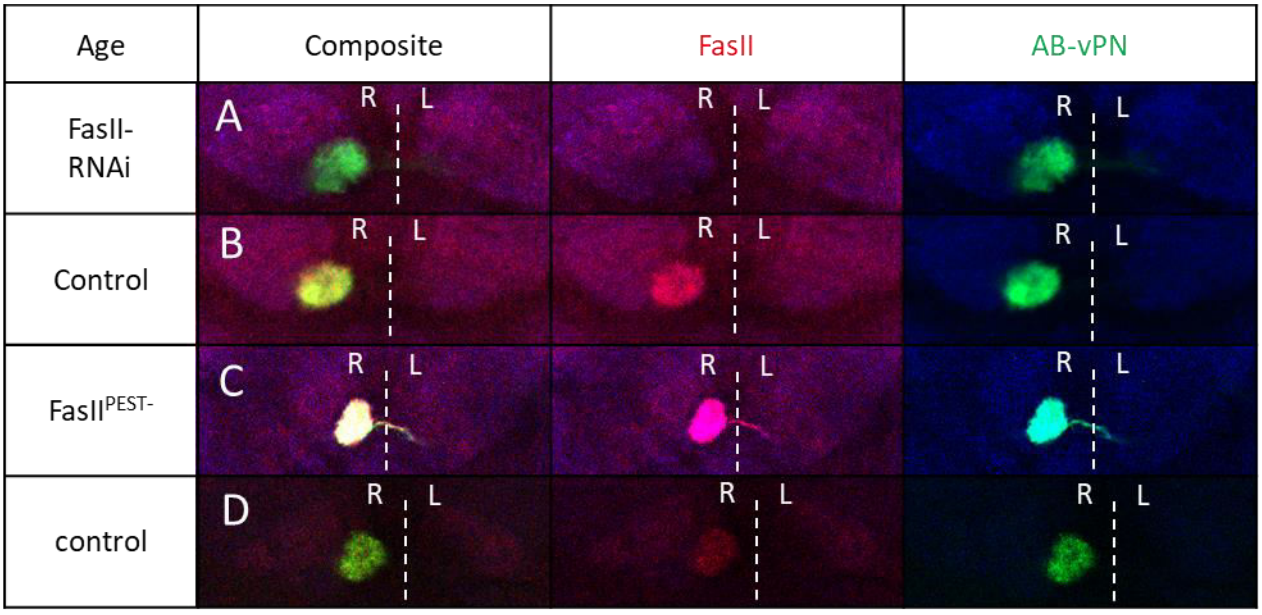
FasII abundance within overexpression flies and controls at different developmental timepoints. Error bars indicate SEM. * p<0.05, ***p<0.001; Mann-Whitney U test.

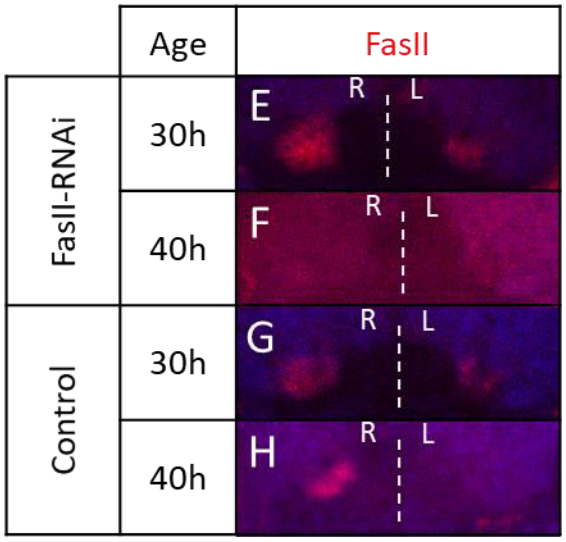
3.5. AB-SLP neuron Intrinsic Alterations of FasII Levels do not Interfere with Asymmetric Remodeling

To test if AB-SLP neuron intrinsic FasII levels are critical for asymmetric remodeling I next used the R502H03-Gal4 to overexpress and knockdown FasII exclusively within AB-SLP neurons. Antibody staining against FasII and R52H03-lexA driven mCD8::GFP expression was used to visualize FasII expression and AB-SLP neuron morphology in knockdown (;R52H03-lexA, LexAop-mcd8::GFP/+ UAS-Fas2 RNAi/R52H03-Gal4) and control flies respectively (R52H03-lexA, LexAop-mcd8::GFP/+ UAS-Fas2 RNAi/TM6) as well as in $FasII^{PEST-}$ overexpression (R52H03-lexA, LexAop-mcd8::GFP/UAS-FasII PEST- ; R52H03-Gal4/+) and control flies (R52H03-lexA, LexAop-mcd8::GFP/Cyo ; R52H03-Gal4/+). FasII abundance as measured by the intensity of FasII antibody fluorescence in relation to background fluorescence was significantly reduced in knockdown flies as compared to controls (1.56 ± 0.27, n= 14; control ± 0.85, n=11, p<0.01; Mann-Whitney U test) (Fig. 7 A, B, I) and significantly elevated in overexpression flies as compared to controls (5.44 ± 0.39, n= 12; control; 2.2 ± 0.16, n=11, p<0.001; Mann-Whitney U test) (Fig.7 C, D).

A comparison of the percentage of brains showing a bilateral AB-SLP neuron innervation revealed no significant differences between knockdown flies and controls (46%, n= 48; control: 33%, n=52; ns; Chi-square test) or overexpression flies and controls. (25%, n= 12; control: 8%, n=12; ns.; Fishers exact test). Within the knockdown I observed a significant reduction of FasII within knockdown flies compared to controls at 40h APF (1.24 ± 0.06, n= 6; control: 2.27 ± 0.14, n=7, p<0.01; Mann-Whitney U test) but not at 30h APF 2.38 ± 0.13, n= 16; control: 2.92 ± 0.32, n=10, ns.; t-test (Fig.7 I). In summary, these results show that in contrast to the broader

FasII-Gal4 driven manipulations, alterations of AB-SLP neuron intrinsic FasII levels do not interfere with asymmetrical remodeling of AB-SLP neurons. However, due to the late onset of the R52H03-Gal4 driven knockdown compared to the FasII-Gal4 driven knockdown in which a depletion of FasII within AB-SLP neurons could already be observed at 30h (Fig.5 H), it is not clear whether the transition from the bilaterally symmetric to the asymmetric state depends on AB-SLP neuron intrinsic FasII or non-autonomous FasII expression within vFB neurons.





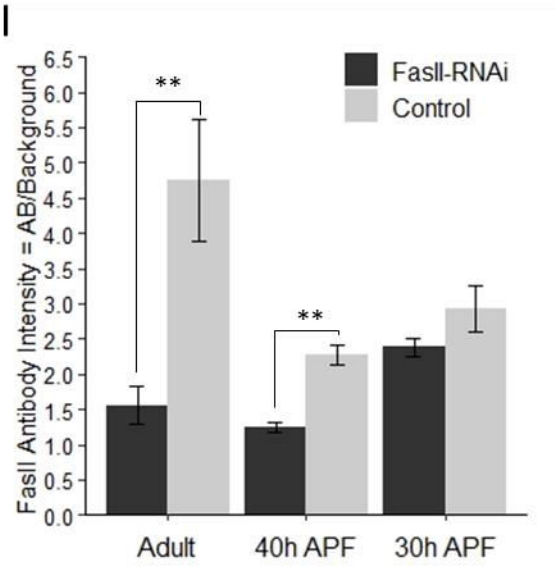


Figure 7. R52H03-Gal4 manipulations of AB-SLP neuron Intrinsic FasII Levels do not Interfere with Asymmetrical Remodeling A, B: R52H03 driven knockdown leads to a depletion of AB-SLP neuron intrinsic FasII (red) but does not interfere with AB-SLP neuron asymmetry (green). Controls show asymmetric FasII expression and a lateralized innervation pattern of AB-SLP neurons. **C, D:** Lateralized morphology and FasII expression of AB-SLP neurons in R52H03- Gal4 driven $FasII^{PEST-}$expressing flies as well as in controls. **E-H:** FasII expression (red) in R52H03 Gal4 driven knockout flies and controls at 30h and 40h APF. **E, G:** At 30h APF FasII is expressed within knockdown flies as well as controls. **F, H:** At 40h APF loss of FasII can be observed within knockdown flies but not in controls. Brains were stained against NCAD (blue) for reference. R52H03-lexA was used to drive mCD8::GFP in AB-SLP neurons. **I:** FasII antibody fluorescence in relation to background fluorescence as a proxy for FasII abundance within overexpression flies and controls at 30h APF, 40h APF, and in the Adult. Error bars indicate SEM, **p<0.01; for adult and 40h APF: Mann-Whitney U test; for 30h APF: t-test.

3.6. Splice variant Specific and Hypomorphic Mutants do not Affect AB Asymmetry

While a recent study indicates a functional redundancy between FasII isoforms (Neuert et al., 2019) others report differential effects of specific isoforms (Ashley 2005; Beck et al., 2012). To investigate if specific isoforms are required for the asymmetric remodeling of AB-SLP neurons I assessed AB-SLP neuron morphology within the splice variant-specific mutants $FasII^{\Delta TM}$, $FasII^{\Delta PB}$, $FasII^{\Delta PC}$ (Neuert et al., 2019). In these mutants membrane anchorage of different isoforms has been removed, resulting in a secreted version of the different variants (Neuert et al., 2019). Compared to the wildtype none of these mutations showed a significant increase in the amount of bilateral AB-SLP neuron innervation (all p>0.05 Chi-square test) (data not shown), suggesting a redundancy of isoforms regarding asymmetric remodeling.

Furthermore, I assessed AB-SLP neuron morphology within hypomorphic FasII alleles of different strengths. $FasII^{rd2}$ is an allele leading to a 50% reduction of wildtype FasII expression (Cheng et al., 2001). The strong hypomorphic mutation $FasII^{e76}$ was described to show 10% of wildtype expression (Grenningloh et al., 1991). $FasII^{eb112}$ is a homozygous lethal null allele (Grenningloh et al., 1991). Analysis of aforementioned alleles, including combinations of $FasII^{eb112}/FasII^{rd2}$ and $FasII^{e76}/FasII^{rd2}$ ($FasII^{e76}$/ $FasII^{rd2}$ was not viable) did not show any significant increase of bilateral AB-SLP neurons compared to the wildtype. (all p>0.05 Chi-

square test) (data not shown). Surprisingly, no difference in FasII levels were found too ($p > 0.05$ Kruskal Wallis Rank sum test) (data not shown). This might be because measuring FasII abundance via antibody staining is not sensitive enough to detect changes in FasII levels.

4. Discussion

Asymmetries between the left and the right hemisphere of the brain are a ubiquitous feature of bilaterian animals, including humans (Aizawa, Goto, Sato, & Okamoto, 2007; Güntürkün & Ocklenburg, 2017; Frasnelli, 2013). Here neural asymmetry has been correlated with behavioral and cognitive functions as well as with several neurological disorders (Herbert et al., 2005; Oertel-Knöchel & Linden, 2011; Postema et al., 2019). Although the study of neural asymmetry in the zebrafish and C.*elegans* have provided much insight into the development of asymmetries in the brain, further genetically accessible model systems are needed to explore different mechanisms leading to the ubiquitous phenomenon of neural asymmetries. In this thesis, I analyzed the formation of a lateralized neural circuit in the Drosophila central complex to gain insights into the developmental processes leading to the establishment of an asymmetrical connectivity pattern.

I could show a progression of AB-SLP neurons from an bilaterally symmetric to an asymmetric innervation pattern. Gain and loss of function experiments revealed a requirement of FasII for the transition from symmetry to asymmetry. Only global, but not AB exclusive suppression or overexpression of FasII lead to a failure of asymmetric remodeling. This suggests a mechanism involving FasII mediated cell-cell interaction, rather then an autonomous effect od AB-intrinsic FasII expression.

4.1. From Symmetry to Asymmetry

The finding that AB-SLP asymmetry develops from a bilaterally-symmetrical ground state reflects previous findings of the development of brain asymmetry. In C. *elegans* AWC neurons stochastically develop L/R differences in gene expression. Axonal contact establishes asymmetric calcium signaling eventually leading to the differential expression of a yet unknown transcription factor (Hobert et al., 2002). ASE neurons develop a L/R differential gene expression non-autonomously after a symmetrical organization has been established, through an early exposure to different cellular environments (Goldsmith et al., 2010; Troemel et al., 1999).

Within the zebrafish developing diencephalon, asymmetric nodal signaling results in a left-sided migration of the parapineal organ, leading to further asymmetries (Gamse et al., 2003). Although here too, asymmetry is established onto an initially symmetric organization, the mechanism for establishing asymmetry in the zebrafish diencephalon seems to be very different form the AB, where an asymmetric Innervation is not achieved through neural migration. Unilateral Neurons in the leech (Shankland & Martindale, 1989) and the hermit crab (Chapple, 1977), arise through an initially bilaterally symmetric population of cells. This is achieved by selective apoptosis. This stands in contrast the AB-SLP neurons where the bilateral clusters remain after the establishment of asymmetry. Here unilateral C. *elegans* neurons remain an exception regarding their establishment through asymmetric cell division without a prior state of bilateral symmetry (Hobert et al., 2002).

4.2. The Role of FasII for the Transition from Symmetry to Asymmetry

Although the Drosophila AB shares the feature of a transition from symmetry to asymmetry with other species, an establishment of asymmetry involving cell adhesion molecules has not been described so far.

Targeted loss- and gain-of-function experiments with FasII lead to specific effects on AB circuit remodeling. Overexpression, as well as knockdown of FasII using FasII-Gal4, which is not expressed in AB-SPL neurons during axon remodeling, led to a significant increase of adult brains showing a symmetrical AB-SLP neuron organization. In contrast, changes AB-SLP intrinsic FasII levels using RH502- Gal4 did not affect asymmetric remodeling. These results speak against a cell- autonomous function of AB-intrinsic FasII expression.

However, the fact that an RH502-Gal4 driven FasII knockdown resulted in a significant FasII protein reduction in developing AB-SLP neurons after 40h APF, at which in wild type AB-SLP neuron remodeling is already completed, does not exclude a cell-autonomous function of FasII for AB-SLP remodeling.

How could loss of FasII affect AB-SLP remodeling in a non-cell autonomous fashion? Before the transition from symmetry to asymmetry transiently FasII positive vFB neurons are located between AB-SLP neurons and their future synaptic partners (FB-AB neurons) (Fig.1 A). During the removal/retraction of AB-SLP neurites in the left hemisphere, vFB neurons downregulate FasII, after which the synaptic partners establish contact. Together with the FasII loss- and gain-of-function described above I propose FasII meditated adhesion to control the opening of a critical window of enhanced synaptic plasticity in which asymmetrical remodeling of AB-SLP neurons takes place.

In mammals, cell adhesion molecules were found to regulate the opening and closing of periods of heightened plasticity in which neural circuits are subject to change (Ribic, Biederer, & Morishita, 2019; Di Cristo et al., 2007; Singh et al., 2016). An increase in cell-cell adhesion through the expression of the cell-adhesion molecule SynCam 1 is required to restrict plasticity in the visual cortex after initial circuit formation (Ribic, Biederer, & Morishita, 2019). Singh et al. (2016) Show that dynamic adhesion between the cell adhesion molecules neurexin-1alpha and

neuroligin-1B controls the opening of permissive developmental periods in which synapses are formed.

Based on this model FasII mediated adhesion between AB-SLP neurons and vFB neurons maintains circuit plastic for axonal remodeling, by preventing the premature formation of synapses between AB-SLP neurons and FB-AB neurons. Only after the removal of left AB-SLP neurites has been initiated vFB neurons downregulate FasII. The resulting loss of adhesion between AB-SLP neurons and vFB neurons enables AB-SLP neurons and vFB neurons to form synapses. A loss of FasII mediated cell adhesion following the FasII-Gal4 driven knockdown leads to a stabilization of the symmetrical organization through the facilitation of premature synapse formation between AB-SLP neurons and FB-AB neurons (Fig. 8 A).

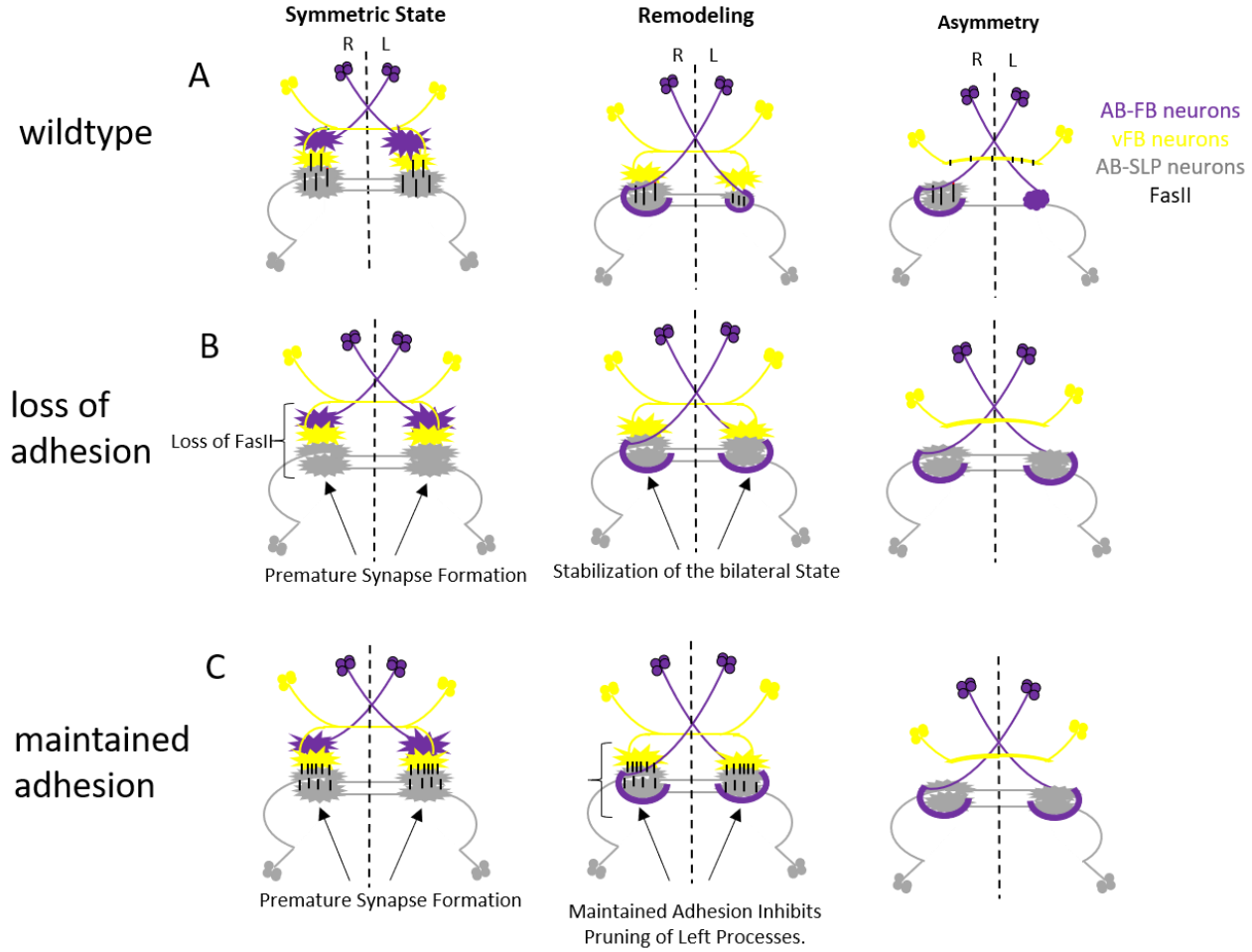


Figure 8. Model for FasII Dependent Asymmetric Remodeling of the AB Circuit
A: wild type development **B:** Loss of FasII within AB-SLP and vFB neurons leads to a failure of adhesion between AB-SLP neurons and vFB neurons leads to a premature formation of synapses between AB-SLP neurons and vFB neurons. The resulting stabilization of the symmetric organization prevents the removal of left AB-SLP neurons, leading to the maintenance of a symmetrical AB-SLP neuron Innervation in the adult. **C:** Following an overexpression, vFB neuron intrinsic FasII expression is maintained. A prolonged adhesion between AB-SLP neurons and FB-ABNs inhibits pruning of left AB-SLP neuron processes leading to a symmetrical organization of the adult circuit.

While overexpression of FasII using FasII-Gal4 led to a loss of FasII in adult AB-SLP neurons, a significant reduction only takes place at 40h APF, suggesting that the observed connectivity phenotype is due to a prolonged and not the loss of FasII expressions . In Drosophila mushroom bodies, downregulation of FasII was found to be critical for the removal of axonal processes during development (Bornstein et al., 2015).

Here I suggest the downregulation of FasII mediated adhesion between AB-SLP neurons and vFB neurons to be a necessary condition for the pruning of left AB-SLP neuron (Fig. 8 B). Therefore FasII expression must not only be maintained to prevent premature synapse formation but then also removed from the neuronal surface to allow the pruning of left AB-SLP neurites. Together the FasII gain and loss of function experiments indicate a dependence of a symmetrical AB-SLP neuron development upon a tight regulation of FasII mediated cell adhesion.

4.3. FasII regulation within AB development

In this thesis, three findings point to a complex regulatory mechanism for FasII expression within AB development. 1. A differential regulation of FasII expression in vFB neurons and AB-SLP neurons. 2. A sensitivity of AB-SLP neuron intrinsic FasII expression to changes in FasII levels within vFB neurons as seen within the FasII Gal4 driven knockdown and overexpression suggesting a non-autonomous effect of vFB FasII levels on FasII expression

within AB-SLP neurons. 3. Downregulation of FasII within vFB neurons, while its enhancer still seems to be active, as seen by a perduring expression of GFP when driven with FasII-Gal4.

4.3.1. Differential Regulation of FasII Expression within vFB neurons and AB-SLP neurons.

Since I found the enhancer trap line FasII-Gal4 to be active in vFB neurons but not in AB-SLP neurons during asymmetry circuit assembly, this points to a differential regulation of FasII expression in the AB. Neuert et al. (2019) suggested the presence of an alternative FasII enhancer located in the intronic region.

Furthermore, Markowitsch (2019) found a considerable variation regarding symmetry and FasII expression within the ABs of various Drosophila species. Altogether my results show FasII to be critical for the establishment of asymmetry. This could point to a process of enhancer evolution within the FasII gene enabling an asymmetrical connection pattern of AB-SLP neurons.

4.3.2. Dependence of of AB-SLP neuron Intrinsic FasII Expression on vFB neuron FasII Levels

Knockdown, as well as overexpression within vFB neurons using FasII-Gal4, led to a loss of FasII expression within AB-SLP neurons. This dependence of AB-SLP neuron intrinsic FasII expression to changes in FasII levels within vFB neurons can be explained in two ways. Neuert et al. (2019) could rescue the lethality of FasII null mutations by the expression of a secreted FasII isoform, indicating a yet unidentified signaling function of FasII. Alternatively, vFB neuron intrinsic FasII could be required for the assembly of FasII within AB-SLP neurons. At the neuromuscular junction of Drosophila, the assembly of FasII at the postsynaptic site is dependent on transsynaptic adhesion provided by presynaptic FasII expression. Here FasII mediated adhesion leads to an accumulation of FasII on the postsynaptic site (Kohsaka, Takasu, & Nose, 2007). A similar scenario could be envisioned for FasII in the AB, where a transient Fas2

expression within vFB neurons induces the assembly of FasII within AB-SLP neurons. While such a model could explain the loss of FasII in AB-SLP neurons following a knockdown within vFB neurons, it is hard to see how an overexpression of vFB neuron intrinsic FasII could have such an effect.

4.3.3. Perdurance of FasII-Gal4 driven GFP expression

While vFB neurons downregulate FasII during the removal of the left AB-SLP neurites, a GFP signal within vFB neurons is still present when driven with FasII-Gal4. This could be explained by the FasII enhancer driving the expression of another gene, a perdurance of GFP while the enhancer is not active anymore, or downregulation of FasII above the transcriptional level. Within the latter case, Gal4 and therefore GFP would still be expressed, while FasII is transcribed but then suppressed after transcription. Such suppression could either take place via a known microRNA binding to the FasII transcript (Niu, Liu, Nian, Xu, & Zhang, 2019) or by the control of FasII membrane stability through different kinases (Bornstein et al., 2015; Hoeffer et al., 2003; Koh et al., 2002) or an endosomal protein known to regulate the membrane cycling of FasII (Nahm et al., 2016).

4.3.4. FasII mutant analysis

While a Knockdown of all FasII Isoforms results in the maintenance of a bilateral AB-SLP neuron innervation, neither hypomorphic FasII mutants nor splice variant-specific mutants elicited a AB circuit phenotype. While some studies reported a requirement of specific FasII isoforms (Ashley, 2005; Beck et al., 2012) my results suggest a redundancy for different FasII isoforms regarding the establishment of AB-SLP neuron asymmetry. Given that a knockdown of FasII leads to a failure of AB-SLP neuron remodeling, it is surprising, that this effect was not observed within the hypomorphic mutants $FasII^{rd2}$ and $FasII^{e76}$. Likely the reduction of FasII

within hypomorphic mutants is not strong enough to elicit a phenotype. This is supported by a strong remaining signal of anti-FasII antibody staining in AB-SLP neuron.

Due to the lethality of FasII null mutants (Grenningloh et al., 1991) an RNAi approach was taken to characterize the role of FasII within AB development. Knockdown does not suppress gene function entirely (Johnston, 2013). Therefore a residual FasII expression could explain why the penetrance of bilateral AB-SLP neuron innervation is not higher than about a third of the brains within the FasII-Gal4 driven knockdown.

Furthermore, I proposed a model in which knockdown and overexpression of FasII leads to bilateral AB-SLP neuron innervation due to premature synapse formation or? failure of pruning, An alternative explanation for these results would be that the depletion of AB-SLP neuron intrinsic FasII following FasII-Gal4 driven overexpression or knockdown leads to a permanent instability of the circuit. Here AB-SLP innervation would switch between unilateral and bilateral innervation in a stochastic fashion. Such a stochastic switch would explain the low penetrance of bilateral AB-SLP neuron innervations and would fit the finding of a previous study where the loss of cell adhesion led to heightened adult plasticity (Ribic, Crair, & Biederer, 2019).

4.4. Future directions

While I could show FasII to be necessary for asymmetric remodeling of AB-SLP neurons, the actual signal leading to the establishment of asymmetry remains elusive. Mutations in the cell adhesion molecule Neuroglian (nrg), which disrupts the contralateral crossing of the AB-SLP neurons due to midline defects, left AB-SLP neuron innervations are still present in the adult brain (Markowitsch, 2019). This could point to midline signaling, causing the retraction of left AB-SLP neurites. However since the nrg mutation also disrupts midline crossing of AB-SLP

neurons, this finding could also be explained through a symmetry-breaking event dependent on direct axonal contact as observed in *C.elegans* AWC neurons (Goldsmith et al., 2010).

Another direction for further research is the relationship between different asymmetries in the AB circuit. In principle, different kinds of asymmetries can arise through independent mechanisms. For instance, in patients with a L/R reversal of organ positions, brain asymmetry remains unaffected, suggesting independent factors determining, neural and internal organ asymmetry (Kennedy et al., 1999; Torgersen, 1950). However, in the case of the AB circuit, such independence is unlikely, since Markowitsch (2019) found a reduction of postsynaptic asymmetry within brains displaying a symmetrical AB-SLP neuron innervation. While this finding suggests some kind of mechanistic coupling between these two asymmetries, the nature of the association of class I and class II asymmetry within the AB circuit remains unclear.

One possibility is that class I asymmetry in postsynaptic neurons directly depends on AB-SLP neuron class II asymmetry. Such a hierarchy of asymmetry has been observed in the zebrafish where class I asymmetries in size, gene expression, and connectivity at least partly depend on the prior establishment of a class II asymmetry in the form of the unilateral migration of the pp (Gamse et al., 2003). Within the AB circuit asymmetry of AB-FB neuron innervation size could be a consequence of a greater number of connections formed on the right hemisphere due to the lateralized innervation pattern of AB-SLP neurons. A similar scenario was observed in mice where lateralized synaptic input leads to L/R size differences in the innervation of postsynaptic neurons (Shinohara et al., 2013). Alternatively, class I and class II asymmetry in the AB could be established independently through the same symmetry-breaking event. An example of such a relationship between different asymmetries can be seen in C.*elegans* ASE neurons where, L/R differences in gene expression as well as in cell body and neurite size independently arise through exposure to a different extracellular environment in the early embryo (Cochella et

al., 2014). In the case of the AB, a symmetry-breaking event leading to AB-SLP neuron class II and AB-FB neuron class I asymmetry could be provided by midline signaling as suggested by Markowitsch (2019).

Finally, the importance of FasII for asymmetry of the AB circuit might have implications for the development of brain asymmetry in humans. Nodal signaling, the cause of diencephalic asymmetry in the zebrafish, is unlikely to be the sole determinant of asymmetries in the human brain (Güntürkün & Ocklenburg, 2017). However, NCAM the vertebrate ortholog of FasII has been associated with autism (Purcell, Hyder, Zimmerman & Pevsner, 2001), a disease correlated with altered cortical asymmetries (Postema et al., 2019).

5. Conclusion

Asymmetries in the nervous systems are ubiquitous in the animal kingdom and have been correlated with a wide range of behavioral and cognitive functions. To determine the molecular basis of brain asymmetries, genetically manipulated model organisms are crucial. Previous studies regarding the genetic pathways leading to brain asymmetry were predominantly studied in the zebrafish and C.*elegans* (Gamse et al., 2005; Hobert et al., 2002). However the developmental basis of these asymmetries remain largely elusive. Given recent advances in the establishment of a Drosophila connectome (Xu et al., 2020), future discoveries of further neural asymmetries within the fruit fly brain can be expected to provide significant insights into the establishment of different forms of asymmetry within this organism. This study set out to further explore the potential of the Drosophila AB as a system for the study of brain asymmetry, by

providing insight about the developmental trajectory of AB asymmetry and the mechanisms by which this asymmetry is established.

This thesis identified a symmetrical ground state undergoing a FasII dependent process of axonal remodeling, leading to asymmetry in the adult brain. While confirming previous findings of a prior symmetrical state upon asymmetry is established, this is the first time cell adhesion has been found to be implicated in such a transition. The commonalities and differences observed with asymmetric brain development of different species show the importance of utilizing different models to distinguish species-specific from general features of this ubiquitous phenomenon of the nervous system.

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