UNIVERSITÀ DEGLI STUDI DI PALERMO

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Behavioral genetics of suicidality in Bipolar Disorder: the interaction between SERT and CLOCK polymorphisms and early-life stress

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Summary

Suicide: a global phenomenon

Epidemiology

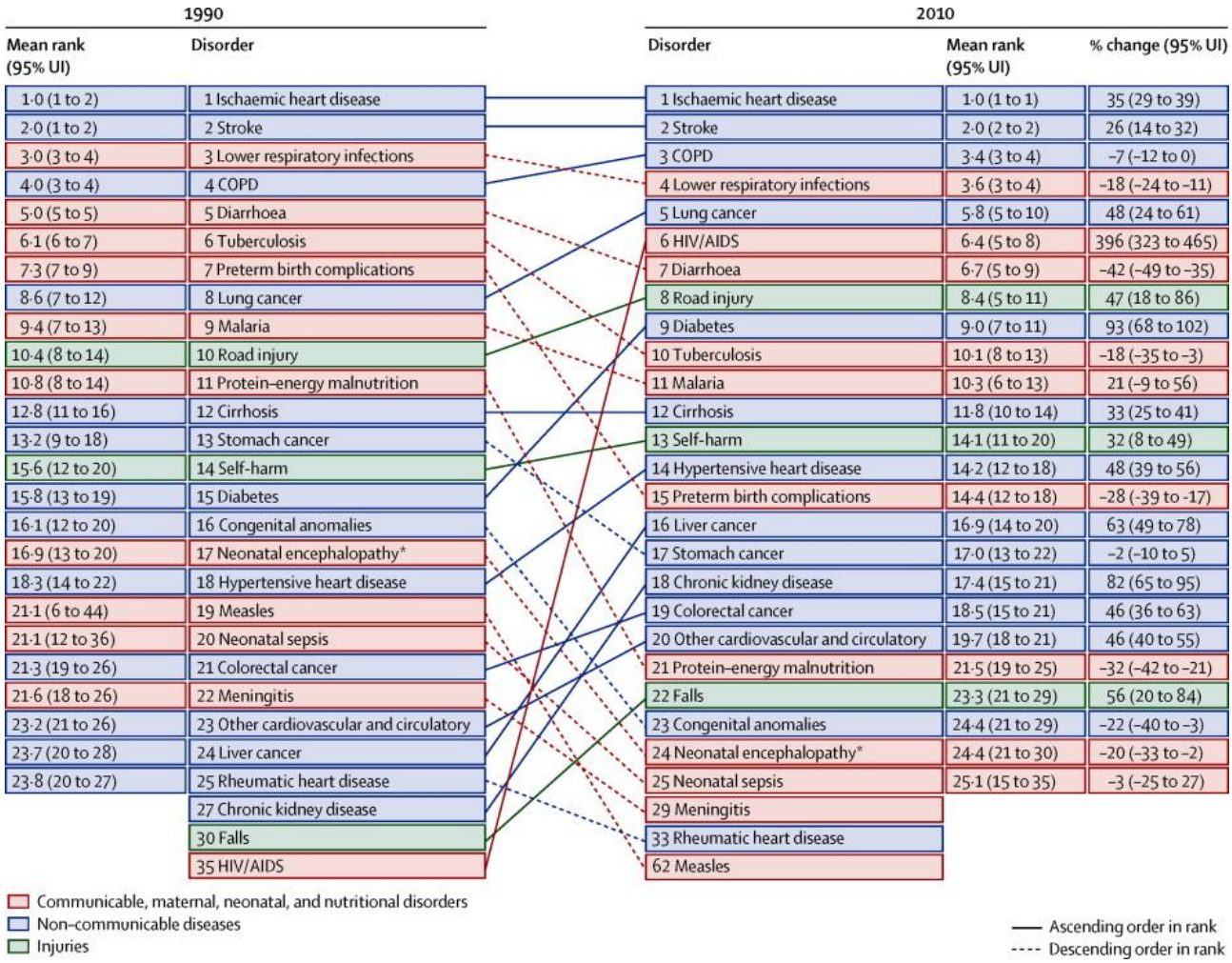
Every 40 seconds, somewhere in the world, someone dies by suicide. Probably he was an old man, who lived in a rich country and had already attempted suicide in the past before he ingested pesticide. He likely was depressed.

Was this loss preventable?

Suicide prevention is an integral part of the Mental Health Action Plan of the WHO (World Health Organization) adopted by Member States in 2013, whose goal is a 10% reduction of suicide rates by 2020. The WHO estimated 804 000 suicide deaths worldwide in 2012, with an annual age-standardized rate of 11.4 per 100 000 population (see the report "Preventing suicide: a global imperative" on the WHO website), but it is very likely that in some countries suicide is under-reported. In 2012, suicide accounted for 1.4% of all deaths and was the 15th leading cause of death worldwide.

To make clear the relevance of suicide as a major health problem is useful to assess its relative contribution to all-cause mortality globally and how it changes in time.

Fig.1



A systematic analysis for the Global Burden of Disease Study 2010 (GBD; all data and publications can be consulted on the Institute for Health Metrics and Evaluation website) estimated the annual global and regional mortality from 235 causes of deaths, by age and sex, for 187 countries, between 1980 and 2010 (Lozano, Naghavi et al. 2012).

Figure 1 shows the top 25 causes of death worldwide in 1990 and 2010 and the percentage change between the periods: in two decades mortality by self-harm raised from the 14th to the 13th cause, increasing by 32%.

In figure 2 it is provided a ranking of cause of deaths according to the years of life lost (YLLs), which quantify the amount of life lost due to premature mortality, along with the percentage change between 1990 and 2010.

Fig.2

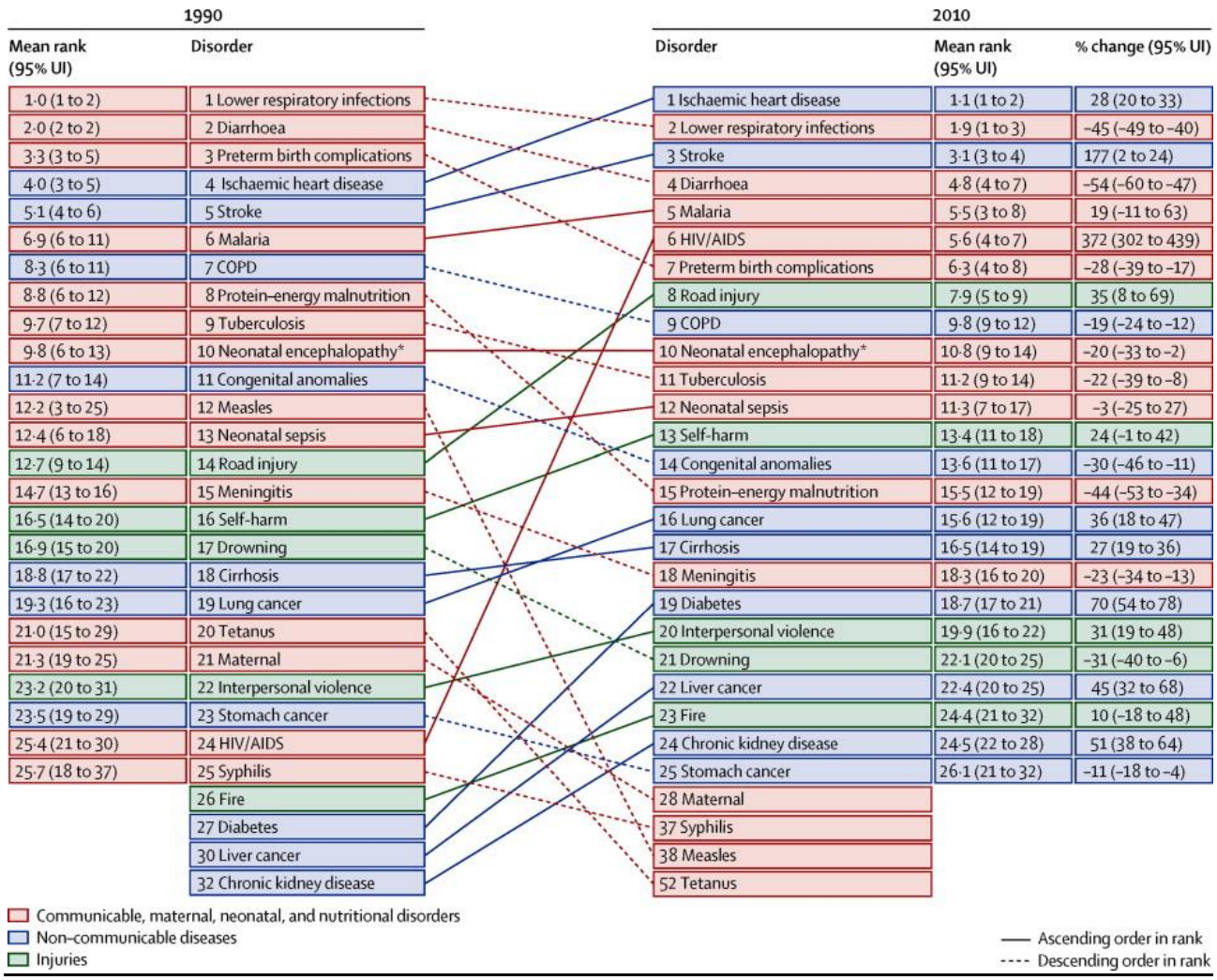
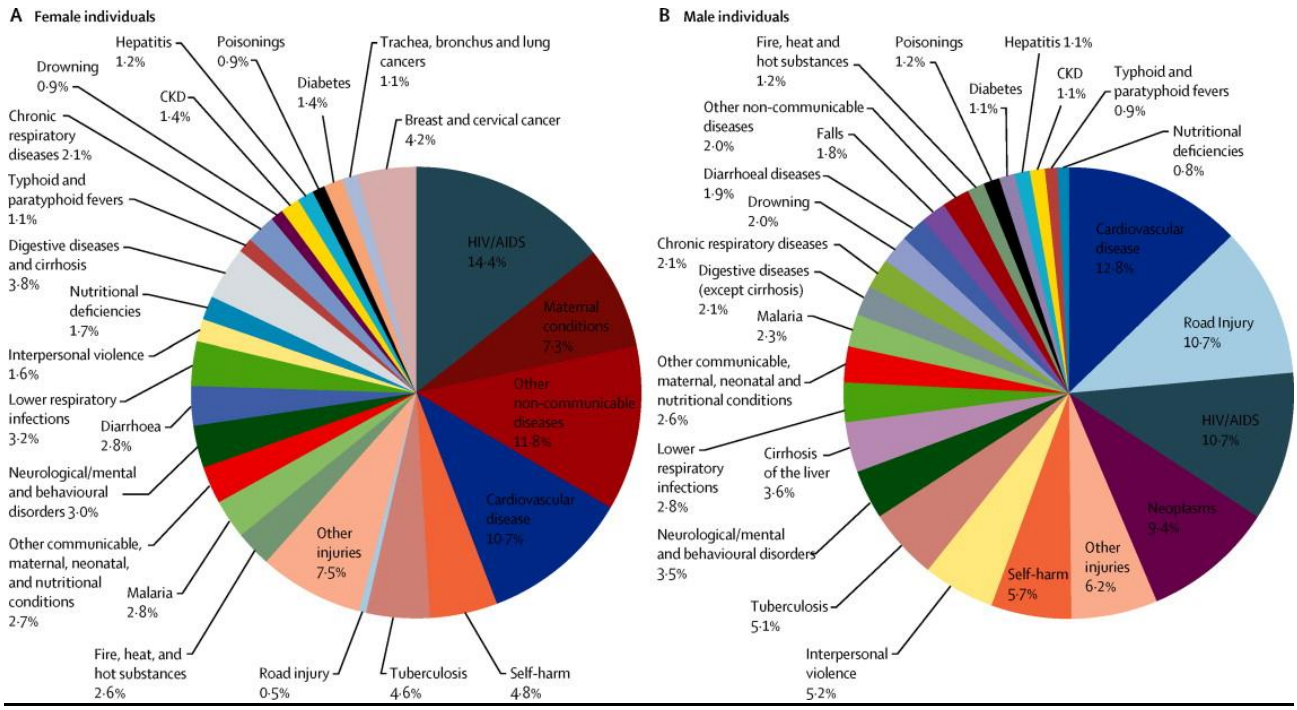


Figure 3 visualizes the percentage number of deaths in 2010 for each cause in individuals aged 15-49, divided according to gender. Self-harm accounts for 4-8% of global deaths in females and 5-7% in males.

Fig.3



The heat maps visualized in fig.4 shows the rank for every cause of death that either was in the global 25 causes of YLLs in 2010 or appeared in the top 25 causes of YLLs for any region. Self-harm is a top ten cause of premature mortality in 10 regions out of 21.

Restricting the analysis to the contribution of suicide to intentional violent deaths only (which include, beside suicide, interpersonal violence and armed conflict), suicides globally account for 56% of all violent deaths, with a peak of 80% in high-income countries (WHO estimate in 2012).

Fig.4

Ranking Legend: 1–10, 11–20, 21–30, 31–50, 51–90, 91–176

Cause	Global	High-income Asia Pacific	Western Europe	Australasia	High-income North America	Central Europe	Southern Latin America	Eastern Europe	East Asia	Tropical Latin America	Central Latin America	Southeast Asia	Central Asia	Andean Latin America	North Africa and Middle East	Caribbean	South Asia	Oceania	Southern sub-Saharan Africa	Eastern sub-Saharan Africa	Central sub-Saharan Africa	Western sub-Saharan Africa
Ischaemic heart disease	1	2	1	1	1	1	1	1	2	2	2	4	1	3	1	2	4	5	11	17	16	17
Lower respiratory infections	2	5	9	14	11	8	3	9	9	5	4	3	2	1	3	4	1	1	2	3	4	2
Cerebrovascular disease	3	1	2	3	3	2	2	2	1	3	10	1	3	5	2	3	9	8	7	14	13	12
Diarrhoeal diseases	4	57	61	76	54	80	55	44	61	23	12	6	14	8	7	7	3	3	3	4	2	3
Malaria	5	119	120	117	120	119	122	119	119	109	108	19	116	98	41	39	36	4	14	2	1	1
HIV/AIDS	6	65	40	56	26	44	20	3	23	8	11	9	20	9	38	5	13	10	1	1	5	4
Preterm birth complications	7	38	30	17	14	24	6	26	18	6	7	7	5	4	4	6	2	7	6	5	6	7
Road injury	8	10	11	6	5	7	4	5	4	4	3	5	7	2	6	11	11	19	12	11	11	9
Chronic obstructive pulmonary disease	9	12	5	7	4	10	11	13	3	11	14	17	11	23	17	27	5	27	27	29	25	32
Neonatal encephalopathy*	10	63	51	32	41	54	28	29	20	12	13	10	4	7	12	13	7	17	9	9	10	10
Tuberculosis	11	32	73	82	82	40	47	15	29	33	31	2	10	14	34	14	8	6	5	8	8	11
Sepsis	12	71	74	68	62	68	29	55	79	13	16	24	35	11	10	10	6	20	18	7	12	5
Self-harm	13	3	6	5	6	5	5	4	8	15	15	20	9	18	22	21	10	22	16	24	30	50
Congenital anomalies	14	24	23	13	19	20	8	17	11	9	9	12	8	6	5	12	12	14	13	15	7	15
Protein–energy malnutrition	15	67	76	79	73	82	52	84	59	38	19	38	50	19	25	19	16	16	29	6	3	6
Trachea, bronchus, and lung cancers	16	4	3	2	2	3	7	6	5	17	21	15	15	27	14	16	34	45	33	66	54	64
Cirrhosis of the liver	17	9	7	18	9	4	9	7	14	10	8	11	6	12	9	20	15	12	28	23	21	20
Meningitis	18	69	69	67	67	61	39	61	51	36	30	26	23	24	19	18	17	9	19	10	9	8
Diabetes mellitus	19	14	13	10	7	14	10	33	15	7	5	8	17	16	11	9	19	2	8	26	26	24
Interpersonal violence	20	49	50	38	12	31	12	8	31	1	1	16	13	10	20	8	27	24	4	18	17	23
Drowning	21	25	54	34	40	29	26	18	12	19	17	22	12	17	24	31	18	28	26	22	18	29
Liver cancer	22	7	20	28	30	21	33	37	6	34	26	18	29	31	29	30	57	21	41	45	47	30
Fire, heat, and hot substances	23	47	66	63	46	53	36	25	52	51	44	40	30	39	28	33	14	26	23	16	19	13
Chronic kidney diseases	24	13	22	21	16	22	16	31	22	16	6	14	16	13	16	22	25	11	17	32	36	31
Stomach cancer	25	6	15	25	34	13	18	12	7	24	18	33	19	15	27	34	43	31	50	52	56	60
Falls	26	21	21	29	29	17	40	21	16	28	27	29	33	33	32	36	23	42	55	27	31	19
Hypertensive heart disease	27	20	18	40	21	9	17	24	17	14	22	21	21	25	13	17	30	52	15	38	39	46
Maternal disorders	28	85	89	88	78	85	61	75	55	48	41	27	51	26	39	29	24	18	20	13	15	14
Colon and rectum cancers	29	8	4	4	10	6	13	14	13	21	28	31	31	34	33	25	55	50	40	56	63	63
Other cardiovascular and circulatory diseases	30	17	10	19	17	11	15	52	21	20	24	23	26	21	8	23	45	39	30	37	35	39
Breast cancer	31	16	8	8	13	12	14	19	24	22	25	30	28	32	26	28	47	35	38	46	55	59
Cardiomyopathy and myocarditis	32	28	24	26	20	15	19	16	34	18	38	37	22	28	15	32	38	40	22	35	32	38
Exposure to forces of nature	33	122	120	117	120	119	58	119	124	125	90	123	124	123	124	1	125	120	126	127	126	124
Exposure to mechanical forces	34	50	64	53	48	50	38	11	35	50	35	36	18	29	21	41	28	32	10	28	22	40
Typhoid and paratyphoid fevers	35	95	110	102	102	113	49	111	39	62	50	13	119	53	36	69	21	76	21	25	37	27
Leukaemia	36	19	19	15	22	23	22	28	19	29	20	32	27	20	23	38	48	23	49	68	62	66
Syphilis	37	102	101	83	101	87	82	98	74	61	57	48	66	30	47	24	41	41	31	12	14	18
Measles	38	106	105	101	108	105	103	110	103	106	93	34	83	58	94	106	20	54	35	19	38	22
Oesophageal cancer	39	18	28	30	33	39	30	41	10	35	61	52	32	66	58	55	46	61	36	44	57	78
Rheumatic heart disease	40	36	41	49	60	36	34	36	28	41	51	28	24	48	18	48	32	36	37	43	44	49
Epilepsy	41	56	48	43	63	48	57	49	43	44	33	39	25	36	42	46	53	30	25	20	24	16
Asthma	42	42	60	50	57	59	65	50	49	54	46	25	41	62	30	45	26	13	32	40	34	33
Poisonings	43	64	65	42	25	47	68	20	25	95	58	57	34	60	40	85	31	15	43	30	28	43
Encephalitis	44	82	96	103	90	84	109	66	99	75	97	69	48	105	45	96	22	91	46	41	42	42
Cervical cancer	46	34	44	48	45	25	24	34	38	27	23	35	40	22	67	35	52	29	34	31	43	41
Pancreatic cancer	47	11	14	12	18	16	21	22	27	37	39	50	43	47	50	40	73	78	53	83	80	87
Brain and nervous system cancers	48	30	17	20	28	19	37	32	26	26	32	42	38	43	31	44	64	88	54	87	70	104
Non-Hodgkin lymphoma	49	23	26	16	24	33	31	51	37	40	40	43	45	35	46	42	51	47	45	48	58	54
Alzheimer's disease and other dementias	50	26	12	9	8	43	27	53	41	45	66	82	79	76	68	37	81	66	58	80	86	75
Tetanus	52	112	113	113	114	110	118	114	86	108	109	64	121	103	79	59	40	51	104	33	45	25
Acute hepatitis A	54	100	103	104	104	99	108	92	78	101	94	91	91	92	54	104	33	25	98	53	64	44
Alcohol use disorders	55	55	31	47	32	18	41	10	73	25	29	59	39	49	108	54	76	72	61	103	99	117
Kidney and other urinary organ cancers	57	29	25	24	23	27	32	27	36	57	47	75	49	63	57	58	85	80	76	84	85	79
Drug use disorders	58	72	34	22	15	57	69	23	64	71	52	70	37	40	35	52	93	56	24	59	72	69
Prostate cancer	63	31	16	11	27	26	23	42	72	30	34	77	59	42	56	26	100	68	47	75	79	74
Whooping cough	67	99	95	84	87	94	87	95	93	105	65	44	63	37	59	43	37	33	56	21	29	21
Gallbladder and biliary tract cancer	68	15	39	51	58	37	25	59	40	49	45	60	60	44	71	67	63	67	89	113	98	84
Iron-deficiency anaemia	72	77	83	90	81	90	74	89	90	63	43	85	73	38	114	15	75	55	44	54	27	26
Interstitial lung disease and pulmonary sarcoidosis	78	22	47	46	38	70	54	76	71	72	59	100	75	46	65	101	62	81	70	77	77	85
Sickle-cell disorders	80	109	94	107	86	104	105	107	120	80	88	122	117	102	64	51	108	115	100	67	20	28
Malignant melanoma of skin	91	73	38	23	44	45	71	56	77	70	77	106	72	84	103	94	117	48	88	116	113	111
African trypanosomiasis	106	122	120	117	120	119	125	119	124	125	127	123	124	123	124	123	125	120	124	76	23	68

According to the 2012 WHO estimates, 75.5% of all global suicides occur in low- and middle-income countries (LMIC) but this is due to the larger proportion of population residing in these regions (82% of global population): the age-standardized rate of suicide is higher in high-income countries (HIC) than in LMIC (figures 5 and 6).

Fig.5 Map of age-standardized 2012 suicide rates for 172 Member States with population≥300 000.

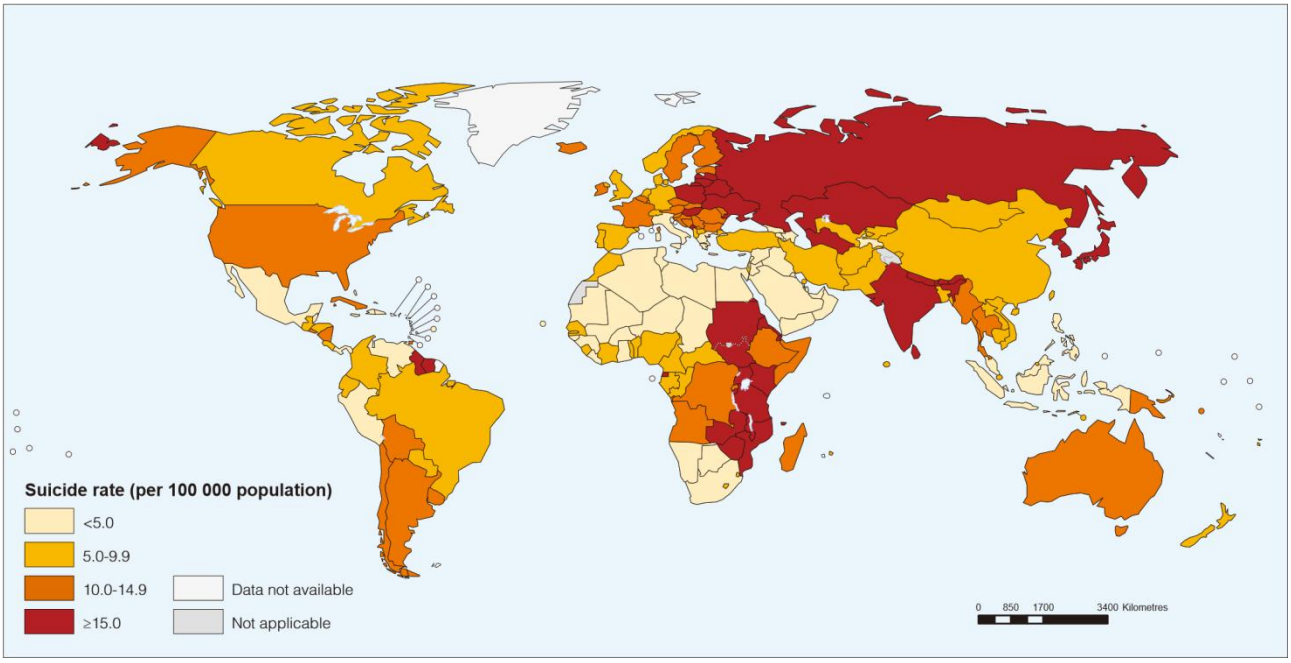
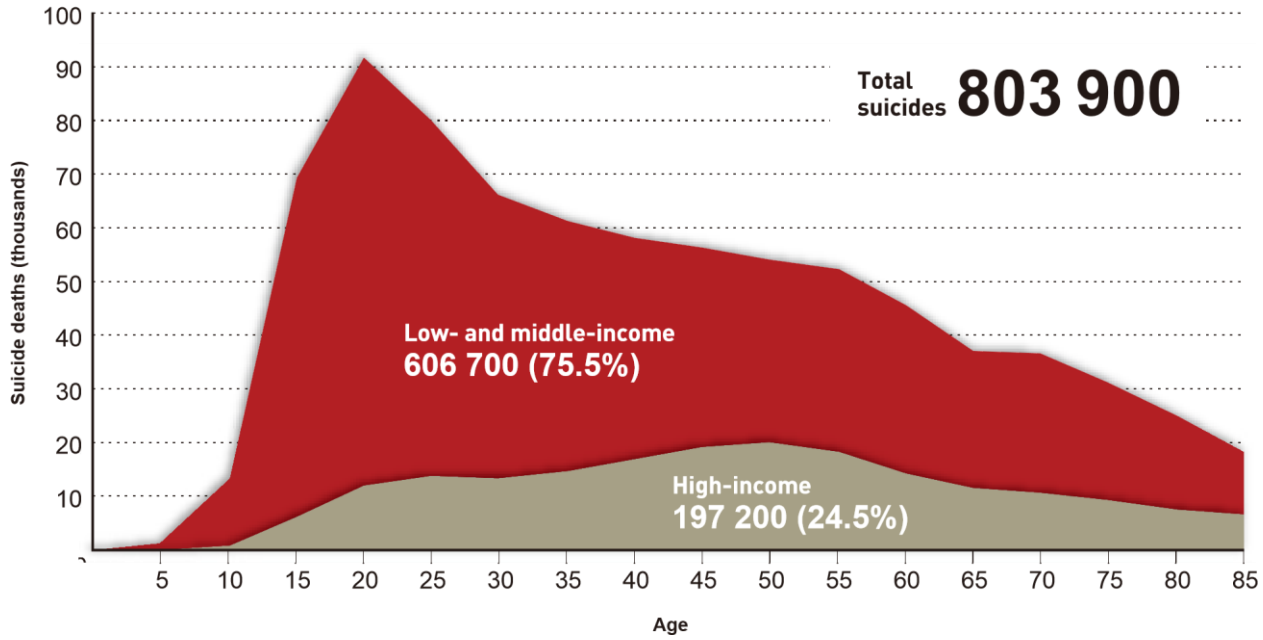


Fig.6 Number of suicides by age and income level of region in 2012.



With respect to age, there is a great variability in suicide rates according to age group in different countries. In almost all regions mortality by suicide has the highest rate in the elderly (aged 70 years or over) and the lowest in younger people (under 15 years aged). Young adults in LMIC have higher suicide rates than young adults in HIC, while middle-aged men in HIC have higher suicide rates than middle-aged men in LMIC. In the 15-30 years old group, suicide globally accounts for

8.5% of all-cause deaths and in the HIC (where it accounts for 18%) is the leading cause of death among young adults.

Suicide incidence varies by gender (fig. 7 and fig.8): males commit suicide more than females but this is particularly true in HIC, where suicide rates are 300% higher in men than women (male-to-female ratio = 3.5 in 2012; 1.6 in LMIC).

Fig.7 Age-standardized suicide rates by sex in 2012.

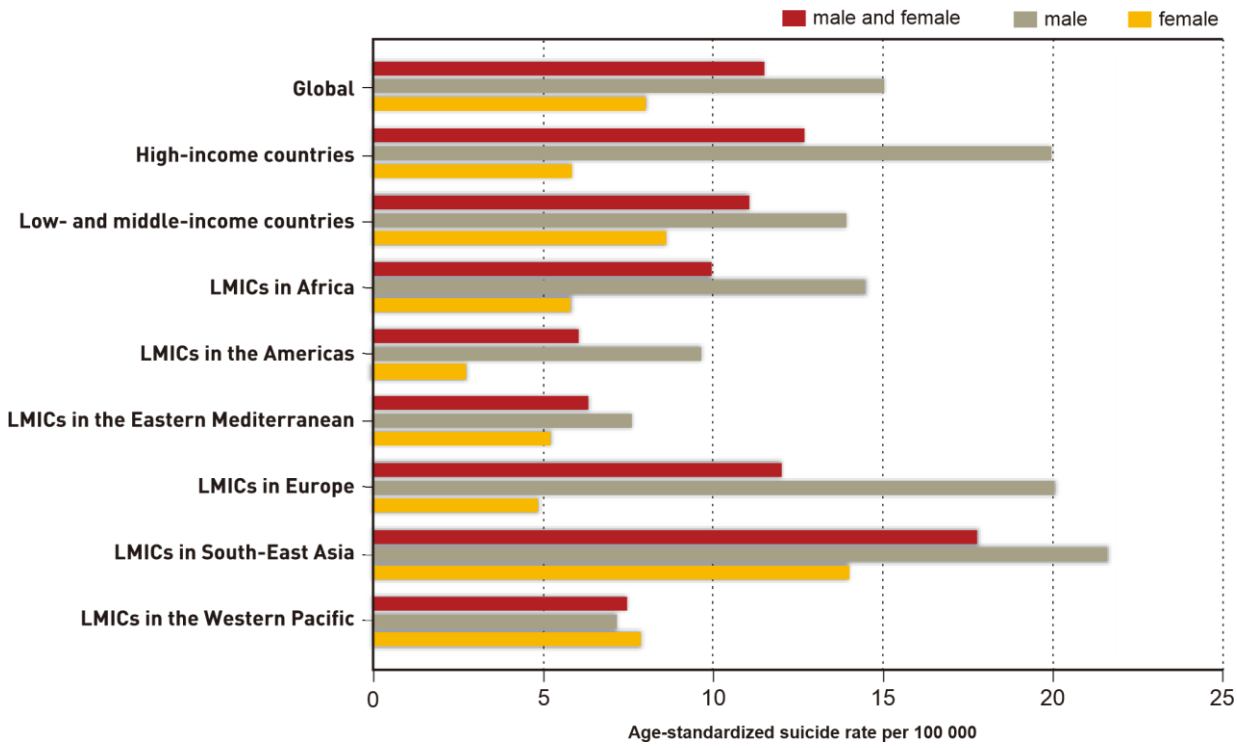
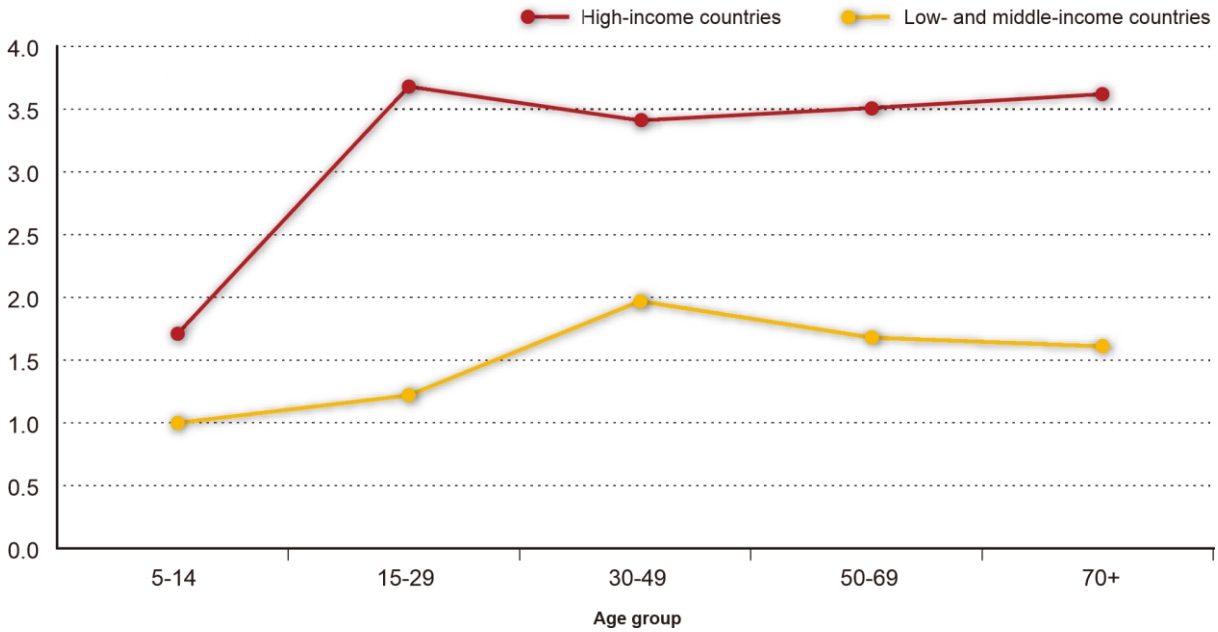


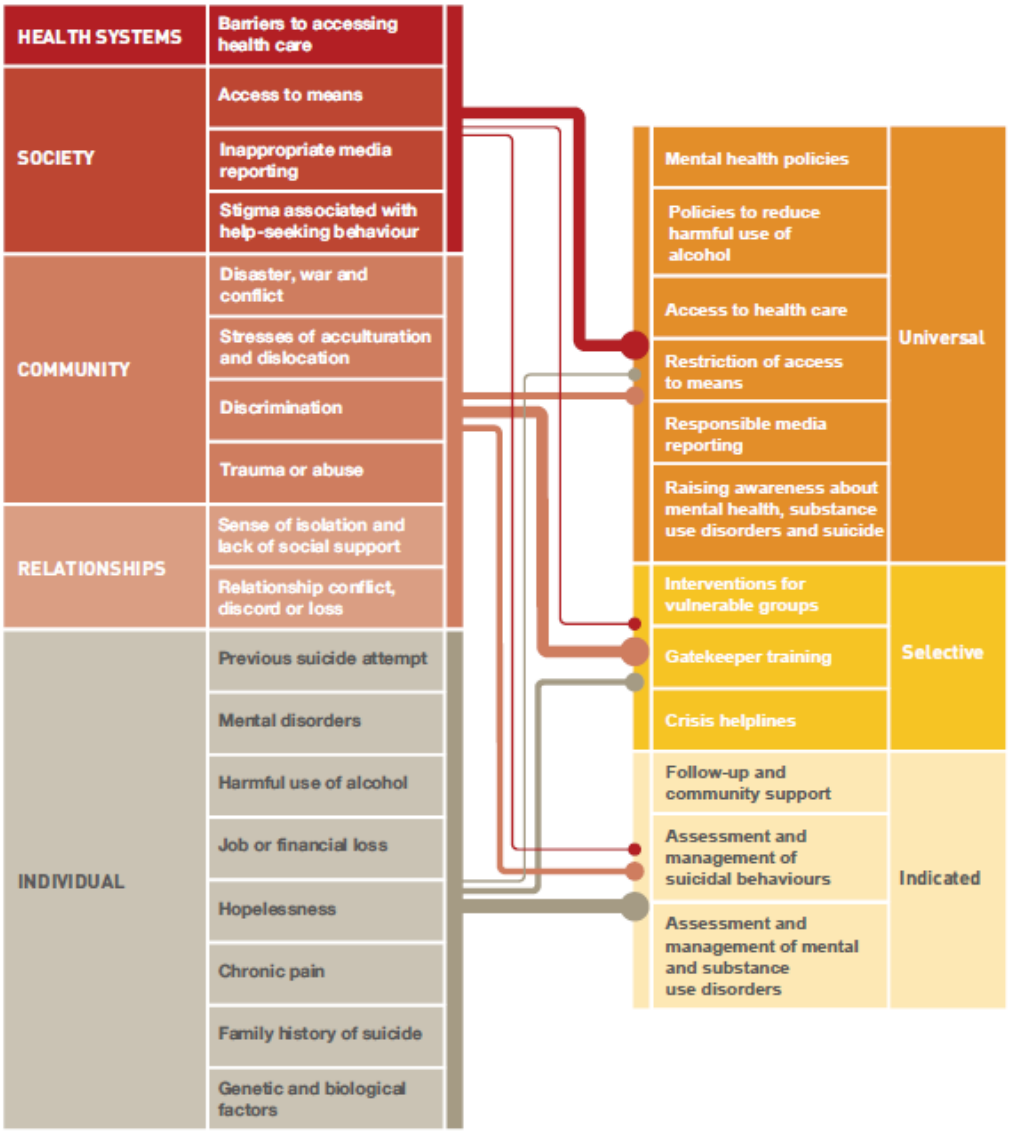
Fig.8 Male-to-female ratio of suicide rates by age group and income level of country in 2012.



Risk Factors

The WHO has identified a list of suicide risk factors that are relevant to the implementation of appropriate interventions and has grouped them in 5 broad categories based on the type of intervention required: health system, society, community, relationships and individual (fig.9).

Fig.9



Health systems and societies can help prevent suicide by allowing a timely and effective access to health care and modulating the availability of the means of suicide. Inappropriate media practices can increase the risk of imitation in vulnerable people by glamorizing or normalizing suicide and social medias have been implicated in inciting and facilitating suicidal behaviour by means of easy access to models of suicide.

Social stigma associated with mental health is still a key risk factor, which prevents vulnerable individuals from help-seeking and their families from providing them with the necessary support.

At the community level, the experience of natural disasters and wars have a destructive impact on well-being, health, housing and finances but there aren't univocal results on suicide rates following disasters or conflicts. Many stressful events act like powerful proximal and distal triggers of suicidal behaviour: acculturation, dislocation and discrimination represent significant suicide risks in vulnerable sub-groups within the population. Trauma, abuse, childhood adversities and psychosocial stressors highly increase the risk to develop a mental disorder or commit suicide.

Also the lack of sources of social support and the sense of isolation that follow aversive life events are contributing variables of suicide, along with violence, conflict, discord or loss in personal relationships.

The stronger predictive variable of death by suicide is a prior suicide attempt; other individual risk factors are a family history of suicide, mental disorders (higher in comorbidity), hopelessness, chronic pain and illness, job or financial loss. Some of these factors will be discussed later in this paper.

Risk factors are often categorised in scientific literature as state-dependent or trait-dependent, or as distal or proximal factors, as visualized in figure 10 (Hawton and van Heeringen 2009).

Fig.10

Panel: **Risk factors for suicide**

Distal

- Genetic loading
- Personality characteristics (eg, impulsivity, aggression)
- Restricted fetal growth and perinatal circumstances
- Early traumatic life events
- Neurobiological disturbances (eg, serotonin dysfunction and hypothalamic-pituitary axis hyperactivity)

Proximal

- Psychiatric disorder
- Physical disorder
- Psychosocial crisis
- Availability of means
- Exposure to models

This kind of categorization is useful to elucidate the relation between risk factors, which will be described later in the section dedicated to the explanatory stress– diathesis models of suicide.

Genes and Environment in Suicide: Serotonin and Stress

Genetics and G x E

Suicidal behaviour is a complex and multifactorial phenotype, and is also familial (Zai 2012). Family, twin and adoption studies showed that a family history of suicide increases the risk of suicidal acts and suggested that genetic predisposing factors interact with environment in the genesis and completion of this behaviour.

Lots of family studies have indicated familial aggregation of suicide, reporting higher rates of suicidal behaviours in relatives of suicide victims or attempters compared to relatives of non-suicidal controls. Studies conducted on data from demographic registries in North-Europe found increased suicide rates in descendants of suicidal parents compared to those of non-suicidal parents (Qin, Agerbo et al. 2002; Qin, Agerbo et al. 2003). Interestingly, individuals who lost their parents to suicide committed more suicide than offspring of homicide or accident victims (Runeson and Asberg 2003), excluding a causal role of grief in increasing suicide rates.

As stated before, a risk factor for suicide is a psychiatric diagnosis, particularly the presence of a Mood Disorder. Nonetheless, relatives of suicide victims who were affected with BD have an higher risk for suicide than relatives of BD patients who weren't suicidal (Tsai, Kuo et al. 2002) and, mood disorder profile being equal, suicide attempts in families of suicide attempters are higher than suicide attempts in families of non-attempters (Brent, Oquendo et al. 2002; Brent, Oquendo et al. 2003). Therefore, studies on suicide familiarity provide evidence for the inheritance of suicidal behaviour (in terms of attempt and completion) and support the partial independence of its familial transmission from that of Mood Disorders.

Whereas family studies explore the extent to which suicide permeates families, twin and adoption studies evaluate the magnitude of the influence of genetic and environmental factors on suicidal phenotype.

Twins investigations usually compare the risk that a twin exhibits suicidal behaviours given that the co-twin died by suicide and compare the suicide risk in monozygotic (MZ) versus dizygotic twins (DZ). In general, the most of the studies report greater concordance in MZ than in DZ pairs both for suicide attempts and for completion, suggesting a shared genetic component (for a review see (Zai 2012)). The heritability of suicide attempt/completion has been recently estimated as 43%. In the large population-based twin studies that have been conducted, it has been

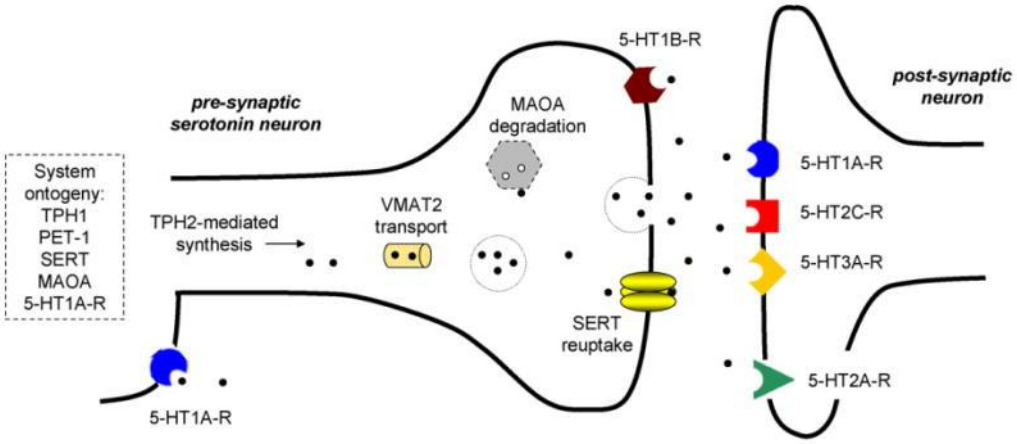
consistently found that psychiatric diagnosis and environmental factors (including childhood abuse) were associated with higher risk of suicide attempt.

In the last decades, the most of published genetic investigations are candidate-gene studies.

The serotonin (5-HT) neurotransmission system has received deep attention in these genetic candidate studies because of its predominant role in mood regulation and suicidal behaviour (role which has been also confirmed by both *post-mortem* data and *invivo* neuroimaging technics; for reviews, see chapter 2 and 9 of "The neurobiological basis of suicide", (AA.VV 2012) and its involvement in a wide range of physiologic functions: emotion, cognition, sensory processing, motor function, pain, neuroendocrine systems and circadian rhythm.

Nonetheless, despite the strong neurobiological evidence implicating serotonergic neuromodulation in suicidality (Mann 2013), none of the genes of the 5HT pathway has been definitively and undoubtedly recognized as having a causal role in suicidal behavior. It is likely that multiple risk alleles works in interactions between them and with environmental factors to produce such a complex phenotype (Judy, Seifuddin et al. 2012). The serotonin system has also been strongly implicated in pathophysiology and therapy of stress-related disorders, such as anxiety and depression (Holmes 2008). Serotonergic modulation of the acute response to stress and the adaptation to chronic stress is mediated by a series of molecules (fig.11) which are responsible for the serotonin neuron development (Pet-1), synthesis (tryptophan hydroxylase 1 and 2 isozymes), packaging (vesicular monoamine transporter 2), actions at presynaptic and postsynaptic receptors (5-HT1A, 5-HT1B, 5-HT2A, 5-HT2C, 5-HT3A, 5-HT4, 5-HT5A, 5-HT6, 5-HT7), reuptake (serotonin transporter), and degradation (monoamine oxidase A).

Fig.11



5-HT producing neurons originate in the midbrain raphe nuclei whose dorsal portion (DRN) extensively innervates most of the brain corticolimbic structures crucially involved in the regulation of stress: the medial prefrontal cortex (mPFC), amygdala and hippocampus.

In rodents, the waking states are characterized by slow and regular tonic activity of serotonin neurons but this activity increases when animals are exposes to a variety of stressor (exposure to a predator, tail pinch, elevated platform exposure, inescapable shock, social defeat, restraint/immobilization), as evidenced by increased immediate gene expression in the DRN and increased extracellular serotonin concentration in corticolimbic structures (probably due to a stress-induced 5-HT release).

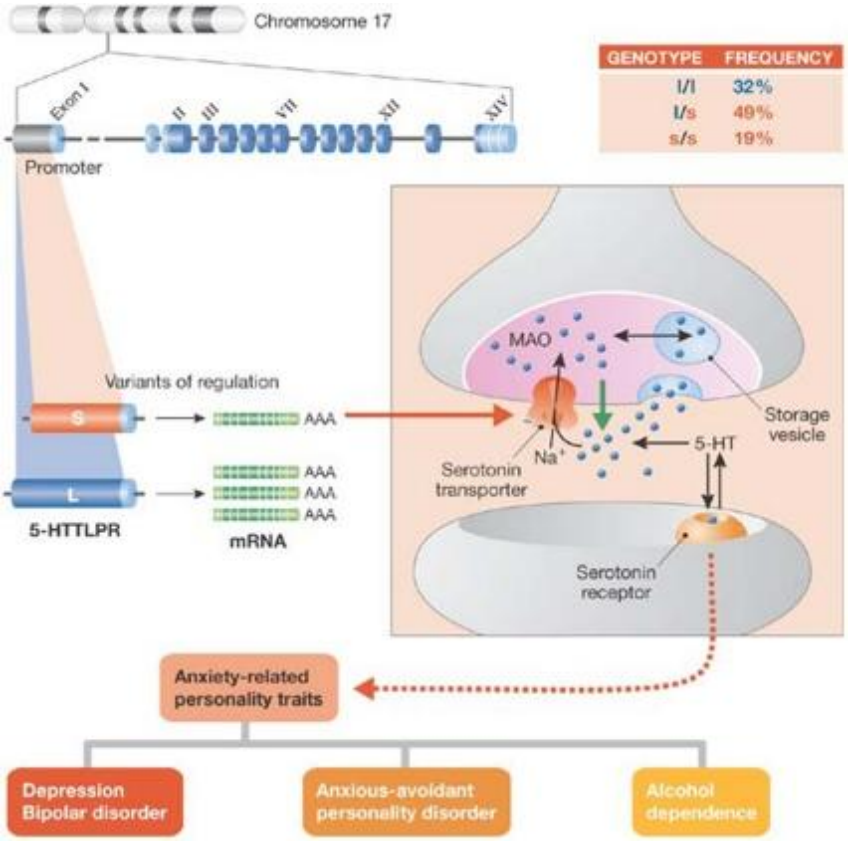
After an acute stress, serotonin will be then removed from the synaptic space by the serotonin transporter (SERT or 5-HTT), which is determinant in the control of magnitude and duration of 5-HT pre- and post- synaptic activity. Even if it is not fully clear how SERT function is altered by stress, 5-HT reuptake is surely an important mechanism by which the serotonin system self-regulates the amount of synaptic serotonin evoked by stress: for example, SERT downregulation (limiting 5-HT clearance in the synaptic cleft) could compromise the dynamic response and adaptation to stress.

Gene knockout of the mouse SERT gene (Slc6a4) produces loss of serotonin clearance and a consequent increase in extracellular levels of serotonin in forebrain; in response to stress, SERT knockout or SERT deficient mice display an exaggerated glucocorticoid catecholamine response, an enhancement of the anxiety-like phenotype and an increased 'depression-related' phenotype. Moreover, they also show elevated levels of rapid eye movement (REM or paradoxical) sleep, an abnormality which has been found to be a correlate of human depression.

Interestingly, the depression-related phenotype in SERT knockout mice appears to be exacerbated by repeated stress exposure and to depend upon the genetic background of the mouse: it could be that the consequences of Slc6a4 deletion are guided by both the chronicity of the stressor and epistatic interactions with other genetic factors that can confer risk or resilience against the loss of this gene.

Also in literature on humans, the gene coding for 5-HTT (the human SERT gene SLC6A, fig.12) has been frequently examined in candidate gene studies. 5-HTT is the primary target of the most prescribed antidepressant medication and its expression has been found to be decreased in the PFC of suicide completers. Researchers have paid close attention, in particular, to a polymorphism in the promoter of the 5-HTT which shows a 44-base pair insertion/deletion polymorphism in the transcriptional control region upstream of the 5-HTT coding sequence (5-HTT-linked polymorphic region or 5-HTTLPR); it is the most widely studied polymorphism among all the variants identified in 5-HT genes.

Fig.12



The short (s) allelic version of the SERT polymorphism is associated in humans with reduced SERT brain expression and lesser serotonin reuptake *in vitro* (Lesch, Bengel et al. 1996) and it is therefore considered a loss-of-function variant. The deletion is associated with reduced transcriptional efficiency and decreased expression of the 5-HTT protein both *in vitro* and *in vivo*. *In vitro* studies showed that the basal transcriptional activity of the long variant (l) of 5-HTTLPR was more than twice that of the short (s) form, with differences in 5-HTT mRNA synthesis resulting in different 5- HTT expression and 5-HT cellular uptake. *In vivo* studies in human subjects confirmed the functional relevance of the 5-HTTLPR genotype by showing that l/l subjects had higher blood 5-HT levels, higher 5-HTT mRNA levels in postmortem brain tissue, higher platelet 5-HT uptake and higher 5-HTT availability in the raphe area on single photon emission computed tomography (SPECT). In patients with Mood Disorders, the l/l genotype has been associated associated with a better response to several antidepressant treatments which target the 5-HT system (Benedetti, Riccaboni et al. 2014).

Similarly to the stress-related phenotype in the SERT knockout mouse, the risk for depression and suicide in individuals carrying the s allele is particularly pronounced, and perhaps even limited to,

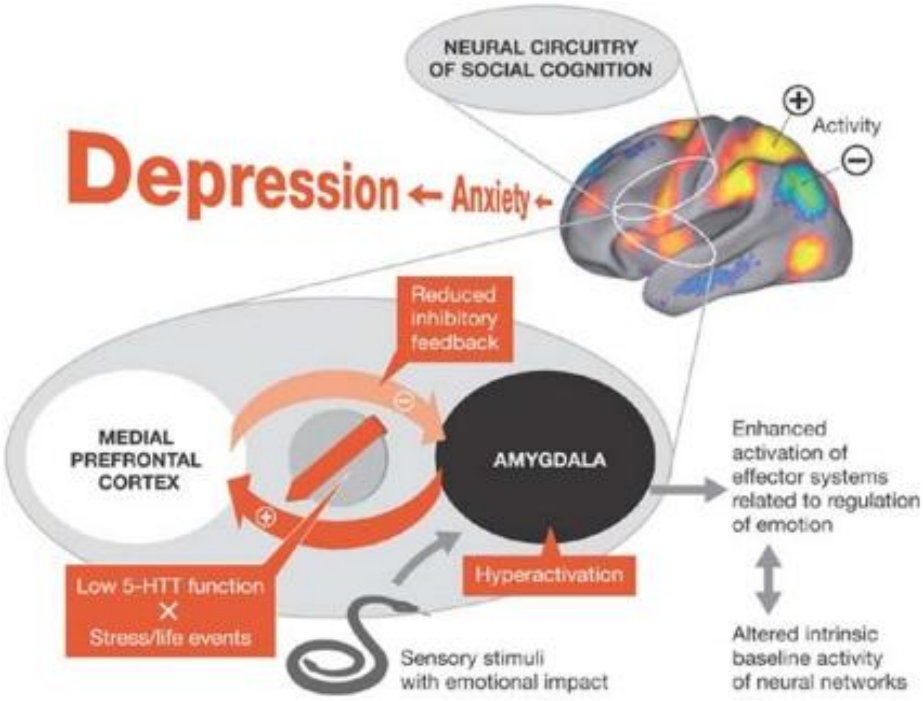
cases in which there is a history of exposure to traumatic life events (Caspi and Moffitt 2006; Hariri and Holmes 2006; Uher and McGuffin 2008).

A higher stress sensitivity in carriers of the short allele of 5-HTTLPR was firstly suggested by the pivotal study by Caspi and colleagues (Caspi and Moffitt 2003), associating the short form, but not the long form, with a positive relationship between the extent of exposure to early and adult stress, and the probability both of developing a major depressive episode and of showing suicidal ideation and attempts.

The hypothesis that 5-HTTLPR moderates the relationship between stress and depression has then been clearly supported by further studies.

Hariri and colleagues, in a seminal fMRI study, demonstrated that exposure to threatening faces, a stimulus that engages the amygdala in humans, produces a greater amygdala response in s allele carriers than long (l) allele carriers (Hariri, Mattay et al. 2002); subsequently, it was found that this amygdala hyperactivity is related to a functional uncoupling between the circuit connecting the amygdala with the anterior cingulate region of the PFC (fig.13). These data support a model in which loss of SERT gene function leads to a possible failure of cortical systems to exert sufficient inhibitory control over the amygdala during stressful situations, thereby catalyzing the development of chronic pathological states such as depression (Pezawas, Meyer-Lindenberg et al. 2005; Hariri and Holmes 2006).

Fig.13



The relationship between the 5-HTTLPR short allele and risk of suicide has been questioned.

Depressed suicide attempters have lower 5-HTT binding compared with depressed non-attempters and controls but the relationship between the 5-HTTLPR genotype and 5-HTT binding in depression has been questioned. While, in healthy subjects, the short allele has been associated with reduced functional coupling and volumes of cortico-limbic structures, the analysis of regional grey matter (GM) volumes of patients with Mood Disorders has associated reduced GM either with the long or short allele. Discrepancies have also been reported for the effects of early stress: notwithstanding the clear detrimental effects on hippocampal volumes, early stress was found to be associated either with increased or decreased cortical GM volumes in healthy subjects. In depressed patients, who show overall reduced GM volumes, early stress had no effects on GM. Moreover, data in healthy humans suggested complex interactions between the effects of specific stressors, in specific brain areas, during specific windows of susceptibility during the life course.

Stress-Diathesis Models

Suicide (as well as any mental disorder) is a complex and multifactorial phenotype that exhibits numerous proximal and distal risk factors. The identified variables that are involved in the development of suicidal behaviour have been categorized in explanatory models, which initially

were restricted to one domain of risk (Van Heeringen 2012). One of such early models recognized stressful life events as a key determinant of suicide and psychopathology in general. Despite the amount of evidence that supports the role of stress in triggering suicide and precipitating mental disorders, not all individuals exposed to stress dies by suicide. There must be a vulnerability that predisposes individuals to suicide when stress is encountered.

In the vulnerability-stress models or Stress-Diathesis models (SDMs), the set of predisposing factors, whether biological or psychological, which makes possible a disorder state is the diathesis (or vulnerability). Vulnerability is a trait, is stable but can change, is endogenous to individuals, and is usually latent. Terms such as *risk* and *vulnerability* (or diatheses) are often used interchangeably but these constructs are not synonymous. Despite the fact that they overlap substantially, risk describes factors that are associated, or correlated, with an increased likelihood of experiencing a disorder and vulnerability is usually defined in such a way that it reflects statements about causal mechanisms.

SDMs can presume additivity or ipsativity (Ingram 2005).In the addictive models (fig.14) stress and diathesis add together to produce the disorder (and competing models may accord a stronger role for one component over the other). In the ipsative models, there is an inverse relationship between factors such that the greater the presence of one factor, the less of the other factor is needed to bring about the disorder. Ipsative models are not necessarily distinct from additive approaches and can thus be considered an additional quality of many diathesis-stress models of psychopathology. More specifically, these models suggest that the diathesis and stress sum together to cause psychopathology, and that whatever this sum is, it reflects an inverse relationship. Thus, the degree of effect of diathesis or stress can be offset or compensated by the other in the summation that is needed for psychopathology.

At present, most diathesis-stress models are ipsative and therefore posit an interaction between stress and diathesis; nonetheless, different investigators have described these models in somewhat varying term.

In the SDM with *dichotomous diathesis* one either has the diathesis or does not; if the diathesis is absent, there is no effect for stress. Hence, even severe stress will not lead to the development of the disorder. On the other hand, when the diathesis is present, the expression of disorder will be conditional on the degree of stress. That is, as stress increases, so does the risk for the disorder in those who possess the diathesis.

In the *quasi-continuous diathesis* model (fig.15) there is a point beyond which a disorder will occur, but there is also a continuous effect of the diathesis once the threshold is passed. In other words, a minimal level of diathesis may be insufficient to produce the disorder even under high stress, but the probability of disorder increases as a function of both level of stress and strength of the diathesis beyond a minimal level.

Fig.14

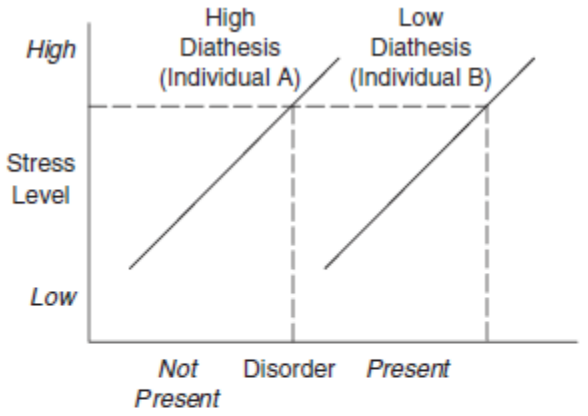


Figure 2.1 Additive Model of Diathesis-Stress Interaction With a Dichotomous Diathesis for Disorder

Fig.15

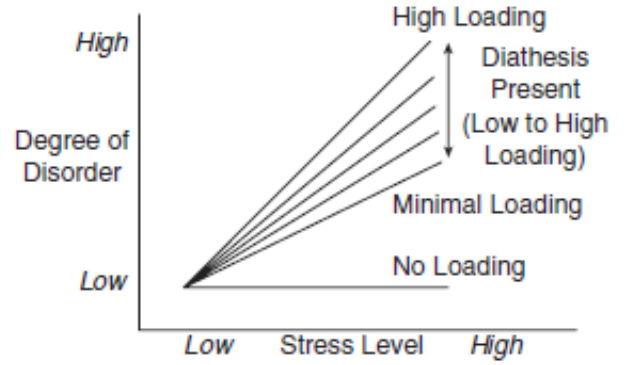


Figure 2.2 Interactive Model of Diathesis-Stress Interaction With Quasi-Continuous Diathesis

Complex diathesis-stress models that represent additive and interactional relationships between variables, as well as threshold effects for the diathesis, have also been proposed. In these

threshold models the diathetic threshold is the point at which the people who fall below the threshold will not develop the disorder, whereas those above this level cross the threshold into disorder. Therefore, the threshold varies from one person to another depending on the degree of vulnerability and the level of stress experienced. For a person who is highly vulnerable, relatively minor stressors may cause the threshold to be crossed. On the other hand, a major stressful event might cause a similar reaction even for a person low in vulnerability.

In the *vulnerability-resilience* model (fig.16), the diathesis continuum interacts with a continuum of stress to produce the possibility that a disordered state will occur. At the most extreme vulnerability end of the spectrum, little life stress is necessary to trigger disorder. At the resilient end, however, a great deal of stress is needed before psychopathology develops.

Fig.16

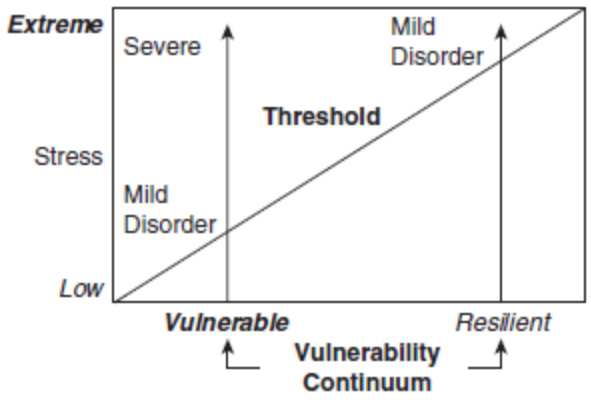


Figure 2.3 The Vulnerability-Resilience Model

Diathesis-stress models are excellent heuristic devices that enable us to potentially understand how predispositional factors from various domains may increase susceptibility to psychopathology and subsequently create the sufficient conditions for the onset of disorder. Nonetheless, there are some outstanding issues that reflect on conceptualizations of diatheses and conceptualizations of stress (Ingram 2005).

For example, diatheses and stress are rarely completely independent of each other, and their interactions can be quite complex. Moreover, the relationship between diathesis and stress can change over time. The phenomenon of kindling suggests that repeated instances of a disorder (and repeated exposure to stress itself) cause neuronal changes that result in more sensitivity to

stress. With heightened sensitivity, less stress becomes necessary to activate the processes that lead to psychopathology. Applying these ideas to SDMs, the relationship between stress and diathesis is dynamic: at some point diatheses change so that less stress becomes necessary to activate the vulnerability factors. Furthermore, early formulations of the SDM, based on biological factors, inferred temporal precedence of stress in the pathogenic process and assumed that the diathesis was unconsequential until stress-activated; on the other hand, complementary influences of the diathesis on stress do exist. Diathesis not only can cause stress in some cases, but it also could be part of the stress. In other cases, stress may affect the development of the diathesis. Typically, diathesis-stress models refer to stressful events that are proximal to the onset of disorder. However, it should be noted that stressors earlier in life may also influence how later stressful events are responded to and thus increase future susceptibility to disorder.

To date, neurobiology, neuropsychology, clinical psychiatry and cognitive psychology heavily support a stress-diathesis model of suicidal behaviour.

It is difficult to differentiate between DSMs of suicide and DSMs of Mood Disorder, because they share the genetic and environmental predisposing factors of the diathesis.

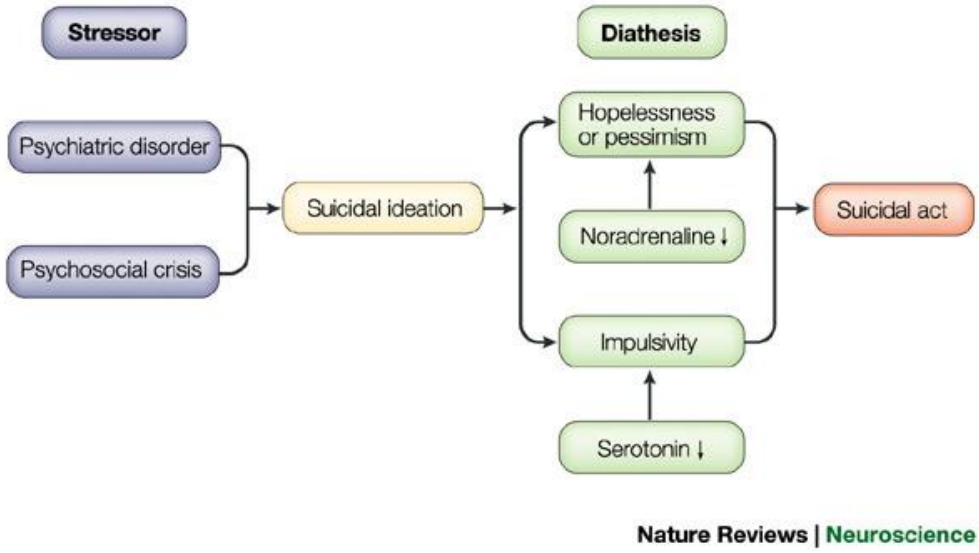
Although there are many paths to suicide and depression is sometimes the final step, not all the depressed individuals attempt or complete suicide and not all the suicide victims or attempters are depressed. Therefore, there must be a diathesis to suicide, which differentiate who will kill himself from who will not among individuals affected with Mood Disorders. This diathesis could be due to a genetic vulnerability that interact with - or add to - the epigenetic effects of childhood adversities and early life stress, with intermediate diathesis constituted by biological and clinical endophenotypes.

Mann and colleagues (Mann 2003) proposed a SDM of suicide that integrates neurobiology and psychopathology and represents a milestone in suicidology research (fig.17)

Briefly: acute psychosocial crises and psychiatric disorders are the proximal stressors leading to suicidal behaviour, while pessimism or hopelessness and aggression or impulsivity are components of the diathesis. Familial or genetic factors, childhood experiences and other factors influence the diathesis. Some intermediate biologic phenotypes have been identified: alterations in the serotonergic and noradrenergic systems are associated with suicidal behavior. Altered functioning of these systems may stem from both genetic and developmental causes: adversity in early-life

has developmental consequences that persist into adulthood. Genetic differences may also contribute to alterations in functioning of neurobiological systems. Moreover, the interaction of early- life experience of adversities and genetic vulnerability is increasing thought to play a role, including via epigenetic mechanisms.

Fig.17



Recently, Malhi and colleagues (Malhi, Bargh et al. 2014) developed a cognitive SD model of suicide in Bipolar Disorder that, even if yet to be tested, seems to be particularly promising. Their Bipolar Suicidality Model (BSM) integrates in a single coherent model up-to-date psychological, neuropsychological and neurobiological findings regarding suicide in BD.

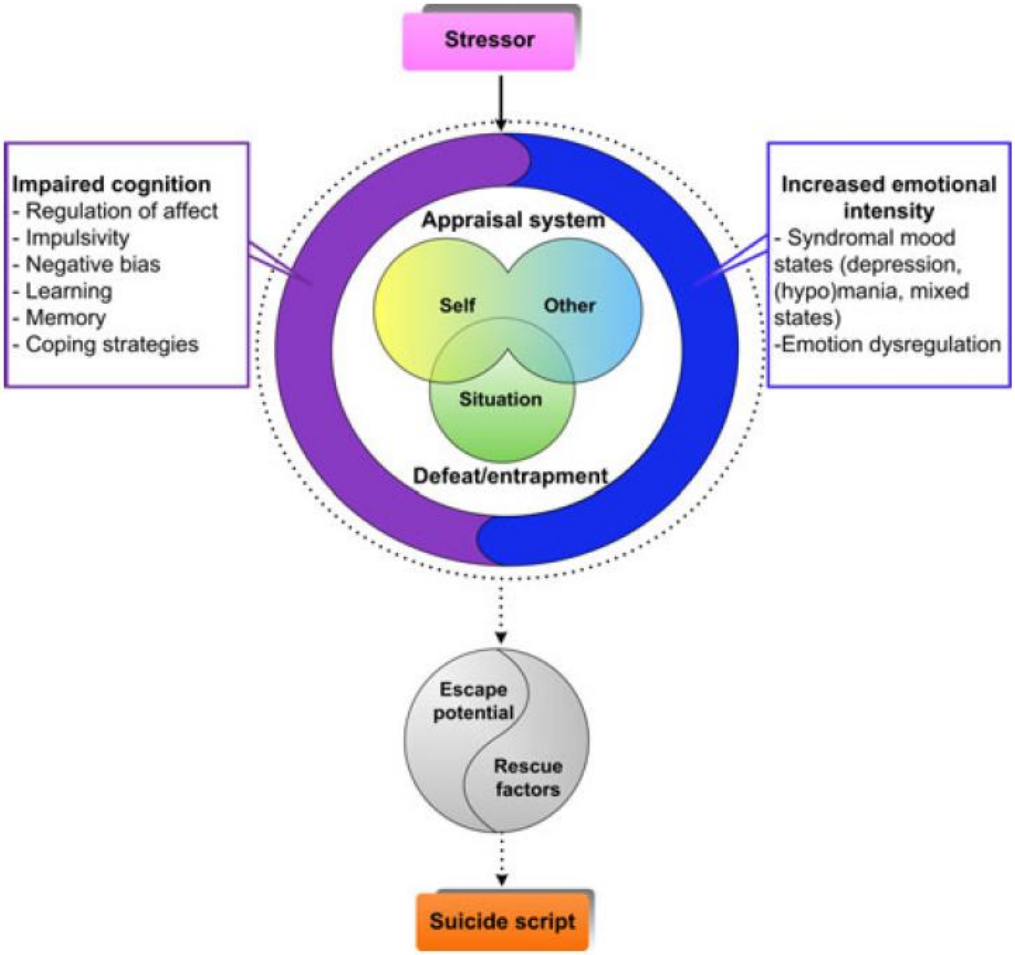
A SDM of Suicide in Bipolar Disorder

BSM has been derived from the CoP (Cry of Pain;(Williams 2001) and the SAMS (Schematic Appraisal Model of Suicide; (Johnson, Gooding et al. 2008)) models of suicide. In the Cop, suicidal behavior is driven by the need to escape from psychological pain: in a stressful situation if defeat, no escape and no rescue are experienced, suicide is motivated by a biologically-mediated mental *helplessness* script. Perceived defeat and entrapment are moreover influenced by negative biases in information processing and memory and by impaired problem-solving. Notably, In the SDM of suicide the *learned helplessness* paradigm (which is used in animal studies of the correspondent human endophenotype of *hopelessness*) is particularly relevant, as it explains how the

generalization of repeated psychological trauma or frustrations result in despair and defeat. The SAMS is an expansion of the CoP model, in which defeat and entrapment are unified in a single construct that depend upon the appraisal system (a concept similar to the Beck's *cognitive triade* of depression and grounded in the *internal working models* of Attachment Theory). Evidently, both self-appraisal (perceived efficacy and mastery) and situation appraisal (perceived defeat and entrapment) are relevant to the response to stressful life events.

In figure18, the BSM is represented.

Fig.18



As predicted by the BSM, when a person affected with BD encounter a stressor, the appraisal system is engaged and the underlying negative models are activated. Appraisal involves considerations about the self, others and situations and in BP disorder it is subjected to impaired cognition and affective dysregulation. The appraisal components, that partially overlap and depend upon experience, produce an estimate of the magnitude of defeat and entrapment; when

this evaluation results in hopelessness, the suicide script is activated. The accessibility to the suicide script is determined by the past experience of suicidal behavior, and the repeated cognitive elaboration of the BSM components enhances this accessibility. Finally, the lowering of escape potential and the lack of social support further increased the likelihood of engagement in suicidal behavior.

Parallel to the cognitive model, authors propose a neurobiological putative model of suicidality in BP disorder, which integrates the anatomical and functional correlates of emotion dysregulation and suicide in BP patients and implicates the fronto-limbic network (graphically schematizes in fig.19)

Fig.19

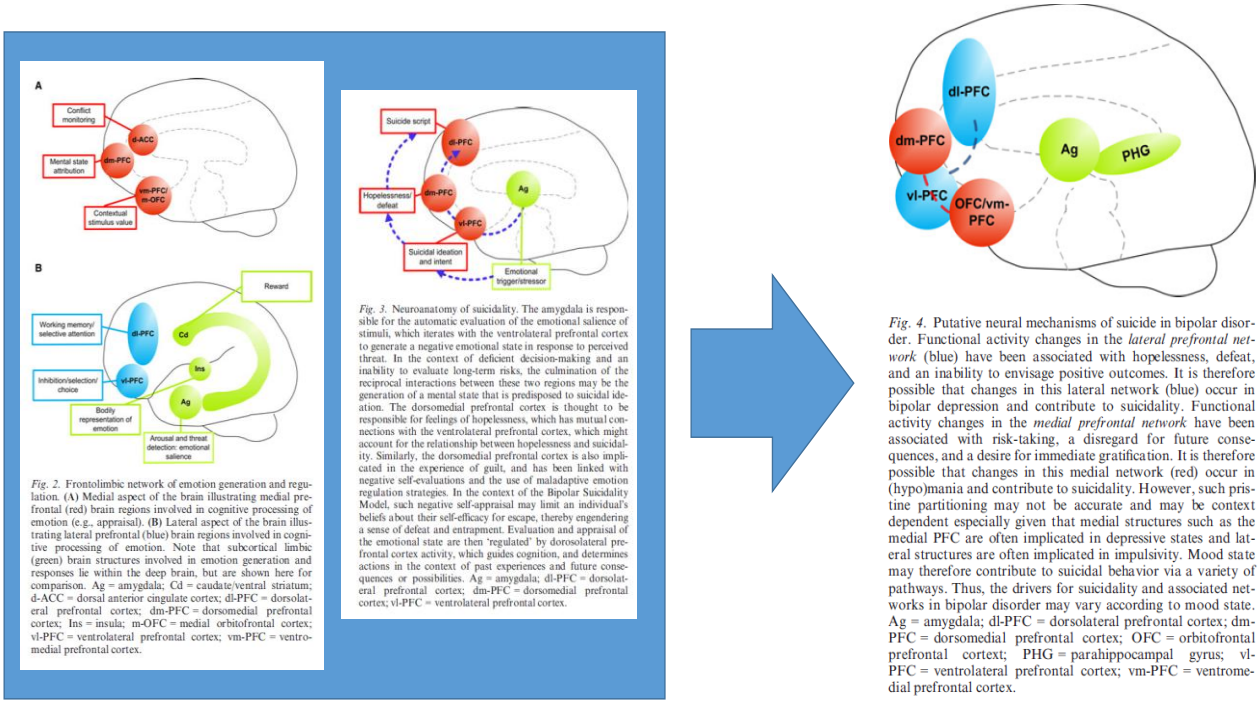


Fig. 2. Frontolimbic network of emotion generation and regulation. **(A)** Medial aspect of the brain illustrating medial prefrontal (red) brain regions involved in cognitive processing of emotion (e.g., appraisal). **(B)** Lateral aspect of the brain illustrating lateral prefrontal (blue) brain regions involved in cognitive processing of emotion. Note that subcortical limbic (green) brain structures involved in emotion generation and responses lie within the deep brain, but are shown here for comparison. Ag = amygdala; Cd = caudate/ventral striatum; d-ACC = dorsal anterior cingulate cortex; dl-PFC = dorsolateral prefrontal cortex; dm-PFC = dorsomedial prefrontal cortex; Ins = insula; m-OFC = medial orbitofrontal cortex; vl-PFC = ventrolateral prefrontal cortex; vm-PFC = ventromedial prefrontal cortex.

Fig. 3. Neuroanatomy of suicidality. The amygdala is responsible for the automatic evaluation of the emotional salience of stimuli, which iterates with the ventrolateral prefrontal cortex to generate a negative emotional state in response to perceived threat. In the context of deficient decision-making and an inability to evaluate long-term risks, the culmination of the reciprocal interactions between these two regions may be the generation of a mental state that is predisposed to suicidal ideation. The dorsomedial prefrontal cortex is thought to be responsible for feelings of hopelessness, which has mutual connections with the ventrolateral prefrontal cortex, which might account for the relationship between hopelessness and suicidality. Similarly, the dorsomedial prefrontal cortex is also implicated in the experience of guilt, and has been linked with negative self-evaluations and the use of maladaptive emotion regulation strategies. In the context of the Bipolar Suicidality Model, such negative self-appraisal may limit an individual's beliefs about their self-efficacy for escape, thereby engendering a sense of defeat and entrapment. Evaluation and appraisal of the emotional state are then 'regulated' by dorsolateral prefrontal cortex activity, which guides cognition, and determines actions in the context of past experiences and future consequences or possibilities. Ag = amygdala; dl-PFC = dorsolateral prefrontal cortex; dm-PFC = dorsomedial prefrontal cortex; vl-PFC = ventrolateral prefrontal cortex.

Fig. 4. Putative neural mechanisms of suicide in bipolar disorder. Functional activity changes in the *lateral prefrontal network* (blue) have been associated with hopelessness, defeat, and an inability to envisage positive outcomes. It is therefore possible that changes in this lateral network (blue) occur in bipolar depression and contribute to suicidality. Functional activity changes in the *medial prefrontal network* have been associated with risk-taking, a disregard for future consequences, and a desire for immediate gratification. It is therefore possible that changes in this medial network (red) occur in (hypo)mania and contribute to suicidality. However, such pristine partitioning may not be accurate and may be context dependent especially given that medial structures such as the medial PFC are often implicated in depressive states and lateral structures are often implicated in impulsivity. Mood state may therefore contribute to suicidal behavior via a variety of pathways. Thus, the drivers for suicidality and associated networks in bipolar disorder may vary according to mood state. Ag = amygdala; dl-PFC = dorsolateral prefrontal cortex; dm-PFC = dorsomedial prefrontal cortex; OFC = orbitofrontal prefrontal cortext; PHG = parahippocampal gyrus; vl-PFC = ventrolateral prefrontal cortex; vm-PFC = ventromedial prefrontal cortex.

Suicide in Bipolar Disorder

Epidemiology

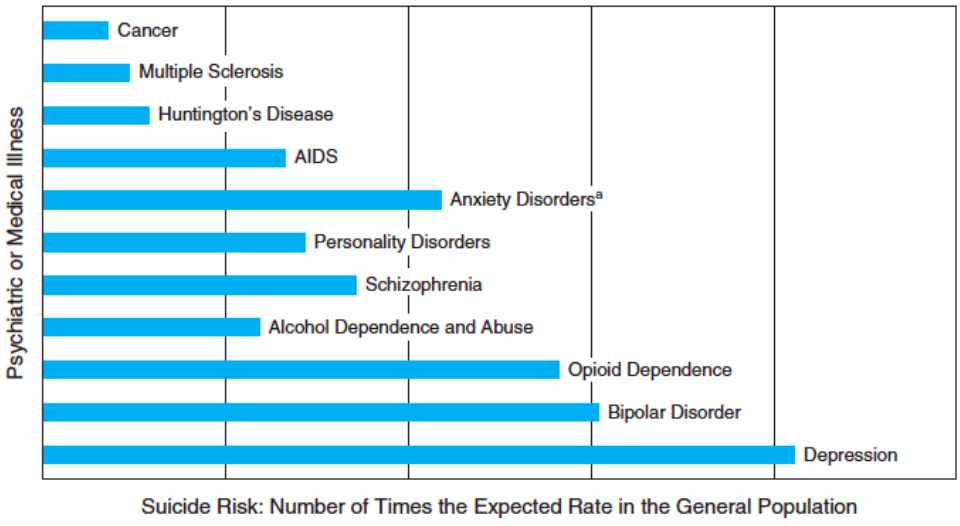
A meta-review (review of systematic reviews) conducted in 2014 (Chesney, Goodwin et al. 2014) explore the risk of all-cause mortality and suicide mortality in 1.7 million of patients affected with mental disorders. All the mental disorders reported had increased risk for all-cause mortality respect to the general population; Bipolar Disorder (BD) had a reduction of life-expectancy (measured in years of life lost) higher than heavy smokers (respectively 8-10 *versus* 9-20) and a risk for suicide greater than 17 times compared with the general population.

Pompili and colleagues (Pompili, Gonda et al. 2013), with a systemic review of the literature, estimated the suicide risk in BD patients as being 20-30 times greater than that for the general population.

Over 90% of suicide victims or suicide attempters have a diagnosable psychiatric illness, most commonly a mood disorder, and about 60% of all suicides occur in relation to mood disorders (Mann 2003).

Patients with depressive and manic-depressive illness are far more likely to commit suicide than those with other psychiatric or medical illnesses. An analysis of nearly 250 studies, reported over a 30-year period, found that mood disorders carry the highest risk of suicide (fig.20) (Goodwin and Jamison 2007).

Fig.20



Yet despite this high risk, the lethality of manic-depressive illness is often underemphasized. Suicide is far too common in untreated, inadequately treated, or treatment-resistant manic-depressive illness. Suicide is often preventable if a correct diagnosis is made, if acute and chronic suicide risk factors are recognized and acted upon, and if appropriate treatment is provided.

Early studies documented a strikingly high lifetime rate of suicide, 15 percent, among patients with manic-depressive illness. The estimated rate today has dropped by about one-half, except for patients early in the course of illness. The higher rates in most early studies (published before the mid-1980s) almost certainly reflect poorer clinical outcomes before the widespread use of lithium and other mood stabilizers, tricyclic antidepressants, and selective serotonin reuptake inhibitors (SSRIs). The figure of 15 percent lifetime risk also reflects suicide risk in clinical populations with severe, untreated manic-depressive illness.

Guze and Robins (Guze and Robins 1970) were the first to document systematically the extent of suicide risk in manic-depressive illness. They reviewed 14 follow-up studies, 2 population surveys, and 1 family study and found that at least 12 percent of all deaths among manic-depressive patients had been the result of suicide (in these early studies, patients with recurrent depressions were diagnosed as manic depressive).

In 9 of the studies, 12 to 19 percent of deaths had been due to suicide, and in the other 8 studies, the suicide rate ranged from 35 to 60 percent. The authors concluded that by the time all the patients in these studies had died, about 15 percent would have committed suicide, a rate at least 30 times that found in the general population.

Subsequent analyses buttressed these early findings. Godwin and Jamison reviewed 30 studies of completed suicide (1937–1988), founding a mean lifetime rate of 19 percent in patients with severe manicdepressive illness (range 9 to 60 percent). In 13 of the 30 studies, the figure was in the 10 to 30 percent range. In one fifth of the studies, at least half of the manic-depressive patients had died because of suicide. Similarly, a study of nearly 500 bipolar patients (mostly untreated or inadequately treated) over a 17-year period (1970–1987) found a suicide rate of 15 percent (Sharma and Markar 1994).

The high suicide rate in manic-depressive illness is also documented by comparison with suicide rates in the general population. Harris and Barraclough (Harris and Barraclough 1997) calculated standardized mortality ratios (SMRs) in a meta-analysis of 14 studies reported from 1945 to 1992

(N= 3,700 bipolar patients). They found that the SMR for suicide in Bipolar Disorder was about 15 times higher than expected (see Fig. 8–1). Increased risk was associated with recent hospital discharge, a suicide attempt within the previous 5 years, and current alcohol abuse. The authors found a somewhat higher SMR, 20.4, for suicide in patients with major depression.

More recent studies, however, have found significantly lower rates of suicide (in the range of 5 percent lifetime risk) among never-hospitalized patients with bipolar disorder of moderate severity (see (Tondo, Isacsson et al. 2003), for a review).

However, these lower suicide rates do not apply early in the course of illness. A study from the United Kingdom found a lifetime suicide rate of 6 percent for patients with affective disorders (depression and bipolar disorder), but the rate increased to 23 to 26 percent early in the course of bipolar illness (Inskip, Harris et al. 1998).

There are several possible explanations for this marked decrease over time in reported suicide rates in bipolar disorder. A major reason alluded to earlier, is most likely the increased use of lithium and other medications in the treatment of manic-depressive illness since 1980. Other reasons for the decline are probably methodological. The findings of newer studies, which often have focused on outpatient populations or mixed inpatient and outpatient populations, reflect suicide rates in less severely ill patients. In their review, Tondo and colleagues (Tondo, Isacsson et al. 2003) suggested that reported rates are lower because most of the patients among whom the rates were ascertained were never hospitalized. They also pointed out that more recent studies, by reflecting the milder range of illness severity, underestimate the risk in more severely ill patients. They noted as well that, compared with a general population of patients, there is a lower ratio of attempts to fatalities in patients with major mood disorders. In their view, this finding suggests a high Suicide 249 lethality and intent in affectively ill patients. The SMR for bipolar patients, based on a review of 28 studies from 1945 to 2000, was 22.1. Rates of suicide averaged about 0.376 percent per year.

Bostwick and Pankratz (Bostwick and Pankratz 2000) directly calculated the rates of suicide in hospitalized affectively ill patients versus other groups. In their meta-analysis, they reanalyzed both the Guze and Robins (Guze and Robins 1970) study and our own analysis of 30 studies (cited earlier), in addition to more recent studies (up to 1998). Using a different metric (case-fatality prevalence) they found lifetime suicide rates of 8.6 percent for patients hospitalized for suicide risk, 4.0 percent for those not hospitalized, and 2.2 percent for mixed inpatient/outpatient

populations; the rate was less than 0.5 percent for the non–affectively ill population. The authors did not separate the risk for bipolar and unipolar disorders.

Identifying diagnostic subgroups with an increased incidence of suicide or suicide attempts is one of the first steps toward identifying individual bipolar patients who are at particularly high risk. Several investigations over the last three decades have found that patients with bipolar-II disorder have a higher risk of suicide attempts than those with bipolar-I. These studies were reviewed by Rihmer and Kiss (Rihmer and Kiss 2002) (see Table 8–1).

In addition, Bulik and colleagues (Bulik, Carpenter et al. 1990) studied 67 patients with a history of recurrent depression and suicide attempts. In comparison with 163 patients with recurrent major depression and no suicide attempts, they found that attempters were distinguished by a history of bipolar-II depression. The findings of these studies suggest that bipolar-II depression confers a particularly high risk of suicide in patients presenting with major depression. A recent retrospective study of 90 bipolar-I and 10 bipolar-II patients, however, did not find a significant difference in rates of suicide attempts between the two groups (Valtonen, Suominen et al. 2006).

250 Clinical Studies In their review, MacQueen and Young (MacQueen and Young 2001) noted a particularly high liability for comorbidity with personality disorders, substance abuse disorders, and anxiety disorders in bipolar-II patients with an elevated risk of suicide. Bipolar-II patients, who have higher rates of comorbid substance abuse and personality disorders than bipolar-I patients, may be at increased risk in large part because of the comorbidity. Rihmer and Kiss (Rihmer and Kiss 2002) found that when lifetime prevalence in the general population is the comparator, bipolar-II patients have the highest prevalence of attempted and completed suicides.

The evidence strongly suggests that bipolar-II patients have—relative to the general population and to those with bipolar-I or unipolar depression—the highest rate of suicide.

With regard to gender prevalence, the patterns of suicidal behaviour among women and men with manic-depressive illness show both similarities to and differences from the general population.

Like the general female population, bipolar women attempt suicide more often than bipolar men do. In contrast to the general population, however, there is no clear predominance of males among bipolar patients who actually commit suicide; the completed suicide rate for males is generally equivalent to or lower than that for females.

Reviewing 28 studies conducted from 1945 to 2001, Tondo and colleagues (Tondo, Isacsson et al. 2003) found that among bipolar patients, the average SMR for suicide was 14.9 for males and 21.1 for females. Evidence of differences by gender among suicide attempters may be distorted by reporting biases, however, when patients are asked about past suicidal behavior, women may be more likely than men to admit to or remember attempts; men, on the other hand, may be more prone to suicidal equivalents, such as extreme risk taking, involvement in car accidents, and substance abuse. These behaviours are less often explored in surveys.

Risk Factors

Suicide is still far too common in manic-depressive illness. Patients diagnosed today face a lifetime suicide risk of at least 5 percent. While this figure is lower than in previous eras—a result in part of better treatments and in part of the inclusion of less severely ill patients in outcome studies—several groups of patients continue to bear a disproportionately high risk burden, including those who are hospitalized or recently discharged and those who are untreated, inadequately treated, or treatment resistant. Further, the suicide rate remains strikingly high early in the course of illness.

Recently, two reviews of articles published since 1980 identified the clinical correlates of suicide in BD. The *International Society for Bipolar Disorders Task Force on Suicide* (Schaffer, Isometsa et al. 2014) investigated the variables associated with suicide attempt and completion, while Pompili et al.(Pompili, Gonda et al. 2013) limited the research to suicide completers. Despite some (also methodological) differences, the results partially overlap, as shown in figures 21 and 22.

Fig.21

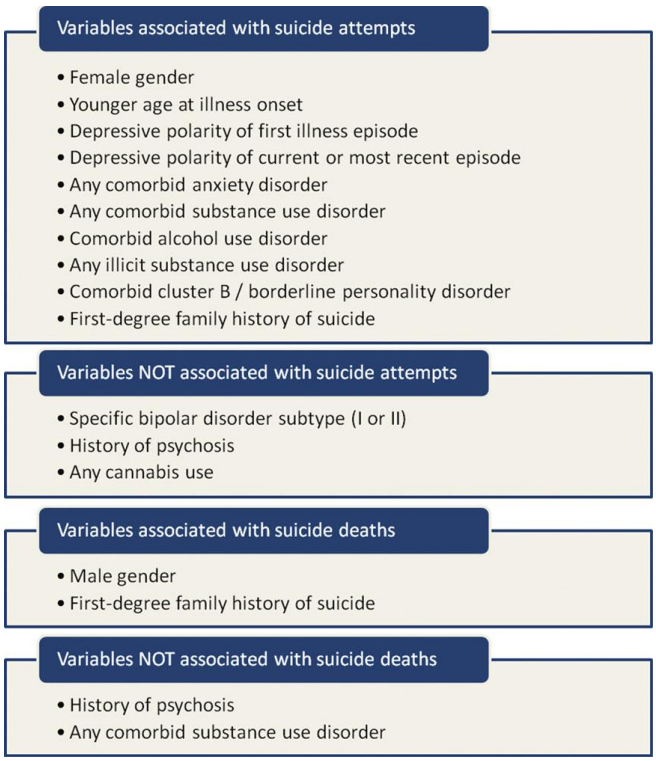


Fig.22

Clinically relevant suicide risk factors in bipolar disorders based on the current literature

Acute mood episodes
- Severe major depressive episode
- Current suicide attempts, plans, ideation
- Hopelessness, guilt, few reasons for living
- Agitation, depressive mixed state (≥ 3 or more intra-depressive hypomanic symptoms)
- Severe anxiety, insomnia
- Psychotic features
- Bipolar II diagnosis
- Comorbid Axis I (anxiety, substance related), Axis II, and serious Axis III disorders
- Lack of medical treatment and family/social support
- First few days of the treatment (particularly if appropriate care and co-medication is lacking), first few weeks and months after hospital discharge
- Mixed affective episode (simultaneously occurring manic and major depressive episode)
- Dysphoric mania (mania and ≥ 3 intra-manic depressive symptoms)

Prior course of the illness
- Previous suicide attempt/ideation (particularly the violent/highly lethal methods)
- Early onset/early stage of the illness/predominantly depressive course
- Rapid cycling course

Personality features
- Aggressive/impulsive personality traits
- Cyclothymic temperament
- Same sex orientation, bisexuality

Personal history and/or family history
- Early negative life events (separation, emotional, physical, and sexual abuse)
- Permanent adverse life situations (unemployment, isolation)
- Acute psychosocial stressors (loss events, financial catastrophe)
- Family history of mood disorders (first- and second-degree relatives)
- Family history of suicide and/or suicide attempt (first- and second-degree relatives)

The precise causes of suicide remain elusive, although they most certainly entail an interaction between the underlying affective illness and additional biological and psychosocial risk factors. But research has not yet found any particular combination of risk factors to be sensitive or sufficient enough to predict a suicide. Salient risk factors include rapid cycling, mixed states, and severe depressive episodes. A significant advance has come with the identification of which of many risk factors operate in the short term (e.g., agitation, severe hopelessness, global insomnia) versus the long term (e.g., past suicide attempt, various comorbidities). Risk factors and their identification, moreover, while far from perfect predictors of suicide, can serve as guideposts for clinicians confronting a complex clinical picture of an illness that encompasses the extremes of human emotions.

There is an extensive literature documenting the strong correlation between a history of suicide attempts and subsequent completed suicide (see, for example, (Nordstrom, Asberg et al. 1995; Nordstrom, Samuelsson et al. 1995; Tsai, Kuo et al. 2002; Joiner, Conwell et al. 2005). Harris and Barraclough, in their extensive meta-analysis, found that a suicide attempt created a 37-fold risk for completed suicide; several large prospective studies also demonstrated a substantial increased risk for suicide in those who had previously attempted it (Fawcett, Scheftner et al. 1990; Marangell, Bauer et al. 2006). In their meta-analysis of 13 studies of completed suicide and 23 studies of attempted suicide, Hawton and colleagues (Hawton, Sutton et al. 2005) found that past suicide attempts were the factor most predictive of future suicidal behavior. In the Finnish Jorvi Bipolar Study, 191 bipolar patients were followed for 18 months, during which time 20 percent attempted suicide (Valtonen, Suominen et al. 2006).

Mixed states are a significant risk factor for suicide. The majority of patients with mixed manicdepressive show the usual depression, numerous self-accusations, ideas of guilt and punishment and a varying degree of hypochondriasis. At the same time there is a mental alertness, associated with tense, apprehensive and restless behavior. The retardation of thought and action that paralyzes the acting out of the wish for death in the average depressed patient is entirely absent in these persons. They are, therefore, the most dangerous types of patients with mental disease, so far as suicide is concerned. The records of the fifteen patients in the group emphasize this strikingly. Three of these patients committed suicide twenty-four hours after leaving the hospital, two within forty-eight hours, another within a week and still another within

two weeks. The patient who was longest outside the hospital lived two months, and the average period for this group was fifteen days ((Jameison 1936). In a series of studies, Dilsaver and colleagues (Dilsaver, Chen et al. 1994; Dilsaver, Chen et al. 1997; Dilsaver and Chen 2003) found that, compared with patients with pure mania, patients with depressive mania had significantly higher rates of suicidality. Suicidality was defined as a severity level of 3 to 7 on the suicide subscale of the SADS, which ranges from mild suicidality (frequent ideation, but without a specific method or plan) to suicide attempts. Sato and colleagues (Sato, Bottlender et al. 2004), using the criteria for dysphoric mania developed by McElroy and colleagues (McElroy, Keck et al. 1992; McElroy, Strakowski et al. 1995) and Strakowski and colleagues (Strakowski, McElroy et al. 1996), studied 576 patients hospitalized with acute mania. They found that mixed mania was strongly associated with suicidality.

It has long been known that depression is strongly correlated with suicide and suicide attempts (Jameison 1933); (Barraclough, Bunch et al. 1974; Weeke 1979).More recent findings strengthen the early clinical observations. Isometsa and colleagues (Isometsa, Henriksson et al. 1994) studied a sample of 31 bipolar patients who committed suicide in Finland within a 12-month period, 74 percent of whom had been receiving psychiatric care at the time of their suicide. Among these patients, 79 percent were depressed at the time of death, 11 percent were in a mixed state, and 11 percent died during or immediately after remission of psychotic mania.The severity of depression is a factor as well (Arato, Banki et al. 1989; Henriksson, Aro et al. 1993; Isometsa, Henriksson et al. 1994).

Keith-Spiegel and Spiegel (Keith-Spiegel and Spiegel 1967) compared the clinician-rated mood states of 61 psychiatric patients immediately preceding their suicides with those of 51 matched control patients of comparable age and diagnoses who had not committed suicide or had no history of a suicide attempt. Those who killed themselves had histories of more frequent and more severe depressions, as well as more suicide attempts, threats, and suicidal ideation. Of particular significance, however, was that just before death, those who committed suicide had been assessed by their clinician as being calmer and in better spirits than members of the control group. An apparently unwarranted mood shift had been observed in those who killed themselves. These findings add weight to the clinical observation, dating back hundreds of years, that improvement in depression is associated with an increased risk for suicide.12 Several factors may account for this counterintuitive observation. The improvement may reflect a sense of calm once the decision

has been made to die, a genuine calm before the storm brought about by biological changes, or a transition from one phase of the illness to another (e.g., from depression to hypomania, mania, or a mixed state). It also may reflect true clinical improvement, with a concomitant level of frustration when symptoms recur. In some instances, an improved clinical state enables a previously indecisive and lethargic patient to become more able to make an unambivalently lethal decision and to act on that decision. Finally, "improvement" may in fact be a patient's deliberate deception of physicians, hospital staff, and family so a suicide plan can be carried out.

Rapid cycling increases the risk of suicide and suicidal behaviour. Brodersen and colleagues (Brodersen, Licht et al. 2000) conducted a 16-year follow-up study of 133 patients with major affective disorders (defined as having two to three affective episodes in a 5-year period) treated with lithium. Most of the 11 suicides occurred among the "atypical" patients (those with schizoaffective disorder, bipolar-II disorder, mixed episodes, and/or rapid cycling). MacKinnon and colleagues (MacKinnon, Potash et al. 2005), in a study of 1,574 family members with bipolar disorder, also found that a history of rapid mood switching was associated with increased suicidality. Two other studies found trends for suicidal behavior and rapid cycling (Bauer, Calabrese et al. 1994; Maj, Magliano et al. 1994).

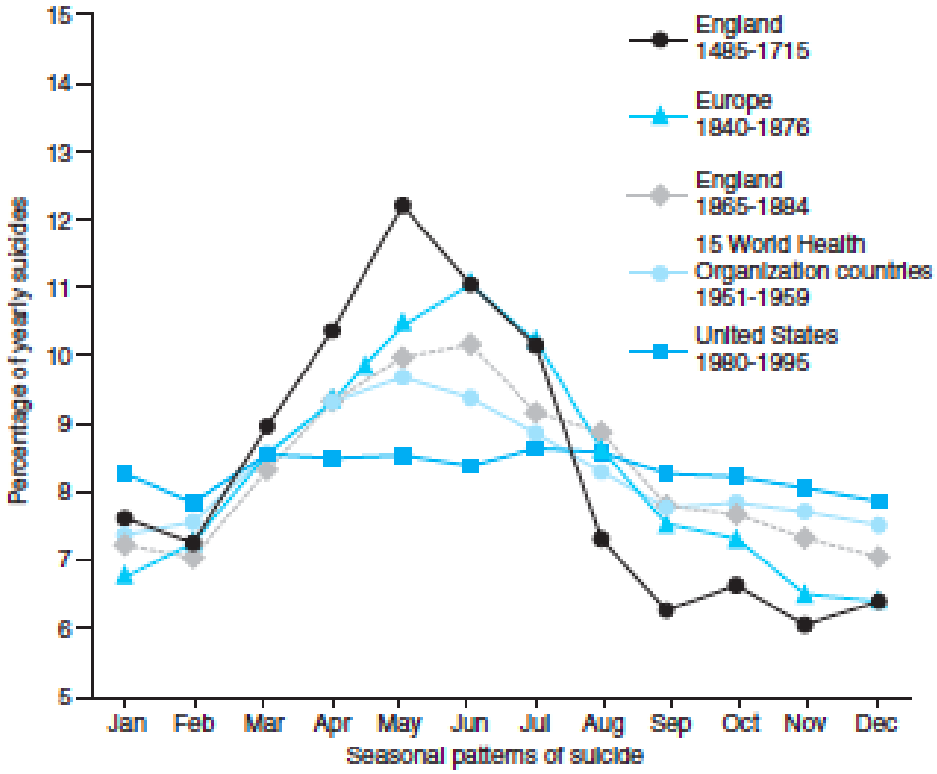
Studies have shown high rates of comorbid anxiety disorder in bipolar patients, often reaching at least 40 percent (McElroy, Altshuler et al. 2001; Simon, Otto et al. 2004). The high incidence of comorbid anxiety disorders in both bipolar-I and bipolar-II patients calls for special attention to severe symptoms of anxiety in the assessment and management of suicide risk in bipolar patients.

Seasonality

Seasonality affects the timing of manic and depressive affective episodes. It also has a profound effect on suicide. There is a robust literature on suicide and seasonality, in part because it is easier to date a suicide than the onset of an affective episode accurately and precisely.

Literature affirms a striking peak incidence of suicide in late spring–early summer. Many studies have also found a smaller peak in October, generally for women rather than men. Both peaks, however, have lessened in amplitude over time (Fig.23).

Fig.23



Findings of more recent studies add considerably more nuance to the overall pattern of a spring-to-summer peak in suicides.

Granberg and Westerberg (Granberg and Westerberg 1999) analyzed suicide data from Sweden (1911–1993) and New Zealand (1975–1995) and found that suicides peaked in the spring month of May in Sweden and November in New Zealand. In other studies, carried out in the southern hemisphere (Takahashi 1964; Parker and Walter 1982), the seasonality of suicide was consistent with the pattern seen in the northern hemisphere (i.e., peaks in the spring), although for New South Wales, Parker and Walter (Parker and Walter 1982) found two peak suicide periods (in May and November) among women rather than one. Chew and McCleary (Chew and McCleary 1995), using timeseries and cross-sectional data for 28 countries and employing bivariate plots and simple correlation techniques, found a sizable spring peak in suicide only in the temperate zones. More recently, Lee and colleagues (Lee, Lin et al. 2006), utilizing the nationwide mortality database in Taiwan 1997–2003, found that suicides peaked in spring, regardless of gender or age. Ambient temperature was positively associated with suicide after adjustment for seasonality.

Fisher and colleagues (Fisher 1997), studying 16,389 nationally registered suicide deaths between 1980 and 1989, found a peak in the spring in South Africa. Retamal and Humphreys (Retamal and Humphreys 1998), reviewing 5,386 suicides in Chile for the period 1979–1994, found the highest rates in the "warm months" (i.e., the southern hemisphere's spring and summer), particularly December, and the lowest rates in the colder months, particularly June. Morken and colleagues (Morken, Lilleeng et al. 2002)studied all admissions in Norway for mania and depression during 1992–1996 (N= 4,341) and all 14,503 suicides in the years 1969–1996. They observed a significant peak in depression for women in November, with a secondary peak in April, and a peak for men in admissions for both depression and mania in April. Both genders showed a trough in admissions in July.

Preti and colleagues (Preti and Miotto 1998; Preti, Miotto et al. 2000), examining violent suicide attempts in Italy, found a peak in the spring months, but only for violent suicide attempts in males. No seasonal trend was observed in nonviolent suicides among males or females during 1984–1995.

Findings reported by Maes and colleagues (Maes, De Meyer et al. 1994) and Linkowski and colleagues (Linkowski, Martin et al. 1992) suggest that violent suicides and perhaps violent attempts may be more common in sunny weather or higher temperatures. It is known that violent suicides and attempts are much more common in males than in females, a fact that is consistent with the greater occurrence of seasonality patterns in males.

There have also been suggestions that seasonal variation is greatest in rural as opposed to urban areas (e.g., (Micciolo, Williams et al. 1991)); one possible explanation for this difference is that urban living conditions, such as less direct exposure to natural light and more exposure to artificial light, may somehow reduce the seasonal effect. In their analysis of seasonal patterns of suicide in farmers and nonfarmers, Simkin and colleagues (Simkin, Hawton et al. 2003) observed no significant seasonal patterns in violent suicides among nonfarmers.

The seasonal pattern of suicide appears to run counter to that of bipolar depression, which is more likely to occur in the winter months in the temperate zone (Goodwin and Jamison 2013).

It may be that the increased activation associated with longer periods of light in the late spring months brings to a suicidal climax depressive episodes that begin in the winter months, particularly among those bipolar patients who are prone to developing dysphoric manic states.

Cassidy and Carroll(Cassidy and Carroll 2002)analyzed the seasonal occurrence of 304 hospital admissions for mixed or manic bipolar states. They found that the frequency of all admissions for mania peaked in early spring, with a nadir in late fall. Admissions for mixed mania had a significantly different pattern, with a peak in late summer and a nadir in late November. Whitney and colleagues (Whitney, Sharma et al. 1999) likewise found that mixed-state admissions in Canada peaked in the summer, but they did not find a seasonal pattern for mania and depression. D'Mello and colleagues (D'Mello, McNeil et al. 1995), studying the admissions of 377 bipolar patients in Michigan over a 6-year period, observed that women had a bimodal seasonal distribution, with peak admission rates in the spring and fall. They also found that aggressive behavior peaked in the spring for both men and women. Several other investigators (Parker and Walter 1982), in New South Wales; (Mulder, Cosgriff et al. 1990), in New Zealand; and (Takei, O'Callaghan et al. 1992), in London) found a peak in admissions for mania in spring and summer.

These findings suggest a seasonal pattern in activation levels in bipolar disorder. Wehr (Wehr 1992) observed that the risk for depression peaked at two opposite times of the year—spring/early summer and fall/early winter.

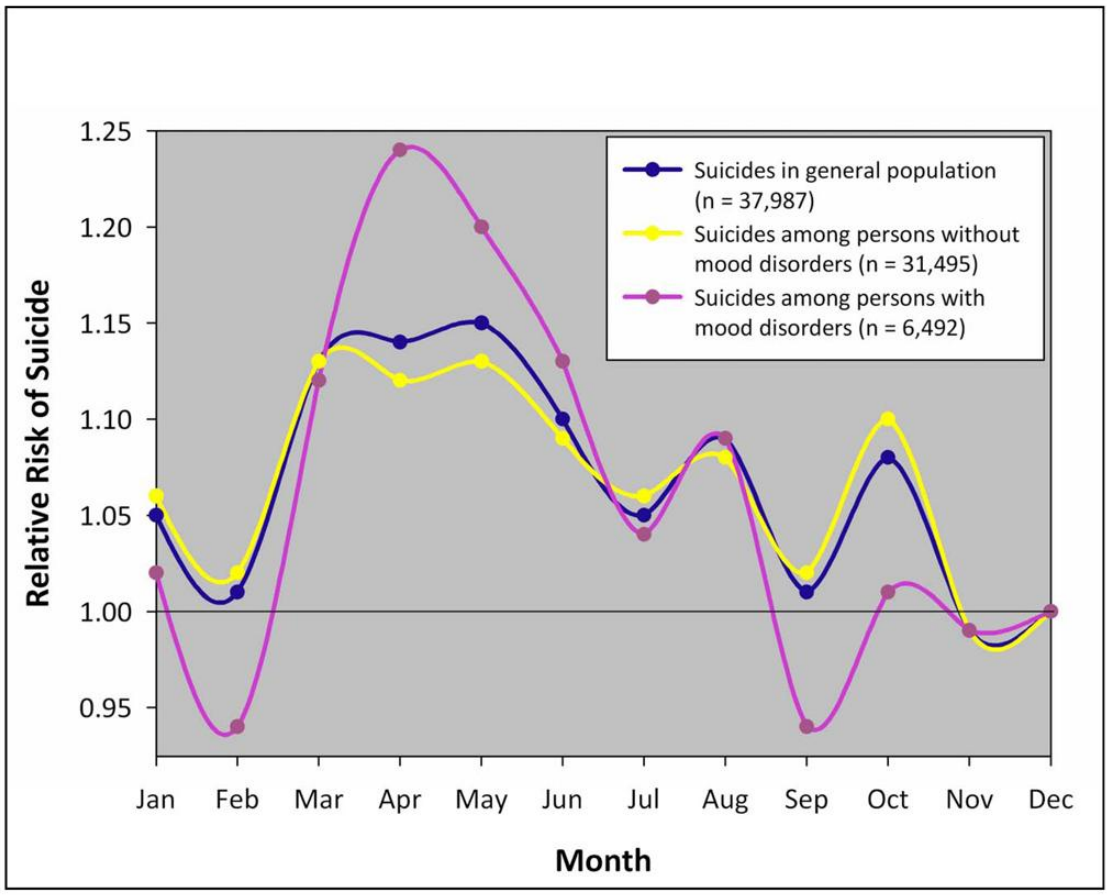
Maes and colleagues (Maes, Scharpe et al. 1995) noted a correlation between seasonal variations in serum l-tryptophan and suicide. Taken together, the studies reviewed here suggest an activation of manic-depressive illness (including mixed states, as well as unipolar recurrent depression) in the spring/summer months that roughly parallels the seasonal increase in suicides reported in the temperate zones, especially in males.

The second suicide peak in October may reflect an increase not only in unipolar depressive episodes, but also in suicidal depressions following the pronounced increase in manic episodes among bipolar patients during the summer months. That is, this second peak may represent suicidal postmanic depressions. It also may reflect the impact of mixed, transitional mood states. As patients recover from summer or autumn hypomania or mania, or as they switch from hypomania or mania into depression, mixed states are not uncommon. A recent study of depressed patients with mixed states found that the depressive episodes peaked in autumn (Sato, Bottlender et al. 2006). In vulnerable individuals, this may lead to periods of an acute, agitated suicidal state.

In 2010, using the data covering the entire national population in Denmark over a 32-year period, Postolache and collegues (Postolache, Mortensen et al.) estimate seasonality of suicide in those

with - versus those without - history of hospitalization for unipolar or bipolar disorder (which is an index of exacerbation of mood disorder) to test the hypothesis that the spring peak in suicide is driven by a seasonal decompensation of mood disorder in spring. Results from this study are visualized in figure 24.

Fig.24



Circadian System and Mood

The Timekeeping System

In humans, most physiological and behavioural functions – including the secretion patterns of hormones (prolactin, corticotrophin, cortisol, growth hormone, melatonin), the sleep–wake cycle, the core body temperature, the thyroid function, the urine output, the blood pressure and the heart rate - show regular daily cycles.

Because of the daily changes in light intensity and temperature due to the rotation of the earth around its own axis, all living organisms, in the course of the evolution, have developed cellular clock mechanisms sensitive to light, organizing their activities in 24-hour cycles.

These endogenous rhythms, arisen from a timekeeping system within the organism, persist however in the absence of any environmental stimuli. Indeed, it has been shown that human beings deprived of any temporal and social cues, still exhibit daily cycles of core body temperature, urinary output and sleep and wakefulness (Pittendrigh 1993).

This framework on which organisms – from single-celled to humans – temporally organize their physiology, allow to accomplish two major tasks. The first task is predicting regularly repeating changes in the environment. Anticipating such changes in the environment can aid even the simplest single-celled photo-synthetic organism in the prediction of daylight hours to optimize energy collection by allowing different biochemical pathways to become active at appropriate times. This then allows potentially incompatible biochemical processes to exist in their own temporal compartments, ensuring they do not interfere with each other.

The second equally important task is the adaptation to unanticipated or less periodic fluctuations in the environment. Indeed, the circadian system allows for stimuli in the environment to “phase shift” the endogenous clock, pushing it forward or backward, in order to optimally adapt to changes in the outside world.

In humans and other mammals, circadian rhythms are generated by an internal clock or pacemaker (fig.25). Anatomically, this circadian internal oscillator is located in the suprachiasmatic nucleus (SCN) of the hypothalamus, which is a cluster of about 10,000 neurons, lying dorsal to the optic chiasma and lateral to the third ventricle (Hastings, Duffield et al. 1997).

Experimental ablation studies, as well as results of disease in humans, show that the absence of SCN disrupts the ability to express any overt circadian rhythm (Klein, Moore et al. 1991). Individual neurons from the SCN, when dissociated and held in vitro, retain a robust circadian rhythm in electrical firing, and this can continuously be recorded for several weeks and shows a slight deviation from 24-hour (usually longer) (Welsh, Logothetis et al. 1995). Furthermore, Inouye and (Inouye and Kawamura 1979), made a series of knife cuts around the SCN in order to isolate these structures from neural, but not hormonal, communication. As a consequence, the rhythm in electrical activity continued inside the "island" containing the SCN, but not in other regions of the nervous system. For these reasons, the SCN seems to be capable of generating circadian oscillations in an organism.

The endogenous clock is synchronized to the solar cycle primarily by retinal light input.

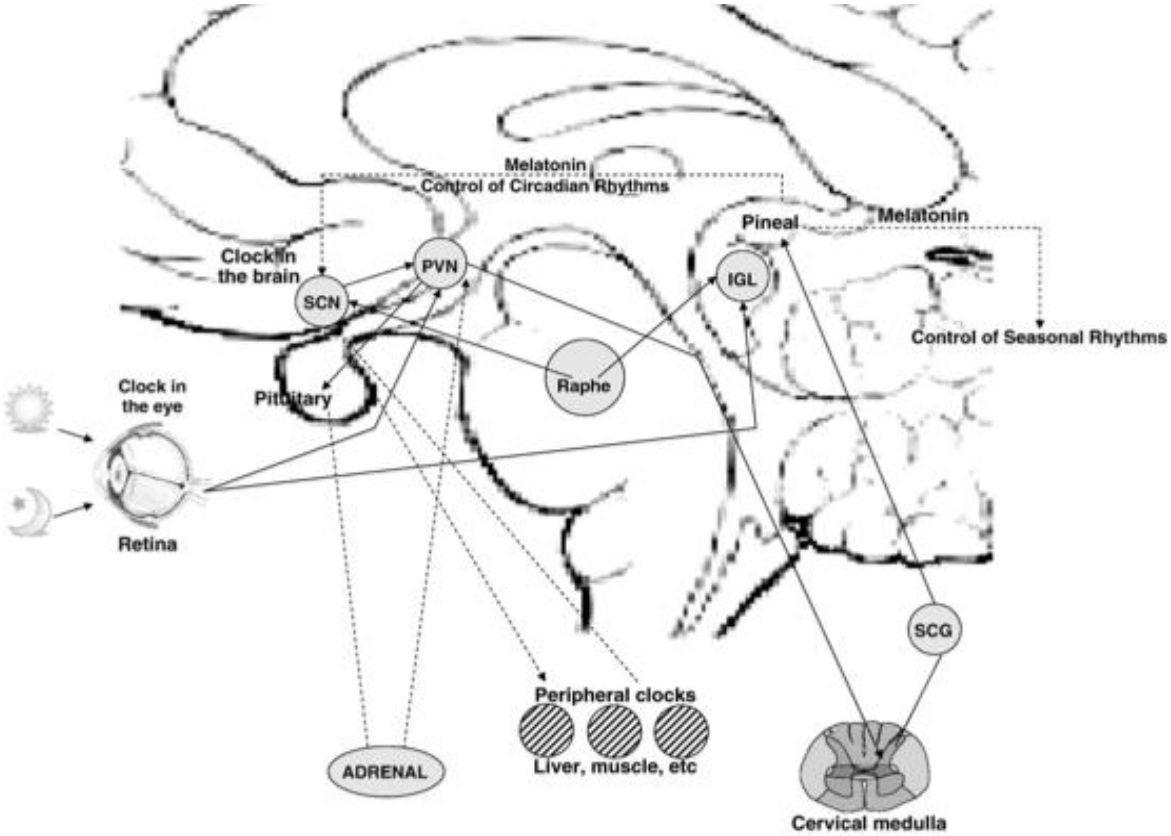
Although, social *zeitgeber* ("time giver"), such as food availability, job or social demands, act directly or indirectly on the SCN to synchronize its rhythmic activity, the primary *zeitgeber* is surely the light. The SCN receives light input *via* a direct retinohypothalamic tract (RHT) from photosensitive retinal ganglion cells expressing the photopigment melanopsin, which responds to selectively short wavelenght blue light energy independently of the classic photoreceptors (rods and cones) (Wirz-Justice, Benedetti et al. 2009). Additionally SCN receives light input indirectly *via* the intergeniculate leaflet of the lateral geniculate complex. The activity of SCN neurons is also modulated by melatonin secreted in the pineal gland (Wirz-Justice 2006) and serotoninergic (5-HT) pathways which ascend from the raphe nuclei (Moore and Speh, 2004).

The SCN then sends signals via direct and indirect projections in the brain, and through coordinated timing of the release of multiple peptides and hormones that circulate throughout the brain and body to synchronize oscillators in various tissues (Reppert and Weaver 2001).

The major output of the SCN is to the paraventricular nucleus (PVN) of the hypothalamus and *via* a multisynaptic pathway, to the pineal gland, where melatonin is synthesized according to the length of the photoperiod. Thereby, melatonin is secreted at night and suppressed by light during the day. Melatonin is a biochemical transducer of photoperiodic information to all cells in the body (including SCN neurons), signalling the seasonal variations of day/night cycle length (Simonneaux and Ribelayga 2003). As the duration and also the amplitude of the nocturnal melatonin peak increases with the lengthening of the dark phase of the day/night cycle, a lengthening melatonin signal from night to night is thought to indicate that the season is moving

from summer to fall/winter, while progressive shortening of night melatonin signal indicates that spring/summer is coming. The PVN is also the site of autonomic neurons, which communicates the time-of-day signal to different body organs, and corticotrophin-releasing factor secreting neurons, which are part of the hypothalamo-pituitary-adrenal (HPA) axis, endowed with a diurnal rhythmicity. Therefore, the SCN signal is translated into hormonal and autonomic signals for peripheral organs mainly within the PVN.

Fig.25



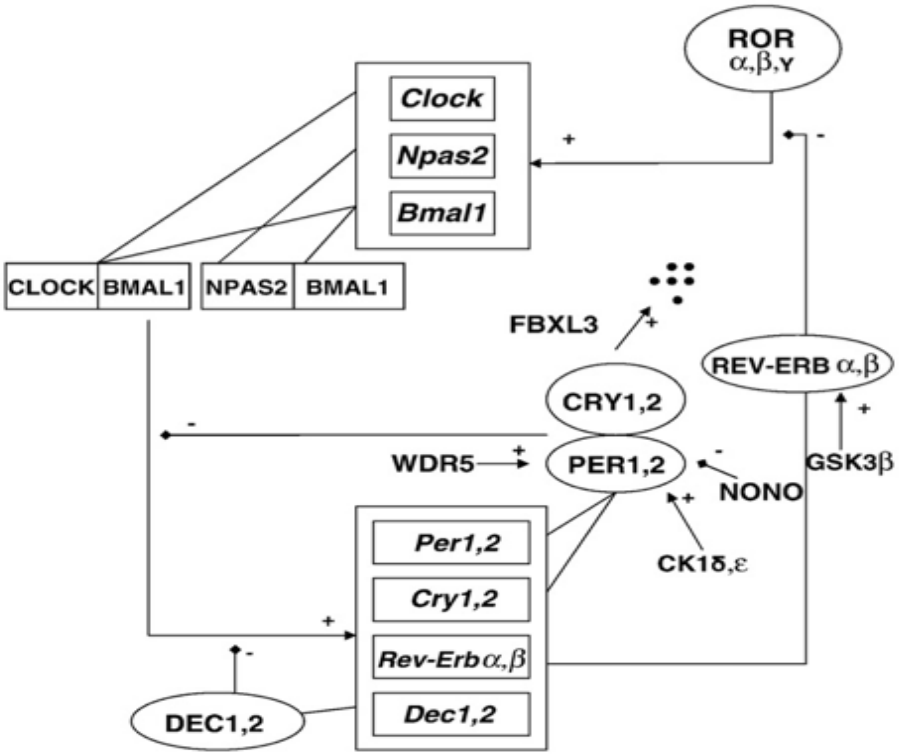
The CLOCK Machinery

The mechanism by which the master pacemaker of SCN synchronize circadian rhythms is primarily controlled by sodium-dependent action potentials regulated by at least two ionic currents: a Ca2+ current and a K+ current which are necessary for the maintenance of proper membrane potential (Pennartz, de Jeu et al. 2002). The circadian regulation of these currents in the SCN is likely through transcriptional or translational control of channel subunits by the core molecular clock

(fig.26). At the cellular level, the clock is a network of “clock genes,” transcriptional regulators that maintain rhythmic expression of their target genes over ~24-h cycles (Takahashi, Hong et al. 2008). This core molecular clock is composed of a series of transcriptional and translational feedback loops that cycle over ~24 h. The basic helix-loop-helix-PAS (Period-Arnt-Singleminded) – containing transcription factors, Circadian Locomotor Output Cycles Kaput (CLOCK) and Brain and Muscle Arnt-Like protein 1 (BMAL1; also called MOP3) heterodimerize and bind to E-box–containing sequences in a number of genes including the three *Period* (*Per*) genes (*Per1*, *Per2* and *Per3*) and the two *Cryptochrome* (*Cry*) genes (*Cry1* and *Cry2*). Over time, the PER and CRY proteins dimerize and are shuttled back into the nucleus where CRY proteins can directly inhibit the activity of CLOCK and BMAL1, thus forming a negative feedback loop (Reppert and Weaver 2001; Ko and Takahashi 2006); Reppert and Weaver, 2002). In addition to this feedback loop, the CLOCK and BMAL1 proteins regulate the expression of the nuclear hormone receptors, Rev-erbα and Rora which in turn can repress or activate *Bmal1* transcription, respectively, through actions at the Reb-Erv/ROR response element in the promoter (Preitner, Damiola et al. 2002; Sato, Yamada et al. 2006). Outside of the SCN, Neuronal PAS-Domain Protein 2 (NPAS2; also known as MOP4) can heterodimerize with BMAL1 and control *Per* and *Cry* gene expression (Reick, Garcia et al. 2001) **(Fig. 2.5)**. NPAS2 can also substitute for CLOCK to regulate rhythms in the SCN when the CLOCK gene has been disrupted (Debruyne, Noton et al. 2006).

Also crucial for period determination are posttranslational modifications of clock components by signaling molecules like casein kinases δ/ε and glycogen synthase kinase 3β (GSK3β) which control the rate of accumulation, association and translocation of PER and CRY (Reischl and Kramer).

Fig.26



The Rhythm in Blues

In view of the wide spectrum of physiological processes driven by the circadian clock, it is not surprising that any disruption of circadian timing can result in physical and mental symptoms and morbidities. There is also compelling evidence of mental and physical malaise from observations of individuals, such as shift workers, airline pilots and medical workers who are subjected to an inappropriate activation of phase shifting processes.

In Mood Disorders there are substantial evidence of generalized deregulations and alterations in circadian rythms as well as in hormones secretion. Circadian-related abnormalities are common in all subtypes of unipolar/bipolar depression, including Seasonal Affective disorder (SAD), Major Depressive Disorder (MDD) and Bipolar Disorder (BPD). Several physiological variables show a clear-cut circadian rhytmicity, which is thought to be due to the influence of biological circadian pacemaker (Barbini, Benedetti et al. 1998). For example, temperature follows a diurnal pattern in

healthy individuals: at dawn, it begins to rise, gradually increase up to the evening and then begin to decline during the night. Depressed patients tend to show low circadian rhythmicity in skin body temperature regulation. They usually show elevated nocturnal core body temperature, higher overall mean temperatures and phase advance (an anticipation) of the daily pattern of body temperature. The evidence of the normalization of temperature rhythms after remission reinforces such observations (Avery, Wildschiodtz et al. 1982).

Regarding diurnal mood fluctuations, usually these patients awaken, in the early morning, with a severe depression which gradually dissipate into almost euthymic state in early evening. These cyclic mood patterns can persist for weeks and months through the course of depressive episode, as it will be explain later, they are a good predictor for determining the probability for an antidepressant response to sleep deprivation (Bunney and Potkin 2008).

Mood Disorders could show a circadian disregulations of hormones too. Stress hormones are elevated in major depressive illness: Gibbons and McHugh, (Gibbons and Mc 1962), measured the plasma cortisol at weekly interval in some depressed patients and they found elevated levels of plasma cortisol. In general, the more severe the depression is, the higher the cortisol level is. Furthermore, Gibbons (Gibbons and Mc 1962) found elevated level of plasma cortisol before treatment, while relief depressed patients were accompanied by a substantial decrease in the secretion rate. Cortisol secretion indeed follows circadian diurnal rhytms. Normal subjects show a peaks of cortisol concentration early in the morning, while a decreased can be observed in the evening and during sleep. Depressed patients instead show elevated levels of cortisol along the duration of the circadian cycle (Linkowski, Mendlewicz et al. 1985): patients have significantly higher mean 24-hour plasma levels of cortisol (Halbreich, Goldstein et al. 1987). It is likely that the cortisol hypersecretion cannot be due to stress or arousal factors alone, but it could also reflects a central endocrine abnormality associated with the illness (Sackar, 1975).

A 2003 review by Tiemeier suggests that the most reliable finding in biological psychiatry is the association between depression and disturbance of the hypothalamic-pituitary-adrenal axis (HPA), which correlates with endocrine dysfunction. Disturbances in HPA axis could explain some symptoms of MDD, including alterations in appetite, concentration, motivation, sleep and psychomotor activity. This is the reason why, several antidepressants act by reducing HPA activity (Bunney and Potkin 2008).On the other hand, bipolar patients have significant cortisol hypersecretion during depressive and manic episodes.

Melatonin has also been observed to be a rhythm regulating factor. Melatonin secretion is indeed stimulated by darkness while light suppresses it. So, melatonin is secreted during the night (Luboshitzky, Yanai et al. 1998) and by feedback it helps to regulate the main circadian clock modifying the rhythms of its own production and of other circadian variables. Many depressed patients have delayed melatonin release, suggesting a phase delay in circadian rhythms (Lewy, Lefler et al. 2006) and, additionally, they could be supersensitivity to light-induced suppression of melatonin (Srinivasan, Smits et al. 2006). Significantly lower melatonin levels have been found in patients affected by bipolar disorder, compared to healthy subjects and, for this reason, the decreased melatonin production is considered a clear marker of bipolar disorder (Kennedy et al., 2007).

In summary, MDD and BPD show a phase advance of circadian rhythms and a reduction in its amplitude (Dallaspezia and Benedetti 2009) and, vice versa, a phase advance of circadian rhythms could be pathognomic for affective disorders (Bloom and Kupfer 1995). Anyway, it is important to remind that exogenous shifts in timing of the sleep-wake cycle, induces, even in healthy subjects, dysphoric mood, poor performance and fatigue, mimicking sleep patterns found in depresses patients (e.g. early morning awakening and short REM latency) (Bloom and Kupfer 1995).

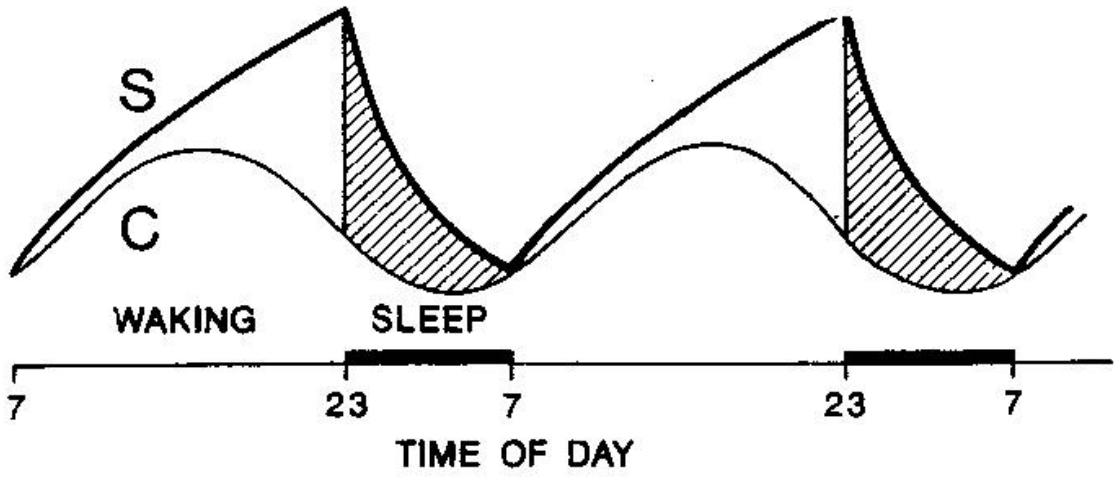
Sleep, like many behavioral and physiological activities, is regulated through both circadian and homeostatic mechanisms (Borbely 1982). The majority adult humans sleeps at night when it is dark and they awake when it is light, according to the circadian rhythms. Molecular mechanism that control circadian rhythms, are highly conserved phylogenetically (Reppert and Weaver 2001). Molecular and behavioural conservation indicates that sleep conferred a selective advantage. Prolonged sleep loss impairs temperature control, dietary metabolism and immune function, and leads ultimately to death (Rechtschaffen and Bergmann 2002).

The homeostatic regulation of sleep refers to the two process model, which postulates that the interaction between the sleep-wake dependent Process S and the circadian Process C accounts for essential aspects of sleep regulatio(Borbely 2009).

The model distinguishes between the circadian and the homeostatic regulation of sleep propensity. The circadian component (process C) describes how sleep propensity changes during the 24h. Process C is well understood, both in its mechanisms, centered in the suprachiasmatic nucleus, and in its function, which is to restrict sleep to a time of day that is ecologically appropriate.

The homeostatic component (process S) accumulates exponentially during wakefulness and is discharged when we sleep, also exponentially, but with a faster time course (fig.27). The time course of process S was derived from a physiological variable, EEG slow wave activity (SWA) in the electroencephalogram (EEG) of non rapid eye movement (NREM) sleep. The homeostatic regulation of SWA suggests that it may reflect some restorative aspect of sleep, but what this aspect may be remains unknown (Tononi and Cirelli 2003).

Fig.27



Thus, the longer one stays awake, the stronger the pressure to go sleep becomes. Thereafter, the exponential decline of the process S, associated with NREM sleep or delta slow wave sleep, intersects with the phase of process C appropriate for waking up. Sleep onset and sleep termination are determined by the level of S and by a gating system consisting of two threshold, under control of the circadian process C, as the Borbèly model explains. Thus, apart from conscious decisions elicited by external social or psychological factors, such as stress, pain, night-shift work, sleep need and circadian pacemaker dictate the timing of NREM sleep (Wirz-Justice and Van den Hoofdakker 1999). In the 1993, Achermann et al. Assumed that process S is characterized by two dynamic components. According to Borbèly model, the first component is proportional to the slow wave sleep, while, in the opposite side, the second component not only declines, but is supposed to increase both during waking and sleep. In particular, process S would increases during NREM interruptions (nocturnal awakenings) and during REM sleep (Achermann, Dijk et al. 1993).

In 1997 Porkka Heiskanen showed what might be the neural mediator of this effect of prior wakefulness, described above. Adenosine, an extracellular substance, is of particular interest as a putative sleep-wakefulness neuromodulator because the production and concentration of adenosine have been linked to neuronal metabolic activity; neural metabolism is greater during wakefulness than during delta slow wave sleep, in the same wave adenosine increases during spontaneous wakefulness as contrasted with slow wave sleep. Furthermore, adenosine exhibits progressive increases during sustained, prolonged wakefulness and declines slowly during recovery sleep (Porkka-Heiskanen, Strecker et al. 1997).

Alteration of the sleep-wake cycle and of the sleep structure are core symptoms of a major depressive disorder, and occur both in course of Bipolar Disorder and of Major Depressive Disorder. About 90% of depressed patients complain of poor quality of sleep, with sleep disturbances appearing weeks before the recurrence of mood episode (Perlis, Giles et al. 1997) and worsening in the days preceding the recurrence (Bauer, Grof et al. 2006). In particular, in patients affected by mood disorders, polysomnographia shows several abnormalities, such as prolonged sleep latency, decreased slow wave sleep, reduced rapid eye movement (REM) latency and increased REM density (Hudson., 1992), characteristics often reversed by most antidepressants.

As told before, sleep-wake cycle, like many behaviors and physiological activities, is regulated through both circadian and homeostatic mechanisms (Borbely 1982). Patients affected by Mood Disorders show indeed a misalignment between the circadian process C and the sleep pressure process S. Normal and stable mood regulaiton during the day, indeed, seems to require good temporal alignment between these two processes, since mood can drop suddenly if they are not in synchrony (Wirz-Justice, Benedetti et al. 2009). In particular, sleep abnormalities such as the shortening of the latency of the first REM stage after sleep onset and the early morning awakening are suggestive of a *phase advance* hypothesis of depression, for which the SCN is phase advanced relative to sleep time (Wehr, Wirz-Justice et al. 1979). Other events consistent with this phase advance of endogenous rhythms in depression are represented by the early morning rise of adrenocorticotropic secretion and the nocturnal elevation of prolactin and growth hormone levels in the blood of depressed individuals (Wehr, Sack et al. 1983; Linkowski, Mendlewicz et al. 1985; Mendlewicz, Linkowski et al. 1985) .

Major depression and insomnia are epidemiologically related, and individuals with insomnia are more likely to develop depression than normal sleepers (Riemann, Voderholzer et al. 2002). Moreover, DSM-V lists, between mood disorder's criteria, insomnia or hypersomnia in major depressive disorder and a reduced need for sleep during manic or hypomanic episodes. Perlis (Perlis, Giles et al. 1997) suggests that sleep disruption is often one of the first signs of a depressive episode preceding the onset of the illness five weeks before, especially in people who have recurrent depression.

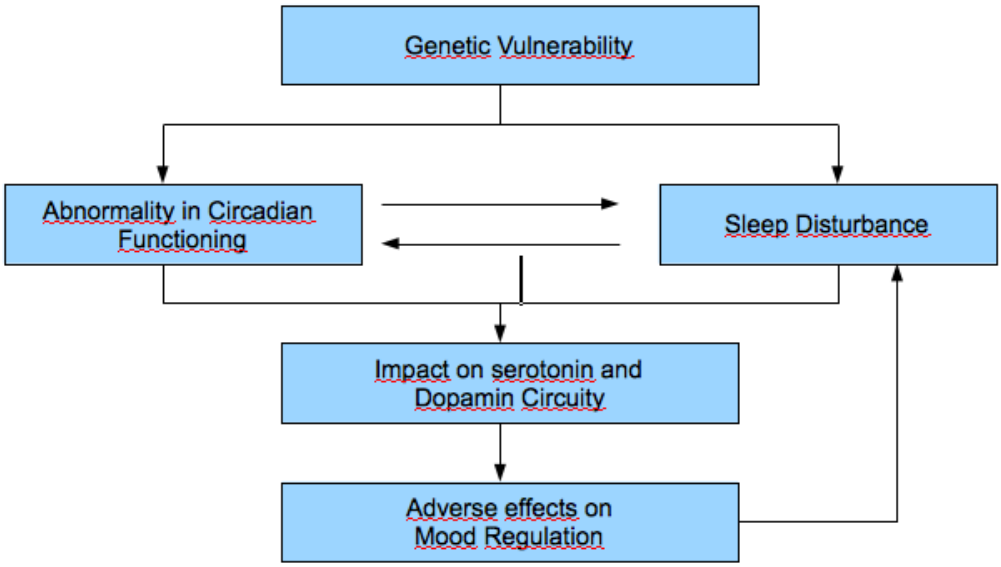
Bipolar patients develop, other than sleep symptoms described for depressed subjects, a decreased need for sleep in their manic periods, up to a reduced total sleep time of 3 or 4 hours (DSM-V, 2013), and a longer sleep onset latency compared to unipolar disorder (deMeartelaer et al., 1987). So, the relationship between sleep disturbances and mood episodes in BD is independent of polarity, and has been documented both for depressive and manic episodes. Most factors are known to precipitate a manic recurrence of illness and could be related to the genesis of mania through a marked reduction in sleep. Moreover, a decreased need for sleep is a fundamental marker of the manic state (Wehr and Goodwin 1987).

Imaging studies (Germain, Nofzinger et al. 2004) have pointed to local brain abnormalities: REM and SWA appear linked to sleep related dysfunctional arousal (decrements in activity) in primary limbic and paralimbic structures (amygdala), and hypofunction in frontal cortical areas during both wakefulness and sleep. It s intriguing to highlight that these sleep disturbances are present also in euthymic periods, therefore, they could be considered a tract rather than a state-like characteristics of the illness.

An interesting model for bipolar disorders proposed by Harvey (Harvey 2008) integrates genetic susceptibility, sleep and circadian functioning, neurotransmitter output and mood deregulation. In this model, some genetic variants of candidate genes (mainly circadian ones) predispose individuals to being relatively less able to properly adapt their circadian rhythms to their environment and, to being prone to sleep disturbances. Since circadian and neurotransmission systems are tightly connected, circadian and/or sleep-related abnormalities may impact the functioning of the dopamine and serotonin circuitry, which in turn affects mood regulation. A vicious cycle is then created in which mood deregulation affects sleep quality and quantity, which thus disturbs rhythm stability (fig.29)

Sleep system and the other circadian rhythms are interconnected by serotoninergic and dopaminergic systems, which, in their turn, are impaired in mood disorder. The role of the serotoninergic system is supported by relevant findings, such as, an increased serotonin turnover immediately after light exposure and the presence of the highest serotonin concentrations in the suprachiasmatic nucleus and in the raphe nucleus. At the same time, sleep deprivation seems to activate limbic dopaminergic pathways, increasing limbic blood flow, dopamine D2 receptor occupancy and eye blink rates, Inthis view, dopamine reuptake inhibitors seem to prevent the antidepressant effect of chronoterapeutics (Harvey 2008).

Fig.28



Finally, sleep disturbances are associated with suicidal behaviour in patients with major depression (Agargun, Kara et al. 1997) and it has been shown that depressed patients can markedly improve their depression by effectively managing their insomnia (Kupfer 1999). All these data support a putative pathogenetic link between sleep–wake cycle disturbances and major depression.

As suggested by Turek (Turek 2007), the expression of most rhythms at behavioural, physiological and biochemical level is regulated by the integration of inputs from the circadian clock and the sleep–wake state of the organism. Thus, the circadian and sleep control centres have evolved together to ensure a timely coordination between internal and external environments in order to optimize the survival of the species. Therefore, it seems likely that alteration in the sleep – wake cycle may desynchronize many endogenous rhythms, which then, in turn, may lead to a depressed state.

Nonetheless, there is ample debate if the effects of disruption in the sleep-wake cycle are merely symptoms of psychiatric disorders, or if they are rather contributing causes. There are two theoretical ways whereby disrupted circadian rhythms might lead to depression. On one hand, alterations of biological clock at molecular level could lead to neurobiological dysfunction, which in turn may generate the depressed state. On the other hand, a primary circadian disturbance of the sleep–wake cycle could lead to insomnia that might facilitate or exacerbate the depressed state.

Another Stress-Diathesis Model

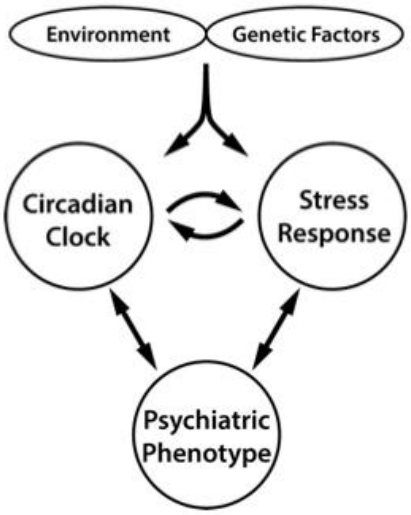
According to Karatsoreos and McEwen (Karatsoreos, Bhagat et al. 2011; Karatsoreos and McEwen 2013), disrupted circadian clocks may make individuals more *susceptible* to the development of neuropsychiatric disorders in a manner similar to the stress-diathesis model, whereby environmental challenges have more severe outcomes due to underlying genetic or experiential differences (Morley, 1983).

One possible model of explanation is that the circadian clock modulates the biological responses to stressful environmental factors that vary with an individual's experience. It is known that the circadian clock and the stress response systems are closely related: circadian clock genes regulate the physiological sensitivity to and rhythmic release of glucocorticoids (GC). In turn, GCs have reciprocal effects on the clock.

In humans the GCs cortisol which is regulated by the hypothalamic-pituitary-adrenal (HPA) axis play major roles in the response to stress. In response to signals from the limbic system, the paraventricular nucleus releases corticotropin-releasing hormone (CRH), signaling the anterior pituitary to release adrenocorticotropic hormone (ACTH). ACTH from the pituitary signals the adrenal gland where it stimulates GC synthesis and release into the systemic circulation to alter

gene expression and physiology throughout the body. In healthy subjects, the increase of GC in response to acute stress leads to increased alertness, mobilization of glucose and fatty acids, and enhanced memory formation. However, after chronic stress and long term elevations of GCs, the effects of GCs are detrimental with neurotoxic, immunosuppressive, and metabolic consequences (Maniam, Antoniadis et al.; Sapolsky, Romero et al. 2000). Accordingly, many neuropsychiatric disorders are associated with abnormalities in the HPA axis, with chronically cortisol and reduced sensitivity to GC, suggesting that stress may contribute to their development. Experiments in rats have shown that rats exposed to early life stress show elevated GCs as adults (Zhang, Li et al. 2013). Similar observations have been made in human subjects who show chronically elevated GCs and altered stress response long after a traumatic event (Maniam, Antoniadis et al. 2014). The effects of stress can therefore be persistent, lasting long after termination of the stressor. Since disturbed circadian rhythms and altered stress responses are involved across many psychiatric conditions, it may be that an individual's genetic and environmental risk factors for a particular illness are subject to modulation by the combined actions of the circadian and stress response systems (fig.29).

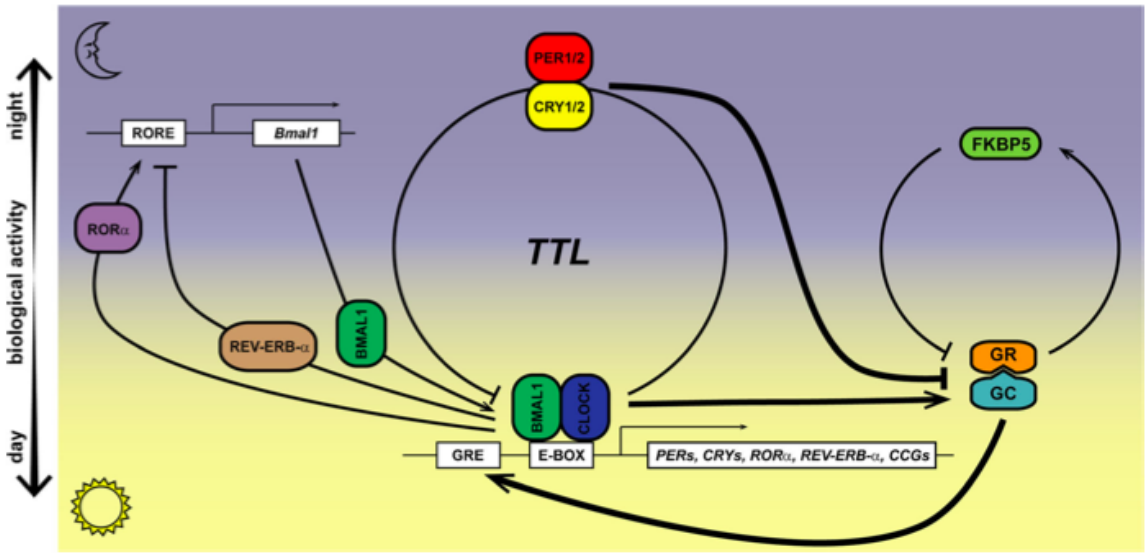
Fig.29



In support of this hypothesis, the circadian clock and the stress response system interact closely (fig.30). During the day BMAL1 and CLOCK proteins are expressed at high levels to activate

transcription of PERs, CRYs, RORα, REV-ERBα, and CCGs, as well as to promote the activity of GRs which, in turn, elevate transcription of GC-inducible genes like FKBP5. An accessory feedback loop consists of RORα, an activator of BMAL1 expression and REV-ERBα, an inhibitor of BMAL1 expression. GCs facilitate the expression of GC-responsive clock genes and clock controlled genes (CCGs) with GRE promoter elements. At night, PER and CRY protein levels are high and repress the activity of BMAL1/CLOCK and the GR. Through feedback inhibition, FKBP5 suppresses GR activity. Due to the tight connection between the circadian clock and the stress response system, manipulations of one system naturally lead to changes in the other.

Fig.30



Circadian clock alteration may be interpreted as an enduring *trait* marker of MDD and BD, encoded by genetic variants in the clock, or as a *state* generated by the influence of environmental stressors on mood. In other words, genetic variants may increase sensitivity to environmental insults, increasing the probability either that the stressors will induce pathological fluctuations in mood, or that mood fluctuations occur autonomously in the absence of stressors (Post 1992).

However, while attractive as the basis for a model, the dissociation of state and trait may prove to be an oversimplification. Epigenetic factors affect transcription by way of gene silencing or activation, typically in response to features of an organism's unique environmental and developmental history (Tsankova, Renthal et al. 2007). As epigenetic states are induced and long lasting, they are neither fully traits nor states. A recent demonstration of imprinting on the clock

is a study showing that photoperiod manipulations during development affect subsequent gene expression and behavior rhythms in inbred adult mice (Ciarleglio, Axley et al.).

Clock Genes Polymorphisms

Of all major mental disorders, the evidence for genetic abnormalities associated with clock genes is strongest in Mood Disorder.

Seasonal affective disorder is associated with Single Nucleotide Polymorhisms (SNPs) in three circadian genes: BMAL1, PER3 and Npas2 (Partonen, Treutlein et al. 2007). An analysis of 46 SNPs in 8 clock genes (*BMAL1, CLOCK, PER 1,2,3, CRY 1,2, TIM*) revealed a significant association of BMAL1 and TIM with Bipolar Disorder. (Mansour, Wood et al. 2006). Also an independent study using haplotype analysis seems to confirm the association between the bipolar disorder and BMAL1 and found a new association with PER3 (Nievergelt, Kripke et al. 2006). Studies examining other genes have found, instead, negative results: no significant associations have been observed with PER2, CKIδ/ε and CRY1 SNP. (Shiino, Nakajima et al. 2003; Nievergelt, Kripke et al. 2005).

The coding region of PER3 gene contains a variable number tandem-repeat (VNTR) polymorphism in which a motif encoding 18 amino acids is repeated either four (PER2) or five times (PER3). These repeated units contain a cluster of putative phosphorilation sites, and this polymorphism can then influence PER3 function (Ebisawa, Uchiyama et al. 2001). The polymorphism has been associated with diurnal preference, sleep structure and sleep homeostasis in healthy subjects; while it influences age at onset in bipolar patients. An earlier age at onset in homozygote carriers of PER3 has been observed, while PER3 homozygotes showed a later age at onset and heterozygotes an intermediate one (Benedetti, Dallaspezia et al. 2008). Patients with the TT genotype of the GSK3β-50 SNP show an earlier age on onset of BD and experience less improvement from lithium therapy than patients with the TC or CC genotypes (Benedetti, Bernasconi et al. 2004; Benedetti, Serretti et al. 2005).

Another important gene of the circadian machinery is the GSK3.

Glycogen synthase kinase 3 is a serine/threorine protein kinase encoded in mammals by two known genes, GSKα and GSKβ. The second one maps to 3q21.1 in the human genome, a region which was reported to be linked with bipolar disorder (Badenhop, Moses et al. 2002). A SNP (-50 T7C) falling in the effective promoter region (nt -171 to +29) of the gene encoding for GSK3 influence the age at onset of bipolar disorder, with homozygotes for the wild variant showing an

earlier age at onset than carriers of the mutant allele (Benedetti, 2004). No association between this mutation and the presence of the illness was found in this study, but a strong link between the T -50 C polymorphism and female with bipolar II disorder was found by Szczepankiewicz and collegues (Szczepankiewicz, Skibinska et al. 2006). Homozygotes for the mutant allele also showed a better acute effect of Total Sleep Deprivation treatment (Benedetti, Bernasconi et al. 2004). The same polymorphism was found to influence the response to lithium treatment: in a group of bipolar patients -50 C/T mutation improved the recurrence index following lithium administration (Benedetti, Serretti et al. 2005). However, subsequent studies found that genotype and allele frequencies did not predict a lithium response in bipolar patients (Szczepankiewicz, Skibinska et al. 2006).

As previously mentioned, CLOCK is an essential positive regulator of the mammalian circadian feedback loop in the SCN. In particular, the T3111C single nucleotide polymorphisms (SNP) of the CLOCK gene has been investigated in both Major Depression and Bipolar Disorder. Although a statistically significant association of Rs1801260 with affective disorders has not be found, it seems to influence some important illness characteristics. This polymorphism influences diurnal preferences both in healthy subjects and in depressed patients. Healthy carriers of the 3111C variant show higher eveningness and a significantly higher evening activity and a delayed sleep onset (on average 79 min. later) were found in a group of bipolar depressed carriers of the same variant (Benedetti, Barbini et al. 2007). While C allele carriers increased their activity levels during the second part of the day, T/T homozygotes decreased them (Benedetti, Dallaspezia et al. 2008)

Among patients affected by mood-disorder, carriers of the 3111C variant had an increased occurrence of insomnia both lifetime and during their depressive episodes, and showed the persistence of sleep complaints, but not of other symptoms, after successful antidepressant treatment.. This effect was unrelated to diurnal mood fluctuations. Moreover, C/C homozygotes experienced a higher lifetime recurrence rate of illness episodes (Benedetti, Serretti et al. 2003). This effect is possibly due to the triggering effect of sleep loss and phase delay on mood episodes.

Rs1801260 could also influence neuropsychological performance and BOLD neural response to a moral valence decision task in patients affected by bipolar depression. During neuropsychological tests and fMRI scanning performed in the afternoon, latencies of response to emotional stmuli were shorter in C allele carriers than in T/T homozygotes, with no difference in response exactness and severity of depression between the two groups. ANOVA analysis between genotype and moral

valence of the stimuli on fMRI data showed maximal activations in dorsal/posterior cingulate cortex; here C carriers showed higher neural responses for positive than negative stimuli, while T/T homozygotes showed an opposite pattern of responses (Benedetti, Serretti et al. 2003). The posterior cingulate areas, where the interaction of genotype and moral valence of the stimuli were detected are known to be involved in moral judgment and in assssment and encoding of self-relevant informations (Vogt 2005). Since in animal models CLOCK is expressed in the cingulate neurons, the differences observed between genotype groups could be due to a direct influence of CLOCK at this molecular level in neurons of the cingulate cortex or to an effect on SCN which influences the reactivity to stimuli in other brain areas. Even an effect of CLOCK on monoaminergic DA and 5-HT projection to cingulate cortex is possible or a combination of all these mechanisms.

Finally, also transgenic mice carrying a mutation in the CLOCK gene showed a mania-like behaviour, which normalized after chronic administration of lithium. These abnormal behaviours could also be normalized by expressing a functional CLOCK protein *via* viral-mediated gene transfer specifically into the ventral tegmental area of mutant mice (Roybal, Theobold et al. 2007). This experiment produced a further proof of CLOCK gene involvement in the genesis of Mood Disorder.

Altogether, these data suggest that clock genes exert their role on human psychopathology by closely interacting with the rhythmic activity of brain neurotransmitter system.

Behavioral Genetics of suicidality in Bipolar Disorder: the interaction between CLOCK and SERT polymorphisms and early life stress

Introduction

Bipolar disorder (BD) strongly associates with the risk of attempting or committing suicide; about 30% of patients with BD attempt suicide, and about 20% eventually die from suicide (Leverich, Altshuler et al. 2003).

Current models of lifetime suicide risk suggest gene–environment (G x E) interactions, and a recent large twin study supported a liability-threshold model, with contributions from additive genetic factors and specific individual environmental influences (Turecki, Ernst et al. 2012).

Adverse childhood experiences can increase the risk of suicidal behaviors more than tenfold (Currier and Mann 2008). Childhood maltreatment is highly prevalent in patients with BD and has been associated with an higher lifetime suicidal ideation and suicide attempts (Leverich, Altshuler et al. 2003).

Suicidality is highly familial, and suicide attempt and completion are heritable, independent of the associated diagnosis (Zai, de Luca et al. 2012). Adding up – or interacting with - genetic risks, early adversities can increase the risk of suicide by influencing the epigenetic regulation of genes involved in stress-response systems (Mann 2003).

Studies on candidate genes have strongly supported a role for gene polymorphisms affecting the serotonergic pathway; in particular, G x E interactions have been described for a polymorphism in the promoter region of the gene coding for the 5-HT transporter (5-HTT or SERT), which shows a 44-base pair insertion/deletion polymorphism in the transcriptional control region upstream of the 5-HTT coding sequence (5-HTT-linked polymorphic region or 5-HTTLPR). A higher stress sensitivity in carriers of the short allele of 5-HTTLPR was suggested by a pivotal study associating the short form (but not the long one) with a positive relationship between the extent of exposure to early and adult stress, and the probability both of developing a major depressive episode and of showing suicidal ideation and attempts (Caspi, Sugden et al. 2003). The specific relationship between childhood stress and suicide in individuals carrying the 5-HTTLPR s allele has been confirmed in patients affected with Bipolar Disorder also (Benedetti, Riccaboni et al. 2014).

Nonetheless, despite the strong neurobiological evidence implicating serotonergic neuromodulation in suicidality (Mann 2003), none of the genes of the 5HT pathway has been definitively and undoubtedly recognized as having a causal role in suicidal behavior. It is likely that multiple risk alleles works in interactions between them and with environmental factors to produce such a complex phenotype.

Disturbed circadian rhythms have been associated with disrupted brain function, and multiple lines of evidence support the idea that the circadian clock is vulnerable and/or disturbed in a variety of mental illnesses.

Systematic review and meta-analysis suggests that sleep disturbances and sleep phase delay are associated with the increased risk of suicidal behaviors, both in the general population and in patients affected with a psychiatric illness (Gangwisch, Babiss et al. 2010; Landgraf, McCarthy et al. 2014)

Since disturbed circadian rhythms and altered stress responses are involved across many psychiatric conditions, it may be that an individual's genetic and environmental risk factors for a particular illness are subject to modulation by the combined actions of the circadian and stress response systems (Landgraf, McCarthy et al. 2014). In support of this hypothesis, the circadian clock and the stress response system are closely connected.

Evidence in patients with Bipolar Disorder suggests a higher level of dependence of behavior on the molecular characteristics of the clock. In healthy humans, polymorphic variations in clock genes are weakly associated with modest changes of circadian behaviors. The same polymorphisms influence core psychopathological features of mood disorders. This system structure supports hypotheses of circadian "misalignment" – or, more generally, unbalanced relationships between the many homeostatic and circadian regulators of biological rhythms in mood illness.

Within the frame of the Diathesis-Stress models, serotonergic abnormalities and circadian system alterations are recognized diathetic factors for suicidal behavior and mood disregulation. Given that: 1) several G x E studies on suicidality in Mood Disorders reported positive findings for the interaction of 5-HTTLPR genotype with early life stress, 2) circadian clock genes and the stress response system interact closely and 3) there are reciprocal connections between serotonin and circadian networks, we modeled a G x G x E study on the interaction between CLOCK and SERT

polymorphisms (respectively: 3111T/C and 5-HTTLPR) and early life stress on history of suicide attempts in patients affected with BD.

Materials and Methods

Sample and data collection

We studied 153 consecutively admitted Caucasian inpatients of Italian descent affected with Bipolar Disorder (BD) type I who had been referred to our hospital for a major depressive episode without psychotic features (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition - DSM-IV criteria; Structured Clinical Interview for DS - SCID I - interview). The sample was composed by 101 females and 52 males, aged from 19 to 72 years. Thirty-four patients (22%) had a positive history of suicide attempts, broadly defined as any behavior aimed at killing oneself during one's life course. Exclusion criteria were: other Axis I diagnoses; mental retarda-tion on Axis II; pregnancy, a history of epilepsy, major medical and neurological disorders; a history of drug or alcohol dependency or abuse within the last six months. Clinical information was obtained by the psychiatrist in charge and by an independent rater using best estimation procedure, taking into account available charts, case notes, and information provided by at least one relative. After complete description of the study to the subjects, written informed consent was obtained. The study was approved by the local ethical committee.

Early life stressful events (ELS) between the ages of 5 and 15 years were scored by trained raters on the Social Readjustment Rating Scale (SRRS), which focuses on occurrences that led to readjustment-requiring changes in usual activities that frequently precede illness onsets, and which has been validated in similar settings. The scale yields two measures of stress: the number of stressful events occurring during the observation period, and a rating score based on the weighted probability that the events are followed by detrimental effects of stress, such as medical illness. For the purpose of this study, in scoring the SRRS, we considered the ELS total score only (the magnitude of each item multiplied by its frequency), which showed to be adequate to detect the relationship between ELS and adult suicidal ideation in patients with BD (Benedetti, Radaelli et al. 2011).

History of attempted suicide (positive *versus* negative) were recorded as clinical outcome measure to test the G x G x E interaction of genotypes and early stress.

Patients were administered with the 21-item Hamilton Rating Scale for Depression (HAM-D; Hamilton, 1967) by the psychiatrist in charge, in order to rate the severity of depression.

5-HTTLPR and CLOCK polymorphisms identification and typing

DNA was extracted from whole blood by a manual technique, using the Illustra blood genomic Prep Midi Flow kit (GE Healthcare, Milan, Italy). Genotyping was performed by personnel blind to the clinical course of illness.

To genotype 5-HTTLPR, polymerase chain reaction (PCR) was performed using the following primers: 50-GGC GTT GCC GCT CTG AAT GC-30 and 50-GAG GGA CTG AGC TGG ACA ACCAC-30. The PCR reaction was carried out in a10 lL volume containing 150 ng genomic DNA,1 lM of each primer, 200 lM of nucleoside triphosphates(including 7-deaza-dGTP), 19 HotMaster Taq Buffer with Mg++ (Eppendorf), 0.025U/lL of Hot Master Taq (Eppendorf), and 5% ofdimethyl sulfoxide. The PCR reaction was performedby ABI 9700 PCR thermal-cycler (AppliedBiosystems - Life Technologies, Monza, Italy) asfollows: after a first step at 94°C for 2 min, steps of94°C for 35 sec, 61°C for 30 sec, and 70°C for65 sec for 35 cycles were carried out. Then, a finalextension step at 70°C for 8 min was added. PCRproducts were separated in 3.5% Seakem agarose gel with ethidium bromide. The bands were visualized by ultraviolet light. The allele including the 44 base pair (bp) insertion ('l' allele) consists of a fragment of 528 bp, while the allele showing the 44 bp deletion ('s') consists of a fragment of 484 bp. Genotyping was performed by staff who were blind to the clinical course.

To genotype CLOCK, polymerase chain reaction (PCR) was performed with the following primers: 5'-TCC AGC AGT TTC ATG AGA TGC -3', and 5'GAG GTC ATT TCA TAG CTG AGC -3'. The PCR reaction was carried out in a 10 µl volume containing 150 ng genomic DNA, 0.1 µl of each primer [50 µM], 1 µl of dNTPs mix [10mM], 1 µl of 10X Hot Master Taq Buffer with Mg++ (Eppendorf), 0.5 U of Hot Master Taq (Eppendorf). PCR was performed as follows: after an initial step of 94°C for 2 min, steps of 94°C for 40 sec, 58°C for 30 sec, 70°C for 75 sec for 5 cycles. Then, steps of 94°C for 40 sec, 57°C for 37 sec, 70°C for 75 sec for 30 cycles. After that, a final extension step at 70°C for 12 min was added. Amplified fragments (221 bp) were digested by use of Bsp 1286I restriction enzyme (New England Biolabs, England, UK). The incubation was performed at 37°C overnight and fragments were separeted in agarose gels. The T allele is the unrestricted PCR product (221 bp), while the C allele produces bands of 125 and 96 bp.

Data Analyses

Demographic and clinical features of the sample were analyzed by means of Analysis of Variance (ANOVA), t-test or Chi-squared test, when appropriate.

The effect of the G x G x E interaction between ELS and SERT and CLOCK polymorphisms on the lifetime risk of attempting suicide was tested using a commercially available software package (StatSoft Statistica 7, Tulsa, OK, USA) and following standard computational procedures.

Given the *a priori* hypothesis that categorical (5-HTTLPR and 3111 T/C) and continuous (extent of exposure to early stress) predictors would interact in affecting the outcome of interest (life-course suicide attempts), the influences of the predictors were modeled using a separate-slopes design instead of the traditional Analysis of Covariance, which is inappropriate when the categorical and continuous predictors interact in influencing the outcome. The analysis was modeled in the context of the Generalized Linear Model (GLZM), the outcome distribution being binomial (positive *versus* negative history of suicide attempts.

Therefore, in order to test their effects on history of suicide attempts (positive versus negative), 5-HTTLPR, 3111T/C and ELS were entered as factors into a GLZM separate-slopes analysis with a logit link function. s/s (for the 5-HTTLPR) and C/C (for the 3111T/C) homozygotes were pooled together with the corresponding heterozygotes (respectively: l/s and T/C), as literature shows they share clinical features, so that the two categorical variables had two levels each.

Parameter estimates were obtained using iterative re-weighted least-squares maximum likelihood procedures. The significance of the effects was calculated using the Likelihood Ratio (LR) statistic, which provides the most asymptotically efficient test known, and using the Wald (W) statistic.

Results

Clinical and demographic data of the sample are reported in tables 1 and 2, divided according to SERT x CLOCK genotypes group (tab.1) and history of attempted suicide (tab.2).

Tab.1 Differences in demographic and clinical features of the sample, divided according to SERT x CLOCK genotypes group.

	Total sample (N=153)	l/l-T/T (n=30; 20%)	l/l-C carr (n=22; 14%)	s carr-T/T (n=52; 34%)	s carr-C carr (n=49;32%)	F/X^2	*p-value*
Age *(years)*	46.54±12.05	44.865±11.56	47.41±11.71	46.90±13.03	46.80±11.69	0.25	0.86
Sex *(males/females)*	52/101	8/22	9/13	19/33	16/33	1.38	0.71
Education *(years)*	11.48±4.01	10.80±3.63	11.72±4.22	11.92±3.81	11.22±4.37	0.58	0.63
Onset *(age)*	29.63±9.73	30.17±9.36	30.18±9.66	30.90±10.60	27.69±8.97	0.99	0.39
HAM-D tot	*22.78±4.34*	*22.07±3.80*	*23.10±3.72*	*21.51±3.85*	*24.44±5.07*	*3.07*	*0.03**
ELS Score	335.80±203.85	360.33±220.63	319.32±209.07	336.80±204.32	327.12±195.26	0.22	0.88
Attempters *(n; %)*	34 (22%)	11 (37%)	6 (27%)	8 (15%)	9 (18%)	5.77	0.12

*(*significant differences)*

Groups didn't differ in age, gender prevalence, years of education and age at onset; also the amount of early life stress and the incidence of suicide attempts didn't differ significantly between groups.

With respect to severity of current depression, ANOVA showed a significant difference (F=3.07, p=0.03) in HAM-D scores: post-hoc Bonferroni correction revealed a significantly more sever simptomatology in s carriers-C carriers respect to s carriers-T/T.

Tab.2 Differences in demographic and clinical features of the sample, divided according to history of suicide attempts (positive versus negative)

	Total sample (N=153)	Attempters (n=34; 22%)	Non-attempters (n=119; 78%)	t/X^2	*p-value*
Age *(years)*	46.54±12.05	43.62±11.79	47.38±12.05	-1.61	0.11
Sex *(males/females)*	52/101	10/24	42/77	0.40	0.52
Education *(years)*	11.48±4.01	11.73±4.03	11.36±4.02	0.47	0.64
Onset (age)	*29.63±9.73*	*26.53±7.80*	*30.51±10.06*	*-2.13*	*0.03**
HAM-D tot	22.78±4.34	22.24±3.19	22.90±4.57	-0.63	0.53
ELS Score	*335.80±203.85*	*431.44±246.26*	*308.48±182.16*	*3.19*	*0.02**
l/l-T/T *(n; %)*	30 (20%)	11 (32%)	19 (16%)	5.77	0.12
l/l-C carr *(n; %)*	22 (14%)	6 (18%)	16 (13%)		
s carr-T/T*(n; %)*	52 (34%)	8 (24%)	44 (37%)		
s carr-C carr *(n; %)*	49 (32%)	9 (26%)	40 (34%)		

*(*significant differences)*

The analyses carried out in attempters versus non-attempters found no significant differences in age, gender, educational level and depression severity. G x G groups were equally represented among attempters and non-attempters.

Suicidal patients had a significant younger age at the onset of the BD illness (t=-2.13, p=0.03) and had been stressed significantly more in childhood than patients who never attempt suicide.

The separate-slopes GLZM analysis, with the proportion of patients who attemped suicide as dependent variable, showed a significant G x G x E interaction (interaction of 5-HTTLPR x 3111 T/C x ELS: LR X^2=11.43, p=0.02). This was due to a significant positive relationship between early life stress and suicide in l/l-C carriers (W=3.92, p=0.047) and in s carriers-C carriers (W=5.51, p=0.018) , but not among heterozygotes l/l-T/T (W=0.15, p=0.702) and s carriers-T/T (W=3.19, p=0.073).

The relationship between lifetime suicidality and ELS is plotted for each group in figure 1.

For a graphical purpose, the median of sample ELS scores has been calculated; the percentage proportion of patients who attempted suicide, divided according to the dichotomous level of childhood exposure to stress, is visually compared between groups in figures 2, 3, 4 and 5.

Fig.1 Predicted probability of suicide as a function of early-life stress in the sample, divided according to the 5-HTTLPR-CLOCK 3111 T/C group.

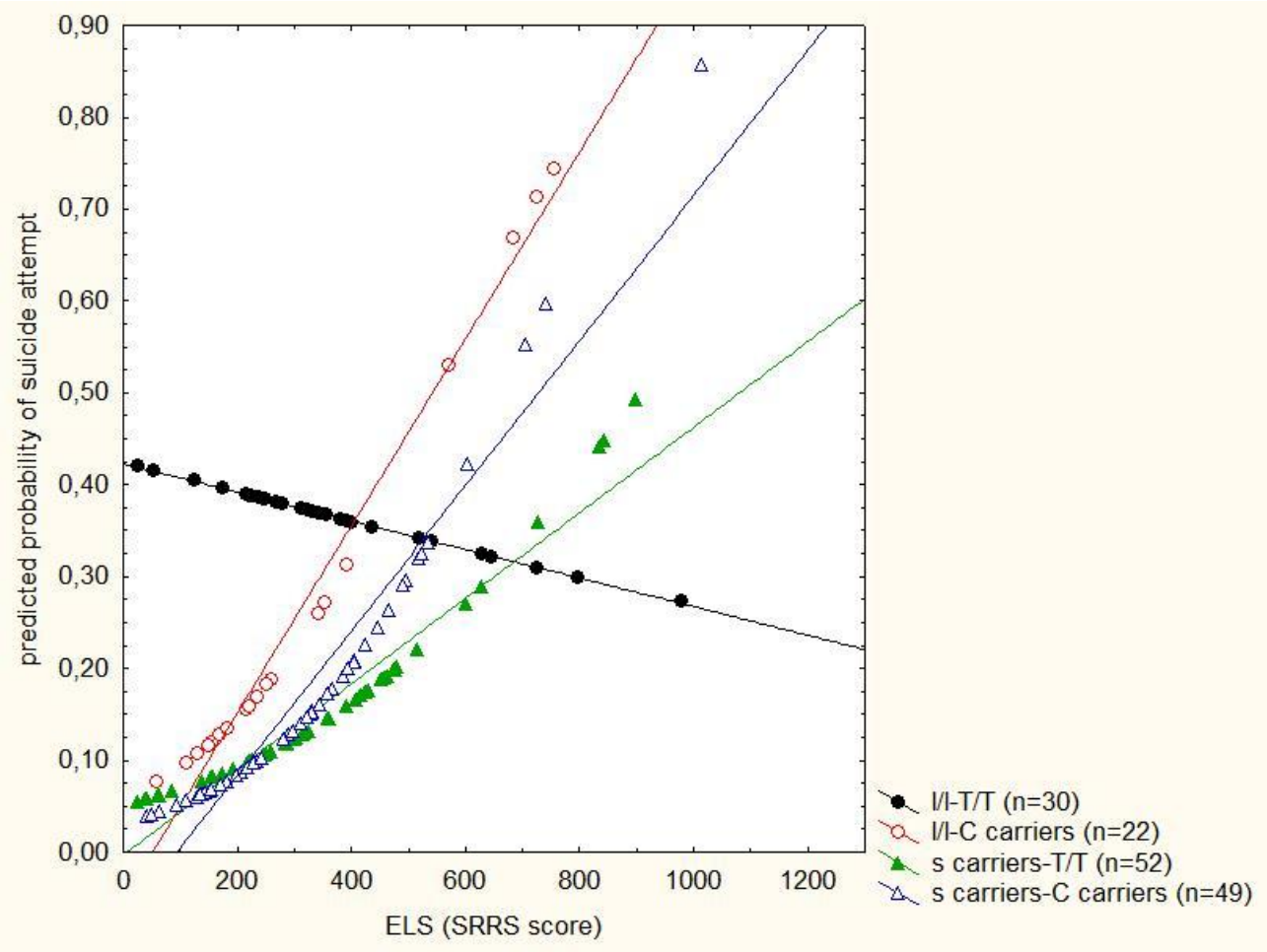


Fig.2 Percentage of attempters and non-attempters in l/l-T/T genotypes group, according to low (left panel) versus high (right panel) early life stress.

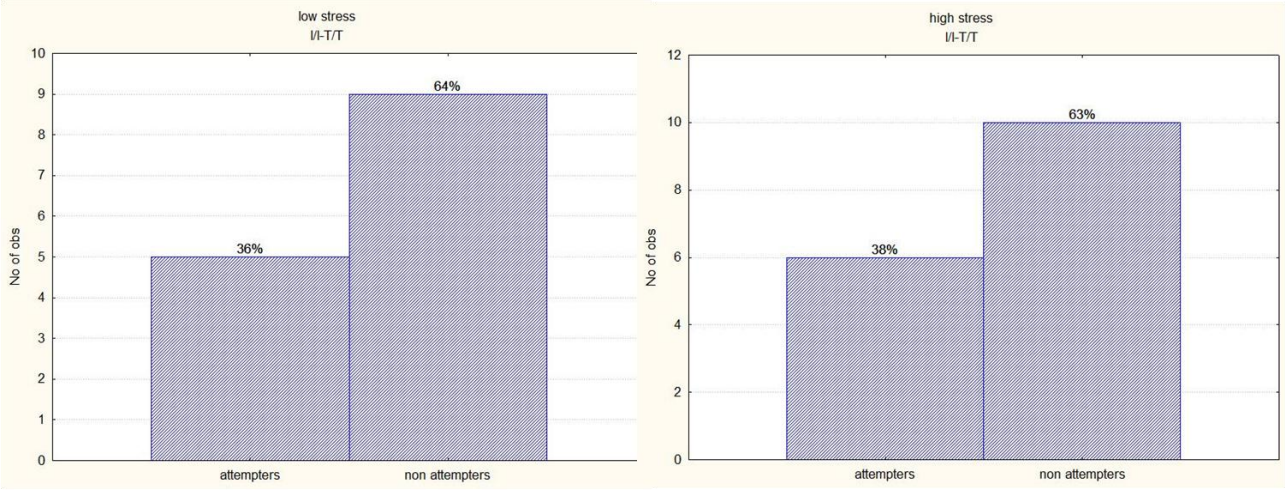


Fig.3 Percentage of attempters and non-attempters in l/l-C carriers genotypes group, according to low (left panel) versus high (right panel) early life stress.

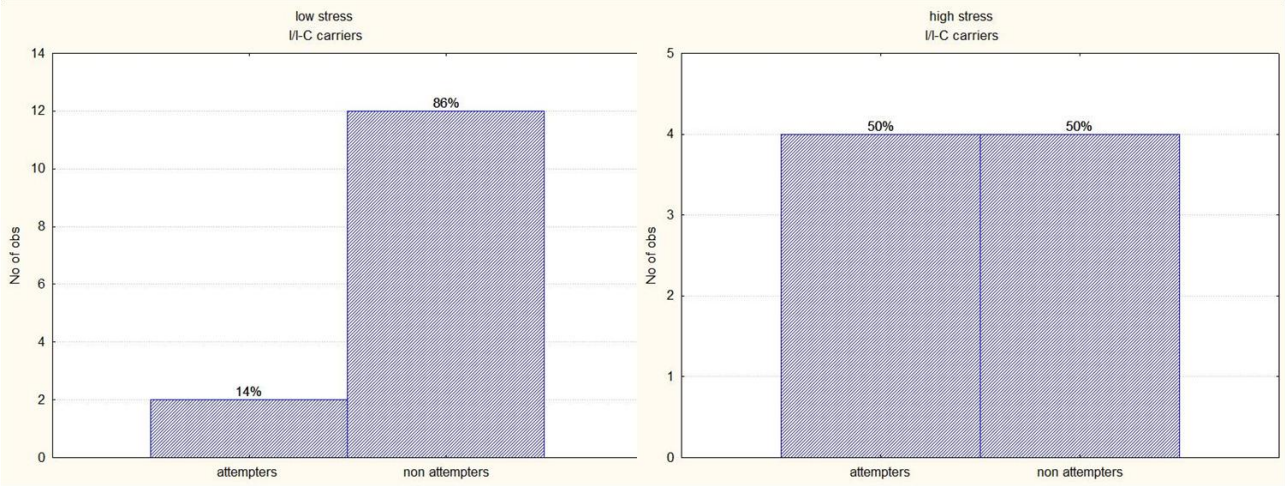


Fig.2 Percentage of attempters and non-attempters in s carriers -T/T genotypes group, according to low (left panel)versus high (right panel) early life stress.

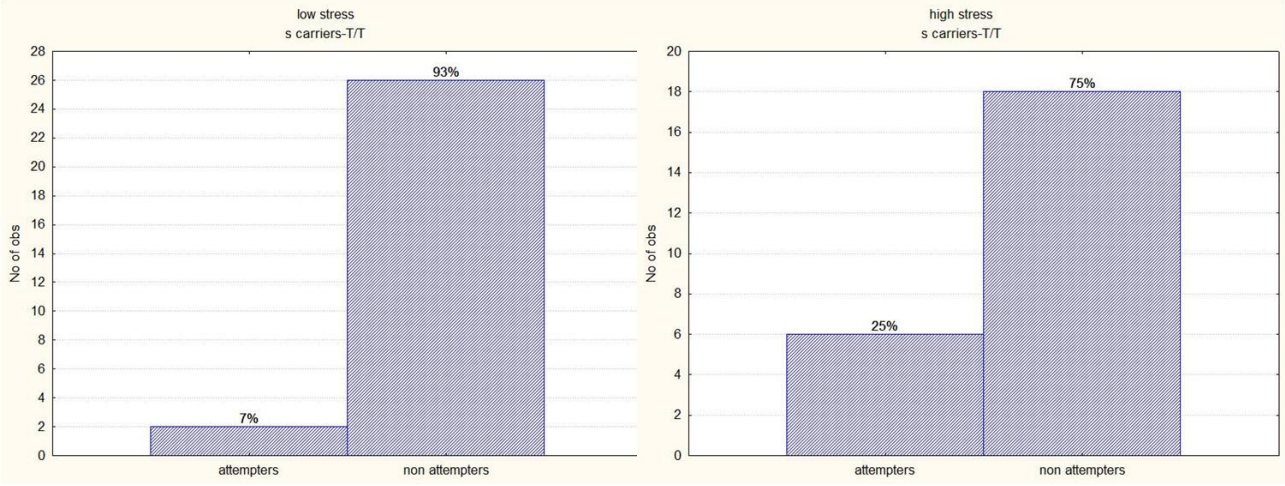
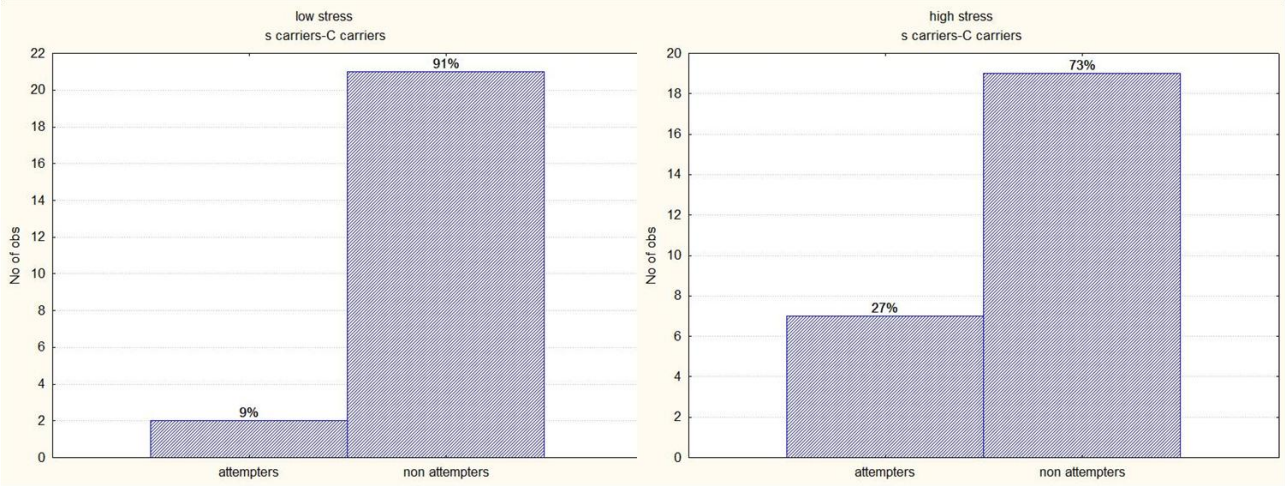


Fig.5 Percentage of attempters and non-attempters in s carriers-C carriers genotypes group, according to low (left panel) versus high (right panel) early life stress.



Discussion

This is the first study which explores the combined effect of the 5-HTTLPR genotype, the CLOCK 3111T/C polymorphism and the stress experienced in early life in conferring vulnerability to suicide in a sample of patients affected with Bipolar Disorder (BD).

The main finding of the present research is the significant association of the modelled G x G x E interaction with the behavioural phenotype of suicide attempt in BD: the relationship between early life stress and the probability to commit suicide is modulated by the action of the genetic background conferred by the 5-HTTLPR and rs1801260 combination of variants.

In individuals who are homozygotes for both the 5-HTTLPR l alleles and the 3111T genotype (l/l-T/T group) , suicidality is not related to the amount of childhood stress, whereas 1) in the l/l-C carriers the relationship between suicide and ELS is significant (p=0.047) and 2) 3111 C carriers (either if l/l homozygotes or s carriers) the likelihood of attempting suicide is directly proportional to the burden of stress suffered in early years of life.

These findings shed some light to the discrepant results of literature about the role of 5-HTTLPR l allele on vulnerability to stress: in l/l homozygotes there is not a relation between ELS and probability to attempt suicide only if they also are homozygotes for the CLOCK 3111 T allele. Conversely, in individuals who are l/l homozyghotes but carriers of the CLOCK 3111 C allele this

relationship does exist, it is positive and it is significant (the more the early stress has been, the higher the suicide probability is).

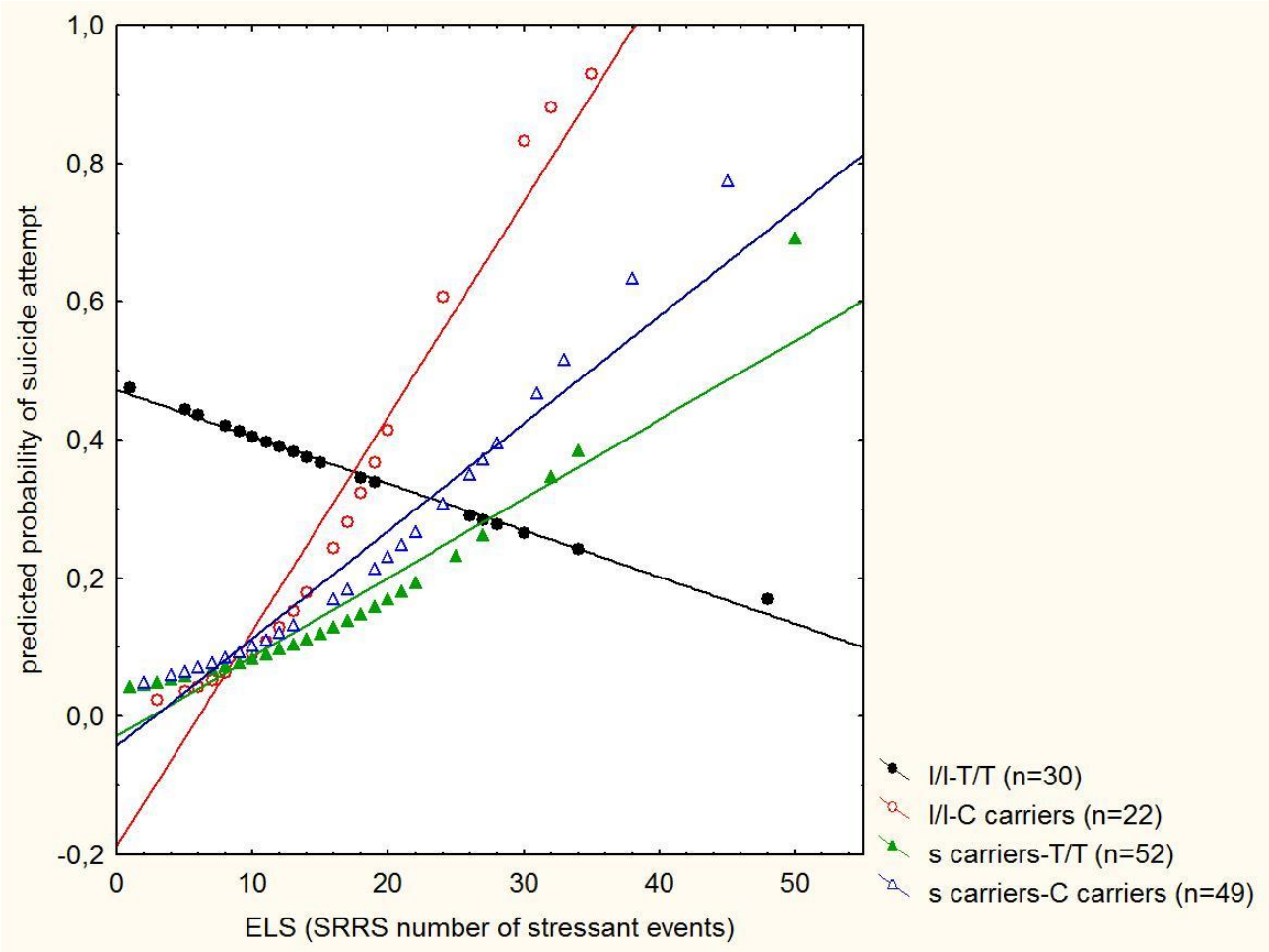
Among rs1801260 C carriers, the 5-HTTLPR s carriers are the subgroup in which this relationship showed the highest significance (*p*=0.018) but suicidality is associated to ELS in l/l-C carriers also, even if to a lesser extent (p=0.047). Overall, the G x G x E interaction seems to follow a continuous and addictive trend, so that the pattern of the stress-suicide association has its *maximum* in l/l-T/T group and its *minimum* in s carriers-C carriers, with the other two combination of variants occupying the intermediate values.

Nevertheless, even though the increasing of suicidality by the increasing of ELS was significant in l/l-C carriers, it failed to reach significance in s carriers-T/T. However it was near the 0.05 significance threshold (*p*=0.073).

To solve this ambiguity, we repeated the separate-slopes G x G x E analysis on history of suicide attempts entering the SRRS number of early life stressors, instead of the SRRS total score, as continuous predictor, with the hypothesis (to be confirmed) that different stressors could affect individuals differently and that this measure could provide a more punctual and objective estimate of ELS.

This analysis yielded similar results to those of the first model (figure 6), with superimposable and enhanced levels of significance (5-HTTLPR x 3111 T/C x ELS: LR X^2=13.40, *p*=0.009; l/l-T/T: *W*=0.5, *p*=0.45; l/l-C carriers: *W*=5.09, *p*=0.02; s carriers-T/T: *W*=4.50, *p*=0.03; s carriers-C carriers: *W*=5.78, *p*=0.01), which lead the s carriers-T/T over the 0.05 threshold.

Fig.6 Predicted probability of suicide as a function of early-life stress (SRRS number of stressors in the sample, divided according to the 5-HTTLPR-CLOCK 3111 T/C group.



However, the issue remains unsolved. It could be that the s carriers-T/T genotype is subjected to the opposite effects of the 5-HTTLPR s allele (which confers vulnerability to stress) and the 3111 T/T variant (whose potential effect on suicide is unrelated to stress), but which one wins is not inferable in our results.

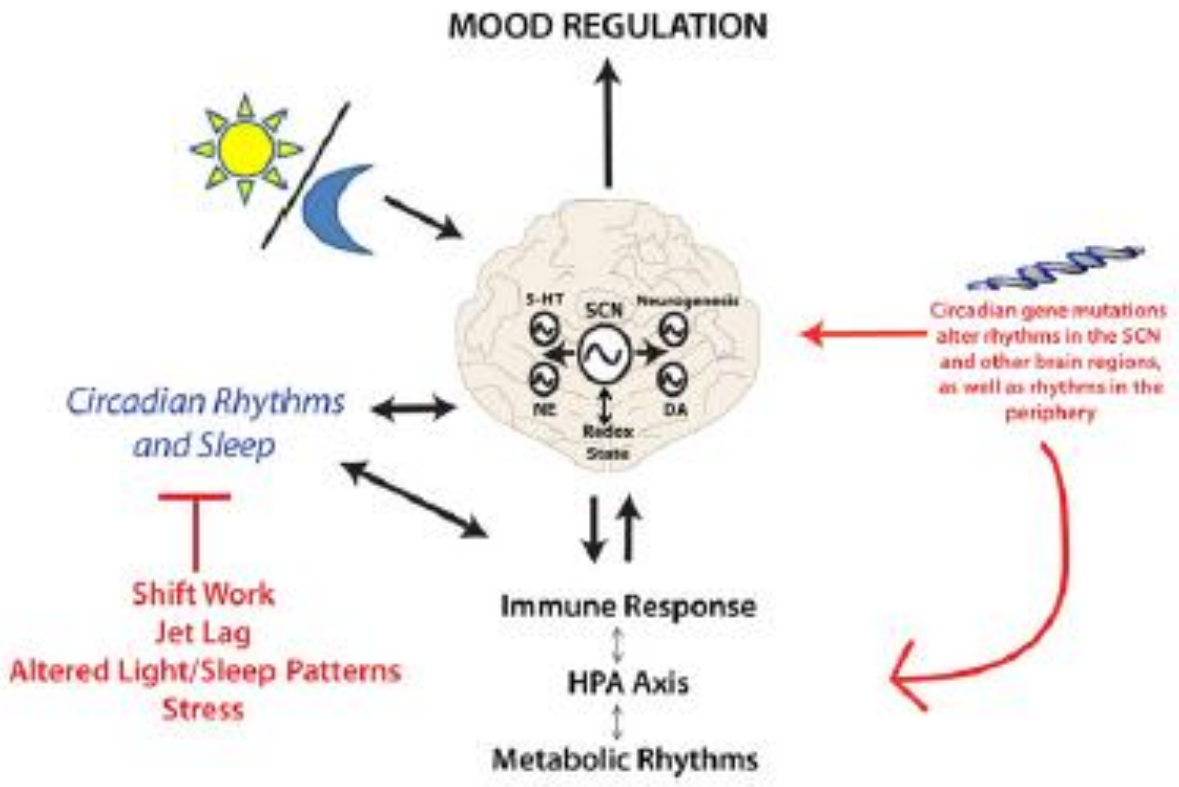
To disentangle the relative contributions of the SERT s allele and of the C CLOCK allele would require a new study with a larger sample. If in s carriers-T/T the role of ELS on suicide probability turn out to be significant, then either the s or the C allele would be sufficient for this relationship to be significant. Alternatively (if in s carriers-T/T the role of ELS on suicide probability would not

turn out to be significant), the C allele alone would be sufficient to the relationship between ELS and suicide.

What clearly results from the current research is that: 1) carrying both the 5-HTTLPR s allele and the 3111 C allele significantly increases the probability of suicide attempt as the ELS exposure increases; 2) on the contrary, in the double homozygosis for the l variant of the 5-HTTLPR and the T variant of the rs1801260, suicide is not associated with childhood stress and 3) there is a dissociation among subjects with the l/l 5-HTTLPR genotype, whereby in the C carriers the relationship between early stress and suicide attempt is significant whereas in the T/T it is not.

The circadian clock influence multiple systems and pathways which are thought to be involved in the ethiopathogenesis of Mood Disorders (McClung) and some of these systems, in turn, regulate the circadian timing (fig.7).

Fig.7 The reciprocal interactions between the circadian clock and the mood regulation system.



The serotonergic and the circadian systems are the principal regulatory network of the nervous system; they influence the development of the brain neural substrates of biological rhythms and

affective functioning and are reciprocally connected within, at both neuroanatomical and genetic level (Ciarleglio, Resuehr et al.) as well as with the stress response system. The central clock receives direct and indirect serotonergic innervations and in turn sends efferences back to the mid-brain serotonergic nuclei. Similarly, at the genetic level, key 5-HT signalling molecules (such as SERT and 5-HT receptors) are expressed in the biological clock, where they exert modulatory influences on rhythms, as well as the clock genes are expressed in serotonergic neurons and regulate their circadian activity.

The serotonergic system comprises 5-HT secreting neurons and a network of genes encoding for segnaling and regulatory protein such as transcriptional factors, enzymes, receptors and the serotonin transporter. Abnormalities in this neural and genetic network are thought to be main contributors to the development and course of stress-related disorder, such as Mood Disorders, and suicidal behaviour. The gene coding for the SERT has been frequently examined in candidate-gene studies: a higher stress sensitivity in carriers of the short form of 5-HTTLPR has been consistently suggested, associating the s allele with a positive relationship between the extent of exposure to early and adult stress and the probability both of developing a major depressive episode and of showing suicidal ideation and attempts.

The brain biological clock is a timing mechanism which regulates the physiology of behavior and mood; its central circadian pacemakers neurons are localized in the hypothalamic SCN (suprachiasmatic nucleus) and its set of clock genes regulates the circadian rhythms by means of auto-regulatory transcriptional and translational feed-back oscillations. As previously mentioned, CLOCK is an essential positive regulator of the circadian feedback loop in the SCN and it seems to influence some important characteristics of Bipolar Disorder, among which sleep disruption is a diagnostic features. 3111 T/C polymorphism influences diurnal preferences in healthy subjects and causes sleep phase delay and insomnia in patients affected by BD. Among patients affected by mood-disorder, carriers of the 3111C variant had an increased occurrence of insomnia both lifetime and during their depressive episodes, and showed the persistence of sleep complaints, but not of other symptoms, after successful antidepressant treatment. This effect is unrelated to diurnal mood fluctuations. C/C homozygotes experienced a higher lifetime recurrence rate of illness episodes also (Benedetti, Serretti et al. 2003). This effect is possibly due to the triggering effect of sleep loss and phase delay on mood episodes. Moreover, CLOCK 3111 T/C SNP has shown

to be associated with non-clock biological and behavioral variables (Benedetti, Radaelli et al. 2008).

The relationship between serotonin neurotransmission and clock timing is clinically evident, too: light therapy accelerates and enhances the therapeutic effect of SSRI (selective serotonin re-uptake inhibitors) antidepressant medication (Benedetti, Serretti et al. 2003), as well as serotonergic antidepressant fluoxetine could change circadian timekeeping by phase advancing the neuronal firing of the SCN and influence CLOCK gene expression in several brain areas (Benedetti, Radaelli et al. 2008).

Moreover, the antidepressant action of Chronotherapeutics (which includes manipulations of the sleep-wake cycle, such as sleep deprivation and sleep phase advance, other than controlled exposure to light and dark) is evident in difficult-to-treat conditions such as bipolar depression, which has been associated with extremely low success rates of antidepressant drugs in naturalistic settings and with stable antidepressant response (Benedetti 2012).

Two other findings of this study need to be commented. Firstly, we found significantly higher HAM-D scores in s carriers-C carriers respect to s carriers-T/T (see table 1). Interestingly, removing from the total score the contribution of sleep disturbance-related items, the same analysis gave negative results, so that this significant difference disappeared. Secondly, attempters had a younger age at the onset of the Bipolar Disorder and have been subjected to more stress in early life than non-attempters (tab.2). As seen elsewhere in this paper, both these results are in line with the previous literature and are coherent with the conceptual frame of the modelled G x G x E interaction.

Strengths of the present study, which was retrospective and correlational in nature, include a focused and well-defined research question and the validation of a gene-gene-environment interactional model with candidate vulnerability factors. However, our results should be viewed in light of several methodological limitations. The sample size was small, compared to other genetic studies, and recruitment was in a single center and involved a single ethnic group, thus raising the possibility of population stratifications limiting the generalizability of the findings. Moreover, patients were not drug-naive, and the drug treatments administered during the course of the illness could have influenced their risk of suicide. Therefore, the present results should be validated in a prospective study with a larger sample size and considering medication.

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