3

GABA and Glutamate Receptors of the Autistic Brain

Agenor Limon, Jorge M. Reyes-Ruiz and Ricardo Miledi
University of California at Irvine
United States of America

1. Introduction

Autism is a severe neuropsychiatric disorder characterized by impaired communication, significant reduction in social interaction, and repetitive and stereotyped behaviour. It is highly hereditable (Hoekstra et al., 2007); however, genomic alterations associated to autism have been found only in less than a fifth of the total number of cases. How those alterations ultimately cause the autistic phenotype is still very poorly understood. Besides genomic abnormalities, environmental and epigenetic factors may also increase the risk of developing autism or autistic traits. Prenatal exposure to rubella virus, cytomegalovirus, or to the chemical substances thalidomide and valproate are among the non-genetic causes linked to autism (Persico & Bourgeron, 2006), however causal relationships are not established. Regardless of the origins of autism, neuropathological observations are consistently found in several areas of autistic brains (Bauman & Kemper, 1985; Ritvo et al., 1986), and abnormal patterns of synaptic connectivity are thought to be at the core of the autistic disorder (Belmonte et al., 2004). Indeed, many of the genes associated with high risk for autism and those increasing susceptibility are directly, or indirectly, involved in axon guidance, neuronal signalling, metabolism, cell differentiation and synaptic homeostasis (Weiss et al., 2009; Autism genome project consortium, 2007; Tabuchi et al., 2007; Toro et al., 2010). Therefore, along with an early diagnosis (Limon 2007), the prevention and correction of the abnormal connectivity, and the modulation of the synaptic function are the main goals of current and future treatments of the pathological characteristics of the autistic disorders.

Glutamate and GABA are the main excitatory and inhibitory neurotransmitters in the human brain and both have important roles during early development of the nervous system, an ontological stage when the evidence indicates that autism begins. Therefore, it is important to analyse the functional status of glutamatergic and GABAergic neurotransmission in the autistic brain. Cumulative evidence indicates that dysfunctional excitatory and inhibitory synaptic activities underlie several of the characteristics of autism and are, consequently, important targets of pharmacological intervention. In this chapter we describe how glutamate and GABA receptors may participate in the aetiology of autistic disorders and will discuss some of the methods that we have developed to study functional and pharmacological properties of human membrane receptors. We include information demonstrating that functional studies of GABA and glutamate receptors from autistic tissue

are feasible and important for the development of new drugs aimed at the manipulation of the GABAergic or glutamatergic systems in the autistic brain.

2. Evidence of glutamatergic dysfunction in autism

Glutamate is the main excitatory neurotransmitter in the vertebrate brain and its effects are exerted mainly through metabotropic (mGlu) and ionotropic (iGlu) glutamate receptors localized in the cellular membranes of neurons and glia. The iGlu receptors are tetrameric proteins that mediate fast synaptic transmission and are grouped into three classes, according to their differential affinity for the agonists N-methyl-D-aspartate (NMDA), kainate (KA), and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) (Dingledine et al., 1999). The metabotropic receptors belong to the G-protein-coupled receptor superfamily, and can be divided in the group I (mGluR1 and mGluR5), group II (mGluR2 and mGluR3) and group III (mGluR4 and mGluR6-8) according to their agonist pharmacology, primary sequence and G-protein effector coupling (Fagni et al., 2004). The increased probability of epilepsy in the autistic population (Tuchman & Rapin, 2002) suggests that abnormally enhanced glutamatergic signalling may contribute to some of the autistic characteristics. Indeed, there is a slight positive correlation between plasma levels of glutamate and the severity of autism that supports this hypothesis (Shinohe et al., 2006). Moreover, the cerebellum of autistic patients had increased expression of mRNAs encoding the excitatory amino acid transporter 1 (EAAT 1) and the AMPA 1 receptor. Increments in those proteins would elevate the extracellular concentration of glutamate and enhance the postsynaptic activity of glutamatergic synapses (Puercell et al., 2001). Genome studies have found associations between autism and *GRM8*, which encodes the metabotropic glutamate receptor 8 (mGluR8) (Serajee et al., 2003). This receptor is localized at or near presynaptic sites and when activated, it negatively modulates the release of glutamate from the presynaptic terminal (Cartmell & Schoepp, 2000); therefore, mGluR8 dysfunction may lead to increased neuronal activity. Interestingly, regional subsets of presynaptic GABAergic and glutamatergic terminals innervating GABAergic interneurons are highly enriched with mGluR8 receptors in rat hippocampus (Ferraguti et al., 2005), suggesting that, if the same pattern is present in the human hippocampus, abnormally expressed mGluR8 would produce aberrant GABAergic activity and disrupt the temporal patterns of synchronic activity generated by inhibitory interneurons. Another important evidence suggesting a central role of glutamate receptors in autism comes from animal models of Fragile X syndrome, a neurological disorder with high prevalence of autism that is caused by silencing of the *FMR1* gene and its translated protein, the fragile X mental retardation protein FMRP (Dölen et al., 2010). This protein inhibits the synthesis of a wide range of proteins by binding to actively translating mRNAs. FMRP is a counterbalance to the synthesis of proteins during mGluR5-mediated neuronal plasticity, therefore, when FMRP is dysfunctional, excessive protein synthesis mediated by activation of mGluR5 leads to synaptic dysfunction in *Frm1* KO mice. Importantly, the phenotype of *Frm1* KO mice can be rescued by 50% reduction of the expression of mGluR5, suggesting that specific antagonists of mGluR5 will have beneficial effects on humans with Fragile X syndrome (Dölen et al., 2010) and encourages the search of pharmacological approaches to treat idiopathic autism. Genome studies have also found several single nucleotide polymorphisms (SNPs) of *GRIK2* associated with risk of autism (Jamain et al., 2002; Shuang et al., 2004). *GRIK2* produces the kainate receptor GluK2 (previously known as GluR6) that participates in processes of learning and memory at

postsynaptic sites and at presynaptic ones, modulates the release of glutamate and GABA from synaptic terminals (Pinheiro & Mulle, 2006). Moreover, one of the reported SNPs changes a methionine to an isoleucine in position 867 (M867I) of the intracellular C-terminus, producing a structural change of the receptor that may be related to the aetiology of autism. Electrophysiological experiments have shown that *Xenopus* oocytes expressing the rat version of M867I-GluK2 elicit larger ion currents than oocytes expressing the wild type receptor, and the increment of current correlates with an enhanced density of the mutated receptors on the plasma membrane of the oocytes (Strutz-Seebohm et al., 2006). Except for a slower rate of desensitization, no major changes in the kinetic properties of the human or rat version of the M867I-GluK2 could account for the observed increment in the ion currents (Han et al., 2010); therefore, the potential link between the M867I and the autistic phenotype could be related mostly to alterations in the normal trafficking of GluK2 in and out of presynaptic and postsynaptic terminals. Interestingly, despite a 99% homology between rat and human GluK2, both receptors had important kinetic and potency differences, highlighting the importance of the validation of animal models with data obtained from the human brain (Halladay et al., 2009; Limon et al., 2011).

3. Evidence of GABAergic dysfunction in autism

GABA is the most abundant and versatile neurotransmitter in the Central Nervous System (CNS). GABA is excitatory in the immature brain and inhibitory in the mature one (Ben-Ari, 2008). At early stages of neural development GABA has a paracrine action on immature neurons. It modulates neuronal migration, stimulating developing networks and exerting a wide range of trophic actions that lead to the correct establishment of neural circuits (Ben-Ari, 2008). In the adult brain, GABA participates in the generation of synchronous rhythms of cortical assemblies. This allows local time-precise communication among neurons and coherent communication with other cerebral centres, thus creating behavioural relevant processes (Somogyi et al., 2005). GABA actions on the membrane potential are mediated mostly by ionotropic receptors. Ionotropic GABA receptors in the CNS are pentameric channels made up by the combination of α (1-6), β (2-3), γ (1-3) and δ subunits in a γ–β–α–β–α arrangement, with δ substituting γ in some extrasynaptic receptors (Olsen & Sieghart, 2008). They are permeable to chloride ions and whether they depolarize or hyperpolarize the cell membrane potential depends on the developmentally regulated expression of the $Na^+/K^+/Cl^-$-cotransporter, NKCC1, and the K^+/Cl^--cotransporter, KCC2. NKCC1 is highly expressed in immature neurons and transports chloride into the neuron producing a high intracellular concentration of chloride, thus making GABA depolarizing. In mature neurons the increased expression of KCC2 and reduction of NKCC1 lowers the concentration of intracellular chloride and makes GABA hyperpolarizing (Mathews, 2007).

Due to the concerted and intertwined activity of GABAergic and glutamatergic neurotransmission, even small net deviances of GABAergic activity could affect the excitation-inhibition balance in the autistic brain. Such an imbalance would reduce the ratio signal to noise of the sensory and procedural information in mild cases (Casanova et al., 2006) and, in the extreme ones, it could lead to epilepsy. Actually, the incidence of epilepsy in one third of people with autism (Tuchman & Rapin, 2002) and the presence of paroxystic activity in the electroencephalogram (EEG) of approximately 68% of autistic people (Kim et al., 2006) is consistent with this hypothesis. These gradual alterations of the EEG traces suggest that aberrant electrical activity in the autistic brain is expressed as a continuum and

it may be present even in cases of autism with normal EEG recordings. Although abnormal electrical activity could have scores of causes, genetic association studies have implicated genes coding for the subunits γ1, α2, α4, β1 and β3 of $GABA_A$ receptors as likely contributors for autism (Blatt et al., 2001; Hussman, 2001; Cook et al., 1998; Ma et al., 2005; Vincent et al., 2006; Kakinuma & Sato, 2008). Indeed, evidence from disorders that share overlapping autistic characteristics like Prader-Willi/Angelman syndrome (AS) and Rett syndrome, supports the idea that GABA receptors are convergent nodes in autistic phenotypes of different genetic origins. Angelman syndrome is an imprinted disorder caused by a maternal deficiency of chromosome 15q11-q13 (Magenis et al., 1987; Lalande, 1996) that includes autistic characteristics and developmental delay, seizures and stereotyped behaviours (Samaco et al., 2005). Because the 15q11-q13 region contains genes for the β3, α5 and γ3 subunits of $GABA_A$ receptors, the chromosomal deficiencies in Angelman syndrome may produce alterations in the expression of these GABA subunits. Knockout mice with deletions in the $GABA_A$ α5 and γ3 subunits did not show a drastic phenotype; but a deletion in the gene for the $GABA_A$ β3 subunit produced a neonatal mortality of 90-95% and the survivors displayed a phenotype resembling severe forms of AS (Sinkkonen et al., 2003). These alterations were associated to a decreased number of GABA receptors in areas like the hippocampus (Sinkkonen et al., 2003) which is commonly affected in autism. Rett syndrome is a pervasive disorder classified within the autism spectrum category. Patients diagnosed with Rett syndrome, in addition to the triad of autistic characteristics, also show cognitive deficits, apraxia, ataxia, seizures and respiratory abnormalities (Chahrour & Zoghbi, 2007). Rett syndrome is the result of mutations of *MECP2*, a gene encoding the transcriptional regulator methyl-CpG-binding protein 2 (MeCp2) (Amir et al., 1999). Mice genetically modified to reduce the function of MeCp2 reproduce much of the phenotype of Rett syndrome. Interestingly these mice also show defects in the expression of the $GABA_A$ β3 subunit (Samaco et al., 2005). Recent studies have shown that selective deletion of *MECP2* in GABAergic neurons reproduces some of the characteristics of regressive autism. Initially, mice with *MECP2* deficiency were indistinguishable from the normal mice; however, after few weeks they started to develop stereotyped movements, compulsive grooming, impaired motor coordination, EEG hyperexcitability and abnormal behavioural patterns (Chao et al., 2010). The EEG abnormalities were tracked down to an impaired production of intracellular GABA[1] and a consequent reduction in the quantal GABA released (Chao et al., 2010). Interestingly, MeCp2 reductions in frontal cortex are frequent in autism and other mental disorders (Nagarajan et al., 2006). Monoallellic or skewed expression of *GABRB3* and a subsequent decrease of $GABA_A$ β3 subunit has been found in 4 out of 8 autistic patients (Hogart et al., 2007), indicating that origins of autism are not exclusively rooted in the genome but any epigenetic, or environmental factor that is able to modify the function of GABA receptors during the development of the nervous system is prone to cause neurodevelopmental disorders and, particularly in the case of GABA β3 subunits, it may lead to autistic phenotypes. Actually, there is strong evidence implicating GABAergic dysfunction in

[1]GABA is produced by the enzymes Gad65 and Gad67 which are respectively coded by the genes *Gad1* and *Gad2*. In *MECP2* deficient mice the expression of *Gad1* and *Gad2* and the immunoreactivity to GABA in interneurons were importantly reduced. These reductions were associated with smaller miniature postsynaptic events (mIPSC) without a change in their frequency (Chao et al., 2010).

anxiety, a common feature of autistic disorders (Amaral & Corbett., 2003). The hippocampus, lateral septum, periaqueductal gray matter and amygdala, which are all cortico-limbic structures involved in modulating anxiety states and have neuroanatomical changes in autism, contain major networks of GABAergic interneurons (Millan, 2003). Many studies focus on $GABA_A$ receptors and anxiety (Yilmazer-Hanke, 2003), but even $GABA_B$ (Mombereau et al., 2004) and $GABA_C$ receptors (Flores-Gracia et al., 2010) have been reported to be involved in anxiety-like behaviours.

The wide number of factors with effects on GABA receptors and the different degrees of changes in GABAergic signalling may explain, at least in part, the high heterogeneity found in the clinical phenotypes within the autism spectrum. However whether changes in animal models apply to humans and what other qualitative changes are present in the human brain is a matter of current research. Blatt et al. (2001) reported a reduced binding of benzodiazepines and muscimol in the hippocampus of autistic brains, suggesting a decrease in the number of $GABA_A$ receptors. Posterior studies using different concentrations of [H^3]flunitrazepan showed that the decrement of benzodiazepine binding was due to reductions of binding sites with no changes in the binding affinity of the receptors (Guptill et al., 2007). Western blot analyses of four $GABA_A$ subunits (α1-3 and β3) showed reductions of all subunits in parietal cortex, of α1 in frontal cortex and of α1 and β3 in cerebellum of post-mortem autistic brains (Fatemi et al., 2009). And recent studies showed reductions in the binding sites to muscimol and benzodiazepines in cingulate cortex and fusiform gyrus of autistic brains (Oblak et al., 2009; Oblak et al., 2011). These authors also found a reduction in the affinity of the binding sites to muscimol, suggesting pharmacological changes in the receptors due to changes in the properties of the same receptors or switching of $GABA_A$ subunits. Indeed, an increment in the expression of α5 has been reported in the autistic brain (Purcell et al., 2001), and a potential remodelling of GABA subunits may explain the several reports of paradoxical benzodiazepine-based sedatives on severe autistic individuals with mental retardation (Marrosu et al., 1987; Sandman & Barron; 1992; Aman & Langworthy, 2000).

Undoubtedly binding experiments are, and will continue, providing important information about the status of GABA receptors in the autistic brain, particularly about density and tissue localization. However, their resolution is limited and the absence of functional information on the receptors is a great drawback compared with electrophysiological studies, where even the ion current through an activated single channel can be detected. Therefore, important differences between $GABA_A$ receptors might be overlooked. Another concern is that the benzodiazepine binding site is not present in all $GABA_A$ receptor isoforms and alterations in GABA receptors containing δ, α4 and α6 subunits, which do not bind classical benzodiazepines but are highly expressed in cerebellum (Sieghart, 2006), have not yet been appropriately addressed. It is worth noting that because of the duplication of the 4p12 chromosome (Ma et al., 2005), a gain of function of the α4 subunits is expected, but not yet explored. Also important is the fact that normal binding does not necessarily mean normal activation; therefore, even though the agonists and antagonists can bind to the receptor, determining whether the receptors are functional or not requires a multidisciplinary approach, using binding, biochemical and electrophysiological methodologies. Because GABA and glutamate are main targets of pharmacological intervention, a detailed analyses of their kinetic and biophysical properties will help to evaluate new drugs and therapeutic treatments.

4. Pharmacology of human receptors beyond binding studies

We have developed two methods that allow in-depth studies of neurotransmitter receptors and ion channels of the **human** brain. The first, now widely used, involves the heterologous expression of human receptors in *Xenopus* oocytes, which, because of their size, sturdiness, and availability, permit experiments that would be very difficult, or impossible, to carry out in the human brain. The second method is the microtransplantation of native receptors that allows the study of native receptors **still embedded in their own lipids and with their associated proteins.**

4.1 Heterologous expression of human receptors

The *Xenopus* oocyte system is a very convenient model for studying the electrophysiological properties of membrane receptors (Kusano et al. 1977). G protein-coupled receptors (GPCRs) that are activated through the phosphatidylinositol signal pathway, and ligand gated ion channels, are particularly easy to study in this system. GPCRs activated through the cAMP signal pathway are also possible, although they are more difficult to study and require co-expression of some of the pathway elements (Gether et al., 2002). Heterologous expression in *Xenopus* oocytes is now a classic method to study the functional impact of mutations in membrane receptors, and has a long tradition on the study of neurological disorders, such as Alzheimer's disease and epilepsy (Dauch et al., 1997; Green et al., 2008; Palma et al. 2002). In autism research, the use of *Xenopus* oocytes is just at the beginning. However, it has already helped to demonstrate the gain of function of the M867I-GluK2 mutation associated with autism (Strutz-Seebohm et al., 2006) and the functional impact of mutations in voltage-gated calcium channels that lead to autistic traits in Timothy syndrome (Splawski et al., 2004). Timothy syndrome is a multisystem disorder characterized by syndactyly, arrhythmias and low survival. A high percentage of the children that survive the first years develop autism (Splawski et al., 2004). Timothy syndrome is caused by a substitution of glycine by arginine at residue 406 (G640R) of the cardiac L-type $Ca_v1.2$ channel and it is expressed in several organs including the brain. The expression of wild type and the mutant versions of $Ca_v1.2$ channels in *Xenopus* oocytes showed that the mutation dramatically impairs the voltage-dependent inactivation of the channel and leads to larger and longer calcium currents (Splawski et al., 2004). The gain of function of the $Ca_v1.2$ channel suggests that impairments of calcium signalling participate in the aetiology of autism. Such central role of calcium in autism is supported by the recent finding that increased levels of calcium enhance the activity of the mitochondrial aspartate/glutamate carrier AGC1, a protein coded by the autism susceptibility gene *SLC25A12* (Palmieri et al., 2010).

The evidence that single mutations of glutamate- and calcium channels can lead to autism indicates that abnormal network excitability and intracellular signalling are critical factors in the generation of autistic phenotypes; and highlights the importance of understanding the electrophysiological properties of the receptors and channels, even in cases where no mutations have been reported. The expression of mRNA or cloned human receptors in *Xenopus* oocytes is a potent tool to evaluate the functional properties of GABA-, glutamate - and calcium-channels in the autistic brain (Limon et al., 2008). Next we describe the method we use to express human receptors in *Xenopus* oocytes.

4.1.1 Methodological insight into heterologous expression in *Xenopus* oocytes

In order to study ion channels and other membrane proteins via the expression approach, the gene must be available in a plasmid. Nuclear injections of plasmidic DNAs can be done,

provided a relative high purity sample is used. We have found that plasmidic DNA isolated with spin columns is good enough to express receptors. The human cytomegalovirus (CMV) promoter is our promoter of choice. We routinely inject 14 nL at a concentration of about 200 ng/mL, aiming at the animal pole and injecting deep to increase the likelihood of reaching the nucleus. Notwithstanding, the rate of successful nuclear injection is not very high, ending always with some oocytes that do not express the protein and with increased oocyte "mortality". We also use the T7 promoter, but the level of expression is considerably lower (see also Geib et al., 2001). Another factor to consider is our observation that the receptor induced membrane currents achieved in the oocytes successfully injected with DNA are lower than those achieved after injecting cRNA. Therefore, our preferred choice for expression of channels is the cytoplasmic injection of synthetic cRNA. If the gene of interest is placed after any RNA Polymerase promoter such as T7, T3, SP6, synthetic cRNA can be produced in large quantities and with a desirable level of purity. One can use the template DNA and add the Polymerase, rNTPs and a reaction of a couple of hours will yield microgram quantities of RNA (Krieg et al., 1984). The ready to use kits are very convenient and even incorporate a 5′ cap analog and have the option of adding an enzyme that will incorporate a Poly(A)+ tail at the 3′ end to resemble more closely a natural RNA.
Of all the kits we have tested, the mMessage mMachine kit from Ambion is the one that has given us the best results. That kit uses ARCA (Anti Reverse Cap Analog) 5′ cap analog (Stepinski et al., 2001), which prevents incorporation in the reverse orientation and maximizes translation efficiency. In such a way, we obtain RNAs that have strong **expressional potency** after injecting 50 nL (at ca. 1 mg/mL) into the equator of the oocyte (Limon et al., 2007; Reyes-Ruiz et al., 2010; Limon et al., 2010). Besides its use for the expression of recombinant proteins, *Xenopus* oocytes can also express membrane proteins after injection of mRNAs directly isolated from human biopsy tissue or from post-mortem brains (Gundersen et al., 1984; Palma et al., 2002; Limon et al., 2008). For the expression of mRNA, the quality of the starting material is important, specially making sure the tissue is not fixed, but snap frozen in liquid nitrogen or CO_2. We have isolated mRNAs that were able to express functional receptors and ion channels from tissue that was obtained several hours post-mortem and was stored frozen for several years (Limon et al., 2008). For the mRNA isolation procedure, it is preferable to start with at least one gram of tissue. We use the TRIzol method for total RNA isolation. The Poly(A)$^+$ RNA is then isolated using an Oligo (dT) resin. A chromatography column is our method of choice because it is more convenient for larger volumes. After overnight precipitation with NaCl, the mRNA is thoroughly washed with 70% ethanol and resuspended in RNase free water at a concentration of 1 mg/mL or higher if possible. By starting with 1 g of tissue we generally recover about 20 or 30 µg of mRNA, suitable for at least 10 injections of 20 oocyte batches. Even though this is the method that has given us the best results, very often the mRNA obtained fails to express large currents after injection, independently of the yield or A260/A280 ratio, although that mRNA is usually good enough for qPCR assays. This methodology extends the amount of information yielded by the mRNA, because functional data can be cross-correlated with data from qPCR analyses of GABA subunits and synaptic markers.

4.2 Microtransplantation of human receptors

Proteins expressed *de novo* in *Xenopus* oocytes have post-translational modifications and membrane lipids that are specific for the oocyte, not for the human brain. Moreover,

microenvironmental effects on the receptor or epigenetic modifications that may have arisen through complex cellular signalling are not necessarily reproduced by the expression of mRNA. To overcome those problems, we have been studying native human receptors using the microtransplantation method which is conceptually depicted in Figure 1. To microtransplant native membranes from human brains, part of the brain is homogenized and the cell membranes are isolated by centrifugation (Miledi et al., 2004).

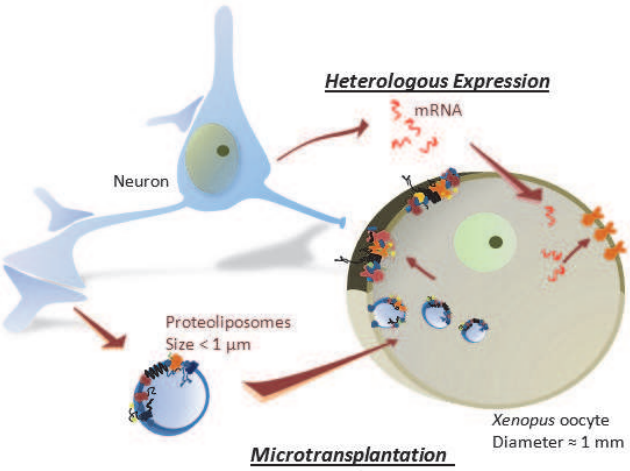


Fig. 1. Methods for evaluating the electrophysiological properties of human neurotransmitter receptors. Diagram showing the heterologous expression of proteins in *Xenopus* oocytes (top) and the microtransplantation of native cell membranes to oocytes (bottom). For the microtransplantation, proteoliposomes isolated from brain tissue specimens, containing the original native receptors, are injected into the oocytes. Within a few hours after injection the proteoliposomes fuse with the oocyte's plasma membrane and expose the native receptors to pharmacological and biophysical experimentation. For the expression of *de novo* the human receptors, mRNA is isolated and injected into the oocytes; and the newly expressed receptors can then be studied in great detail.

Cell membranes, mostly in the form of small vesicles, are adjusted to a protein concentration of 1-2 mg/mL and injected into an oocyte. Within a few hours the membranes, carrying their original neurotransmitter receptors and ion channels, begin to fuse with the oocyte plasma membrane. Voltage-clamp recording is then used to study the functional characteristics of the transplanted receptors. Oocytes with transplanted receptors can be studied up to several days post-injection (Miledi et al., 2004; Limon et al., 2008).

We have assessed whether autistic brains with long post-mortem intervals still contain functional neurotransmitter receptors and voltage-operated ion channels that could be microtransplanted into *Xenopus* oocytes. For that purpose, we chose the cerebellum and temporal cortex, because neuroanatomical and biochemical studies have shown abnormalities in the neuronal organization of those areas (Bauman & Kemper, 1985; Bailey et al., 1998; Purcell et al., 2001). Our initial studies of six autistic cerebella showed that the amounts of microtransplanted receptors differed from their respective controls in all cases.

Four of them yielded responses to GABA, kainate, and glutamate that were smaller than their respective paired controls. The other two cases generated larger responses. In contrast, when the receptors of the temporal cortex were microtransplanted, the amplitudes of the currents induced by kainate, glutamate, and GABA by the receptors from the autistic membranes were larger than their controls in two of the three autistic cases (Limon et al., 2008). Interestingly, even though the number of patients tested is low, deviations from the controls were already found, supporting the hypothesis of an altered GABAergic signalling in the autistic brain, thus encouraging further studies.

It will be interesting to microtransplant receptors from other areas of the brain. The hippocampus is important in memory and learning processes; and together with the amygdala and prefrontal cortex participates in the negative feedback of the hypothalamic-pituitary-adrenal (HPA) axis which in turn controls the body's response to stress (Morris, 2007). The hippocampus and amygdala have clear neuroanatomical alterations in autistic brains (Bauman & Kemper, 2005) and there is a hypothesis that prenatal stress may affect the HPA axis during development and increase the risk of developing autism (O'Donnell et al., 2009). Accordingly we evaluated if neurotransmitter receptors from hippocampi with long post-mortem intervals can be transplanted to *Xenopus* oocytes.

4.2.1 Microtransplantation of native receptors from the hippocampus of autistic brains

For these experiments we used the anterior hippocampus from two autistic brains and two matching control brains (Table 1). Cell membranes were prepared as previously reported (Limon et al., 2008) and then injected into the equator of *Xenopus* oocytes. Ligand-activated ion currents were observed after 24 h post-injection in oocytes voltage-clamped at -80 mV, clearly indicating the successful transplantation of functional GABA and glutamate receptors (Fig. 2). Oocytes injected with cell membranes derived from both autistic brains showed smaller GABA-currents than their respective controls (Fig. 3); suggesting that the number of GABA receptors is decreased in the hippocampus of autistic brains. However, for the moment we have not discounted the possibility that changes in the properties of the receptors, or in the efficiency of membrane fusion, produce a decrease of the currents without a decrease of receptor density. Kainate induced currents were reduced in the autistic sample from the older patient and unaffected in the other autistic sample. Evidently further studies are needed including a statistically meaningful number of samples. However, it is already evident that the microtransplantation of receptors from hippocampus, even with long post-mortem intervals is possible.

Case	Dx	Age (years)	Gender	PMI (h)
B6076	Control	38	Male	25.47
B6401	Autism	39	Male	13.95
B6207	Control	16	Male	26.16
B5666	Autism	8	Male	22.16

Table 1. Characteristics of the tissue used. Dx, diagnosis; PMI, post-mortem interval.

An important advantage of the microtransplantation method is that the biophysical studies are done directly on native receptors that were once in the human brain and are still embedded in their original lipids and with their own cohort of associated proteins. Another advantage is the

rapid and high yield of functional and pharmacological information, obtained from minimal amounts of protein. For comparative purposes, consider for example that to prepare a 2-dimensional gel ranges of 2-2000 µg of protein are needed (Adams & Gallagher, 2005); for a Western Blot about 40 µg is recommended. Even for mass spectrometry-based proteomics few µg of total protein is used. In contrast, with the microtransplantation method 50 ng of protein are injected into a single oocyte and a full dose response plus several pharmacological experiments can be done with such a small amount of protein.

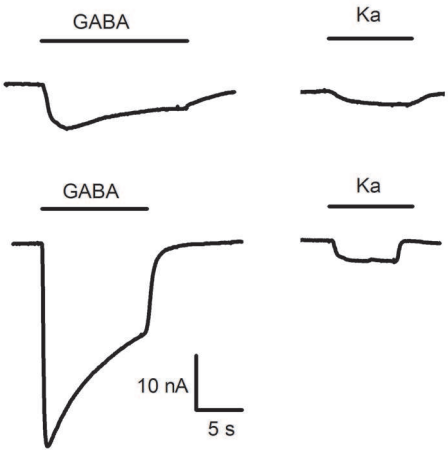


Fig. 2. Sample responses to 1 mM GABA and 100 µM kainate of an oocyte injected with membranes from an autistic hippocampus (above; case B6076, PMI = 25.47 h) and of another oocyte injected with its matching control (below, case B6401, PMI = 13.95 h).

5. Conclusion

Strong evidence indicates that autism is a developmental synaptic disorder that affects the processing of behavioural relevant information. Although the causes of autism are still not known, GABAergic and glutamatergic synapses appear to be convergent nodes of genetic, epigenetic, and probably environmental factors causing the autistic phenotype. Even small alterations in GABAergic or glutamatergic signalling produce autistic characteristics in animal models, and it is highly probable that a similar phenomenon is present in the human brain. GABA and glutamate receptors are also important targets of pharmacological interventions and a detailed knowledge of their function in the human brain will improve the use and design of molecules with therapeutic activity. The microtransplantation of the original receptors from postmortem brains coupled to the expression of receptors by post-mortem mRNAs will help to determine in great detail the type, number, and functional properties of autistic neurotransmitter receptors and channels. These procedures will help to decipher the functional impact of genetic and epigenetic factors in autism. Furthermore, the microtransplantation method will help to determine the mode of action of the medicines presently used to treat autism, and help to develop new medicines and evaluate their pharmacological activity.

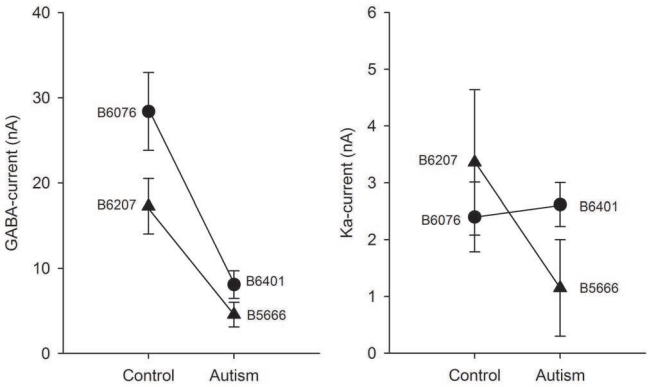


Fig. 3. Responses to 1 mM GABA and 100 μM kainate in oocytes injected with membrane preparations from anterior hippocampus of autistic and control brains (n=5-6 oocytes/case). Data is mean ± SEM.

6. Acknowledgment

We are extremely grateful to all the persons that donated their brains for scientific research. Human brain tissue samples were generously provided by the Autism Tissue Program via the Harvard Brain Tissue Resource Center (Belmont, MA). This work was supported by the King Abdul Aziz City for Science and Technology, (Riyadh, SA; Grant KACST-46749).

7. References

Adams, L. D., and S. R. Gallagher. "Two-Dimensional Gel Electrophoresis." Curr Protoc Immunol Chapter 8 (2005): Unit 8 5.

Aman, M. G., and K. S. Langworthy. "Pharmacotherapy for Hyperactivity in Children with Autism and Other Pervasive Developmental Disorders." J Autism Dev Disord 30, no. 5 (2000): 451-9.

Amaral, D. G., and B. A. Corbett. "The Amygdala, Autism and Anxiety." Novartis Found Symp 251 (2003): 177-87; discussion 87-97, 281-97.

Amir, R. E., I. B. Van den Veyver, M. Wan, C. Q. Tran, U. Francke, and H. Y. Zoghbi. "Rett Syndrome Is Caused by Mutations in X-Linked MeCp2, Encoding Methyl-Cpg-Binding Protein 2." Nat Genet 23, no. 2 (1999): 185-8.

Autism genome project consortium. "Mapping Autism Risk Loci Using Genetic Linkage and Chromosomal Rearrangements." Nat Genet 39, no. 3 (2007): 319-28.

Bailey, A., P. Luthert, A. Dean, B. Harding, I. Janota, M. Montgomery, M. Rutter, and P. Lantos. "A Clinicopathological Study of Autism." Brain 121 (Pt 5) (1998): 889-905.

Bauman, M., and T. L. Kemper. "Histoanatomic Observations of the Brain in Early Infantile Autism." Neurology 35, no. 6 (1985): 866-74.

Belmonte, M. K., E. H. Cook, Jr., G. M. Anderson, J. L. Rubenstein, W. T. Greenough, A. Beckel-Mitchener, E. Courchesne, L. M. Boulanger, S. B. Powell, P. R. Levitt, E. K. Perry, Y. H. Jiang, T. M. DeLorey, and E. Tierney. "Autism as a Disorder of Neural

Information Processing: Directions for Research and Targets for Therapy." Mol Psychiatry 9, no. 7 (2004): 646-63.

Ben-Ari, Y. "Neuro-Archaeology: Pre-Symptomatic Architecture and Signature of Neurological Disorders." Trends Neurosci 31, no. 12 (2008): 626-36.

Blatt, G. J., C. M. Fitzgerald, J. T. Guptill, A. B. Booker, T. L. Kemper, and M. L. Bauman. "Density and Distribution of Hippocampal Neurotransmitter Receptors in Autism: An Autoradiographic Study." J Autism Dev Disord 31, no. 6 (2001): 537-43.

Bauman, M. L., and T. L. Kemper. "Neuroanatomic Observations of the Brain in Autism: A Review and Future Directions." Int J Dev Neurosci 23, no. 2-3 (2005): 183-7.

Cartmell, J., and D. D. Schoepp. "Regulation of Neurotransmitter Release by Metabotropic Glutamate Receptors." J Neurochem 75, no. 3 (2000): 889-907.

Casanova, M. F., I. A. van Kooten, A. E. Switala, H. van Engeland, H. Heinsen, H. W. Steinbusch, P. R. Hof, J. Trippe, J. Stone, and C. Schmitz. "Minicolumnar Abnormalities in Autism." Acta Neuropathol 112, no. 3 (2006): 287-303.

Chahrour, M., and H. Y. Zoghbi. "The Story of Rett Syndrome: From Clinic to Neurobiology." Neuron 56, no. 3 (2007): 422-37.

Chao, H. T., H. Chen, R. C. Samaco, M. Xue, M. Chahrour, J. Yoo, J. L. Neul, S. Gong, H. C. Lu, N. Heintz, M. Ekker, J. L. Rubenstein, J. L. Noebels, C. Rosenmund, and H. Y. Zoghbi. "Dysfunction in Gaba Signalling Mediates Autism-Like Stereotypies and Rett Syndrome Phenotypes." Nature 468, no. 7321 (2010): 263-9.

Cook, E. H., Jr., R. Y. Courchesne, N. J. Cox, C. Lord, D. Gonen, S. J. Guter, A. Lincoln, K. Nix, R. Haas, B. L. Leventhal, and E. Courchesne. "Linkage-Disequilibrium Mapping of Autistic Disorder, with 15q11-13 Markers." Am J Hum Genet 62, no. 5 (1998): 1077-83.

Dauch, P., G. Champigny, J. E. Ricci, and F. Checler. "Lack of Effect of Presenilin 1, Betaapp and Their Alzheimer's Disease-Related Mutated Forms on Xenopus Oocytes Membrane Currents." Neurosci Lett 221, no. 2-3 (1997): 85-8.

Dingledine, R., K. Borges, D. Bowie, and S. F. Traynelis. "The Glutamate Receptor Ion Channels." Pharmacol Rev 51, no. 1 (1999): 7-61.

Dölen, G., R. L. Carpenter, T. D. Ocain, and M. F. Bear. "Mechanism-Based Approaches to Treating Fragile X." Pharmacol Ther 127, no. 1: 78-93.

Fagni, L., F. Ango, J. Perroy, and J. Bockaert. "Identification and Functional Roles of Metabotropic Glutamate Receptor-Interacting Proteins." Semin Cell Dev Biol 15, no. 3 (2004): 289-98.

Fatemi, S. H., T. J. Reutiman, T. D. Folsom, and P. D. Thuras. "GABA(A) Receptor Downregulation in Brains of Subjects with Autism." J Autism Dev Disord 39, no. 2 (2009): 223-30.

Ferraguti, F., T. Klausberger, P. Cobden, A. Baude, J. D. Roberts, P. Szucs, A. Kinoshita, R. Shigemoto, P. Somogyi, and Y. Dalezios. "Metabotropic Glutamate Receptor 8-Expressing Nerve Terminals Target Subsets of Gabaergic Neurons in the Hippocampus." J Neurosci 25, no. 45 (2005): 10520-36.

Flores-Gracia, C., A. Nuche-Bricaire, M. Crespo-Ramirez, R. Miledi, K. Fuxe, and M. Perez de la Mora. "GABA(A) Rho Receptor Mechanisms in the Rat Amygdala and Its Role in the Modulation of Fear and Anxiety." Psychopharmacology (Berl) 212, no. 4 (2010): 475-84.

Geib, S., G. Sandoz, E. Carlier, V. Cornet, V. Cheynet-Sauvion, and M. De Waard. "A Novel Xenopus Oocyte Expression System Based on Cytoplasmic Coinjection of T7-Driven Plasmids and Purified T7-Rna Polymerase." Receptors Channels 7, no. 5 (2001): 331-43.

Gether, U., F. Asmar, A. K. Meinild, and S. G. Rasmussen. "Structural Basis for Activation of G-Protein-Coupled Receptors." Pharmacol Toxicol 91, no. 6 (2002): 304-12.

Green, K. N., A. Demuro, Y. Akbari, B. D. Hitt, I. F. Smith, I. Parker, and F. M. LaFerla. "Serca Pump Activity Is Physiologically Regulated by Presenilin and Regulates Amyloid Beta Production." J Cell Biol 181, no. 7 (2008): 1107-16.

Guptill, J. T., A. B. Booker, T. T. Gibbs, T. L. Kemper, M. L. Bauman, and G. J. Blatt. "[3h]-Flunitrazepam-Labeled Benzodiazepine Binding Sites in the Hippocampal Formation in Autism: A Multiple Concentration Autoradiographic Study." J Autism Dev Disord 37, no. 5 (2007): 911-20.

Gundersen, C. B., R. Miledi, and I. Parker. "Messenger Rna from Human Brain Induces Drug- and Voltage-Operated Channels in Xenopus Oocytes." Nature 308, no. 5958 (1984): 421-4.

Halladay, A. K., D. Amaral, M. Aschner, V. J. Bolivar, A. Bowman, E. DiCicco-Bloom, S. L. Hyman, F. Keller, P. Lein, I. Pessah, L. Restifo, and D. W. Threadgill. "Animal Models of Autism Spectrum Disorders: Information for Neurotoxicologists." Neurotoxicology 30, no. 5 (2009): 811-21.

Han, Y., C. Wang, J. S. Park, and L. Niu. "Channel-Opening Kinetic Mechanism for Human Wild-Type Gluk2 and the M867i Mutant Kainate Receptor." Biochemistry 49, no. 43 (2010): 9207-16.

Hoekstra, R. A., M. Bartels, C. J. Verweij, and D. I. Boomsma. "Heritability of Autistic Traits in the General Population." Arch Pediatr Adolesc Med 161, no. 4 (2007): 372-7.

Hogart, A., R. P. Nagarajan, K. A. Patzel, D. H. Yasui, and J. M. Lasalle. "15q11-13 $GABA_A$ Receptor Genes Are Normally Biallelically Expressed in Brain yet Are Subject to Epigenetic Dysregulation in Autism-Spectrum Disorders." Hum Mol Genet 16, no. 6 (2007): 691-703.

Hussman, J. P. "Suppressed Gabaergic Inhibition as a Common Factor in Suspected Etiologies of Autism." J Autism Dev Disord 31, no. 2 (2001): 247-8.

Jamain, S., C. Betancur, H. Quach, A. Philippe, M. Fellous, B. Giros, C. Gillberg, M. Leboyer, and T. Bourgeron. "Linkage and Association of the Glutamate Receptor 6 Gene with Autism." Mol Psychiatry 7, no. 3 (2002): 302-10.

Kakinuma, H., and H. Sato. "Copy-Number Variations Associated with Autism Spectrum Disorder." Pharmacogenomics 9, no. 8 (2008): 1143-54.

Kim, H. L., J. H. Donnelly, A. E. Tournay, T. M. Book, and P. Filipek. "Absence of Seizures Despite High Prevalence of Epileptiform Eeg Abnormalities in Children with Autism Monitored in a Tertiary Care Center." Epilepsia 47, no. 2 (2006): 394-8.

Krieg, P. A., and D. A. Melton. "Functional Messenger Rnas Are Produced by Sp6 in Vitro Transcription of Cloned Cdnas." Nucleic Acids Res 12, no. 18 (1984): 7057-70.

Kusano, K., R. Miledi, and J. Stinnakre. "Acetylcholine Receptors in the Oocyte Membrane." Nature 270, no. 5639 (1977): 739-41.

Lalande, M. "Parental Imprinting and Human Disease." Annu Rev Genet 30 (1996): 173-95.

Limon, A. "Importance of Early Detection in Autism Spectrum Disorder." Gac Med Mex 143, no. 1 (2007): 73-8.

Limon, A., J. M. Reyes-Ruiz, F. Eusebi, and R. Miledi. "Properties of Glur3 Receptors Tagged with GFP at the Amino or Carboxyl Terminus." Proc Natl Acad Sci U S A 104, no. 39 (2007): 15526-30.

Limon, A., J. M. Reyes-Ruiz, and R. Miledi. "Microtransplantation of Neurotransmitter Receptors from Postmortem Autistic Brains to Xenopus Oocytes." Proc Natl Acad Sci U S A 105, no. 31 (2008): 10973-7.

Limon, A., J. M. Reyes-Ruiz, R. G. Vaswani, A. R. Chamberlin, and R. Miledi. "Kaitocephalin Antagonism of Glutamate Receptors Expressed in Xenopus Oocytes." ACS Chem Neurosci 1, no. 3 (2010): 175-81.

Limon, A., J. M. Reyes-Ruiz, and R. Miledi. "GABAergic drugs and Alzheimer's disease." Fut Med Chem 3, no. 2 (2011): 149-53.

Ma, D. Q., P. L. Whitehead, M. M. Menold, E. R. Martin, A. E. Ashley-Koch, H. Mei, M. D. Ritchie, G. R. Delong, R. K. Abramson, H. H. Wright, M. L. Cuccaro, J. P. Hussman, J. R. Gilbert, and M. A. Pericak-Vance. "Identification of Significant Association and Gene-Gene Interaction of GABA Receptor Subunit Genes in Autism." Am J Hum Genet 77, no. 3 (2005): 377-88.

Magenis, R. E., M. G. Brown, D. A. Lacy, S. Budden, and S. LaFranchi. "Is Angelman Syndrome an Alternate Result of Del(15)(Q11q13)?" Am J Med Genet 28, no. 4 (1987): 829-38.

Marrosu, F., G. Marrosu, M. G. Rachel, and G. Biggio. "Paradoxical Reactions Elicited by Diazepam in Children with Classic Autism." Funct Neurol 2, no. 3 (1987): 355-61.

Mathews, G. C. "The Dual Roles of GABA in Seizures and Epilepsy Generate More Excitement." Epilepsy Curr 7, no. 1 (2007): 28-30.

Miledi, R., Z. Duenas, A. Martinez-Torres, C. H. Kawas, and F. Eusebi. "Microtransplantation of Functional Receptors and Channels from the Alzheimer's Brain to Frog Oocytes." Proc Natl Acad Sci U S A 101, no. 6 (2004): 1760-3.

Millan, M. J. "The Neurobiology and Control of Anxious States." Prog Neurobiol 70, no. 2 (2003): 83-244.

Mombereau, C., K. Kaupmann, W. Froestl, G. Sansig, H. van der Putten, and J. F. Cryan. "Genetic and Pharmacological Evidence of a Role for Gaba(B) Receptors in the Modulation of Anxiety- and Antidepressant-Like Behavior." Neuropsychopharmacology 29, no. 6 (2004): 1050-62.

Morris, R. "Stress and the hippocampus.", In: *The hippocampus book*, P. Andersen, R. Morris, D. Amaral, T. Bliss, J O'Keefe. (Ed), (2007): pp. 751-68.

Nagarajan, R. P., A. R. Hogart, Y. Gwye, M. R. Martin, and J. M. LaSalle. "Reduced MeCp2 Expression Is Frequent in Autism Frontal Cortex and Correlates with Aberrant Mecp2 Promoter Methylation." Epigenetics 1, no. 4 (2006): e1-11.

Oblak, A., T. T. Gibbs, and G. J. Blatt. "Decreased $GABA_A$ Receptors and Benzodiazepine Binding Sites in the Anterior Cingulate Cortex in Autism." Autism Res 2, no. 4 (2009): 205-19.

Oblak, A. L., T. T. Gibbs, and G. J. Blatt. "Reduced GABA(A) Receptors and Benzodiazepine Binding Sites in the Posterior Cingulate Cortex and Fusiform Gyrus in Autism." Brain Res 1380 (2011): 218-28.

O'Donnell, K., T. G. O'Connor, and V. Glover. "Prenatal Stress and Neurodevelopment of the Child: Focus on the HPA Axis and Role of the Placenta." Dev Neurosci 31, no. 4 (2009): 285-92.

Olsen, R. W., and W. Sieghart. "International Union of Pharmacology. Lxx. Subtypes of Gamma-Aminobutyric Acid(a) Receptors: Classification on the Basis of Subunit Composition, Pharmacology, and Function. Update." Pharmacol Rev 60, no. 3 (2008): 243-60.

Palma, E., V. Esposito, A. M. Mileo, G. Di Gennaro, P. Quarato, F. Giangaspero, C. Scoppetta, P. Onorati, F. Trettel, R. Miledi, and F. Eusebi. "Expression of Human Epileptic Temporal Lobe Neurotransmitter Receptors in Xenopus Oocytes: An Innovative Approach to Study Epilepsy." Proc Natl Acad Sci U S A 99, no. 23 (2002): 15078-83.

Palmieri, L., V. Papaleo, V. Porcelli, P. Scarcia, L. Gaita, R. Sacco, J. Hager, F. Rousseau, P. Curatolo, B. Manzi, R. Militerni, C. Bravaccio, S. Trillo, C. Schneider, R. Melmed, M. Elia, C. Lenti, M. Saccani, T. Pascucci, S. Puglisi-Allegra, K. L. Reichelt, and A. M. Persico. "Altered Calcium Homeostasis in Autism-Spectrum Disorders: Evidence from Biochemical and Genetic Studies of the Mitochondrial Aspartate/Glutamate Carrier Agc1." Mol Psychiatry 15, no. 1: 38-52.

Persico, A. M., and T. Bourgeron. "Searching for Ways out of the Autism Maze: Genetic, Epigenetic and Environmental Clues." Trends Neurosci 29, no. 7 (2006): 349-58.

Pinheiro, P., and C. Mulle. "Kainate Receptors." Cell Tissue Res 326, no. 2 (2006): 457-82.

Purcell, A. E., O. H. Jeon, A. W. Zimmerman, M. E. Blue, and J. Pevsner. "Postmortem Brain Abnormalities of the Glutamate Neurotransmitter System in Autism." Neurology 57, no. 9 (2001): 1618-28.

Reyes-Ruiz, J. M., L. D. Ochoa-de la Paz, A. Martinez-Torres, and R. Miledi. "Functional Impact of Serial Deletions at the C-Terminus of the Human GABArho1 Receptor." Biochim Biophys Acta 1798, no. 5: 1002-7.

Ritvo, E. R., B. J. Freeman, A. B. Scheibel, T. Duong, H. Robinson, D. Guthrie, and A. Ritvo. "Lower Purkinje Cell Counts in the Cerebella of Four Autistic Subjects: Initial Findings of the Ucla-Nsac Autopsy Research Report." Am J Psychiatry 143, no. 7 (1986): 862-6.

Samaco, R. C., A. Hogart, and J. M. LaSalle. "Epigenetic Overlap in Autism-Spectrum Neurodevelopmental Disorders: MeCp2 Deficiency Causes Reduced Expression of UBE3A and GABRB3." Hum Mol Genet 14, no. 4 (2005): 483-92.

Sandman, C.A., and J. Barron. "Paradoxical response to sedative/hypnotics in subjects with self-injurious behavior and stereotypy". J Dev Phys Disabil 4, no. 4 (1992):307-16.

Serajee, F. J., H. Zhong, R. Nabi, and A. H. Huq. "The Metabotropic Glutamate Receptor 8 Gene at 7q31: Partial Duplication and Possible Association with Autism." J Med Genet 40, no. 4 (2003): e42.

Sinkkonen, S. T., G. E. Homanics, and E. R. Korpi. "Mouse Models of Angelman Syndrome, a Neurodevelopmental Disorder, Display Different Brain Regional GABA(A) Receptor Alterations." Neurosci Lett 340, no. 3 (2003): 205-8.

Shinohe, A., K. Hashimoto, K. Nakamura, M. Tsujii, Y. Iwata, K. J. Tsuchiya, Y. Sekine, S. Suda, K. Suzuki, G. Sugihara, H. Matsuzaki, Y. Minabe, T. Sugiyama, M. Kawai, M. Iyo, N. Takei, and N. Mori. "Increased Serum Levels of Glutamate in Adult Patients with Autism." Prog Neuropsychopharmacol Biol Psychiatry 30, no. 8 (2006): 1472-7.

Shuang, M., J. Liu, M. X. Jia, J. Z. Yang, S. P. Wu, X. H. Gong, Y. S. Ling, Y. Ruan, X. L. Yang, and D. Zhang. "Family-Based Association Study between Autism and Glutamate

Receptor 6 Gene in Chinese Han Trios." Am J Med Genet B Neuropsychiatr Genet 131B, no. 1 (2004): 48-50.

Sieghart, W. "Structure, Pharmacology, and Function of $GABA_A$ Receptor Subtypes." Adv Pharmacol 54 (2006): 231-63.

Somogyi, P., and T. Klausberger. "Defined Types of Cortical Interneurone Structure Space and Spike Timing in the Hippocampus." J Physiol 562, no. Pt 1 (2005): 9-26.

Splawski, I., K. W. Timothy, L. M. Sharpe, N. Decher, P. Kumar, R. Bloise, C. Napolitano, P. J. Schwartz, R. M. Joseph, K. Condouris, H. Tager-Flusberg, S. G. Priori, M. C. Sanguinetti, and M. T. Keating. "Ca(V)1.2 Calcium Channel Dysfunction Causes a Multisystem Disorder Including Arrhythmia and Autism." Cell 119, no. 1 (2004): 19-31.

Stepinski, J., C. Waddell, R. Stolarski, E. Darzynkiewicz, and R. E. Rhoads. "Synthesis and Properties of Mrnas Containing the Novel "Anti-Reverse" Cap Analogs 7-Methyl(3'-O-Methyl)Gpppg and 7-Methyl (3'-Deoxy)Gpppg." RNA 7, no. 10 (2001): 1486-95.

Strutz-Seebohm, N., G. Korniychuk, R. Schwarz, R. Baltaev, O. N. Ureche, A. F. Mack, Z. L. Ma, M. Hollmann, F. Lang, and G. Seebohm. "Functional Significance of the Kainate Receptor Glur6(M836i) Mutation That Is Linked to Autism." Cell Physiol Biochem 18, no. 4-5 (2006): 287-94.

Tabuchi, K., J. Blundell, M. R. Etherton, R. E. Hammer, X. Liu, C. M. Powell, and T. C. Sudhof. "A Neuroligin-3 Mutation Implicated in Autism Increases Inhibitory Synaptic Transmission in Mice." Science 318, no. 5847 (2007): 71-6.

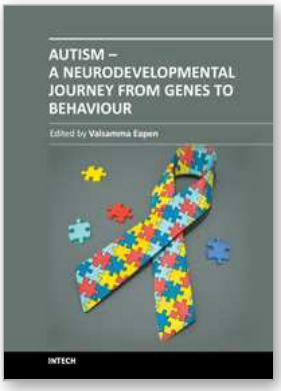
Tuchman, R., and I. Rapin. "Epilepsy in Autism." Lancet Neurol 1, no. 6 (2002): 352-8.

Toro, R., M. Konyukh, R. Delorme, C. Leblond, P. Chaste, F. Fauchereau, M. Coleman, M. Leboyer, C. Gillberg, and T. Bourgeron. "Key Role for Gene Dosage and Synaptic Homeostasis in Autism Spectrum Disorders." Trends Genet 26, no. 8 (2010): 363-72.

Vincent, J. B., S. I. Horike, S. Choufani, A. D. Paterson, W. Roberts, P. Szatmari, R. Weksberg, B. Fernandez, and S. W. Scherer. "An Inversion Inv(4)(P12-P15.3) in Autistic Siblings Implicates the 4p Gaba Receptor Gene Cluster." J Med Genet 43, no. 5 (2006): 429-34.

Weiss, L. A., D. E. Arking, M. J. Daly, and A. Chakravarti. "A Genome-Wide Linkage and Association Scan Reveals Novel Loci for Autism." Nature 461, no. 7265 (2009): 802-8.

Yilmazer-Hanke, D. M., T. Roskoden, K. Zilles, and H. Schwegler. "Anxiety-Related Behavior and Densities of Glutamate, Gabaa, Acetylcholine and Serotonin Receptors in the Amygdala of Seven Inbred Mouse Strains." Behav Brain Res 145, no. 1-2 (2003): 145-59.



Autism - A Neurodevelopmental Journey from Genes to Behaviour

Edited by Dr. Valsamma Eapen

ISBN 978-953-307-493-1

Hard cover, 484 pages

Publisher InTech

Published online 17, August, 2011

Published in print edition August, 2011

The book covers some of the key research developments in autism and brings together the current state of evidence on the neurobiologic understanding of this intriguing disorder. The pathogenetic mechanisms are explored by contributors from diverse perspectives including genetics, neuroimaging, neuroanatomy, neurophysiology, neurochemistry, neuroimmunology, neuroendocrinology, functional organization of the brain and clinical applications from the role of diet to vaccines. It is hoped that understanding these interconnected neurobiological systems, the programming of which is genetically modulated during neurodevelopment and mediated through a range of neuropeptides and interacting neurotransmitter systems, would no doubt assist in developing interventions that accommodate the way the brains of individuals with autism function. In keeping with the multimodal and diverse origins of the disorder, a wide range of topics is covered and these include genetic underpinnings and environmental modulation leading to epigenetic changes in the aetiology; neural substrates, potential biomarkers and endophenotypes that underlie clinical characteristics; as well as neurochemical pathways and pathophysiological mechanisms that pave the way for therapeutic interventions.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Agenor Limon, Jorge M. Reyes-Ruiz and Ricardo Miledi (2011). GABA and Glutamate Receptors of the Autistic Brain, Autism - A Neurodevelopmental Journey from Genes to Behaviour, Dr. Valsamma Eapen (Ed.), ISBN: 978-953-307-493-1, InTech, Available from: http://www.intechopen.com/books/autism-a-neurodevelopmental-journey-from-genes-to-behaviour/gaba-and-glutamate-receptors-of-the-autistic-brain

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike-3.0 License, which permits use, distribution and reproduction for non-commercial purposes, provided the original is properly cited and derivative works building on this content are distributed under the same license.