RESEARCH NOTE

Do mood disorders play a role in pig welfare?

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ABSTRACT

The work examines the hypothesis that the behavioural disorders found in pigs under conditions of stress may also be attributable to inherent conditions of alteration in mood.
In light of new evidence that links the biochemical characterization of human depression to a particular profile of fatty acids in platelets, in particular the Palmitic Acid, Linoleic and Arachidonic, the decision was made to investigate platelet fatty acids of different animal species (pig, cattle, cat, sheep), together with the same data found in literature for rats and guinea pigs.
The results obtained from normal and depressed human subjects have made it possible to achieve a particular Artificial Neural Network called the Self Organizing Map (SOM).
This network, which is also used in the classification of some species (pigs, cattle, cats and sheep), has been utilised to distribute and classify all the animals studied, in agreement with the fatty acid markers of depressive disorder and the degree of saturation of membrane lipids.
In agreement with this approach, the pig is comparable to humans that present a clinical diagnosis of depression. A critical analysis of specific references indicates the existence of a wide range of similarities between human beings suffering from depression and pigs. All the results we obtained on platelets, together with bibliographic evidence make plausible, in our view, the hypothesis that the pig is an animal intrinsically prone to depression. This tendency, which is probably genetically predetermined, must be taken into account in studies on the welfare of this animal and could also serve as a good model for the study of antidepressant molecules for humans.

Key words: Depression, Platelets, Fatty acids, Pig, Animal welfare.

RIASSUNTO

I DISORDINI DELL'UMORE GIOCANO UN RUOLO SUL BENESSERE DEL SUINO?

Il lavoro analizza l'ipotesi che i disordini comportamentali noti per il suino sottoposto a condizioni di stress, possano essere anche riferibili a intrinseche condizioni di alterazione dell'umore. Alla luce di nuove evidenze che riconducono la caratterizzazione biochimica della depressione umana a un particolare profilo degli acidi grassi delle piastrine, con specifico riferimento all'Acido Palmitico, Linoleico e Arachidonico, si è voluto verificare il profilo degli acidi grassi piastrinici di diverse specie animali (suino, bovino, gatto, pecora),

unitamente agli stessi dati reperiti in letteratura per il ratto e per la cavia. I risultati ottenuti su soggetti umani, normali e depressi, hanno consentito di realizzare una particolare Rete Neurale Artificiale, denominata Self Organizing Map (SOM). Questa rete, utilizzata per la classificazione degli animali sulla base degli acidi grassi individuati, ha distribuito e classificato tutti gli animali studiati in accordo con gli acidi grassi markers del disordine depressivo e con il grado di saturazione/insaturazione dei lipidi di membrana. In conformità a quest'approccio il suino è classificabile in modo corrispondente agli umani che presentavano diagnosi clinica di depressione. Una disamina critica della bibliografia specifica indicherebbe l'esistenza di un'ampia gamma di similitudini fra esseri umani affetti da depressione e suini.

L'insieme dei risultati sperimentali da noi ottenuti sulle piastrine, congiuntamente ai riscontri bibliografici, rende, a nostro avviso, plausibile l'ipotesi che il suino sia un animale intrinsecamente tendente alla depressione.

Di quest'attitudine, verosimilmente geneticamente predeterminata, occorrerà tenere conto nell'effettuazione di ricerche sul benessere di tale animale che potrebbe anche configurarsi come un ottimo modello per lo studio di molecole ad azione antidepressiva per l'uomo.

Parole chiave: *Depressione, Piastrine, Acidi grassi, Suino, Benessere Animale.*

Introduction

Recent studies have demonstrated the existence of significant differences in fatty acids composition of cell membranes of platelets in depressed humans when compared to apparently healthy subjects (Cocchi *et al*., 2008b). These differences have been pointed out through the use of advanced mathematical tools. In particular an Artificial Neural Network (ANN), the Self Organizing Map (SOM) described by Kohonen *et al.* (1998) and Kohonen (2001) was employed.

Kohonen (1982) has defined an ANN as being a massively parallel, interconnected network of simple (usually adaptive) elements and their hierarchical organizations, intended to interact with the objects of the real world in the same way as the biological nervous systems do. The most important types of ANNs are: a) signal-transfer networks, in which the output signal values depend uniquely on input signals, so that these circuits are designed for signal transformations; b) state-transfer networks, which are based on relaxation effects, in which the feedbacks and non-linearity are strong enough to cause the activity state to rapidly converge to a stable value (the attractor); c) competitive-learning networks, in the simplest structures of which cells compete upon receiving identical input information. The type of ANN we found to be best, namely the Self-Organizing Map (SOM), belongs to the last group.

The SOM is an unsupervised competitive-learning network algorithm which was invented by Kohonen (1982). According to Kohonen *et al*. (1998): "The central property of the SOM is that it forms a nonlinear projection of a high-dimensional data manifold on a regular, low-dimensional (usually 2D) grid. In the display, the clustering of the data space as well as the metric-topological relations of the data items is clearly visible. This kind of combined display has been found very useful for the understanding of the mutual dependencies between the variables, as well as of the structures of the data set." In the context of this definition, a manifold refers to a topological space with well-defined mathematical properties. A particular strength of the SOM displays lies in enabling relevant information to be 'found' rather than 'searched for'.

As a result, the SOM isolated three fatty acids: the Arachidonic Acid (AA), the Linoleic Acid (LA) and the Palmitic Acid (PA), (Cocchi *et al.,* 2008b), and was able to map the two different populations (normal and depressive humans) recognizing as similar

the subjects belonging to the same population (Cocchi and Tonello, 2008). The results are shown in Figure 1.

The data obtained brought us to understand that the whole phenomenon was strictly linked to a different saturation degree of platelet membrane fatty acids (Cocchi *et al*., 2008b). Briefly, each subject had a specific degree of saturation which was expressed by means of a specific index, called the B2 index, based on the percentages of AA, LA and PA, which represent the majority of the total fatty acids, according to the following formula:

$$B_2 = \sum_{i=1}^{3} (A_i \frac{mp_i}{mw_i})$$

Where:
A_i = percentage of i-th Fatty Acid
mw_i = molecular weight of i-th Fatty Acid
mp_i = melting point of i-th Fatty Acid

i	Name	
1	Palmitic A.	C 16:0
2	Linoleic A.	C 18:2
3	Arachidonic A.	C 20:4

The distribution of the chemical index over a mono-dimensional map and a not bi-dimensional one as the SOM is, revealed a strong difference in the levels of saturation of platelet cell membrane between pathological and normal subjects, i.e. the platelet membrane of depressed humans were characterized by a higher un-saturation degree when compared to the group of clinically healthy subjects (Figure 2), (Cocchi and Tonello, 2008). The map of B2 provides guidance on the comparability of the degree of saturation of the platelet membrane in the cases studied.

Full properties of biochemical characterisation of the disease studied can not be assigned to the B2 map , as is the case of the SOM.

These results are in line with the well known use of platelets as a model for the serotonergic neurons of patients with mood disorders (Coleman, 1971; Takahashi, 1976; Sthal, 1977; Kim *et al.,* 1982; Dreux and Launay, 1985; Arora and Meltzer, 1989; Thompson, 1999; Camacho and Dimsdale, 2000; Plein and Berk, 2001).

On the whole these studies indicate the existence of specific supra molecular mechanisms which are probably genetic-based (Donati *et al.*, 2008; Poulter *et al.*, 2008; Niculescu *et al.*, 2009). If we accept the hypothesis of a genetic-based aetiology of depression in humans, differences between species, with respect to an intrinsic species-specific aptitude to depression, can also be envisioned.

Animal experiment

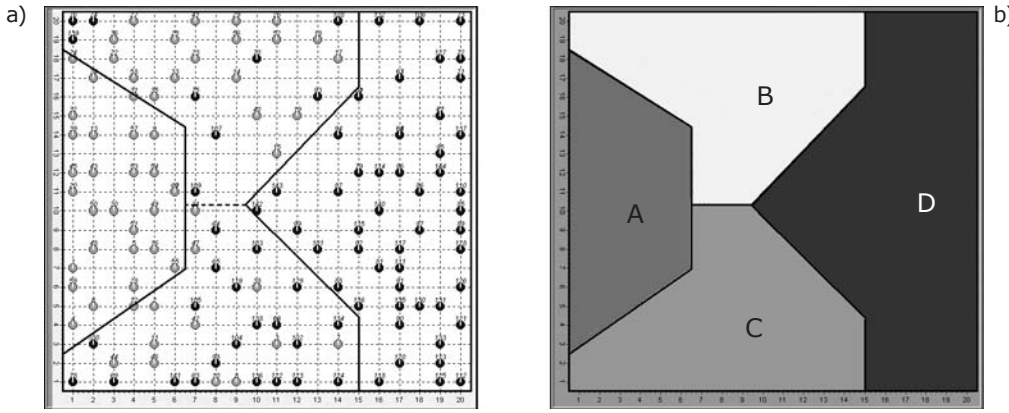
According to the results obtained we have carried out research in order to evaluate the positioning within the SOM of some animal species, i.e. cattle, sheep, cats and pigs, according to the PA, LA and AA values.

From each animal species [14 cows; 10 sheep; 9 cats (3 pools, 3 cats each); 80 Duroc x Large White pigs] platelet fatty acids have been analyzed (Cocchi *et al.,* 2007a). Based on literature evidence of platelet fatty acids profile of rats (Berdeaux *et al.,* 1996) and guinea pigs (Schick and Schick, 1981), the PA, LA, AA values of rats and guinea pigs were considered, together with all the other animals investigated, for the classification in the SOM (Cocchi *et al.,* 2009a).

In Figure 3, it is possible to see the distribution of each animal species fatty acid triplet over the human depression map.

The results indicated a clear positioning of guinea pigs in the D depression area while rats and pigs fell within the C area (an area characterized by a high density of depressive subjects, see Figure 1); thus, it can be envis-

Figure 1. Distribution of the human subjects over the SOM.
a) The distribution of the 144 subjects (60 apparently healthy (light grey) and 84 diagnosed as depressed (dark grey)) affected by the SOM has allowed us to identify four areas: two specific ones (exclusively normal and exclusively pathological) and two mixed with different concentrations of pathological subjects and apparently normal subjects of the sample. The two intermediate areas (B and C) have been interpreted as a misleading diagnosis of Major Depression, as described by literature (Bowden, 2001).
b) SOM areas: A=normal, D=Depressive, B=high density of normal subjects, C=high density of pathological subjects.



aged that rats and pigs may be considered as animals strongly prone to depression.

It is well known that both rats and guinea pigs are animals for which the depressive condition is possible and they are extensively used for research on anti depressant drugs (el Mansari *et al.,* 1995; Green *et al.,* 2005; Owen and Matthews, 2007; Brenes *et al.,* 2008; Caldwell *et al.,* 2008; Malkesman *et al.,* 2008; Overstreet *et al.,* 2008; Rex *et al.,* 2008; Spiacci *et al.,* 2008).

Fatty acids from megakaryocytes to platelets

As is well known, platelets are formed by fragmentation of the cytoplasm and plasma membrane of the megakaryocyte in the bone marrow. Schick and Schick (1981), Schick *et al*. (1990) and Schick and He (1990) have studied the lipid metabolism in megakaryocytes and platelets and compared the lipid composition of guinea pig platelets and megakaryocytes. The major fatty acid compositions of the individual platelet phospholipids reflected those of the megakaryocyte counterparts. However, the increased arachidonic acid and decreased oleic acid in platelets relative to megakaryocytes were found in all four glycerophospholipids. According to Schick and Schick (1981), the differences between the megakaryocytes and platelets fatty acids have been graphed in Figure 4. How is it possible, if the platelet is a bud of the megakaryocyte? It must have the same composition. This aspect of the fatty acids (higher Arachidonic Acid and lower Oleic Acid) has been respectively found in human subjects with depressive disorder (Cocchi *et al*., 2008b) and in human subjects

Figure 2. Distribution of the B2 index over a mono-dimensional map respecting the details of the SOM. The coefficient was calculated for all the 144 subjects studied. Unsaturation increases from the left to the right.

B2 Platelets chemical index

20	2.24	1.17	1.92	1.89	1.92	2.04	2.11	1.85	1.68	1.59	1.58	1.56	1.64	1.60	1.30	0.99	0.50	0.02	-0.49	-1.02
19	1.92	1.94	2.25	2.08	1.98	1.98	2.00	1.71	1.53	1.44	1.37	1.53	1.70	1.54	1.32	0.96	0.48	0.10	0.12	-0.21
18	2.34	2.28	2.21	2.20	2.11	2.00	1.91	1.83	1.55	1.34	1.36	1.50	1.51	1.33	1.29	0.52	0.39	0.36	0.35	0.44
17	2.36	2.37	2.27	2.19	2.22	2.12	2.04	1.85	1.77	1.55	1.39	1.32	1.31	1.30	1.23	0.49	0.39	0.38	0.43	0.51
16	3.03	2.57	2.35	2.38	2.31	2.18	2.09	2.03	1.71	1.47	1.29	1.23	1.31	1.27	1.25	0.97	0.40	0.43	0.46	0.47
15	3.21	3.19	2.59	2.47	2.53	2.39	2.21	2.41	1.67	1.40	1.27	1.14	1.22	1.21	1.22	0.72	0.53	0.47	0.47	0.15
14	3.13	3.24	2.76	2.55	2.79	2.73	2.55	2.57	2.27	1.36	1.15	1.13	1.16	1.16	1.08	0.55	0.54	0.48	0.11	-0.49
13	2.99	3.05	2.53	2.44	2.53	2.47	2.42	2.53	2.17	1.17	1.09	1.11	1.02	1.01	0.67	0.49	0.51	0.18	-0.01	-0.24
12	2.91	2.83	2.35	2.28	2.34	2.18	2.01	2.06	1.97	1.63	1.55	1.48	0.79	0.67	0.60	0.43	0.49	0.12	-0.21	-0.27
11	2.39	2.54	2.44	2.31	2.22	2.12	1.93	1.97	2.22	2.20	2.01	1.91	0.79	0.73	0.63	0.48	0.31	0.07	-0.18	-0.38
10	2.49	2.55	2.45	2.41	2.28	2.13	2.01	1.97	2.27	2.40	2.15	1.68	1.07	0.69	0.55	0.50	0.35	0.05	-0.30	-0.42
9	2.83	2.78	2.54	2.52	2.46	2.26	2.05	1.91	2.02	2.21	1.81	1.57	1.37	0.69	0.56	0.51	0.22	0.04	-0.24	-0.40
8	3.28	3.10	2.78	2.53	2.57	2.43	2.23	2.03	2.01	2.05	1.91	1.41	1.23	0.87	0.58	0.51	0.32	0.19	-0.41	-0.57
7	3.45	3.33	2.88	2.69	2.57	2.50	2.22	1.93	1.97	1.82	1.66	1.38	1.19	0.85	0.63	0.64	0.38	0.32	-0.37	-0.51
6	3.78	3.74	3.07	2.86	2.88	2.59	2.43	2.06	2.07	1.54	1.50	1.40	1.11	0.83	0.76	0.44	0.26	0.08	-0.36	-0.46
5	4.05	3.80	3.40	3.13	2.93	2.76	2.54	2.49	1.99	1.81	1.83	1.57	1.12	0.87	0.75	0.33	-0.05	-0.05	-0.35	-0.54
4	4.60	4.20	3.75	3.18	3.18	2.91	2.85	2.71	2.20	1.99	1.89	1.85	1.23	1.01	0.91	0.10	-0.03	-0.14	-0.70	-0.80
3	4.45	4.20	4.19	3.68	3.45	3.36	2.90	2.69	2.42	2.09	1.93	1.77	1.51	1.34	1.18	-0.01	-0.16	-0.82	-1.07	-1.01
2	7.10	4.47	4.14	3.96	3.63	3.40	3.34	2.91	2.75	2.43	2.08	2.05	1.66	1.19	0.73	-0.14	-0.29	-1.00	-1.53	-1.95
1	8.23	6.58	4.26	3.98	3.36	3.06	3.89	3.07	2.90	2.50	2.16	2.34	1.72	1.03	0.50	-0.03	-0.19	-1.48	-1.79	-2.64
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20

with ischemic cardiovascular disease (Cocchi *et al.,* 2007b; Cocchi *et al.,* 2009b).

Because of the guinea pig position in the SOM of depression and because the depressive state was not induced artificially, it was very clear that the guinea pig was born already depressed.

Neither Schick nor anybody else could know this, because they could not know the SOM for depression.

We tried to understand the possible origin of the metabolic error. It could not come from the megakaryocyte because it is very unlikely that the platelet gained such a specific fatty acid difference directly, so we started to think that the error might be at a higher level, probably wrong genetic information to platelets fatty acids concentration of the membrane, perhaps in the stem cell from which megakaryocytes come.

Considering the same fatty acids (PA, LA, and AA) from megakaryocytes (Schick and Schick, 1981) and their position in the SOM, we produced Figure 5. Clearly a different SOM position from that in the platelet characterizes the megakaryocyte fatty acid triplet.

The very recent work of Poulter *et al.* (2008) gives a possible explanation. They have demonstrated that the DNA methylation activity in brain cells of depressive suicide victims is ten times higher than in people that died from other causes. Poulter *et al.* (2008) have also found that the gene that was being shut down was a chemical message receptor which plays a major role in regulating behaviour and this finding suggests that this reprogramming could contribute to the protracted and recurrent nature of the major depressive disorder.

Figure 3. SOM of the mean PA, LA and AA values of the platelets of the different animal species. Areas A,B,C,D: see Figure 1.

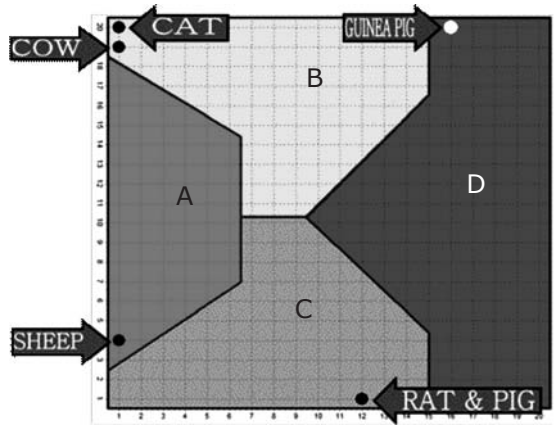
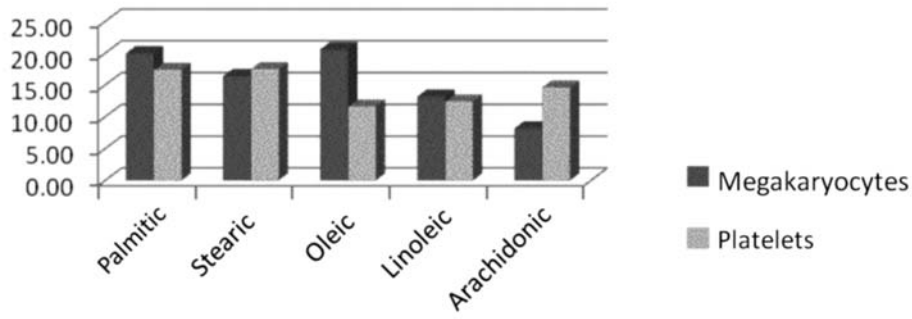


Figure 4. Megakaryocytes and platelets fatty acids comparison.



They hypothesize that, because the neurons are very stable cells and do not divide during the course of their life, it is very difficult to think that there are still epigenetic mechanisms going on.

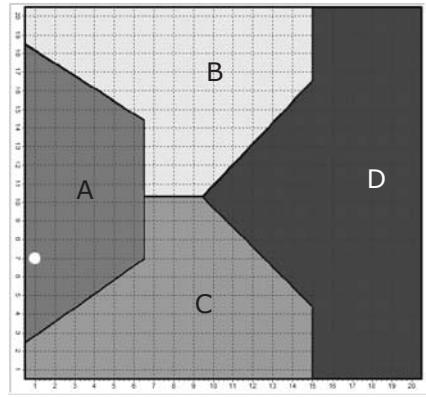
With respect to our studies on swine (Cocchi *et al.,* 2007a; Cocchi *et al.,* 2008a), the fact that pigs were not exposed to any stressful condition led us to the supposition of a genetic influence, as it has been hypothesised for the guinea pig and the rat.

Similarities between depressed humans and "normal" pigs

The effects of photoperiod

It is well known that humans can suffer from Seasonal Affective Disorders (SAD) (Rosenthal *et al.,* 1984). SAD is a form of depression most often associated with seasonal reductions in daylight, with remission linked to a subsequent seasonal increase in daylight. It has, indeed, been shown that

Figure 5. Position of the guinea pig megakaryocyte fatty acids triplet (PA, LA, and AA) in the SOM. Areas A,B,C,D: see Figure 1.



winter-SAD may respond to brightlight therapy (Lewy *et al*., 1989; Willeit *et al*., 2008). These authors have recently demonstrated that changes in mood are associated with alterations in the efficiency of the serotonin (5-hydroxytryptamine) transporter in the patients' blood platelets. Similar findings concerning the possibility of inducing depression-like behaviours in gerbils and rats by means of a reduction of lighting duration have been recently reported (Einath *et al*., 2007; Prendergast and Kay, 2008).

With respect to pigs, swine display a diurnal behavioural pattern (Hafez and Signoret, 1969). The dimensions of the pig's eye are comparable with those of humans, having the same hypermetropia value, i.e. the same ability to see at a distance. Consequently, pigs have good visual capabilities (Piggins, 1992). Furthermore pigs do not have the *tapetum lucidum*, a biologic reflector system of the eye enhancing visual sensitivity in non diurnal species (Ollivier *et al.,* 2004). Although some inconsistencies occurred in specific literature, it can be stated that pigs have a specific requirement in terms of environmental lighting, i.e. photophase duration and light intensity (EFSA, 2007). This is the reason why present EU legislation on pig protection (EU, 2001) prescribes that pigs must be kept in light with an intensity of at least 40 lux for a minimum period of eight hours per day. From a general standpoint, within a range of moderate lighting (i.e. not exceeding 80 lux), pigs show a better welfare level when they receive the higher (40 vs. 20 lux and 80 vs. 40 lux) light intensities (Mattiello *et al.,* 2004; Sardi and Martelli, 2008). Other recent studies have demonstrated that lighting periods, longer than the minimum prescribed by the law, can improve the welfare level of fattening pigs (Martelli *et al.,* 2005). Based on these studies finishing pigs receiving a 14-hour lighting regime grew better than control animals (kept at 8 hours of lighting per day) and, likewise with humans suffering from SAD subjected to brightlight therapy were calmer (longer resting periods) and showed fewer abnormal behaviours.

Tryptophan effects on humans and pigs

Pigs show a clearly positive behavioural response to the oral administration of tryptophan through feed and this amino acid shows many well known anti-depressant

properties. Tryptophan is, in fact, the precursor of serotonin, which in turn is involved in the adaptive response of the organism. The serotonin deficiency syndrome of humans has been shown to manifest itself as a broad array of emotional and behavioural problems. Although serotonin depletion cannot be considered the only cause of depression *per se*, it is well documented that tryptophan depletion can lead to mood disorders in healthy humans (memory impairment and increase in aggression) and produce a worsening of symptoms in depressed patients who responded to serotonergic antidepressant drugs (Bell *et al.,* 2001).

Piglets reject diets low in L-tryptophan (0.11% as fed-basis) and prefer diets containing adequate levels of this amino acid (Ettle and Roth, 2004). As regards behaviour, tryptophan supplementation (via feed or drinking water) has been reported to reduce the duration of fighting among unfamiliar pigs (Li *et al.,* 2006) and increase the time spent lying in pigs subjected to transport simulation (Peeters *et al.,* 2004). Dietary supplementation with tryptophan induces a reduction in the plasma concentrations of noradrenaline (Koopmans *et al.,* 2005), while there is disagreement as to the responses induced with respect to cortisolemia (Peeters *et al*., 2004; Koopmans *et al.,* 2005, 2006). In the latter case, the inconsistency of findings may depend either on the different doses of tryptophan used in the various experiments or on an inner depressed-aptitude of swine which is not necessarily related to external stress agents or noxiae.

Other similarities/differences between depressed humans and pigs

A further very interesting similarity between humans and pigs in terms of mutual inclination to mood disorders is the recently investigated field (Quilter *et al.,* 2008) concerning puerperal psychosis. Parturition can trigger extreme behavioural disturbances in both women and sows and this can lead, in extreme cases, to infanticide/piglet savaging. Studies have pointed out the existence in both species of a pool of possible genes responsible for the disease.

Among environmental factors other than lighting, noise can also play a role on mood disorders of humans and swine. As it has recently been extensively reviewed (Woo and Postolache, 2008), exposure to noise is an important stressor and predicts irritability, somatic complaints, anxiety, and depression in humans. Although intense noise is difficult to bear for practically anyone, even mild or intermittent noise may affect certain vulnerable subjects with "noise annoyance" (the emotional reaction to noise at exposure). Noise annoyance is associated with "noise sensitivity" (the physiological reaction to noise), an individual trait quite stable over time which may predict depression (Stansfeld, 1992). With respect to animal species, it is well known that noise exposure is a potential stressor for laboratory and farm animals. With respect to pigs there is only little information about acute or chronic noise effects. The different sources and levels of noise of piggeries, as well as the effects of noise on pig welfare, have very recently been extensively reviewed by Scipioni *et al.* (2009). According to Kanitz *et al.* (2005), even moderate noise stress (2h/day, 90 dB) can induce an alteration of the adrenal cortex and medulla with potential consequences that include endocrine dysfunction. If these findings are confirmed, the high susceptibility of swine to noise stress may represent a further element of linkage between depressed humans and pigs.

Another interesting aspect that can be considered is segregation. It is difficult to determine whether social isolation leads to depression or whether depression leads to a

lack of social engagement in humans. With respect to pigs, some attempts have been made to demonstrate the roles of isolation in the depressive-like symptoms arise. According to van der Staay *et al.,* (2008), no data are at present available clearly indicating that isolation induces depressive effects in pigs and the effects of isolation seem to have more to do with post-traumatic stress disorders than with depression (Kanitz *et al.,* 2004).

Contrary to isolation, stress induced by the social defeat of male rodents forms a robust animal model of depression (Palanza, 2001). Nevertheless, it has been concluded that pigs exposed to repeated social defeat do not represent a valid model of depression for humans (van der Staay *et al.,* 2008).

In any case, if a genetic presetting of an inner tendency of pigs to depression is confirmed, such studies may need to be reconsidered in light of a new perspective based on a "new normal" (i.e. prone to depression or depressed) condition of swine.

Relationships between biochemical and bio molecular aspects of depression and consciousness

If we consider the literature about the relationships among biochemistry, molecular biology, fatty acids and serotonin, some reflections can be made according to our results.

It has been demonstrated that membrane viscosity is involved because observation has been made of an altered distribution of Gs alpha protein in the TX-100-resistant and the non raft TX-100-soluble membrane domains in the Pre Frontal Cortex (PFC) and cerebellum of depressed suicide subjects compared with control subjects. (Donati *et al.,* 2008).

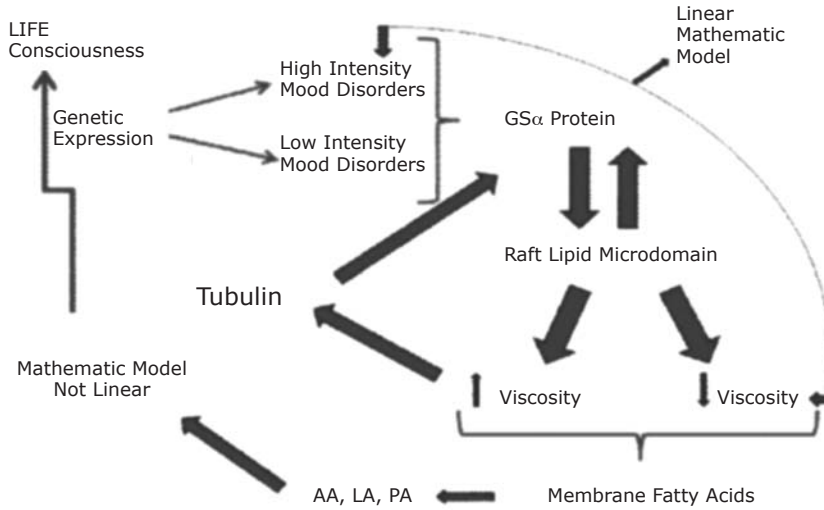
The concentration of AA in brain tissue from various areas of Flinders Sensitive Line (FSL) rats (model of depression) was higher than in the corresponding brain areas of controls (Green *et al.*, 2005), as we found in platelets of depressive subjects with respect to normal subjects (Cocchi *et al.*, 2008b). The possible involvement of AA in depression is being increasingly recognized because of the demonstrated effects of mood-regulating drugs on the turnover of this fatty acid. Serotonin has been demonstrated to be low in the brain (Young and Leyton, 2002) and in platelets of depressed subjects (Maurer-Spurej et *al.,* 2007).

In a recent interview, Hameroff (2008), the father of the studies on consciousness (Hameroff and Penrose, 1996), stated: "Indeed, recent advances in psychiatry suggest mental disorders are related to molecular-level problems inside neurons involving its cytoskeleton, for example as suggested by prof. Mark M. Rasenick, (Donati *et al.*, 2008). Such problems may occur not just in the brain, but also in other parts of the body including blood. Italian researchers Massimo Cocchi and Lucio Tonello, supported by Nobel Laureate Kary Mullis, have shown that mental depression correlates with measurable changes in blood cells known as platelets".

In this framework the study of consciousness modifications, which may correspond to different behaviours and mood disorders, carried out by gamma synchrony investigation, i.e. high frequency EEG (Electro-Encephalogram) brain waves, can forecast the pig as the animal model (Hameroff, 2009).

On the basis of research done it has been possible to hypothesize a possible pathway of the depressive disorder to the consciousness (Figure 6). Figure 6 describes the molecular depression hypothesis made according to the experimental findings of Cocchi (Cocchi *et al.,* 2008b), Rasenick (Donati *et al.,* 2008), and Hameroff (Hameroff and Penrose, 1996). Because of the possible simi-

Figure 6. Scheme of the possible bio-molecular pathway which, according to the cell (neuron and platelet) membrane viscosity, through Protein Gs alpha and tubulin modification can modify the consciousness state in psychiatric disorders.



larity of platelets to neurons, the membrane viscosity can modify the Gsα protein status. The Gsα protein is connected with Tubulin. Tubulin, depending on local membrane lipid phase concentration, may serve as a positive or negative regulator of phosphoinositide hydrolysis (PIP2) such as G proteins do.

Tubulin is known to form high-affinity complexes with certain G proteins. The formation of such complexes allows tubulin to activate Galpha and fosters a system whereby elements of the cytoskeleton can influence G-protein signalling. Rapid changes in membrane lipid composition or in the cytoskeleton might modify neuronal signalling through such a mechanism. Briefly, the biological mechanisms described in Figure 6 permit the consideration that it is possible, through the registration of the brain gamma synchrony (Flynn *et al.*, 2008) and its modification, by induction of substantial behaviour changes, to demonstrate that the state of consciousness can be modified through the bio-molecular pathway described.

Conclusions

Evidence shows that some animals, particular guinea pigs, are prone to the depressive condition. Rats and pigs, like the guinea pig, seem to be born as animals already predisposed to mood disorders.

On this issue there is certainly a need for additional research that, in our view, sees the pig as an ideal model because of the many similarities that the literature provides between this model and the human one (Linda *et al.,* 2007).

Although pigs used in the above-described experiments were reared under conventional intensive conditions and they were not exposed to any particular stress, in the framework of future research it would be of interest to investigate the platelet fatty acids profile of pigs raised under extensive conditions (i.e. animals living in a more "natural" way, closer to the situation of their wild ancestors) and/or belonging to non selected genotypes (ancient local breeds).

Beyond the existence of a link among the various bio-molecular sequences of events leading to human depression, our results may indicate an inherent predisposition to mood disorders of some animal species able to modify their behaviour. In the case of pigs, this hypothesis can lead to a reconsideration of criteria defining its welfare with particular regard to the so called "design criteria" (environmental and managerial criteria). In the case of "animal criteria" used for swine welfare assessment, we must also bear in mind that behavioural outcomes are obtained on a species that is possibly inherently predisposed to mood disorders.

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