Functional brain imaging: a brief overview of imaging techniques and their use in human and canine anxiety research

Angststoornissen: de verschillende technieken van functionele hersenbeeldvorming en de bevindingen bij mens en hond met angststoornissen

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ABSTRACT

When used in combination with specific radioactive markers, functional imaging modalities such as Single Photon Emission Computed Tomography (SPECT) and Positron Emission Tomography (PET) enable the visualization of several neurotransmitter receptors and transporters, as well as of the perfusion and metabolism of the brain.

This paper gives an overview of the functional imaging techniques, as well as of the studies that have been performed on humans and canines with anxiety disorders. Thus far, most of the research in this field has been focused on brain perfusion and the serotonergic and dopaminergic neurotransmitters, and less on gamma-aminobutyric acid (GABA), glutamate, norepinephrine and the hypothalamic-pituitary-adrenal (HPA) axis.

SAMENVATTING

Aan de hand van functionele beeldvormingstechnieken, zoals *Single Photon Emission Computed Tomography* (SPECT) en *Positron Emission Tomography* (PET), en de bijpassende radioactieve merkers kunnen receptoren en transporters van neurotransmittersystemen in beeld gebracht worden. Ook de hersenperfusie en het hersenmetabolisme kunnen met diezelfde modaliteiten bepaald worden.

In dit artikel worden de technische specificaties van de verschillende beeldvormingsmodaliteiten kort aangekaart. In tweede instantie worden de functionele beedvormingsstudies die handelen over angststoornissen bij mens en hond, besproken. Hierbij wordt voornamelijk over hersenperfusie en het serotonerge en dopaminerge systeem gerapporteerd en minder over gamma-aminobutyraat (GABA), glutamaat, norepinefrine en de hypothalamische-hypofysaire bijnieras (HPA).

INTRODUCTION

Research on abnormal behavior in small animals, and especially in dogs (Cyranoski, 2010), has become increasingly important in the last couple decades due to the growing public interest in companion animals, which parallels the increasing demand for more fundamental veterinary health care. This has paved the way to introducing new tools such as functional imaging modalities in canine behavioral medicine, in addition to the standard diagnostic tools such as anamnesis, behavioral consultation, blood work, behavioral questionnaires and structural imaging modalities. The functional imaging modalities of Single Photon Emission Computed Tomography (SPECT) and Positron Emission Tomography (PET), which have recently entered the scene of veterinary behavioral medicine, offer the possibility to demonstrate relationships between disturbances in certain brain regions and neurotransmitter systems and certain behavior disturbances (Peremans *et al.*, 2003a; Vermeire *et al.*, 2010; Irimajiri *et al.*, 2010). Research focusing on physiological brain alterations leads to insights into the pathophysiology of the disease, thus enabling correct diagnosis and treatment.

This manuscript aims to give a brief overview of the available functional imaging techniques and to review present research findings in human and animal anxiety disorders based on functional brain imaging modalities.

Functional brain imaging modalities

The available functional imaging modalities are not invasive, yet they are *in vivo* techniques that can look beyond the structural abnormalities and detect disturbances at the neuron level by imaging brain systems

such as the neurotransmitter systems, cerebral blood flow and brain glucose metabolism.

The three main neuroimaging modalities are briefly explained below.

Single Photon Emission Computed Tomography (SPECT)

After intravenous injection of a gamma ray (photon) emitting radiopharmaceutical, SPECT cameras can register the distribution of these photons within the patient. This tomographic nuclear imaging modality consists of gamma ray detectors which rotate around the patient's axis. Each detector consists of a collimator, a sodium iodine crystal and photomultiplier tubes (Clifford and Daniel, 2006). Collimators, which are made of lead and exist in different shapes and thicknesses, allow only photons coming from a specific direction to enter the crystal. Photons not coming from the specific direction are absorbed by the collimator and consequently do not reach the crystal. The crystal is a crucial part of the apparatus, as it registers the photons and converts them into light signals. Finally, the photomultiplier tubes convert the light signals into a measurable electric current which, after reconstruction, results in a 3-dimensional image that can be viewed as multiple 2-dimensional images in the 3 spatial planes (horizontal, transversal and sagittal) (Figure 1).

^{99m}Technetium (^{99m}Tc) is the most often used isotope in SPECT imaging (Kowalsky, 2006). The short half-life (6 hours), the emission of only gamma rays, the gamma ray energy of 142 keV, the availability of ready-to-use pharmaceutical kits and the easy access to it (through the Molybdeen generator) makes ^{99m}Tc the ideal radionuclide for SPECT imaging. In Table 1 the commercially available radiopharmaceuticals for brain single photon emission computed tomography and positron emission tomography are presented, as well as the advantages and disadvantages of both techniques. As can be seen in Table 1, radiopharmaceuticals systematically have two compounds: a chemical substance (e.g. ethyl cysteinate dimer (ECD) or fluorodeoxyglucose (FDG)) which directs the radiopharmaceutical to a certain target, and a radioactive marker (e.g. ^{99m}Tc or ^{18}F) which enables the radiopharmaceutical to be imaged. For instance, labeling the chemical substance FDG with the radioactive marker ^{18}F enables the analysis of the glucose metabolism in the regions of interest.

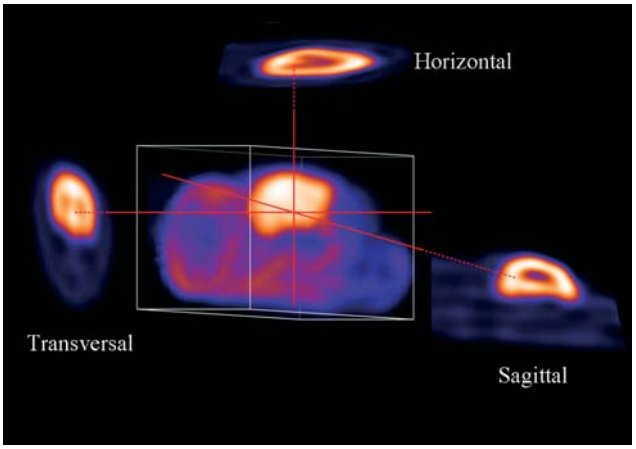

Figure 1. A canine brain is displayed in the 3 spatial planes (horizontal, sagittal and transverse).

^{99m}Tc-ethyl cysteinate dimer (ECD; Neurolite, Bristol-Myers Squibb) is an example of a SPECT tracer used to assess the regional cerebral blood flow (rCBF) *in vivo* (Leveille, 1992). This lipophilic radiopharmaceutical undergoes rapid uptake in the brain (i.e. within the first minutes after intravenous injection) through the blood-brain barrier by transcellular lipophilic diffusion. Once intracellular, it is trapped within the brain (by de-esterification into a hydrophilic compound) in local concentrations proportional to the local cerebral blood flow (Leonard *et al.*, 1986), and this brain distribution remains stable for at least two hours (Leveille *et al.*, 1992). Since the rCBF is closely linked to the regional brain metabolism and regional neural activity (i.e. in the event of an increase in brain activity, a higher oxygen and metabolite supply is required, which is provided for by increased perfusion), imaging of the rCBF using SPECT ^{99m}Tc-ECD makes it possible to study the regional neuronal function (Warwick, 2004).

Positron Emission Tomography (PET)

Positron emission tomography is also a tomographic nuclear imaging technique, but it uses positron emitting radiopharmaceuticals. PET imaging is based on the positron-electron annihilation reaction, which induces the production of two 511keV gamma rays traveling in opposite directions (Matwichuk-Bassett and Berry, 2006). It is only when two gamma rays are thus detected traveling simultaneously in opposite directions that an event is registered. The use of such a coincidence registration method makes collimation superfluous, thereby increasing the sensitivity of PET over SPECT. Due to the unknown distance the positron will travel before the positron-electron annihilation occurs, i.e. the so-called positron range, the precise origin of the radiopharmaceutical cannot be localized with conventional PET cameras (Figure 2). However, even taking this intrinsic limitation into account, PET spatial resolution (3-4mm) remains better than that obtained with conventional SPECT systems (7-9mm) (Rahmin and Zaidi, 2008). The high cost and the need for a nearby cyclotron due to the very short half-life of PET isotopes are the main disadvantages (Table 1).

Functional Magnetic Resonance Imaging (fMRI)

Functional magnetic resonance imaging is a very recent functional imaging modality that is based on the blood oxygenation level and concurrent changes in magnetic signals that are observed while the patient is presented with a stimulus or is required to perform a specific task (Malhi and Lagopoulos, 2008). Hemoglobin can occur either in an oxygenated or in a deoxygenated

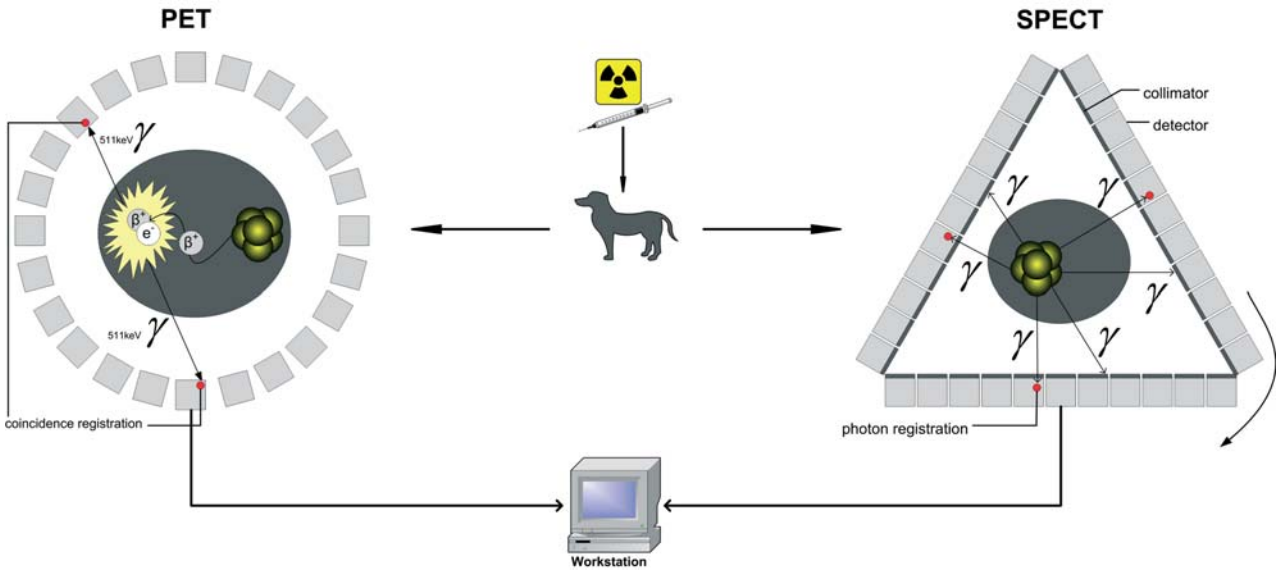

Figure 2. Depicting PET and SPECT.

state in the blood, with the two states having different magnetic properties. Because it is known that changes in neuronal activity result in changes in blood oxygenation, the blood oxygenation level dependent (BOLD) signal changes visualized by *f*MRI can be used to assess neuronal activity. The main advantages of *f*MRI are that no radioactive or other contrast agents are required, and that concurrent high resolution structural MRI images can be obtained. The price and the applicability, which is limited to activation studies, are the primary disadvantages. The introduction into veterinary medicine is impracticable due to the anesthesia required to immobilize the animal, which hinders any task performances or measurements of reactions on the stimuli presented. Therefore, this technique is beyond the scope of this article. The reader is referred to a review by Di Salle et al. for more detailed information regarding *f*MRI (Di Salle *et al.*, 1999).

Functional imaging of anxiety disorders

Major developments in the field of neuroimaging in the last decades have created opportunities to investigate neurocircuitry models of anxiety disorders in order to acquire crucial information for the understanding of these disorders and to investigate the pharmacological effects of drugs on them (Kent and Rauch, 2003). Functional brain imaging techniques like positron

Table 1. Main radiopharmaceuticals for SPECT and PET, and the advantages and disadvantages of both functional imaging modalities (Peremans *et al.*, 2003b, Rahmim and Zaidi, 2008, Waelbers *et al.*, 2010).

SPECT	**PET**		
Isotope	**$T_{1/2}$**	**Isotope**	**$T_{1/2}$**
^{99m}Tc	6.0h	^{15}O	2.05 min
^{123}I	13.3h	^{18}F	109.8 min
		^{11}C	20.4 min
		^{13}N	9.98 min
Radiopharmaceuticals	**Target**	**Radiopharmaceuticals**	**Target**
^{99m}Tc-ECD	rCBF	^{15}O-H2O	rCBF
^{99m}Tc-HMPAO	rCBF	^{18}F-FDG	glucose metabolism
^{123}I-FP-CIT	DA transporters	^{18}F-DOPA	DA receptors
^{123}I-beta-CIT	5-HT & DA transporters	^{11}C-raclopride	DA D_2 receptors
Advantages	**Disadvantages**	**Advantages**	**Disadvantages**
relatively low cost	limited spatial resolution	good spatial resolution	expensive
long $T_{1/2}$ of isotopes	only semi-quantification	absolute quantification	short $T_{1/2}$ of isotopes
^{99}Mo generator on site	moderate sensitivity	high sensitivity	need of nearby cyclotron
easy tracer labeling			complex tracer labeling

$T_{1/2}$: half life; rCBF: regional cerebral blood flow; 5-HT: serotonin; DA: dopamine

emission tomography (PET), single photon emission computed tomography (SPECT) and functional magnetic resonance imaging (*f*MRI) can be used to study the relationships between cerebral blood flow, cerebral metabolism and behavior.

Regional Cerebral Blood Flow (rCBF)

Regional cerebral blood flow is strongly related to regional brain metabolism and regional neural activity (Leonard *et al.*, 1986; Warwick, 2004). ^{99m}Tc-ECD SPECT, ^{99m}Tc-HMPAO SPECT, [^{15}O]-CO_2 PET, [^{15}O]-H_2O PET and *f*MRI are all techniques that reflect the regional neuronal function.

Neuroimaging studies in human anxiety disorders have revealed disturbed activity mainly in the medial prefrontal cortex, the thalamus, the hippocampus and the amygdala, which are the brain regions involved in the normal fear circuitry (Figure 3). Most studies describe a consistently decreased rCBF in the cortical areas and increased rCBF in the subcortical amygdala (Bremner, 2004; Etkin and Wager, 2007). Results for the thalamus and hippocampus are less consistent with elevated rCBF in obsessive-compulsive disorder (OCD), but both elevated and decreased rCBF have been observed in the thalamus of post-traumatic stress disorder patients (Tillfors *et al.*, 2001; Kent and Rauch, 2003; Lanius *et al.*, 2003; Bremner, 2004; Shin *et al.*, 2004; Kim *et al.*, 2007).

Asymmetric changes in rCBF involving mainly the right amygdala and hippocampus, and accompanied by altered rCBF in the (ventromedial) prefrontal cortex, are typical for panic disorders (Eren *et al.*, 2003; van den Heuvel *et al.*, 2005; Lee *et al.*, 2006; Domschke *et al.*, 2008). Functional MRI studies in generalized anxiety disorder patients reveal heightened amygdala activity, which also positively correlates with the symptom severity, thus revealing that the amygdala and the prefrontal cortex play an even greater role. In addition, right amygdala and right ventrolateral prefrontal cortex activation exhibit a negative connectivity during the presentation of angry faces (Monk *et al.*, 2006; Monk *et al.*, 2008). Moreover, other activation *f*MRI studies in social anxiety disorder, panic disorder and post-traumatic stress disorder also show greater activation of the right amygdala (van den Heuvel *et al.*, 2005; Etkin and Wager, 2007; Domschke *et al.*, 2008; Stein and Stein, 2008). A recent canine study found decreased rCBF in the left frontal cortex, combined with decreased rCBF in the subcortical area (including the thalamus) and increased rCBF in the temporal cortex (including the amygdala and hippocampus) of dogs with an anxiety disorder (Vermeire *et al.*, 2009a).

Overall, both canine and human neuroimaging studies confirm the hypothesis of failure of the prefrontal cortex to deliver an appropriate response, and of hyper-responsiveness of the elementary and more primitive amygdaloid danger recognition system. A top-down disinhibition involving the absence of compensatory modulation by the prefrontal cortex, leading to hyperactive amygdalar response, could explain the underlying mechanism (Kent and Rauch, 2003).

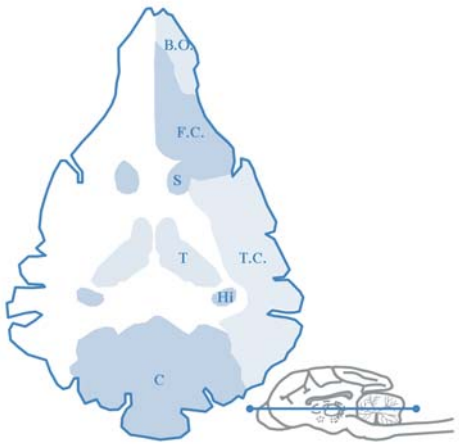

Figure 3. Transverse slice of the canine brain. (B.O.: bulbus olfactorius; F.C.: frontal cortex; T.C.: temporal cortex; C: cerebellum; S: striatum; T: thalamus; Hi: hippocampus).

Serotonin (5-HT)

Concerning affective disorders, it has been shown that especially the 5-HT2A receptor is of great importance (Frokjaer *et al.*, 2008). Decreased frontal 5-HT2A receptor bindings were observed in deliberate self-harm (Audenaert *et al.*, 2001) and depressed patients (Biver *et al.*, 1997; Messa *et al.*, 2003), and similar results were found in dogs with anxiety disorders (Vermeire *et al.*, 2009b). Decreased frontal 5-HT2A receptor bindings were also noted in humans and dogs with (obsessive) compulsive behavior (Perani *et al.*, 2008; Vermeire *et al.*, 2010). In another human [^{18}F]-alteserin PET study, significantly higher striatal 5-HT2A receptor bindings were observed in the striatum of drug-free OCD patients, and this was interpreted as a 5-HT2A receptor up-regulation compensation for a lack of serotonin in the cortico-striatal-thalamic-cortical loops (Adams *et al.*, 2005).

In addition to the 5-HT2A receptor disturbances, evidence shows disturbed serotonin 1A receptor and serotonin transporter (SERT) densities in both human and canine anxiety studies. Using PET and [^{11}C]WAY-100635, different studies have shown both increased and decreased 5-HT1A receptor binding in the raphe nuclei in depressed patients (Parsey *et al.*, 2006; Drevets *et al.*, 2007; Sullivan *et al.*, 2009), and 5-HT1A receptor binding in multiple (para)limbic areas of individuals with social anxiety disorder, including the amygdala (Lanzenberger *et al.*, 2007). One study, using PET, described reduced 5-HT1A receptor binding in the cingulated and raphe nuclei in patients with panic disorder (Neumeister *et al.*, 2004).

SERT binding indices were lower in dogs with compulsive behavior (Vermeire *et al.*, 2010) and in human OCD patients (Hesse *et al.*, 2005; Zitterl *et al.*, 2007; Reimold *et al.*, 2007), as well as in human patients with panic disorder (Maron *et al.*, 2004), but in-

creased in humans with general anxiety disorder (van der Wee *et al.*, 2008), thus showing that symptomatic differences (e.g. obsessive-compulsive versus generalized anxiety disorder) can be associated with different underlying neuro-pathologies.

Dopamine

By comparing drug-naive OCD patients with controls using SPECT [^{123}I]-β-CIT, a significantly reduced availability of striatal dopamine transporters (DATs) was found in the OCD patients (Hesse *et al.*, 2005). By contrast, another study, also using drug-naive OCD patients and the same radiopharmaceutical, found increased striatal DAT binding (van der Wee *et al.*, 2004). Altered DAT availability was also noted in generalized anxiety disorder patients (increased) (van der Wee *et al.*, 2008) and in social anxiety disorder patients (decreased) (Tiihonen *et al.*, 1997a). A recent canine study observed both increased and decreased DAT densities in a group of compulsively behaving dogs (Vermeire *et al.*, 2010).

Imaging of the dopamine system is also feasible using (semi)-quantifying dopamine receptors. With this approach, low levels of striatal D_1 receptors have been observed using PET [^{11}C]-SCH23390, a dopamine D_1 receptor antagonist, in drug free (but not all drug naive) OCD patients (Olver *et al.*, 2008). Low levels of striatal D_2 receptor binding have been observed in OCD patients using [^{123}I]-iodobenzamide (IBZM) (Denys *et al.*, 2004; Schneier *et al.*, 2008) and [^{11}C]-raclopride (Perani *et al.*, 2008), and also in (generalized) social anxiety disorder patients using [^{123}I]-IBZM (Schneier *et al.*, 2000; Schneier *et al.*, 2008). The finding of downregulation of both D_2 and D_2 receptors in the striatum suggests increased nigrostriatal dopaminergic drive in OCD and social phobia patients.

Gamma Aminobutyric Acid (GABA)

Overall decreased $GABA_A$ receptor bindings have been noted in different anxiety disorders, with the highest reduction of frontal and temporal cortex in panic disorder and generalized anxiety disorder (Malizia *et al.*, 1998; Nutt, 2001; Cameron *et al.*, 2007; Nikolaus *et al.*, 2010), even though other functional imaging studies have found no alterations or even increased binding indices (Nikolaus *et al.*, 2009).

Several $GABA_A$ receptor subtypes (so-called benzodiazepine receptors) are targets of benzodiazepines, which enhance the postsynaptic phasic inhibition and consecutively result in an anxiolytic effect. Different neuroimaging studies have indicated a reduction in GABA levels and $GABA_A$ –benzodiazepine receptor binding, which can be interpreted as a decrement of the inhibitory GABAergic input in patients with panic disorders, posttraumatic stress disorder and generalized anxiety disorder (Tiihonen *et al.*, 1997b; Bremner *et al.*, 2000a; Bremner *et al.*, 2000b; Goddard *et al.*, 2001; Nutt, 2001).

Norepinephrine and glutamate

Up to now neither PET nor SPECT radiopharmaceuticals are commercially available for imaging brain glutamate receptors such as the N-Methyl D-aspartate (NMDA) receptor subtype or adrenoceptors, and development is still ongoing (Sobrio *et al.*, 2010). A few anecdotal functional imaging studies exist on the noradrenergic system using experimental radioligands. For instance, Meana *et al.* noted increased density and affinity of α_2-adrenoreceptor in the hypothalamus and, to a lesser extent, in the frontal cortex of depressed suicidal patients (Meana *et al.*, 1992).

Hypothalamic-Pituitary-Adrenal (HPA) Axis

No radiopharmaceuticals exist for imaging corticotropin releasing factor, adrenocorticotropin-releasing hormone or glucocorticoids such as cortisol. However, by combining rCBF PET imaging and salivary cortisol measurements in social anxiety disorder patients performing a public speaking task, a positive correlation between hypothalamic rCBF and salivary cortisol was observed, in addition to a negative correlation between medial prefrontal cortex and salivary cortisol (Ahs *et al.*, 2006). Such results indicate that stress-induced cortisol excretion appears to be enhanced by activation of the hypothalamus and inhibited by medial prefrontal cortex activation.

CONCLUSION

Functional imaging enables *in-vivo* visualization and (semi)quantification of multiple brain neurotransmitters. A review of the literature on the functional imaging of anxiety disorders in humans and canines reveals major alterations in the regional cerebral brain perfusion, the serotonergic system and the dopaminergic system. Furthermore, the fact that important analogies exist between humans and dogs regarding these alterations suggests that research in dogs with anxiety disorders can also be of use in human psychiatry.

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Uit het verleden

ZEER MODERNE MAATREGELEN TEGEN PAARDENSNOT IN 1746

Oorlogen brachten in het verleden steeds grote concentraties paarden en vee in beweging, met steevast geweldige uitbraken van besmettelijke ziekten tot gevolg. Toen de Franse legers in 1746 tijdens de Oostenrijkse successieoorlog weer eens ‘op bezoek’ kwamen in onze streken, werden de militaire paarden (al of niet opgeëist, m.a.w. gestolen bij de burgers) aangetast door de gevreesde snotziekte. Vermoedelijk was dat droes verwekt door *Streptococcus equi* infectie al of niet in combinatie met virale luchtwegeninfecties.

Maatregelen werden genomen door de Franse militaire overheid in de veroverde gebieden van Vlaanderen en Brabant. Alle eigenaars van stallen waarin zieke dieren hadden verbleven, werden verplicht dat aan te geven bij de dorpsschepenen. Deze laatsten moesten lijsten met besmette stallen overhandigen aan de Fransen en aanplakbrieven laten ophangen met de tekst *Stal besmet met de quale vande snotte*.

Verder werd bevolen binnen de vijftien dagen de kribben en ruiven te verbranden in aanwezigheid van een wethouder. Nog belangrijker en verrassend modern aandoend is het bevel de vloeren (waarschijnlijk de muren: ‘surfaces’ vertaald als ‘vloeren’?) driemaal te witten met ongebluste kalk. Blijkbaar was men in militaire kringen op de hoogte van het ontsmettende vermogen van dat product lang voor de introductie van desinfectie in de humane verloskunde en chirurgie door Semmelweiss en Lister en de ontdekkingen van Pasteur en Koch. Uit die tijd stamt de gewoonte kazernemuren te kalken en stallen te witten.

De methode aangewend om deze bevelen te doen opvolgen was ook niet mis. De opbrengst van de zeer zware boete (meer dan een half jaarloon in het bouwvak uit die tijd) bij overtredingen mocht namelijk voor de helft te goede komen aan de armen van het dorp en voor de andere helft aan de aanbrenger!

Bron: Nederlandstalig afschrift van deze maatregelen opgenomen in het ‘*Boek der Voorgeboden*’ van de stad Gent (Stadsarchief Gent, reeks 108 nr. 7, folio 104 recto - folio 105 verso).

Luc Devriese