General Introduction

The actions of neurons can alter the output of the neural circuits that generate the many different patterns in movement used by animals during their normal behavior. In order to accomplish the orchestrated action and response properties and the precise tuning of the many participants in a neural network, the key players have to be well equipped with cellular properties such as ion channels. To investigate intrinsic properties underlying behavior, isolated cells have proven a good choice for the examination of the neuron`s repertoire of electrical equipment in insects (Heidel and Pflüger, 2006; for review see Grolleau and Lapied, 2000) but also in vertebrates (for review see Fry *et al*., 2006; Shi *et al*., 2003)

Identified insect neurons allow relating cellular properties to behavioral function

In insects many neurons are well characterized and easy to reach. Because of their relatively small number and the possibility to identify certain neurons via retrograde labeling from target tissues such as muscles insect neurons are valuable targets for investigating their involvement in behavior. Several years ago, Hammer (1993) stated that the reward stimulus in associative olfactory learning in the honey bee *Apis mellifera* can be mimicked by current injection into one identified DUM neuron. In crickets an identified auditory interneuron is used to create an efference copy for inhibiting sensory afference that beckons the cricket its own song, so that the animal can discriminate between self-generated and external stimuli during behavior (Poulet and Hedwig, 2006). In the moth, *Manduca sexta*, postembryonic changes in the dendritic structure and the excitability of an identified motoneuron has been related to changing behavioral requirements during its postembryonic change in function when developing from a slow crawling into a fast flight motoneuron (Duch and Levine, 2000). Therefore, identified insect neurons have proven good models to relate individual neuron properties and firing to behavioral function, although the activity patterns of these neurons can not be understood without detailed knowledge about the network.

Ventral nerve cord neurons are easy to identify by their efferent projections onto their target muscles. First, these neurons are fast transmitting motoneurons with type I terminals that cause muscle contraction upon spiking or, second, these are modulatory neurons which release of neuromodulators via type II terminals (for review see Pflüger, 1999). The most prominent population of efferent modulatory neurons is DUM (doral unpaired median) neurons (for review see Pflüger and Bräunig, 2001)

Efferent DUM neurons

DUM is short for dorsal unpaired median and describes the location of these particular neurons along the dorsal midline of many ganglia of the insect ventral nerve cord (Plotnikova, 1969; Hoyle *et al*., 1974). Locust DUM neurons project bilaterally symmetric on efferent targets on both sides of the body and can be divided into sub-populations depending on the nerve they are projecting through (Baudoux and Burrows, 1998). The efferent DUM neurons contain the neurotransmitter octopamine. Octopamine is released directly onto the target tissues such as muscle (Evans and O`Shea, 1977, 1978; O`Shea and Evans, 1979) and modulates its activity when the DUM neuron itself is activated. Octopamine release from DUM neurons onto skeletal muscle leads to increases in the amplitude and speed of twitch contractions as well as increases in the relaxation rate (O´Shea and Evans, 1979; Whim MD and Evans PD, 1988). In contrast to previous suggestions DUM neurons are not recruited as a homogeneous group during behavior (Duch *et al*., 1999; Baudoux and Burrows, 1998). The different sub-populations, DUM neurons are divided into, are selectively recruited during certain behaviors such as locomotion or take-off for flight, whereas others are specifically inhibited during flight (Pflüger and Duch, 2000; Mentel *et al*., 2003; for review see Pflüger *et al*., 2004). In addition to their modulatory effects on synaptic transmission they also cause metabolic changes in muscles (Mentel *et al*., 2003). DUM neurons are capable of generating overshooting somatic action potentials (Goodman and Spitzer, 1981) and are equipped with a rich bouquet of ion channels. To investigate intrinsic properties of neurons it is administrable to work with isolated somata. Isolated DUM neuron somata express various calcium channels as well as sodium channels and also at least five different types of potassium channels (Heidel and Pflüger, 2006 and for review see Grolleau and Lapied, 2000). The various potassium channels have been reported to take part in initiation (hyperpolarization activated potassium current) and termination (sodium-dependent, A-type, delayed rectifier and calcium-dependent potassium current) of action potentials as well as setting interspike intervals and also in stabilizing the resting membrane potential (inwardly rectifying and A-type potassium current; Grolleau and Lapied, 1995, and for review see Grolleau and Lapied, 2000). Locust DUM neuron somatic action potentials strongly depend on sodium and calcium (Goodman and Spitzer, 1981). Some ion channels are known to be activated and/or modulated by intracellular calcium. Wicher *et al*. (2004) reported an intracellular mechanism that activates voltage-independent calcium entry into cockroach DUM neurons via neurohormone D mediated intracellular cascades. Calcium is a unique molecule that acts as a charge carrier and also as a second messenger which is involved in various intracellular cascades that are, for

example, involved in apoptosis (Szalai *et al*., 1999). In this study, however, we report an intracellular mechanism that is voltage-dependent but calcium-independent and most likely acts via a voltage activated G-protein located in the DUM soma membrane. Depolarization of the isolated DUM neuron membrane under calcium-free conditions led to increased intracellular calcium concentrations. This mechanism was mediated by an intracellular cascade involving calcium release from internal stores via inositol-1,4,5-triphosphate (IP_3) receptors (Ryglewski *et al*., 2007). Calcium release from internal stores is known to be mediated by synthesis of IP_3 by hydrolysis of phosphatidylinositol (PIP_2) by phospholipase C. IP_3 then binds to IP_3 receptors located in the membrane of the endoplasmic reticulum (ER) and calcium is released from the ER into the cytoplasm.

The role of ionic currents and other cellular properties for behavior

In order to overcome the descriptive level and unravel the functions of cellular properties of identified neurons for behavior one needs to work with a system that fulfils two requirements: First, one has to work with identified neurons with well defined functions. Second, one has to be able to selectively manipulate the cellular properties of these identified neurons, ideally without affecting the rest of the circuitry. Both requirements are pretty well fulfilled by flight motoneurons in the adult fly, *Drosophila melanogaster*. First, insect flight is a very well investigated and well described behavior. Groundbreaking work on flight behavior in locusts set the generally accepted concept of central pattern generation (Wilson, 1961 and 1966; Edwards, 2006). In Drosophila the giant fiber mediated jump and flight escape pathway is a valuable system for investigations on behavior (Levine and Tracey, 1973; Tanouye and Wyman, 1980). About one decade ago, Engel and Wu (1996, 1998) linked the escape pathway to physiology by using gene mutations. Another approach for understanding the modulatory control of flight behavior is to genetically alter enzymes regulating biogenic amine levels in the Drosophila flight system (Brembs *et al*., 2007). And second, Drosophila is one of the best genetic model systems. Expression of specific genes in Drosophila can be achieved by using enhancer trap lines (O´Kane and Gehring, 1987; Bellen *et al*., 1989). Together with the GAL4-UAS expression system the enhancer trap technique is a powerful tool for targeted genetic manipulation. To activate genes specifically at a special time temperature sensitive alleles can be used. Therefore, we use this system to unravel the role of intrinsic cellular properties for the behavioral function of identified motoneurons.

The identified flight motoneurons MN1-5

The Drosophila flight system is well described. The dorsolongitudinal flight muscle (DLM) in Drosophila is an indirect asynchronous flight muscle that consists of six muscle fibers. The muscle fibers 1-4 are ipsilaterally innervated by the DLM motoneurons MN1-4 whereas MN5 innervates the fibers 5 and 6 contralaterally (Ikeda and Koenig, 1988). The five DLM flight motoneurons are born embryonically, but only MN1-4 innervate the larval precursor of the DLM until dendritic regression occurs at the onset of metamorphosis followed by outgrowth to innervate the developing DLM. MN5 is not involved in embryonic or larval innervation but starts to grow out during early pupal stages and joins MN1-4 (Consoulas *et al*., 2002). The MN1-5 obtain their behavioral function and physiological properties while the development from the larva to the adult fly occurs (Consoulas *et al*., 2000). The dendritic development of MN5 until maturation is well described (Consoulas *et al*., 2002). Due to its location close to the midline of the adult Drosophila ventral ganglion and contralateral to the DLM, MN5 is individually identifiable. MN5 is well characterized with regard to morphology and dendritic development and it is involved in the Drosophila giant fiber mediated escape response which is an important behavior. Therefore, we were particularly interested in the intrinsic properties of MN5. According to Fayazzuddin *et al*. (2006) synaptic transmission onto MN5 via an interneuron is blocked by genetic alteration of the Dα7 acetylcholine receptor subunit, as demonstrated by stimulation of the escape pathway *in vivo*. In order to set the bedrock for interpreting targeted genetic manipulation of identified neuron properties in Drosophila a detailed description of the wildtype properties is necessary. Therefore, the second aim of this study was to describe potassium currents and their related genes in MN5 *in situ*.

On the basis of our understanding of the wildtype properties of MN5, we can now use genetic manipulations to address a number of important questions of modern neuroscience, such as what are the functions of specific ion channel proteins for the generation of motor behavior, what are the functions of genetic manipulations of intrinsic or synaptic activity for dendritic growth or synaptogenesis, what are the roles of transcription factors or other signals for the developmental acquisition of the adult neurons properties? As a first start step into such analysis in this thesis we asked whether targeted manipulations of intrinsic excitability of MN5 affect its dendritic architecture during postembryonic development. Therefore, we tested the effects of genetic alterations in potassium channel genes onto intrinsic excitability of the MN5, and we tested whether genetically altered intrinsic excitability affected dendritic growth and flight motor performance.

The results are presented in three chapters based on three manuscripts, one of which is already published and two of which are ready for submission.

Chapter 1: Ryglewski S, Pflüger HJ, Duch C (2007) Expanding the Neuron`s Calcium Signaling Repertoire: Intracellular Calcium Release via Voltage-Induced PLC and IP3R activation. PLoS Biol 5(4): e66. doi:10.1371/journal.pbio.0050066

Chapter 2: Ryglewski S, Duch C (ready for submission) Potassium currents of an identified adult Drosophila motoneuron *in situ*.

Chapter 3: Duch C, Vonhoff F, Ryglewski S (ready for submission) Dendrite elongation and dendritic branching are separately affected by different forms of intrinsic motoneuron activity.

References

Baudoux S, Burrows M (1998) Synaptic activation of efferent neuromodulatory neurones in the locust Schistocerca gregaria. J Exp Biol 201: 3339-3354

Bellen HJ, O`Kane CJ, Wilson C, Grossniklaus U, Pearson RK, Gehring WJ (1989) P-element-mediated enhancer detection: a versatile method to study development in Drosphila. Genes Dev 3(9): 1288-1300

Brembs B, Christiansen F, Pflüger HJ, Duch C (2007) Flight initiation and maintenance deficits in flies with genetically altered biogenic amine levels. J Neurosci 27(41): 11122-11131

Consoulas C, Duch C, Bayline RJ, Levine RB (2000) Behavioral transformations during metamorphosis: remodeling of neural and motor systems. Brain Res Bull 53(5): 571-583

Consoulas C, Restifo LL, Levine RB (2002) Dendritic remodeling and growth of motoneurons during metamorphosis of Drosophila melanogaster. J Neurosci 22(12): 4906-4917

Duch C, Mentel T, Pflüger HJ (1999) Distribution and activation of different types of octopaminergic DUM neurons in the locust. J Comp Biol 403(1): 119-134

Edwards JS (2006) The central nervous control of insect flight. J Exp Biol 209: 4411-4413

Engel JE, Wu CF (1996) Alterations of non-associative conditioning of an identified escape circuit in Drosophila memory mutants. J Neurosci 16: 3486-3499

Engel JE, Wu CF (1998) Genetic dissection of functional contributions of specific ppotassium channel subunits in habituation of an escape circuit in Drosophila. J Neurosci 18(6): 2254-2267

Evans PD, O`Shea M (1977) The identification of an octopaminergic neurone which modulates neuromuscular transmission in the locust. Nature 270: 257-259

Evans PD, O`Shea M (1978) The identification of an octopaminergic neurone and the modulation of myogenic rhythm in the locust. J Exp Biol 73: 235-260

Fayyazuddin A, Zaheer MA, Hiesinger PR, Bellen HJ (2006) The nicotinic acetylcholine receptor Dα7 is required for an escape behavior in Drosophila. PloS 4(3): e63

Fry CH, Sui G, Wu C (2006) T-type Ca^{2+} channels in non-vascular smooth muscles. Cell Calcium 40(2): 231-239

Goodman CS, Spitzer NC (1981) The mature electrical properties of identified neurones in grasshopper embryos. J Physiol 313: 369-384

Grolleau F, Lapied B (1995) Separation and identification of multiple potassium currents regulating the pacemaker activity of insect neurosecretory cells (DUM neurons). J Neurophysiol 73(1): 160-171

Grolleau F, Lapied B (2000) Dorsal unpaired median neurones in the insect central nervous system: towards a better understanding of the ionic mechanisms underlying spontaneous electrical activity. J Exp Biol 203: 1633-1648. Review.

Hammer M (1993) An unidentified neuron mediates the unconditioned stimulus in associative olfactory learning in honeybees. Nature 366:59–63

Heidel E, Pflüger HJ (2006) Ion currents and spiking properties of identified subtypes of locust octopaminergic dorsal unpaired median neurons. Eur J Neurosci 23(5): 1189-1206

Hoyle G, Dagan D, Moberly B, Colquhoun W (1974) Dorsal unpaired median insect neurons make neurosecretory endings on skeletal muscle. J Exp Zool 187: 159-165

Mentel T, Duch C, Stypa H, Wegener G, Müller U, Pflüger HJ (2003) Central modulatory neurons control fuel selection in flight muscle of migratory locust. J Neurosci 23(4): 1109-1113

Ikeda K, Koenig JH (1988) Morphological identification of the motor neurons innervating the dorsal longitudinal flight muscle of Drosophila melanogaster. J Comp Neurol 273(3): 436-444

Levine J, Tracey D (1973) Structure and function of the giant motoneuron of Drosophila melanogaster. J Comp Physiol 87: 213-235

Mentel T, Duch C, Stypa H, Wegener G, Müller U, Pflüger HJ (2003) Central modulatory neurons control fuel selection in flight muscle of migratory locust. J Neurosci 23(4): 1109-1113

O`Kane, C.J. & Gehring, W.J. (1987) Detection in situ of genomic regulatory elements in Drosophila. PNAS 84(24): 9123-9127

O`Shea M, Evans PD (1979) Potentiation of neuromuscular transmission by an octopaminergic neurone in the locust. J Exp Biol 79: 169-190

Pflüger HJ (1999) Neuromodulation during motor development and behavior. Curr Opin Neurobiol 9: 683-689. Review.

Bräunig P; Pflüger HJ (2001) The unpaired median neurons of insects. Adv Insect Physiol 28, 185-266

Pflüger HJ, Duch C (2000) The functional role of octopaminergic neurons in insect motor behavior. Acta Biol Hung 51(2-4): 343-348

Pflüger HJ, Duch C, Heidel E (2004) Neuromodulatory octopaminergic neurons and their function during insect motor behavior. The Ernst Florey memory lecture. Acta Biol Hung 55(1-4): 3-12

Plotnikova SN (1969) Effector neurones with several axons in the ventral nerve cord of the Asian grasshopper Locusta migratoria, J Evol Biochem Physiol 5: 276-277

Poulet JF, Hedwig B (2006) The cellular basis of a corollary discharge. Science 311(5760): 518- 522

Ryglewski S, Pflueger HJ, Duch C (2007) Expanding the neuron`s calcium signaling repertoire: intracellular calcium release via voltage-induced PLC and IP3R activation. PLoS Biol 5(4): e66.doi:10.1371/journal.pbio.0050066

Shi YL, Bai JP, Wang WP (2003) Ion-channels in human sperm membrane and contraceptive mechanisms of male antifertility compounds derived from Chinese traditional medicine. Acta Pharmacol Sin 24(1): 22-30. Review.

Szalai G, Krishnamurthy R, Hajnoczky G (1999) Apoptosis driven by IP3 linked mitochondrial calcium signals. EMBO J 18: 6349-6361

Tanouye MA, Wyman RJ (1980) Motor output of giant nerve fiber in Drosophila. J Neurophysiol 44: 405-421

Whim MD, Evans PD (1988) Octopaminergic modulation of flight muscles in the locust. J Exp Biol 134: 247-266

Wicher D, Messutat S, Lavialle C, Lapied B (2004) A new regulation of non-capacitative calcium entry in insect pacemaker neurosecretory neurons. J Biol Chem 279(48): 50410-50419

Wilson DM (1961) The central nervous control of locust flight. J Exp Biol 38: 471-490

Wilson DM (1966) Central nervous mechanisms for the generation of rhythmic behavior in arthropods. Symp Soc Exp Biol 20: 199-228