On the role of volume transmission and receptor–receptor interactions in social behaviour: Focus on central catecholamine and oxytocin neurons

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ABSTRACT

This article is focused on understanding the mechanisms for the interactions between the central catecholamine (CA) and oxytocin (OXY) neurons and their relevance for brain function especially social behaviour in the field of pair bonding. Such a topic is analysed under two perspectives namely the intercellular communication modes between CA and OXT neurons and the molecular integrative mechanisms at the plasma membrane level between their respective decoding systems. As a matter of fact, recent observations strongly indicate a major role of volume transmission and receptor–receptor interactions in the CA/OXT neuron interplay in the brain control of social behaviour and pair bonding. This article is part of a Special Issue entitled: Brain Integration.

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1. Introduction

The aim of this review is to understand the possible human relevance of the biochemical correlates of social behaviour obtained in other mammals. Several findings have indicated that some brain regions are especially involved in the control of cognitive processes controlling social behaviour in mammals (Barbas et al., 2002; Levine, 2009; Phan et al., 2004; Salzman and
Fusi, 2010). On this basis Anderson suggested the fascinating theory of the creative reuse of existing neural components that may have played a significant role in the evolutionary development of cognition (Anderson, 2007, 2010). In fact, it may be possible to broaden up Anderson’s theory that is focused on the problem of regional brain specialization under an evolutionary pressure to suggest that also chemical signals and integrative molecular mechanisms can have been subjected to creative reuses.

As a matter of fact, our proposal takes into account in some way the tinkering principle proposed more than three decades ago by Jacob (1977). Thus, Jacob claimed that evolution tinkers together contraptions and novelties come from previously unseen associations of already available material. Already early investigations did have as one aim the detection of possible morphological (mapping) and functional differences between the rat, monkey and human brain (see Battista et al., 1972) as far as the monoamine systems were concerned to discover a possible reuse in the human brain of evolutionary older neurochemical signals. However, the fundamental structural organization of the central monoamine neurons remained fundamentally the same from rat to human.

From the 1960 another main emphasis was in the attempt of building up a new psychopharmacology on the basis of studies on the behavioural correlates of the experimental manipulations of the monoamine transmission in the brain (Anden et al., 1966; Arbuthnott et al., 1970a,b; Battista et al., 1974; Everitt et al., 1974, 1975; Fuxe et al., 1977; Hole et al., 1976; Lidbrink and Fuxe, 1973). Later on, the peptide systems were mapped out and their functional roles studied. This new field of the transmitter identified neuronal systems that could be tackled in a much easier way on the basis of the knowledge already acquired in the monoamine field (see Fuxe et al., 1979). Against this background, we will describe the main features of the monoamine systems in the rat brain of mammals pointing out possible differences between mammalian brains. Furthermore, the peculiar intercellular communication modes and molecular mechanisms allowing integration of monoamine and peptide signalling at plasma membrane level will be mentioned (see Agnati et al., 2010; Fuxe et al., 2010a,b). The major focus will be put on Dopamine (DA), Noradrenaline (NA), oxytocin (OXY) signals.

2. The architecture of central catecholamine and oxytocin neurons

The principle architecture of the dopamine (DA), noradrenaline (NA) and serotonin (5-HT) pathways in the rat brain was described in 1965 (Fuxe, 1965a,b; see Fuxe et al., 2007). The long ascending mono-synaptic monoamine pathways from the lower brain stem to the tel- and di-encephalon play a major role in modulating directly the functions of inter alia the cerebral cortex, subcortical regions like the ventral and dorsal striatum and the hypothalamus (see Fuxe et al., 2007). A similar nuclear parcellation of the DA, NA and 5-HT nerve cell groups can be found in all rodents and in other orders of mammals (Moon et al., 2007).

A major action of the meso-limbic DA afferents from the ventral tegmental area involves mainly dopamine D2/D3 receptor mediated effects in the ventral striatal complex, including the ventral pallidum, amygdala, extended amygdala and the septal area (de la Mora et al., 2012; Heimer, 2000). The role may be to regulate the emotional impact of the subcortical limbic networks on the cerebral cortex, especially the prefrontal cortex, involving the ventral striatum-ventral pallidum-mediodorsal thalamic nucleus circuit to the prefrontal cortex. The meso-limbic DA pathways (Fig. 1) will therefore have an impact on social behaviour which can also involve D1 receptors (de la Mora et al., 2010). In line with this view, parts of the meso-accumbens DA system represent a reward system able to also

Fig. 1 – Major ascending dopamine pathways and their relationship to oxytocin neurons in the CNS. Basic framework of proposed interactions between dopamine and oxytocin projections in the human brain. Sagittal view of a human brain illustrating potential neural pathways involving dopamine and oxytocin terminal interactions. Oxytocin (OXT) is mainly synthesized in magnocellular neurons in the paraventricular (PVN) and supraoptic nuclei (SON) of the hypothalamus but also in parvocellular nerve cells in the anterior hypothalamic and preoptic areas. Oxytocin is processed along the axon projections to the posterior lobe of the pituitary, where it is stored in secretory vesicles and released into peripheral circulation (inset). Furthermore, there is a dendritic and terminal release of OXT as a VT signal into the extracellular space, resulting not only in local actions but also in diffusion in the brain to reach distant brain targets. In the brain, axonal OXT projections from parvocellular neurons of the hypothalamus go to different areas, including the amygdala (AMG), nucleus accumbens (NAc), dorsal striatum (STRd), bed nucleus of striae terminalis and medulla oblongata, where oxytocin via VT acts as a neurotransmitter, and thereby influences neurotransmission in these areas. Dopamine (DA) pathways from the substantia nigra (SN) cell bodies innervate several brain regions but mainly the STRd. In addition, the mesocortical and mesolimbic dopamine pathways originate in the VTA and project inter alia to the PFC, NAc and other subcortical and cortical limbic regions. Oxytocin and DA nerve terminal networks in view of their extensive codistribution in particular brain regions, may therefore interact and have an impact on social behaviour via short and long distance VT. In the inset the D2R-OXTR heteromer is indicated, demonstrated to exist in cellular models (Borroto-Escuela et al. and Romero Fernandez et al., unpublished data).
predict the time of future rewards (Montague et al., 1996; Schultz, 2002) and mediate natural rewards like food.

Many of the non-locus coeruleus NA cells in the pons and medulla oblongata give rise to ascending NA axons, forming a ventral NA bundle in the pons and ventral midbrain richly innervating inter alia the hypothalamus, the preoptic area, the septal area and nucleus interstitialis striae terminalis but not the ventral and dorsal striatum (Fuxe, 1965a; Fuxe et al., 1970a, b; Ungerstedt, 1971). This non-LC NA system like the mesolimbic DA system may therefore also have an impact on social behaviour in view of its rich innervation of these hypothalamic and subcortical limbic structures known to modulate emotional behaviour but without directly innervating the striatum.

The non-LC NA system (Fig. 2) via collaterals may innervate also brain stem nuclei like the nucleus tractus solitarius, an important visceral center in the dorsal medulla oblongata (Fuxe, 1965a,b). Thus, these types of NA neurons may be important coordinators of autonomic and neuroendocrine functions in central autonomic networks at the hypothalamic-limbic and lower brainstem levels (Fuxe et al., 1970a,b,c, 2007; Olson and Fuxe, 1972). Non-LC NA systems controlling visceral and neuroendocrine function can also modify the alertness and attention via an indirect influence on the LC-cortical NA (Fuxe et al., 1970c, 2007; Ungerstedt, 1971) involved in tonic arousal (Jouvet, 1972; Lidbrink and Fuxe, 1973) linking autonomic activities to arousal and cognitive performance. In addition, dysfunction of the LC and non-LC NA neuron systems has been suggested to contribute to stress and attention deficit hyperactivity disorders (Oades et al., 2005).

High densities of alpha2 adrenoceptors (α2AR) which can exist in a high and low affinity states are mainly linked to the non LC NA terminal networks in the hypothalamus (Fig. 2), preoptic area and the subcortical limbic forebrain (Unnerstall et al., 1985). They may therefore have a special role in modulating emotional behaviours including stress via this NA system. Much lower densities of these receptors are found in the cerebral cortex with the exception of the entorhinal and piriform cortex, which in fact are innervated by the LC locus coeruleus system as the rest of the cerebral cortex.

Evidence indicates that DA mainly acts as a short distance volume transmission (VT) transmitter through its release into the extracellular fluid of brain where the D1-like and D2-like receptors are predominantly extra-synaptic (Fuxe et al., 2007, 2010a). This appears to be especially true for the D2 mediated DA transmission in view of the increased affinity of dopamine for D2-like versus D1-like receptors (Marcellino et al., 2012). The noradrenergic receptors have also been shown to be extra-synaptically located in relation to the NA nerve terminal networks in the cerebral cortex in addition to being located in postsynaptic densities of dendritic spines (Aoki et al., 1998). Thus α2ARs were demonstrated in axons, dendritic shafts and astrocytic processes indicating their involvement in short distance VT. Their extra synaptic location was first demonstrated in smooth muscle tissue by Langer et al. (1980).

The oxytocin neuron systems mainly originate from the paraventricular and supraoptic nuclei (Fig. 1). Magnocellular components mainly give rise to the terminals in the neurohypophysis releasing oxytocin as a hormone into the circulation. The parvocellular components mainly give rise to the descending oxytocin systems to autonomic regions in the lower brain stem and the NA cell groups in the medulla and pons, some of them giving rise to the ascending non-LC NA system. Neurophysin II-oxytocin terminal networks e.g. exist in the nucleus tractus solitarius (NTS) of the rat with innervation also of the dorsal motor nucleus of the vagus (Kalina et al., 1984). The major ascending oxytocinergic pathways largely arise from the paraventricular nucleus (PVN) and some minor groups in the medial preoptic area innervating the subcortical limbic structures and the ventral and dorsal striatum (De Vries and Buijs, 1983; McEwen, 2004). The oxytocin immunoreactivity terminals in the ventral and dorsal striatum are very sparse in the rat compared with
the high density of DA terminal networks in these regions. The same is true also for the oxytocin terminal networks in the hypothalamus, preoptic area and associated subcortical limbic areas compared with their rich plexa of NA terminals of the non-LC NA system.

The high affinity Gq coupled oxytocin receptors (OXTRs) have a variable distribution in the brain of mammals (Gimpl and Fahrenholz, 2001; McEwen, 2004). In the rat brain the labelling is mainly found in the forebrain (Gimpl and Fahrenholz, 2001; McEwen, 2004). There exist marked differences in the OXTR distribution between rat brain and the guinea pig brain.

In the rat brain they have been found in low to high densities in the olfactory and limbic system, the cortical areas, the basal ganglia including the ventral and dorsal striatum and in hypothalamic and thalamic areas. They clearly overlap with the distribution of D2-like receptors in the dorsal and ventral striatum and with α2ARs in the hypothalamus in the nucleus interstitialis striae terminais, in the subcortical limbic structures and in the nucleus tractus solitarius. Thus OXTRs may interact with D2-like and α2AR receptors in discrete areas and modulate the receptor function of D2 like and α2AR receptors. It is also likely that D2-like and α2AR receptors can modulate OXTRs through a reciprocal receptor–receptor interaction.

3. OXTR-α2AR receptor–receptor interactions

Oxytocin can induce anti-stress-like effects such as reduction of blood pressure and cortisol levels. It increases pain thresholds, exerts an anxiolytic-like effect and stimulates various types of positive social interactions (Uvnas-Moberg and Peterson, 2005). It has been indicated as a “cuddle” transmitter. NA actions via alpha2 adrenergic receptors, in contrast to alpha1 adrenergic receptors, have in many respects similar actions. We have therefore been interested to study receptor–receptor interactions between oxytocin receptors and α2ARs as a way to integrate their signals in favouring similar types of positive social interactions (Fig. 2). One possibility was that the monoamine and peptide signals became integrated through direct peptide receptor–monoamine receptor interactions in the plasma membrane (Agnati et al., 1980; Fuxe et al., 1981, 1983).

Our novel view emphasized the existence of direct receptor–receptor interactions (RRIs). As a logical consequence for the indication of direct physical interactions between neuropeptide and monoamine receptors, the term heteromerization was introduced by us in 1993 to describe a specific physical interaction between different types of GPCRs (Zoli et al., 1993). The term heteromerization sometimes can involve an adapter protein and sometimes require the assistance of scaffolding proteins to allow the direct interaction to occur. This has been our working hypothesis. Thus, this work was the first one to lead the GPCR field from monomers into dimers and especially heterodimers (Agnati et al., 2010; Fuxe et al., 2010b).

As we have repeatedly discussed over the years the allosteric RRIs in receptor heteromers make possible a marked rise of the repertoire of GPCR recognition and decoding signalling (Fuxe et al., 2010b). This is achieved through modulation of the orthosteric and allosteric binding sites of the adjacent protomer, and its G protein activation, its G protein selectivity, of its signalling cascades with among others switching from G proteins to β-arrestin and through appearance of novel allosteric sites that may alter for instance G protein coupling and selectivity.

3.1. Studies on food intake

The current work started with analysis of food intake. The α2AR agonist clonidine has also been shown to potentiate feeding when injected systemically, intraventricularly or directly into the PVN area of the rat (Diaz-Cabiale et al., 2000b). Oxytocin is involved in the reduction of food intake. Can it modulate the α2AR mechanism in the PVN?

An acute antagonistic interaction in food intake was observed after intracerebroventricular administration of oxytocin and clonidine in satiated rats (Diaz-Cabiale et al., 2000b). With receptor autoradiography antagonistic OXTR-α2ARs interactions in the medial hypothalamus were demonstrated. Thus, a rise of the Kd value of the alpha2 agonist binding sites was found together with an increase in the Bmax values. A U shaped concentration curve in the nanomolar range was found. The reduction of alpha2 receptor binding was substantial. An even more convincing effect was observed in the amygdala where also a codistribution of OXTR and α2AR exists. A partial oxytocin agonist-antagonist 1-deamino-2-D-Tyr-(OEt)-4-Thr-8-OH-Orn-oxytocin (CAP 527) could partially block the actions of oxytocin.

The reduction by oxytocin in the α2AR agonist affinity and increase in Bmax values may involve heterodimerization with possible associated allosteric changes leading also to reduce transduction efficacy (reduced G protein coupling) of the α2AR. In fact, the increase in Bmax of the α2AR may be explained by a reduced G protein activation under oxytocin, leading to increased G protein association with the receptor and therefore an increase in high-affinity binding.

3.2. Studies on central cardiovascular control. Focus on the nucleus tractus solitarius

Similar results have been observed in the NTS. Effects of microinjections into the NTS of an ED50 dose (left) or of a threshold dose (right) of clonidine were made alone or together with a threshold dose of oxytocin and artificial cerebrospinal fluid (aCSF). Effects on mean arterial blood pressure and heart rate were determined. Oxytocin counteracted at 1 pmol the clonidine action (Diaz-Cabiale et al., 2000a). Again these functional antagonistic interactions had an in vitro antagonistic receptor–receptor interaction correlate with a reduction of alpha2 agonist affinity associated with an elevation of Bmax values of the alpha2 agonist binding sites and the effects were partially counteracted by CAP (Diaz-Cabiale et al., 2000a).

The current findings suggest the existence of an acute antagonistic effect of OXTRs on the hypotensive and bradycardic responses mediated by α2ARs in the NTS, likely due to an antagonistic intramembrane receptor–receptor interaction (OXTR/α2AR) within heteromers of these receptors. Again the increase in Bmax value of the α2AR could be explained by a reduced G protein activation under oxytocin with an increase α2ARs in the high affinity state (Fig. 3).
3.3. Systemic subchronic OXT treatment and effects on possible α(2A)AR-OXTR heteromers

Interestingly systemic subchronic oxytocin treatment (as seen 5 days after the last injection) increased the density of α(2A)AR agonist binding sites without a change of affinity. The effects are very clear-cut in the receptor autoradiograms and the rise is only seen in areas of codistribution of the two receptors (hypothalamus, amygdala and NTS) and therefore not present in the lateral posterior thalamic nucleus (Diaz-Cabiale et al., 2000c).

OXT administration favours the appearance of a pattern of anti-stress or energy conservation characterized by behavioural calm, reduction of arterial blood pressure and changes in the pattern of vagally controlled gastrointestinal hormones. The OXT induced increase of α(2A)ARs may be part of the neurochemical substrate for these autonomic and behavioural effects. Thus, long-term modulation of autonomic functions and emotional behaviours elicited by endogenous brain OXT may involve enhancement of central α(2A)AR function known for their anti-stress activity.

The mechanism for the subchronic OXT treatment induced rise of α(2A)AR density without change of affinity after systemic treatment may inter alia involve an increased recycling of the α(2A)AR to the plasma membrane due to increased formation of α(2A)AR-OXTR heteromers leading to increased co-trafficking. The rapid recycling of the activated OXT receptor (rapidly recycling endosomes) after co-internalisation may be true also for the α(2A)AR since it is physically linked to the OXTR via the receptor interface in the heteromer. Alternatively, the oxytocin treatment via activation of OXTRs may lead to an increase in α(2A)AR mRNA levels due to activation of the oxytocin signalling pathways. An interesting mechanism may also be an increase in α(2A)AR homodimers since OXTR monomers and homodimers are also internalized by oxytocin treatment and targeted for breakdown leading to a deficit in available OXTRs for heteromerization with α(2A)ARs.

![Fig. 3 - Signalling and trafficking events engaged by alpha2 adrenoceptor (α(2A)AR) and oxytocin receptor (OXTR) interactions.](image-url)
homodimers are also internalized by oxytocin treatment and targeted for breakdown leading to a deficit in available OXTRs for heteromerization with α2(2A)ARs. This may also explain the failure to see affinity changes in the α2(2A)AR agonist binding sites after the subchronic oxytocin treatment with a disappearance of the antagonistic OXTR-α(2A)AR interaction.

3.4. Effects of ovariectomy (OVX)

We see similar effects of a 10-day oxytocin treatment in OVX rats on α(2A)AR binding studied with the α(2A)AR agonist [3H]UK14.304 in the hypothalamus, the amygdala and the NTS 5 days after the last oxytocin injection in OVX rats (Petersson et al., 2005).

These findings further support an interaction between OXTRs and α(2A)ARs and show that oxytocin treatment may increase α(2A)AR density without change of the affinity; probably leading to an increase in α(2A)AR signalling in several parts of the brain of the OVX rats. Thus, this increase in α(2A)AR function may be one major mechanism underlying the long-lasting anti-stress and calming actions of oxytocin both in the female and male rat.

Molecular mechanisms can involve increases of α(2A)AR mRNA, increased formation of α(2A)AR homodimers and/or increased recycling of α(2A)ARs to the plasma membrane through co-trafficking with OXTRs in rapidly recycling endosomes as discussed above (Fig. 3).

Other results provide evidence that consolidation of inhibitory avoidance memory depends critically on prolonged activation of the noradrenergic system in the basolateral nucleus of the amygdala and indicate that this modulatory influence is mediated, in part, by α(2A)ARs (Ferry and McGaugh, 2008).

3.5. Long-term postnatal effects of oxytocin

Anti-stress activity of postnatal oxytocin has been demonstrated in adult life in a food restriction model (Sohlstrom et al., 2000). Thus, oxytocin treatment may ameliorate some of the adverse effects of food restriction in utero when given postnatally involving inter alia increased body weight and reduced corticosterone levels. In fact, systemic postnatal treatment with oxytocin has been suggested to also act as an anti-stress and calming agent with an effect that lasts till adulthood.

Persistent modulation by postnatalOXTR of α(2A)AR agonist binding sites was discovered in adulthood in NTS and the role of prenatal stress has been evaluated involving prenatal food-restriction of dams (Diaz-Cabiale et al., 2004).

Stress alone in the NTS increases the affinity of the α(2A)AR agonist binding sites and this action is counteracted by the postnatal oxytocin. Daily treatment with oxytocin was performed (1 mg/kg) during the first 14 days of life. Analysis was made at 4 months of age in the NTS postnatal oxytocin mimicked to some degree what we have seen after subchronic oxytocin treatment in adulthood. There was a rise of the Bmax values favouring α(2A)ARs signalling and vasodepressor activity.

In the hypothalamus and amygdala the modulations are different. There is no effect by prenatal stress alone but there is an impressive rise of the Bmax values of the α(2A)AR agonist binding sites induced by postnatal oxytocin treatment after prenatal stress with no effects on the affinity of these α(2A)AR agonist sites in the hypothalamus and the amygdala (Diaz-Cabiale et al., 2004). These effects on the alpha2 adrenergic receptors can contribute to the anti-stress actions of postnatal oxytocin. However, the hypothalamic results after postnatal oxytocin alone are more difficult to interpret since there is in this case a marked reduction of the affinity of the α(2A)AR agonist binding sites associated with an increase in Bmax values. The results also show that in contrast postnatal oxytocin alone induces persistent reductions in alpha(2A)AR affinity in the NTS in the absence of an increase in Bmax values demonstrating regional heterogeneity in the response to oxytocin.

After prenatal stress postnatal oxytocin produces potentially persistent increases in α(2A)AR signalling via selective increases of receptor density without changes of affinity in the α(2A)AR agonist binding sites in the hypothalamus and the amygdala. The mechanisms underlying these persistent changes are unclear. We may have persistent increases of α(2A)AR mRNA expression through changes in epigenetic programming. An additional mechanism may also be oxytocin induced increases in formation of α(2A)AR-OXTR heteromers which become long-lasting through an associated increased and persistent formation of unique adapter proteins binding to and stabilizing these heteromers. Such a mechanism which may vary among brain regions may help explain the persistent and variable changes in the affinity of the α(2A)AR agonist binding sites.

3.6. Maternal behaviour and epigenetics

It is of interest that naturally occurring variations in maternal behaviour in the rat are associated with differences in oestrogen-inducible central OXTRs as shown by Meaney’s group (Champagne et al., 2001). Increased pup licking and grooming and arched-back nursing by rat mothers were found to alter the offspring epigenome at a glucocorticoid receptor (GR) gene promoter in the hippocampus. Central infusion of a histone deacetylase inhibitor removed the group differences in histone acetylation, DNA methylation, NGFI-A binding, GR expression and hypothalamic-pituitary-adrenal (HPA) responses to stress, suggesting a causal relation among epigenomic state, GR expression and the maternal effect on stress responses in the offspring. An epigenomic state of a gene can be established through behavioural programming, and it is potentially reversible (Weaver et al., 2004).

Naturally occurring differences in maternal care are associated with the expression of oxytocin and vasopressin (V1a) receptors and gender differences exist (Francis et al., 2002). The mechanism by which maternal stimulation regulates neuropeptide receptor expression in offspring has yet to be elucidated, but these results demonstrate that the effects are sexually dimorphic and are evident in systems previously implicated in the expression of social behaviour. So epigenetic mechanisms can be involved in the discoveries we have made on α(2A)AR-OXTR interactions after postnatal oxytocin and prenatal stress since e.g. permanent increases in expression of OXTRs may produce long-lasting changes in the formation of α(2A)AR-OXTR heteromers with associated long-lasting changes in their RRIs.
3.7. Maternal care as a model for experience-dependent chromatin plasticity (Meany and Szfy, 2005)

This model is a beautiful example of epigenetic mechanisms. It shows how increased maternal licking-grooming can activate the nerve growth factor-inducible protein A (NGFI-A) expression (via 5-HT, cAMP and PKA). Although the affinity of NGFI-A to its recognition sequence on the GR promoter is reduced by the methylation of this element on postnatal-day 1, binding occurs through the increased levels of NGFI-A associated with enhanced tactile stimulation derived from maternal licking-grooming. Binding of NGFI-A results in recruitment of histone acetyltransferases (HATs) leading to an increase of histone acetylation (Ac), which in turn facilitates the access of demethylase and demethylation of the GR promoter. The unmethylated promoter will maintain high affinity to NGFI-A throughout adulthood, resulting in greater NGFI-A-induced activity of the GR promoter in adult offspring of high-licking-grooming mothers. Instead the methylated GR promoter exhibits reduced affinity for NGFI-A, resulting in low activity of the GR in adult offspring of low-licking-grooming mothers.

4. The essence of volume transmission (VT)

We and our colleagues proposed in 1986 based on a number of observations the existence of VT as a new major mode of communication in the CNS complementary to the well known Wiring Transmission (WT) in the brain (Agnati et al., 1986; Fuxe et al., 1988). Of special importance for us was our demonstration of relative transmitter-receptor mismatches in the opioid peptide systems. Thus, there was no correlation in their distribution patterns (see Agnati and Fuxe, 2000; Jansson et al., 2002). Volume transmission is a widespread mode of intercellular communication that occurs in the extracellular fluid and in the cerebrospinal fluid (CSF) of the brain.

Volume transmission signals are neurotransmitters, modulators, trophic factors, gases and ions that move from the source cells to the target cells as a consequence of energy gradients leading to diffusion and convection and pressure waves generated by the rhythmic cardiac activity that pervade and deform the entire mass of the brain favouring the movements of the extracellular fluid in the extracellular space (Agnati et al., 1994, 2005). A fundamental difference between VT and WT is the characteristics of the communication channel connecting the cell source of the signal with the cells target of the signal. In the case of VT is basically a ‘private’ (physically well delimited) channel being represented by axons. In the case of VT is a ‘public’ channel being represented by open diffusion pathways in the extracellular space or in the ventricular space. This is the fundamental distinction. However, it should also be mentioned that while safety of signal transmission is high for WT, on the contrary it is usually low in the case of VT since the signal can be altered during its migration. At the level of the cell target of the VT signal privacy is retained since a specialized receptor apparatus is needed to decode the message as is the case also for synaptic transmission. The connectivity is dynamic since the connection between the source and target can be rapidly formed and/or removed allowing some kind of learning based on a sort of B-type unorganised Turing machine (Agnati et al., 2006; Fuxe et al., 2007).

Previous diffusion experiments in brain using real time iontophoretic techniques have generated three classical diffusion parameters that describe the structural and dynamic properties of the extracellular space (ECS) (Chen et al., 2002; Fuxe et al., 2007; Hoistad et al., 2002; Nicholson and Sykova, 1998). Volume fraction is the relative size of the ECS compared to total brain tissue volume. It is in the order of 20% tortuosity is the increase in path length that is imposed on a migrating molecule by cellular structures and extracellular matrix components and can be determined by measuring the apparent diffusion coefficient in relation to the free diffusion coefficient determined in dilute agar. Clearance is a constant that reflects the removal or disappearance of a compound from the ECS. An example of structural features of VT can be found in the 5-HT-T2A relationships. We have studied the 5-HT2A immunoreactivities pyramidal cell bodies and dendrites and 5HT terminal-like varicosities in the same sections of the parietal cortex. The morphometrical analysis in horizontal sections shows that the shortest mean distance of a 5-HT terminal varicosity to a 5-HT2A receptor positive dendrite is 7.7 μm (Jansson et al., 2001).

Rice and Cragg (2008) have modelled DA spillover after quantal release based on a large number of experimental data (Rice and Cragg, 2008). In the updated DA synapse the DA release is unconstrained by the extra-synaptic DA transporter (DAT), the diffusion process being too fast for the extra-synaptic DAT which mainly increases clearance of DA and thus reduces its half-life in ECF. So a cloud of DA is formed and can reach the extra-synaptic DA receptors which are in vast majority. Thus, the primary mode of DA, NA and 5-HT communication is VT. The effective radius for low affinity DA receptors is 2 μm and for high affinity DA receptors is 7–8 μm. The spheres obtained, respectively would encompass tens to thousands of synapses. The functionally relevant monoamine receptors are likely those in the high affinity state. Thus, in the case of monoamine VT we are mainly dealing with a short distance diffusion taking place at the local circuit level (Fuxe et al., 2010a; Marcellino et al., 2012). This type of short distance VT is often referred to as extra-synaptic transmission. Indications for the existence of striatal D2-like receptor-mediated DA VT at the local circuit level have even been obtained in vivo (Marcellino et al., 2012) based on PET findings using PET D2 radioligands in combination with amphetamine induced DA release (Seneca et al., 2006). DA shows an increased affinity for D2 like receptors vs D1 like receptors (Marcellino et al., 2012).

We have also proposed that changes in the mixture of VT and WT can give rise to changes in the outputs of polymorphic networks. As shown in this example changes in local VT signalling can give rise to three different types of outputs from this network demonstrating the impact of changes in VT signalling on activity of brain circuits connecting cellular networks with each other (Agnati and Fuxe, 2000). It seems that the important role of our brain stem monoamine neurons may be to act as VT signals in polymorphic cellular networks representing functional modules to modulate large numbers of functions like mood, cognition, arousal, attention, reward, motor control, central autonomic control and neuroendocrine...
control. In this way we can best grasp their global modulation of brain circuits since they are through connections between functional modules with impact on behaviour like social interactions e.g. pair bonding.

An important question is how VT and WT integrate their signals at the molecular level. It may take place to a major degree by RRIs in heteromers. As an example we show here how DA via VT may reach up to glutamate synapses where it modulates the signalling of NMDA receptors since both D1 and D2 receptors form heteromers with NMDA receptors where e.g. D2 receptors reduce NMDA signalling via direct RRIs involving D2-NR2B subunit interactions as shown by Liu et al. (2006). Thus, DA VT modulates glutamate WT via RRIs with NMDA receptors in D2-NMDA heteromers and also in D1-NMDA heteromers involving NR2A and NR1.1 subunits (Lee et al., 2002). However, the major role of DA receptors is to participate in the integrative processes of extra-synaptic heteromers built up of GPCRs, GPCR-ion channel receptors or GPCR-RTK complexes (Borroto-Escuela et al., 2010; Fuxe et al., 2007). Higher order heteromers are likely also involved called receptor mosaics (Agnati et al., 2003; Fuxe et al., 2008).

5. Oxytocin neurons and pair bonding in the prairie vole female. Role for VT and D2-oxytocinR interactions

5.1. Volume transmission

To understand the role of VT in behaviours it becomes interesting to look at the pioneering work from the Insel group on the role of oxytocin and its receptors on social attachment in the monogamous prairie vole female (Young and Wang, 2004; Young et al., 2001). The prairie but not the polygamous montane voles show a high density of OXTRs in the nucleus accumbens and dorsal striatum. After mating, female prairie voles spend more time in the partner’s chamber than in the neutral or stranger’s chamber, indicating a partner preference. By contrast, mated montane voles show no preference for either the partner or the stranger, indicating the absence of a mating-induced social attachment. Partner preference formation is blocked in the mating female prairie vole by infusions of OXTR antagonist (OTA) into the nucleus accumbens.

It is highly interesting that in the monogamous prairie vole this marked increase in oxytocin densities is not associated with any clear increase in the number of oxytocin nerve cell bodies in the hypothalamus and in the medial preoptic area projecting to these areas nor in oxytocin nerve terminal networks in e.g. the nucleus accumbens vs polygamous voles (Lim and Young, 2004; Lim et al., 2004a). We therefore propose that monogamy in the female prairie vole is brought about through an increase in oxytocin VT especially in nucleus accumbens.

The mating induced oxytocin release from the sparse plexus of oxytocin terminals can thus through long distance diffusion and flow of oxytocin in critical limbic regions of the female reach and activate increased numbers of widespread extra-synaptic high affinity OXTRs leading to an increased impact of oxytocin transmission on these networks and favour the development of social attachment in the female (long distance volume transmission) (Fuxe et al., 2010a). Thus, species differences in social attachment in the same genus can develop through increases in OXTR densities and thus in oxytocin VT in the presence of an unchanged sparse oxytocin nerve terminal network within the nucleus accumbens and dorsal striatum. For the first time we have indications for a role of VT in social behaviour.

5.2. D2-oxytocinR interactions

DA has also been shown to regulate pair bonding in female prairie voles (Young and Wang, 2004). The partner preference was blocked by a D2 antagonist but not by a D1 antagonist. Also intra accumbens infusion of a D2 antagonist blocked the mating induced partner preference in estrous female voles associated with a substantial increase in extracellular DA levels in the nucleus accumbens. It is also of interest that infusion of a D2 agonist into the nucleus accumbens could induce partner preference in the absence of mating blocked by a D2 antagonist or an OXTR antagonist. The results suggest that coactivation of D2 and OXTRs in the nucleus accumbens is of importance for pair bond formation and maintenance with rostral nucleus accumbens shell being of special importance (Aragona et al., 2003; Gingrich et al., 2000; Young and Wang, 2004; Young et al., 2001) (Fig. 4).

In view of above it is of substantial interest that we recently have been able to demonstrate with the BRET technique in cellular models that co-transfection of D2 and OXTRs leads to the formation of D2-OXTR heteromers where oxytocin induced OXTR activation causes increases in D2 signalling with increased inhibition of the cAMP-PKA-CRE pathway (Borroto-Escuela et al. unpublished observations). Also oxytocin induced increases in D2 recognition in biochemical experiments on 3H-raclopride binding (Romero-Fernandez et al. unpublished observations). Thus, the molecular mechanism for the need of concurrent activation of D2 and OXTRs in the nucleus accumbens for pair bond formation may involve enhancement of D2 recognition and signalling in D2-OXTR heteromers via the existence of facilitatory allosteric RRIs (Fig. 4).

6. Vasopressin neurons and pair bonding in the prairie vole male. Role for VT

There exists a marked increase in the density of the vasopressin receptor V1a in the ventral pallidum of the monogamous male prairie vole vs the polygamous montane vole which appears to play a key role for monogamy in the male prairie vole (Winslow et al., 1993; Young et al., 1999, 2001). Microinjections of a vasopressin V1a antagonist into the ventral pallidum block mating induced pair bonding. In view of the lack of a corresponding rise of the vasopressin immunoreactive terminals and axons in this region (Lim and Young, 2004; Lim et al., 2004a) the evidence favours the view that an increase in vasopressin volume transmission in the ventral pallidum mediates monogamy in the male prairie vole. Vasopressin receptors in the ventral pallidum exist also in monogamous mice and primates, whereas they are absent in this region in related rodent and primate species that do not form pair bonds (Insel and Young, 2001). Important evidence on the
The crucial role of the V1a receptor mediated vasopressin volume transmission in ventral pallidum has been obtained in gene transfer experiments. Viral vector-mediated gene transfer to over-express V1aR in the ventral pallidum in the socially promiscuous male meadow vole essentially recreates an evolutionary event namely the development of monogamy known to occur in prairie but not in montane voles (Liu et al., 2004b; Pitkow et al., 2001).

Fig. 4 - Schematic illustration of D₄R-OXTR heterodimer and their cross-talk signalling pathways. Left panel. The striato-pallidal GABAergic neurons may co-express D₂R and OXTR homo- and heterodimers at the plasma membrane. Oxytocin (OXT) and dopamine (DA) can potentially interact with both receptor homo- and heterodimers of OXTR and D₂R. Right panel. In the D₂-OXTR heteromer the D₂ inhibitory control of adenylyl cyclase and the OXTR stimulatory control of phospholipase C (PLC) can become integrated. Facilitatory allosteric D₂-OXTR interactions have been shown both in the inhibition of the adenylyl cyclase and in the activation of the PLC through demonstration of enhanced inhibition of CREB activity and enhanced activation of SRE activity in gene reporter assays.

7. On the complexity of social behaviour

Very likely many neurochemical signals are involved in regulating social behaviour, which is the result of the proper integration of memories, capabilities of detecting the best strategies, efficient motor control and subtle emotional tuning. Furthermore, the choice of the sexual partner and/or the adoption of the monogamy rather than the polygamy habit have heavy consequences not simply for the fate of single animal but of the species. Thus, we believe that the available data are of an extraordinary importance, but they are only a part of a complex story which becomes more and more so in moving from simple vertebrates to mammals and finally to humans.

Furthermore, oxytocin and vasopressin neurons are neuroendocrine systems and act both as hormones in the blood and as neurotransmitters in the ECF and CSF. It will be highly relevant to investigate how their peripheral and central actions become coordinated (see Nephew et al., 2005; Neumann et al., 2006).

8. Conclusions

The data presented in this review article are discussed according to a conceptual frame that derives from our previous research demonstrating that monoamines and peptides can mainly operate as VT signals (Agnati et al., 1986; Fuxe et al., 2007, 2010a) and the relevance of RRIs for their integrative actions (Agnati et al., 2010; Fuxe et al., 2008). Thus, the main focus of the present paper is the relevance of the interplay
between WT and VT and the possible modulatory action of VT signals allowing the appearance of polymorphic networks, which can favour completely different integrations in networks regulating behaviour and hence also different types of social interactions. Results have been mentioned from experiments in progress in our labs demonstrating that RRIs in D2-OXTR heteromers can be a basic mechanism for the polymorphism and thus differential outputs of neuronal networks playing a crucial role on brain circuits regulating social attachment.

8.1. Pair bonding may be linked to oxytocin and vasopressin volume transmission with involvement of facilitatory accumbens D2–OXTR interactions

Based on the work of Insel and colleagues pair bonding may be brought about by increases in vasopressin (male) and oxytocin (female) volume transmission in critical limbic regions: ventral pallidum (AVP; male) and nuc accumbens shell (OT; female). Species differences in oxytocin and vasopressin staining were subtle relative to the profound species differences previously reported for receptor binding. These results are consistent with the hypothesis that neuroendocrine systems may evolve by changes in receptor distribution rather than by restructuring the presynaptic pathway (Wang et al., 1996).

The increase of oxytocin and vasopressin VT is brought about by a rise of oxytocin and vasopressin receptor densities without a change in the density of oxytocin and vasopressin terminal networks. Thus, behavioural differences like pair bonding found in different species of the same genus may be caused by increasing the extra-synaptic receptor densities in critical circuits for the diffusing VT signals in this case the diffusing oxytocin and vasopressin signals. These results demonstrate that a rise of oxytocin and vasopressin volume transmission in critical circuits mediates pair bonding and give evidence for a behavioural role of volume transmission in social interactions.

Behavioural differences like pair bonding found in different species of the same genus may thus be caused by increasing the extra-synaptic receptor densities in critical limbic circuits for the diffusing volume transmission signals in this case the diffusing oxytocin and vasopressin signals. It therefore seems possible that these peptide VT signals may have a modulatory influence on social interactions also in other orders of mammals.

The evidence obtained in our laboratory in cellular models suggests that facilitatory D2–OXTR receptor interactions in putative D2–OXTR heteromers in the nucleus accumbens shell may be a major molecular mechanism in pair bonding for the female prairie vole. In view of the codistribution of D2 and OXTRs in the lateral and capular subnuclei of the central amygdaloid nucleus these RRIs may also have a role in fear with oxytocin exciting and D2 inhibiting the GABA interneurons in these subnuclei projecting into the medial subnucleus (de la Mora et al., 2010; Huber et al., 2005).

8.2. Anti-stress actions of oxytocin may be linked to interactions between oxytocin and noradrenaline nerve terminal networks and their oxytocin receptors and alpha(2A)AR in central autonomic nuclei and cortical limbic regions

Alpha(2A)AR-OXTR RRIs in postulated heteromers may through unknown molecular mechanisms lead to increased alpha(2A)AR signalling likely playing a major role in mediating anti-stress effects and calming actions of repeated oxytocin treatment (Diaz-Cabiale et al., 2000e). It will be of substantial interest to evaluate if this alpha(2A)AR-OXTR interaction mechanism is also linked to the maternal actions of oxytocin as well as its actions on the female reproductive tract.

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