



**Electrophysiological studies on the impact of repeated
electroconvulsive shocks on catecholamine systems in the rat brain**

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ABSTRACT

Electroconvulsive therapy (ECT) effectively treats depression by administration of repeated seizure-inducing electrical stimuli. Sprague-Dawley rats were administered 6 electroconvulsive shocks (ECS) over 2 weeks, and *in vivo* single unit extracellular electrophysiological activity was recorded after 48 hours. Overall firing activity in the locus coeruleus and ventral tegmental area was unchanged, suggesting the therapeutic efficacy of ECT may not be attributed to increased norepinephrine and dopamine release. There were more spontaneously active neurons in the substantia nigra pars compacta (SNc), indicating greater dopamine tone in the nigrostriatal motor pathway, which may contribute to alleviation of psychomotor retardation. In the facial motor nucleus (FMN), locally administered norepinephrine, but not serotonin, facilitated greater glutamate-induced firing, which may contribute to improved facial motricity. Current results indicate that repeated ECS enhances postsynaptic norepinephrine neurotransmission in the FMN and SNc dopamine neurotransmission, which could represent the mechanism behind the alleviation of depressive symptoms including psychomotor retardation.

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LIST OF ABBREVIATIONS

β-OH	hydroxypropyl-beta-cyclodextrin
λ	lambda
5-HIAA	5-hydroxyindoleacetic acid
5-HT	5-hydroxytryptamine, serotonin
5-HTTLPR	serotonin transporter promoter
6-OHDA	6-hydroxydopamine, oxidopamine
AD	antidepressant
ANOVA	analysis of variance
AP	anterior-posterior
APA	American Psychiatric Association
CBT	cognitive behavioural therapy
COMT	catechol-O-methyltransferase
DA	dopamine
DALY	disability-adjusted life year
DAT	dopamine transporter
DOPA	dihydroxyphenylalanine
DRN	dorsal raphe nucleus
DSM-IV-TR	Diagnostic and Statistical Manual of Mental Disorders 4 th Edition Text Revision
DV	dorsal-ventral
ECS	electroconvulsive shocks
ECT	electroconvulsive therapy

EEG	electroencephalography
FMN	facial motor nucleus
GABA	γ -aminobutyric acid
HRSD	Hamilton Rating Scale for Depression
HVA	homovanillic acid
i.p.	intraperitoneal
i.v.	intravenous
ICD-10	International Statistical Classification of Diseases and Related Health Problems 10 th Revision
ISI	interspike interval
L-DOPA	L-3,4-dihydroxyphenylalanine
LC	locus coeruleus
MADRS	Montgomery-Åsberg Depression Rating Scale
MAO	monoamine oxidase
MAOI	monoamine oxidase inhibitor
MDD	major depressive disorder, unipolar depression, clinical depression
MDE	major depressive episode
MDL-100,907	R-(+)- α -(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4- piperidinemethanol
MHPG	3-methoxy-4-hydroxyphenylglycol
ML	medial-lateral
NE	norepinephrine
NET	norepinephrine transporter

NRI	norepinephrine reuptake inhibitor
PET	positron emission tomography
rs6295	C(-1019)G (a single nucleotide polymorphism)
S+2D	single ECS followed by 2 days before recording
S+14D	single ECS followed by 14 days before recording
S30V	subconvulsive ECS (30 V)
SB-242,084	6-chloro-5-methyl- <i>N</i> -{6-[(2-methylpyridin-3-yl)oxy]pyridin-3-yl}indoline-1-carboxamide
SEM	standard error of the mean
SERT	serotonin reuptake transporter
SNc	substantia nigra pars compacta
SNRI	serotonin-norepinephrine reuptake inhibitor
SSRE	selective serotonin reuptake enhancer
SSRI	selective serotonin reuptake inhibitor
VTA	ventral tegmental area
TCA	tricyclic antidepressant
TPH	tryptophan hydroxylase
TRD	treatment resistant depression
VMAT	vesicular monoamine transporter
WHO	World Health Organization
YLD	years lived with disability

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INTRODUCTION

1. Major depressive disorder

1.1. Background

Major depressive disorder (depression, MDD) is a mental disorder characterized mainly by persistent depressed mood and loss of pleasure, and is often accompanied by psychomotor retardation. Affecting one in six people during their lifetime (Kessler *et al.* 2003), the World Health Organization (WHO; 2008) designates MDD as the leading medical condition in middle- and high-income countries contributing to overall disease burden worldwide, measured by disability-adjusted life year (DALY), which represents the number of years lost due to disability or early death. Depression also leads in years lived with disability (YLD). According to WHO, it is estimated that by 2030, depression will become the leading cause of DALY. Furthermore, amongst all mental disorders, depression has the highest risk of suicide (WHO 2001) with 15-20% of depressed patients ending their lives by suicide (Guze and Robins 1970, Goodwin and Jamison 1990). Given the heterogeneity of depressive symptoms, a particular treatment that acts primarily on one system may not alleviate a major depressive episode (MDE). There is no panacea for the treatment of depression. Treatments that act on different regions of the central nervous system or exhibit dissimilar mechanisms of action may complement one another and optimize treatment outcome. Therefore, it is of considerable interest to understand how brain regions associated with the etiopathology, symptoms, and treatment of MDD are affected by antidepressants, including the highly effective psychiatric treatment electroconvulsive therapy (ECT).

Given that there are no concrete markers or tests to determine the presence of MDD, diagnoses of the disorder is based solely on subjective self-reported experiences, behaviour reported by friends and family, and assessment of symptoms by a physician or psychologist, in line with diagnostic criteria set by the American Psychiatric Association's (APA's) Diagnostic and Statistical Manual of Mental Disorders 4th Edition Text Revision (DSM-IV-TR; APA 2000a). Described in the DSM-IV-TR are three levels of MDD severity, including mild, moderate, and severe. Another classification system that uses similar conditions for the diagnosis of depression is the International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10; WHO 1992), which is less descriptive in terms of symptoms. Thus, the ICD-10 is less preferred compared to the DSM-IV-TR when studying depression.

For an individual to be diagnosed with MDD, one must exhibit a single or recurrent MDEs, as opposed to brief depressed mood as experienced in everyday life. An individual experiencing a MDE must display five or more symptoms over a period of two weeks, as described by the DSM-IV-TR, including persistent depressed mood, anhedonia (defined as the inability to attain pleasure from activities that were once pleasurable), changes in eating and/or weight, changes in sleep, changes in motor activity (including psychomotor retardation), fatigue, diminished self-worth, impaired concentration, and thoughts of death. Psychomotor retardation, a symptom which may or may not be present in depression, can consist of a slowing down of thought, impaired motor control of the body and facial expressions, and difficulty performing ordinary physical activities such as walking (Tryon 1991). The Montgomery-Åsberg Depression Rating Scale (MADRS) and Hamilton Rating Scale for Depression (HRSD) are types of questionnaires that clinicians

can use to determine the severity of a MDE by scoring the significance of the symptoms. For instance, the level of psychomotor retardation can range from zero (i.e., normal speech and thought) to four (i.e., complete stupor) in the HRSD. Along with the other symptoms, all of which should be outside the parameters of the individual's normal behaviour, persistent depressed mood and/or anhedonia must be present.

When an individual does not remit after two adequate trials of antidepressants from different classes, they are diagnosed with treatment resistant depression (TRD; Souery *et al.* 1999, Fava 2003). With each subsequent failed antidepressant (AD) treatment, fewer individuals achieve remission (Rush *et al.* 2006b). The urgency of treating depression before it becomes more difficult furthers the need for an adequate understanding of the mechanism of action of antidepressant treatments. Response to an antidepressant treatment is described as a greater than 50% reduction of symptoms, whereas clinical remission is defined as the absence of symptoms for two months (APA 2000a, Rush *et al.* 2006a). Relapse is the return of a MDE which interrupts remission. Currently, ECT is more efficacious in the treatment of depression than any other antidepressant treatment (Pagnin *et al.* 2004). However, its mechanism of action has yet to be fully elucidated.

In this dissertation, the effects of electroconvulsive shocks (ECS) on components of the monoamine systems implicated in the pathophysiology and therapeutics of MDD were assessed. Repeated ECS in Sprague-Dawley rats was studied in relation to its effects on the electrophysiological activity of norepinephrine (NE) and dopamine (DA) neurons of the locus coeruleus (LC) and ventral tegmental area (VTA), respectively. Given the alleviation of psychomotor retardation with ECT treatment, also assessed were the effects of repeated ECS on the electrophysiological activity of DA neurons of the substantia

nigra pars compacta (SNc) and on the responsiveness of facial motor nucleus (FMN) neurons to locally administered NE and 5-hydroxytryptamine (5-HT, serotonin). Furthermore, the receptor subtypes that mediate monoamine facilitation of FMN activity were characterized. The clinical relevance of repeated ECS was also investigated by observing its effects in relation to the effects of a single ECS and repeated subconvulsive shocks, both of which are not clinically efficacious.

1.2. Pathophysiology

The cause of MDD is multifactorial as both genetics and environment mediate the risk of developing and influencing the severity of the disorder (Kendler *et al.* 1995, Caspi *et al.* 2003, Farmer *et al.* 2005). It is a combination of genetic variation and diverse environmental conditions that may also explain the disparity in treatment response between depressed individuals. An example of an environmental factor that poses a strong risk of initiating a major depressive episode is stress (Williamson *et al.* 1998, Caspi *et al.* 2003).

A strong predictor of TRD and indicator of poor prognosis is a family history of depression (Keller 2005, Berlim and Turecki 2007). In a family study linking heritability to risk of MDD, first degree relatives of depressed patients were shown to have a 2-4 times higher risk of MDD, and twin studies showed heritability to be between 31% and 42% (Sullivan *et al.* 2000, Lesch 2004, Kendler *et al.* 2006, Levinson 2006). Heritability, as well as prevalence of MDD, is higher in women compared to men (Marcus *et al.* 2005). However, not only are there many studies reporting conflicting results regarding an association between particular genetic polymorphisms and the etiology and treatment

of depression, there has been little linking treatment response to ECT with genetic variation.

A positron emission tomography (PET) study showed an increase in monoamine oxidase-A (MAO-A) density during depression (Meyer *et al.* 2006), an enzyme that preferentially deaminates 5-HT, DA, NE, epinephrine, and melatonin. In turn, monoamine oxidase inhibitors (MAOIs) help to maintain synaptic concentrations of monoamines, which are associated to their antidepressant response, by inactivating the activity of MAOs.

2. Monoamine hypothesis

2.1. Background

Monoamine neurotransmitter deficiency is the leading hypothesis in the biological etiology of depression. The monoamine systems, including the indolamine 5-HT, and the catecholamines NE and DA (Figure 1), have been implicated in MDD because many antidepressants interfere with their inactivation processes via the inhibition of intraneuronal reuptake or metabolism, or by increasing their release or ligand-receptor sensitivity, thereby increasing neurotransmission (Blier and de Montigny 1994, Feighner 1999, Delgado 2000, Leyton *et al.* 2000, Elhwuegi 2004, Dunlop and Nemeroff 2007). Along with evidence indicating that most ADs enhance monoamine neurotransmission, it was shown that reserpine, a monoamine depleter, produces depression-like effects (Baumeister *et al.* 2003). It is suggested that impairment to one or more of these monoamine neurotransmitter systems may lead to depression, and elevating neurotransmission in one or more of these systems with AD treatments and/or ECT may reverse depressive symptoms. Furthermore, given the extensive reciprocal interactions

between the monoamine systems, a change in even a single element of one system will likely impact the neurotransmission of another monoamine system (Figure 2; Guiard *et al.* 2008). It is important also to assess the effects of antidepressant treatments, including ECT, on postsynaptic components of monoamine transmission in regions involved in the causation and/or symptomatology of depression, such as the hippocampus and facial motor nucleus, both of which are known to receive strong serotonergic and noradrenergic innervation (Menkes *et al.* 1980, de Montigny 1984). In particular, monoaminergic transmission in the facial motor nucleus serves as a model for the lower motor neurons of spinal cord, given that both populations of neurons are similarly modulated by 5-HT and NE (White and Neuman 1980, Commissiong 1981).

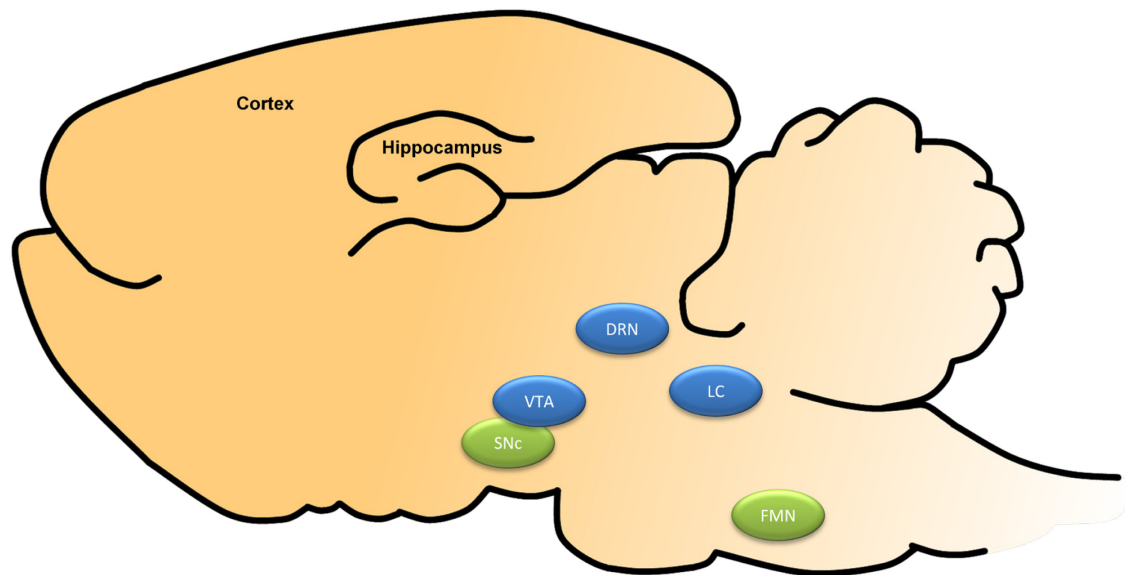


Figure 1. Monoamines implicated in the pathophysiology of major depressive disorder. Implicated in the pathophysiology of major depressive disorder are the monoamines serotonin of the raphe nucleus, norepinephrine of the locus coeruleus, and dopamine of the ventral tegmental area and substantia nigra pars compacta, which project widely throughout the central nervous system, including to the cortex, hippocampus, and facial motor nucleus. Darker (or blue) ellipses are regions in which the monoamine neurotransmitters implicated in depression are synthesized. Lighter (or green) ellipses are regions involved in motor control.

2.2. Serotonin system

Serotonin is an indolamine neurotransmitter synthesized from the amino acid tryptophan, a process regulated by tryptophan hydroxylase-2 (TPH2) in the brain and TPH1 in the periphery (Côté *et al.* 2003, Walther and Bader 2003). In the brain, serotonin is produced in the raphe nuclei, located in the brainstem, and is important in the regulation of mood, appetite, and sleep (Belmaker and Agam 2008, aan het Rot *et al.* 2009). Impairment of this system is implicated in MDD, anxiety, and dementia (Serretti *et al.* 2007). The 5-HT neurotransmitter is stored in presynaptic vesicles, mediated by the vesicular monoamine transporter (VMAT). Upon release into the synapse, the serotonin reuptake transporter (SERT) drives 5-HT from the synaptic cleft back into the presynaptic neuron. Excess 5-HT is metabolized by MAO-A at the cleft and MAO-B within the presynaptic neuron. The primary metabolite produced is 5-hydroxyindoleacetic acid (5-HIAA). In direct correlation to the monoamine hypothesis, individuals with MDD and very low levels of 5-HIAA (< 15 ng/ml), as opposed to depressed individuals with higher 5-HIAA levels, attempted and successfully committed suicide more often and by more violent means (Asberg *et al.* 1976).

Selective serotonin reuptake inhibitors (SSRIs) block SERT activity. Long-term treatment with SSRIs causes increased neurotransmitter concentration in the extracellular space and ultimately leads to an adaptive desensitization of somatodendritic 5-HT_{1A} and terminal 5-HT_{1B} autoreceptors (Piñeyro and Blier 1999). In the raphe nuclei, 5-HT_{1A} autoreceptors negatively mediate 5-HT transmission, acting as brakes on this system presynaptically by controlling neuronal firing rate. Although there is an initial decrease in 5-HT firing activity due to the increased activation of autoreceptors, the inhibitory 5-

HT_{1A} receptors desensitize after long-term treatment and allow for the normalization of neuronal firing rate (Blier and de Montigny 1983). Furthermore, the function of the reuptake transporters is continually inhibited by the SSRI, eventually resulting in an increase in 5-HT levels at the synapse and an overall augmentation in serotonergic neurotransmission. Along with postsynaptic 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors, other 5-HT receptors mediate the effects of 5-HT throughout the body. Given that 5-HT neurons of the raphe nuclei project widely and are involved in reciprocal interactions between the other two monoamine systems (Guiard *et al.* 2008), a change to the 5-HT system may lead to altered activity and overall neurotransmission of the DA and/or NE systems.

Several gene polymorphisms have been associated with MDD, including the serotonin transporter promoter gene polymorphism which affects SERT function (5-HTTLPR), and the 5-HT_{1A} receptor rs6295 polymorphism C(-1019)G (Smits *et al.* 2004, Arias *et al.* 2005, Bozina *et al.* 2006, Hong. *et al.* 2006, Yu *et al.* 2006, Kato *et al.* 2009). Given that SERT is the primary known target of most ADs, polymorphisms to 5-HTTLPR have been studied at length. Compared to the long l allele of SERT, the short s allele is associated with lower function of the transporter. That is, SERT uptakes twice as much 5-HT when there are two l alleles compared to two s alleles (Lesch *et al.* 1996). However, studies on the function of either allele in relation to the susceptibility of developing depression and attempted suicides have produced mixed conclusions (Du *et al.* 1999, Moreno *et al.* 2002, Neumeister *et al.* 2002, Courtet *et al.* 2005). The rs6295 polymorphism for the 5-HT_{1A} receptor appears to mediate over-expression of presynaptic 5-HT_{1A} autoreceptors on neurons of the raphe nuclei and reduce 5-HT neurotransmission,

but evidence supporting this concept is also mixed (David *et al.* 2005). Nonetheless, it was found that the 5-HT_{1A} receptor polymorphism is associated with MDD and completed suicide attempts (Lemondé *et al.* 2003), regardless of the level of 5-HT_{1A} receptor expression.

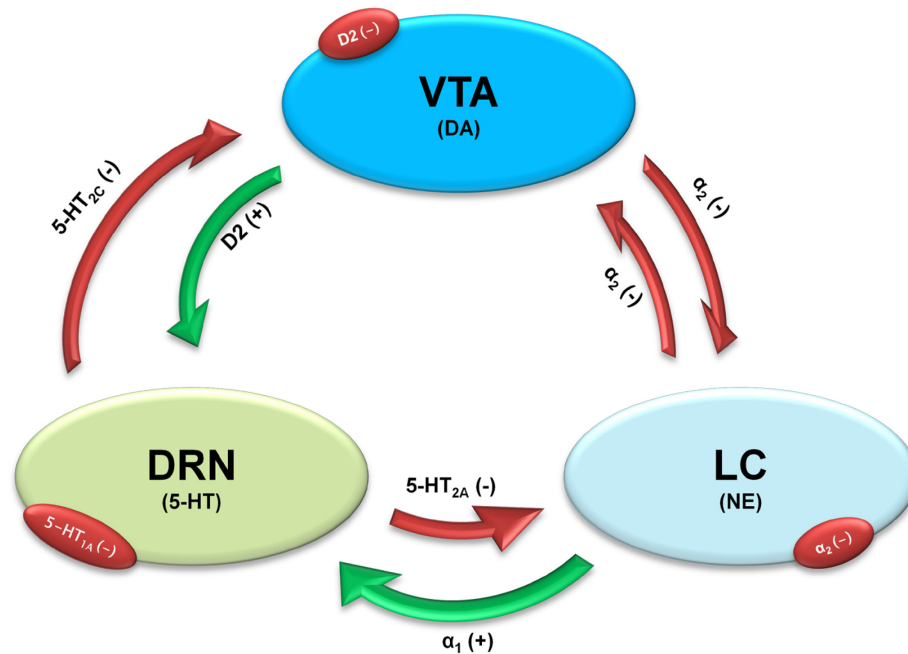


Figure 2. Schematic representation of the reciprocal interactions between the ventral tegmental dopamine, locus coeruleus norepinephrine, and dorsal raphe nucleus (DRN) serotonin neurons. Not shown are the high affinity reuptake transporters on the neurons. A positive sign (+) indicates a stimulatory pathway and a negative sign (-) indicates an inhibitory pathway. The inhibitory autoreceptors on DA, NE, and 5-HT neurons are the D2, α_2 -adrenoceptor, and 5-HT_{1A} receptors, respectively. Given the reciprocal interactions between the monoamine systems, an antidepressant treatment could affect all three systems both presynaptically and postsynaptically. Adapted from Guiard *et al.* 2008.

2.3. Dopamine system

Dopamine is a catecholamine neurotransmitter synthesized from the amino acid tyrosine, a process regulated by tyrosine hydroxylase and dopamine decarboxylase, and is produced primarily in the VTA and substantia nigra, both located in the midbrain (Figure 3; Shiroma *et al.* 2010). DA neurons of the VTA are highly implicated in the compulsion, pleasure, and natural reward pathways of the brain and are important in motivation. DA

neurons of the SNc, as part of the nigrostriatal pathway, primarily mediate motor control, in which an 80% loss of these neurons leads to Parkinson's disease (Kish *et al.* 1988). On the other hand, impairments to DA neurons of the VTA are implicated in the pathophysiology of psychiatric disorders such as MDD (Dunlop and Nemeroff 2007). Similarly to 5-HT, DA is stored in vesicles by VMAT. The dopamine transporter (DAT) inactivates DA transmission by removing it from the synaptic cleft (Torres 2006), but due to the structural similarity between DA and NE, the norepinephrine transporter (NET) may also transport synaptic DA into the presynaptic neuron. Catechol-O-methyltransferase (COMT) and MAO-A degrade DA at the cleft and MAO-A/B catabolize the DA neurotransmitter in the presynaptic neuron.

Presynaptic D2 autoreceptors mediate DA release through negative feedback regulation. Compared to other DA receptors that mediate DA neurotransmission, the D2 receptor is the best understood because most antipsychotics and agonists for the treatment of Parkinson's disease bind to this receptor. Pramipexole is an example of a D2/D3 receptor agonist that has also demonstrated therapeutic effectiveness in the treatment of depression in comparison to placebo (Corrigan *et al.* 2000).

2.4. Norepinephrine system

Norepinephrine is a stress-related hormone and neurotransmitter synthesized from the dopamine neurotransmitter, a process regulated by dopamine β -hydroxylase (Figure 3; Belmaker and Agam 2008). NE in the brain is primarily produced in the LC, located in the brainstem. Stored in presynaptic vesicles via VMAT, and released into the synapse from noradrenergic neurons of the LC, NE largely has an excitatory effect on most regions of the brain. In the synapse, NE is catabolized by COMT and MAO-A. The

action of NE is also terminated when the NET removes NE from the synapse, followed subsequently by the degradation of the catecholamine by MAO-A/B in the presynaptic neuron. When not degraded by MAOs, NE is stored presynaptically for further release. Presynaptic α_2 -autoreceptors inhibit NE firing and release through negative feedback regulation, while α_1 -, α_{2A} -, α_{2B} -, α_{2C} -, and β -adrenergic receptors mediate the noradrenergic response throughout the brain and body.

Similarly to the effects of SSRIs on terminal 5-HT_{1B} autoreceptors, prolonged administration of norepinephrine reuptake inhibitors (NRIs) increases extracellular levels of NE which desensitize α_2 -autoreceptors on LC neuron terminals, ultimately increasing NE levels in postsynaptic regions, such as the hippocampus and frontal cortex (Invernizzi *et al.* 2001). Given that there is no desensitization of somatodendritic α_2 -autoreceptors, NE levels increase despite a lack of recovery in neuronal firing activity (Lacroix *et al.* 1991; Parini *et al.* 2005). The disparity in NRI-induced autoreceptor desensitization is explained by the receptor subtype. Somatodendritic receptors belong to the α_{2A} subtype in comparison to terminal receptors which are α_{2C} -adrenoceptors (Callado and Stamford 1999; Esteban *et al.* 1999). Considering how depressed patients are often resistant to particular antidepressant options, it is

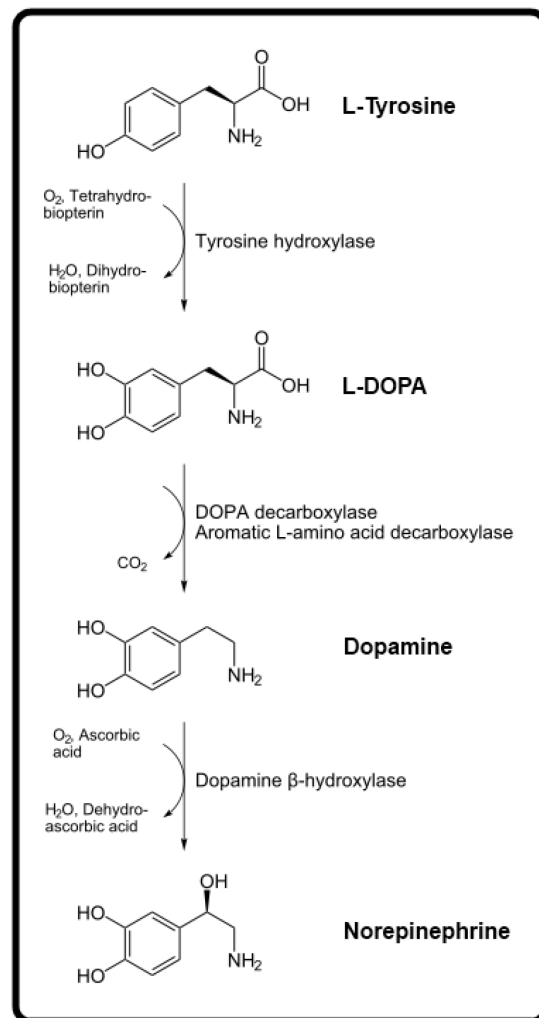


Figure 3. Dopamine and norepinephrine synthesis. The catecholamine norepinephrine is synthesized from the dopamine neurotransmitter, which is in turn synthesized from L-DOPA (L-3,4-dihydroxyphenylalanine).

possible that each monoamine system may be components of different depression subtypes, in which mood is mediated by 5-HT, while drive and motivation is affected by NE, and pleasure associated with DA (Montgomery and Schatzberg 1998).

2.5. Issues not addressed by the monoamine theory

Despite the strong evidence supporting the role of monoamines in the pathophysiology of depression, other systems may be involved. This idea is supported by studies that investigated monoamine depletion by means of decreasing the availability of a particular monoamine. 5-HT depletion induced by a low tryptophan diet has been shown not to cause depressive symptom in both people who are healthy and those who have MDD and have not remitted (Bell *et al.* 2001). NE and DA depletion also do not cause symptoms indicative of depression (Delgado 2000).

Several drugs exhibit the antidepressant response without elevating monoamine neurotransmission. Many tricyclic antidepressants (TCAs), if not all, enhance the responsiveness of postsynaptic 5-HT receptors in the hippocampus and many inhibit SERT-mediated 5-HT reuptake (Blier *et al.* 1987). However, trimipramine, an effective TCA, does not display any inhibition of 5-HT reuptake and its greatest affinity is for the H₁ histamine receptor (Hyttel 1982, Cournoyer *et al.* 1987). Tianeptine, another atypical TCA that also effectively treats depression, challenges the monoamine theory because it is a selective serotonin reuptake enhancer (SSRE; Kasper and McEwen 2008), yet the efficacy of tianeptine in the treatment of MDD has been shown to be at least equivalent to that of SSRIs, the most commonly used medication for clinical depression (Kasper and Olié 2002, McEwen and Olié 2005). In opposition to the monoamine theory, long-term administration of tianeptine does not increase or decrease 5-HT levels or transmission in

corticolimbic structures (Piñeyro *et al.* 1995, Malagié *et al.* 2000). In addition, tianeptine has no affinity for NE or DA receptors (Kato and Weitsch 1988, Svenningsson *et al.* 2007). It is hypothesized that the antidepressant effect of tianeptine may be attributed to its action on the glutamatergic system (McEwen *et al.* 2010). Given the heterogeneity and multifactorial nature of MDD, the monoamines may not be the only systems impaired in depression. Nevertheless, antidepressants acting on the monoamine systems have been efficacious in the alleviation of depressive symptoms and are still the primary therapies for this widespread illness.

3. Antidepressant treatments

3.1. Pharmacological medications

The monoamine theory took form in the 1960s with the monoamine-acting TCAs and MAOIs which both displayed antidepressant effects (Schildkraut 1965). Although efficacious in the treatment of MDD, TCAs and MAOIs are less frequently prescribed due to alternative medications that are more selective and elicit less adverse side effects. These alternatives include the SSRIs, serotonin-norepinephrine reuptake inhibitors (SNRIs), and augmentation agents when necessary. TCAs are a family of reuptake blockers for 5-HT and/or NE neurotransmitters, with side-effects correlating to the altered transmission of 5-HT and/or NE, including drowsiness and increased heart rate. MAOIs, although potentially fatal when interacting with foods that contain tyramine, effectively treat depression by blocking MAOs that degrade the neurotransmitters 5-HT, NE, and DA.

The most commonly used antidepressant agents are from the SSRI class. Long-term treatment with SSRIs leads to a desensitization of somatodendritic 5-HT_{1A} and terminal

5-HT_{1B} autoreceptors over at least a couple of weeks and helps to enhance 5-HT neurotransmission, which is believed to be the primary mechanism of the antidepressant response of SSRIs (Piñeyro and Blier 1999). Although fewer adverse side effects are presented with SSRI treatment, effects inherent to increased 5-HT transmission may occur, including anxiety and decreased appetite.

A class of antidepressants that enhance synaptic levels of NE is the NRI. Antidepressants that enhance both 5-HT and NE transmission are the SNRIs, which block the activity of SERT and NET. SNRIs also enhance DA in the prefrontal cortex and hippocampus given that DA reuptake in these regions is mediated more by NET than DAT (Bymaster *et al.* 2002, Guiard *et al.* 2008). SNRIs, although not as extensively used in the clinical treatment of MDD compared to SSRIs, given that SSRIs exhibit adequate balance efficacy and tolerability (Cipriani *et al.* 2009), are also preferential because of their greater selectivity and fewer adverse side effects compared to TCAs and MAOIs. Treatments other than pharmacotherapy for depression include ECT and psychotherapy, such as cognitive behavioural therapy (CBT).

3.2. Electroconvulsive therapy

3.2.1. Brief history

The early days of convulsive therapy for the treatment of mental illnesses involved induced epileptic seizures for the treatment of psychotic symptoms associated with schizophrenia. The belief was that epilepsy and schizophrenia are mutually exclusive and that both disorders rarely existed together. In the 1930s, camphor- and later pentylenetetrazol-induced seizures were used in the treatment of schizophrenia (Meduna 1936). Along with chemically induced seizures, it appeared that electrically induced

seizures, or ECT, were also effective in the treatment of schizophrenia (Cerletti and Bini 1938). However, following the introduction of ECT and its rise in popularity in the western world, it was shown to be more efficacious in the treatment of MDD than schizophrenia (Smith *et al.* 1943). Although there was a decline in the use of ECT due to the introduction of pharmacotherapies in the 1950s, interest in ECT was restored in the 1980s, in part because of its greater efficacy in comparison to antidepressant drugs (Weiner 1979, Fink 1993, Thompson *et al.* 1994).

3.2.2. Efficacy and implications

Despite the many types of antidepressant treatments, a third of patients display only a partial response to first-line pharmacological monotherapies and a third show no improvement (Thase *et al.* 2001, Lam *et al.* 2002, Thase 2003, Trivedi *et al.* 2006). ECT, a non-pharmacological psychiatric treatment developed in the late 1930s, has demonstrated consistently high rates of improvement for depression, up to 95%, especially in patients with psychotic or psychomotor symptoms (Flint and Rifat 1998, Stoudemire *et al.* 1998, Sackeim *et al.* 2000, Petrides *et al.* 2001, Husain *et al.* 2004, Greenberg and Kellner 2005), and up to 60% in TRD (Devanand *et al.* 1991, Prudic *et al.* 1996, Khalid *et al.* 2008). Indeed, no study has ever shown an antidepressant response to pharmacological agents to be greater than ECT, although a comparison with lithium augmentation has shown equal effectiveness in patients not responding to TCAs alone (Dinan and Barry 1989, Kho *et al.* 2003, Pagnin *et al.* 2004). However, ECT is not used in clinical practice as a first-line therapy unless there is a high risk of suicide or catatonia.

Along with the efficacy of ECT in the treatment of MDD, especially in cases of antidepressant treatment resistance, high risk of suicidality, or depression with psychotic

or psychomotor retardation symptoms, ECT has been shown to be beneficial in severe non-responsive mania, non-responsive schizophrenia, and Parkinson's disease comorbid with clinical depression. However, the applications of ECT for illnesses other than MDD are less frequent (Rasmussen and Abrams 1991, Wengel *et al.* 1998, Kennedy *et al.* 2003, Mohan *et al.* 2009, Buyukdura *et al.* 2011). Along with the rapid alleviation of Parkinson's disease comorbid with depression, ECT improves motor function, which may be associated to depression (Friedman and Gordon 1992, Aarsland *et al.* 1997). Unlike with pharmacological antidepressants, the alleviation of particular symptoms and the precise mechanism of action of ECT have yet to be fully elucidated. Nonetheless, along with its greater efficacy, evidence suggests the ECT leads to an early improvement for all subtypes of depression (Sobin *et al.* 1996).

ECT side effects, including temporary short-term memory loss, confusion, nausea, muscle aches and headaches, are in part due to the anaesthesia applied during the procedure. Often patients will experience longer lasting memory problems, but rarely changes in blood pressure or heart rhythm. As for brain damage, the electricity passing through the scalp and skull is dampened and is therefore too small to cause any lasting brain damage. For example, in people with epilepsy, who experience convulsions under less controlled conditions, no lasting brain damage is detected unless the seizures are prolonged. Kindling is a phenomenon in which seizures become more spontaneously frequent and/or intense in terms of convulsive symptoms. There is evidence suggesting that ECT results in kindling (Pinel and Van Oot 1975). Other studies argue the opposite by showing that ECT exerts an anticonvulsant effect (Babington and Wedeking 1975, Handforth 1982). For instance, seizure threshold appears to increase over the course of

ECT treatment (Coffey *et al.* 1995, Kales *et al.* 1997). Kindling is associated with neuronal loss in the hippocampus, as opposed to neurogenesis in other parts of the rat brain, but no hippocampal atrophy occurs with ECS (Dam and Dam 1986, Dalby *et al.* 1996). Rather, repeated ECS is associated with increased sprouting of mossy fibers and a higher quantity of bromodeoxyuridine-stained cells in the hippocampal dentate gyrus, suggesting the occurrence of neurogenesis (Scott *et al.* 2000, Vaidya *et al.* 2000). Therefore, repeated ECS may protect against kindling and is perhaps associated with the therapeutic action of ECT.

Furthermore, although the death rate of ECT is between 1 and 10 in 100 000 people, it is similar to that of minor surgery under general anaesthesia (APA 2001, Shiwach *et al.* 2001). There are no absolute contraindications for ECT (APA 2001). However, conditions with an elevated risk of worsening with ECT include recent myocardial infarction, recent cerebral infarction, congestive heart failure, severe pulmonary conditions, and aneurysms.

About 50% of patients relapse within twelve months of receiving ECT where there is no continuation or maintenance therapy (Sackeim *et al.* 2000, Birkenhäger *et al.* 2004, Greenberg and Kellner 2005). Continuation ECT refers to the administration of ECT in order to prevent relapse. During continuation ECT, treatments are administered about once a month, for up to 6 months following the initial full course of ECT. Maintenance ECT refers to administration of ECT past these six months in order to prevent the recurrence of a new MDE. Given that many patients receiving ECT are non-responsive to other antidepressant therapies and are inherently more susceptible to depression, risk of relapse with ECT treatment is particularly high (Sackeim *et al.* 2001). The treatment

during continuation and maintenance ECT, in which the treatments are administered at the same dose as during the acute ECT phase, lowers relapse rates at 2 years to 7% (APA 2000b, Gagné *et al.* 2000, Andrade and Kurinji 2002). In comparison, treatment regimens including pharmacotherapies alone, such as with nortriptyline-lithium combination, has a relapse rate of 39% (Sackeim *et al.* 2001). The relapse rate with placebo treatment is 84%.

3.2.3. Administration

ECT treatments are given 2 or 3 times weekly for a total of between 6 and 12 treatments, more if response is not achieved, with the patient likely being hospitalized during their treatments. Prior to administration of each electrical stimuli, the patient receives general anaesthesia (e.g., methohexital, propofol) intravenously (i.v.), to make the patient unaware of the seizure and to minimize, if not completely, eliminate pain and discomfort. A muscle relaxant (e.g., succinylcholine) is used to minimize body convulsions, thereby preventing injuries such as bone fractures and bruising (APA 2001). Heart rate, blood pressure, and breathing are monitored throughout each session. Once asleep, electrodes are placed either bilaterally, right unilaterally, or bifrontally, through which a seizure-inducing electrical shock is applied. An electrical dose that exceeds the seizure threshold is required for the clinical efficacy of ECT, with seizure threshold increasing over a course of ECT (Coffey *et al.* 1995, Kales *et al.* 1997, Sackeim *et al.* 2000). Stimuli last between 1 and 2 sec, sometimes up to 8 sec, and cause a minimum 25 sec brain seizure measured by electroencephalography (EEG; Beyer *et al.* 1998, Andrade 2010). Other recommended parameters include 0.5-1.0 msec for the pulse width, 100-200

Hz for the pulse frequency, and 0.5-1.0 A for the pulse amplitude. The only evidence of a convulsion is a slight twitching of the toes, with the patient awaking within 5-10 min.

3.2.4. Biological mechanism involving monoamines

ECT effectively treats severe and resistant depression through repeated administration of seizure-inducing shocks (Cronholm and Ottosson 1960). Studies have shown that with subconvulsive shocks, or with the administration of a single shock, there is a lack of remission (Fink 1978), suggesting that a regimen of repeated seizure-inducing stimulations for at least 2 weeks may be responsible for the therapeutic efficacy of ECT. One possible theory regarding the mechanism of action of ECT is that the carefully controlled seizures cause monoamine neurotransmission in the brain to reset. Although blood levels of NE are elevated and blood brain barrier permeability are increased with ECT (Weinger *et al.* 1991), changes to CSF, plasma, and urinary levels of monoamine metabolites have been inconsistently reported. The main metabolite of NE degradation in the brain, 3-methoxy-4-hydroxyphenylglycol (MHPG), the main metabolite of 5-HT, 5-HIAA, and the major catecholamine metabolite homovanillic acid (HVA), have been shown either to increase, decrease, or not change following ECT (Lerer and Belmaker 1982, Linnoila *et al.* 1984, Devanand *et al.* 1989, Lykouras *et al.* 1990, Hofmann *et al.* 1996, Nikisch and Mathé 2008). In rats, repeated ECS has been shown to increase 5-HT neurotransmission by sensitizing postsynaptic 5-HT_{1A} receptors, particularly in the hippocampus (de Montigny 1984, Chaput *et al.* 1991, Mongeau *et al.* 1994, Piñeyro *et al.* 1995, Haddjeri *et al.* 1998, Dong *et al.* 1999). This enhancement in 5-HT_{1A} receptor sensitivity was selective, given that repeated ECS did not affect the responsiveness of NE and γ -aminobutyric acid (GABA) receptors in the hippocampus (de Montigny 1984). The

effects of repeated ECS on other components of monoamine neurotransmission, such as the firing activity of LC and VTA neurons, are uncertain (Grant and Weiss 2001, West and Weiss 2010).

Similarly to long-term TCA treatment, ECS-treated rats exhibit enhanced forebrain responsiveness to 5-HT (Figure 4; de Montigny and Aghajanian 1978, de Montigny 1984). Long-term TCA treatment has also been shown to sensitize FMN neurons to 5-HT and NE (Menkes *et al.* 1980), indicating that the FMN might be a common target for some antidepressant treatments. In the event that ECT acts on similar components of the central nervous system as TCAs, a comparable sensitization of FMN neurons may occur with repeated ECS treatment. Furthermore, depressed patients often have symptoms of psychomotor retardation, characterized in part by the slowing down of physical movement, the loss of postural control, and reduced facial motricity (Lemke *et al.* 1999, APA 2000a), such that an augmented sensitivity of FMN neurons to 5-HT and NE may indeed contribute to the antidepressant response of ECT. Therefore, it is of interest to assess the effects of repeated ECS on monoaminergic transmission in the FMN. Ultimately, the therapeutic efficacy of ECT may be attributed to an overall enhancement in monoamine neurotransmission, which can result from increases in firing patterns, synaptic monoamine levels, or receptor sensitivity.

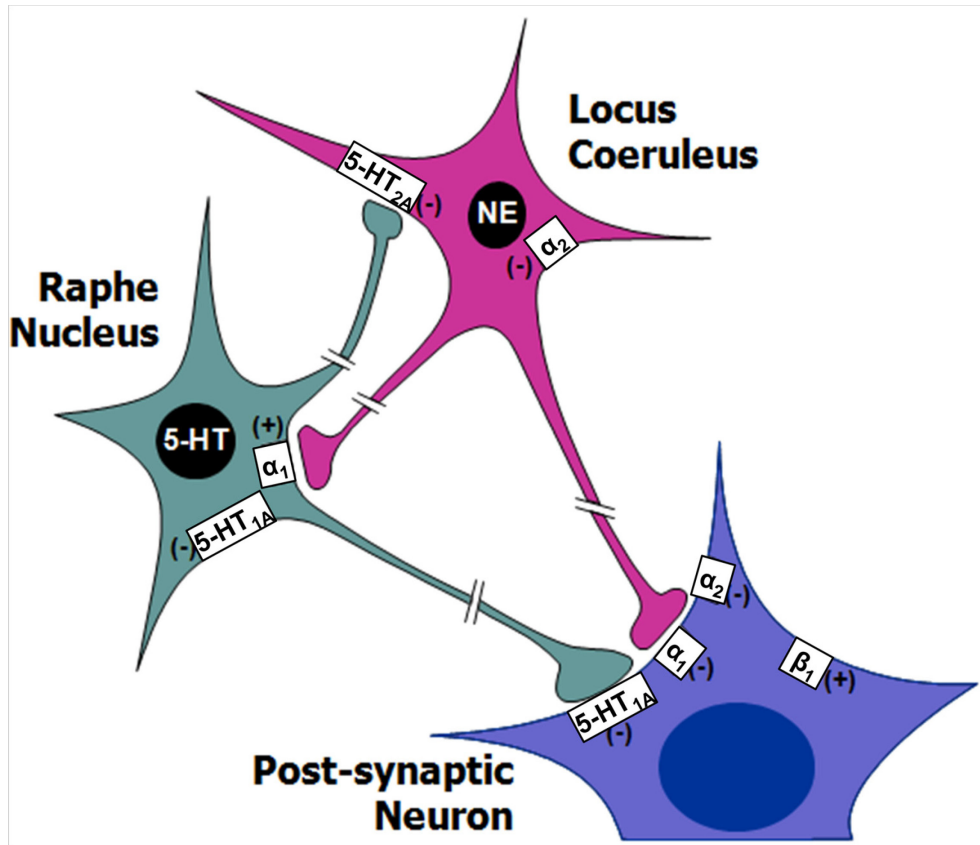


Figure 4. Sensitization of 5-HT_{1A} receptors in hippocampus with repeated ECS. Following administration of repeated electroconvulsive shocks in animals, there is an increase in sensitivity of pyramidal neurons of the CA3 region of the hippocampus to serotonin, but not norepinephrine or GABA. Adapted from Blier 2001.

MATERIALS AND METHODS

1. Animals

Adult male Sprague-Dawley rats (Charles River, St. Constant, Canada), weighing 275-350 g at the time of electrophysiological recordings, were used to study the effects of repeated ECS. They were kept under standard laboratory conditions (i.e., artificially illuminated 12:12 hour light-dark cycles with voluntary access to food and water). Rats were housed in groups of two, consisting of the ECS-treated animal and negative control (naïve, sham, subconvulsive, or single ECS with recordings after 2 or 14 days). Animals were handled in strict accordance to the guidelines of the Canadian Council on Animal Care, and experimental protocols were approved by the local Animal Care Committee (University of Ottawa, Institute of Mental Health Research, Ottawa, Canada).

2. Administration of electroconvulsive shocks

Treated animals were administered a seizure-inducing shock (10 msec pulses of 150 V at 50 Hz for 1 sec) 3 times weekly, over a 2 week period, for a total of 6 ECS.

Stimulations were generated from an S45 stimulator (Grass Technologies, West Warwick, USA) and were administered through needles, which served as electrodes, inserted subcutaneously and bilaterally in the temporal region between the eye and ear (Figure 5). Light

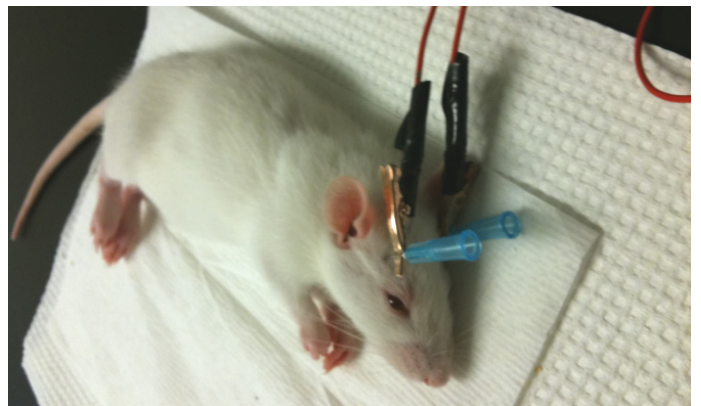


Figure 5. Administration of ECS in Sprague-Dawley rats. Electroconvulsive shocks were administered in animals three times weekly for two weeks, separated by at least one day, with the electrical current induced by needles placed subcutaneously in the temporal region.

isoflurane anaesthesia (Benson Medical Industries, Markham, Canada) was used to assist in the placement of the needles. Each ECS of 150 V induced a generalized convulsion lasting 5-15 sec followed by distinct attenuated motility not observed in negative control animals. Animals in the subconvulsive group received the same treatment regimen but voltages were lowered to 30 V. Animals that received a single ECS were administered a single stimulation at 150 V. Sham animals underwent placement of the needles under anaesthesia but did not receive the ECS. Naïve animals did not receive any handling.

3. Electrophysiological recordings

In vivo electrophysiological activity was recorded 48 hours following the final stimulation in chloral hydrate (400 mg/kg, intraperitoneal, i.p.) anaesthetized animals; Sigma-Aldrich, St. Louis, USA) secured to a stereotaxic apparatus. Chloral hydrate was used because its anaesthetic effect is rapid and levels remain high longer than agents such as isoflurane (Beland *et al.* 1998). Supplemental doses of anaesthesia were applied (100 mg/kg, i.p.) to maintain a full anaesthetic state, demonstrated by a lack of nociceptive reaction to hind paw or tail pinching. Body temperature was maintained at 37°C by a thermostatically controlled water heating pad (Seabrook Medical Instruments, Saint-Hyacinthe, Canada). A burr hole was drilled through the skull directly overlying the desired neuronal nucleus, according to stereotaxically defined coordinates (Paxinos and Watson 1998).

Extracellular single-unit electrophysiological activity was recorded using glass micropipettes filled with 2 M NaCl with impedances ranging from 3-6 MΩ. Each descent in or around the nucleus of interest, whether successful in locating a neuron or not, is referred to as a track. Tracks used in the assessment of the effects of repeated ECS were

those between the first and last successful track. In the LC, VTA, and SNc, spontaneous firing activity was recorded for at least 1-2 min and only cells with a stable firing rate and uniform spike amplitude were used. Neurons were identified by action potential shape, action potential duration, frequency of firing, and sound of electrical activity as projected by the audio monitor, described later. Electrode potentials were passed through a high-input impedance monitor and were recorded with Spike2 (Cambridge Electronic Design, Cambridge, UK).

4. Identification of neurons

4.1. NE neurons of the LC

Single-barrelled glass micropipettes were positioned using the following coordinates, in mm from lambda (λ , a physical landmark on the rat skull in which coordinates for underlying brain regions are based), according to Paxinos and Watson (1998): anterior-posterior (AP) -1.0 to -1.2; medial-lateral (ML)

± 1.0 to ± 1.3 ; and dorsal-ventral (DV) +5.0 to +7.0. Spontaneously active NE neurons were identified using the following criteria *in vivo*, as summarized by Ramirez and Wang (1986): a regular firing rate of 1.0-4.0 Hz; a biphasic (positive-negative) spike waveform with duration of about 2.0 msec; an occasional notch in the initial rising deflection; and a response to noxious stimulation, such as a pinch to the contralateral hind paw, which consists of a volley of action potentials followed by a short quiescent period with no firing activity (Figure 6).

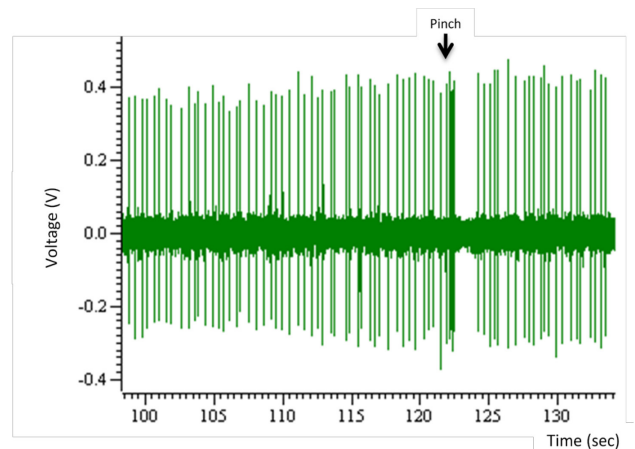


Figure 6. Example of an electrophysiological recording of a NE neuron recorded from the LC. Action potentials appear as spikes. A distinct characteristic of NE neurons is an induced volley of action potentials followed by quiescence in response to a pinch of the contralateral hind paw.

4.2. DA neurons of the VTA

Single-barrelled glass micropipettes were positioned using the following coordinates, in mm from λ : AP +3.0 to +3.6; ML \pm 0.6 to \pm 1.0; and DV +6.5 to +9.0. Spontaneously active DA neurons were identified using well-established criteria *in vivo* (Figures 7 and 8; Bunney *et al.* 1973, Ungless *et al.* 2004): a firing rate of 0.5-7.0 Hz; a biphasic or triphasic (positive-negative-positive) spike waveform greater than 3.0 msec; a broad initial positive phase which is greater than 1.1 msec from the start of the action potential to the negative trough; an occasional notch in the initial rising deflection; slow bursting activity characterized by spike amplitude decrement; and a distinct low-pitch sound as projected by the audio monitor.

4.3. DA neurons of the SNc

Single-barrelled glass micropipettes were positioned using the following coordinates, in mm from λ : AP +3.4 to +4.0; ML \pm 1.8 to \pm 2.2; and DV +7.6 to +8.0. DA neurons were identified using the same criteria *in vivo* as DA neurons of the VTA (Figures 7 and 8).

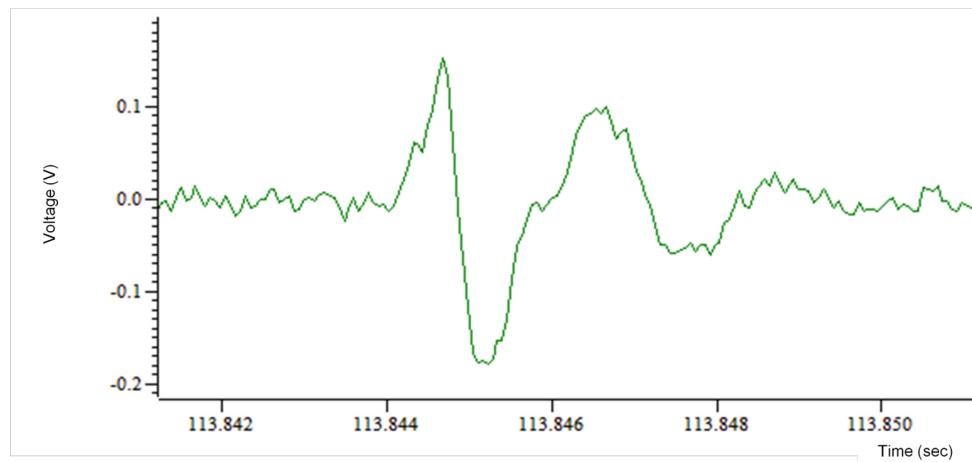


Figure 7. Enlarged view of a single action potential from a recording of a DA neuron. Action potentials of DA neurons are similar in shape in both the VTA and SNc.

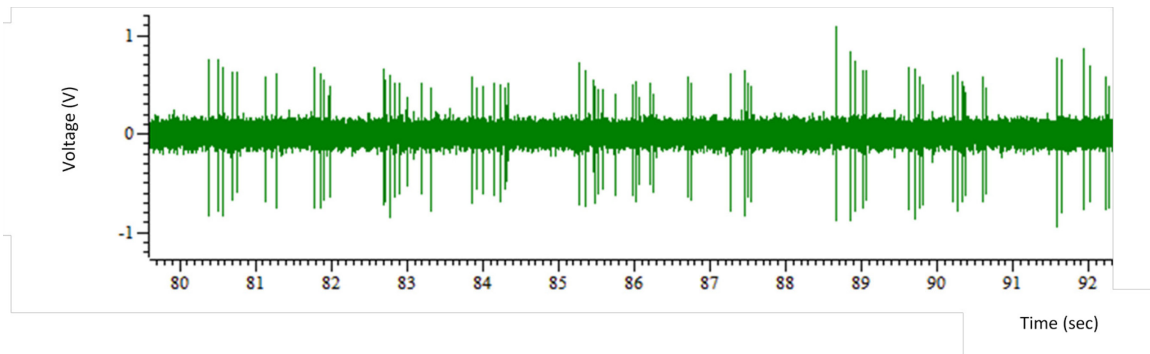


Figure 8. Example of an electrophysiological recording of a DA neuron recorded from the VTA. Action potentials appear as spikes, showing burst-like firing. DA neurons in the VTA and SNc have similar firing rates but measures of burst activity are higher in the VTA. NE neurons recorded from the LC exhibit lower firing rates and burst activity.

4.4. Lower motor neurons of the FMN

The sensitivity of FMN neurons to 5-HT and NE was analyzed following repeated ECS. Given that lower motor neurons of the FMN are quiescent in anaesthetized animals, neurons were activated with locally administered glutamate (0.1 M, pH 8.0) via multi-barrelled iontophoretic pipettes (Figure 9). Individual neurons required different concentrations of glutamate to induce firing activity. To accommodate for this variability, the current used to eject glutamate was adjusted for each neuron. The remaining side barrels were filled with 2 M NaCl for automatic current balancing, 5-HT (10 mM in 200 mM NaCl, pH 4.0), and NE (10 mM in 200 mM NaCl, pH 4.0). To promote filling, the 5-HT and NE barrels were preloaded with fibreglass strands. The central recording barrel was loaded with 2 M NaCl. The duration of the iontophoretic ejections of 5-HT and NE was kept constant at 60 sec for all experiments. Micropipettes were positioned using the following coordinates, in mm from λ : AP -2.3 to -2.5; ML \pm 2.2 to \pm 2.6; and DV +8.0 to +8.5.

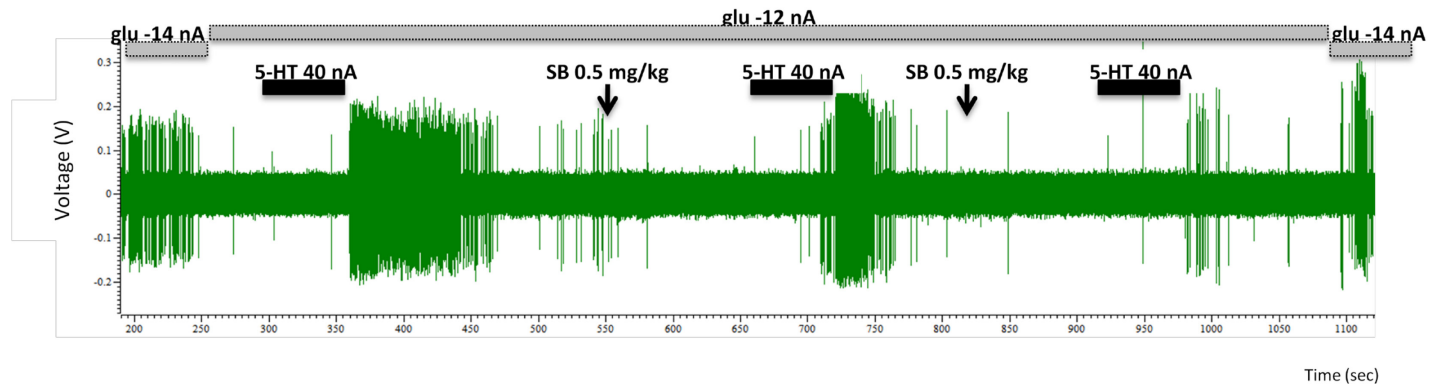


Figure 9. Example of an electrophysiological recording of a FMN lower motor neuron. Locally applied glutamate induces excitation of FMN neurons and 5-HT and NE facilitate this excitation. To characterize the serotonergic and noradrenergic receptors in the FMN, the levels of 5-HT- and NE-induced facilitation of glutamate excitation is assessed before and after systemic injection of selective antagonists at increasing doses.

5. Pharmacological agents and administration

5.1. Administration

Prior to electrophysiological recordings, a catheter was inserted into the lateral tail vein for systemic i.v. injection of pharmacological agents when applicable.

5.2. Characterization of 5-HT and NE receptors in the FMN

Once a lower motor neuron of the FMN was identified and deemed stable, the 5-HT and NE receptors that facilitate excitation of FMN neurons were tested to identify their subtype with the use of i.v. tail vein injections of selective antagonists for the receptor of interest (Figure 9). Antagonists included MDL-100,907 which is selective for 5-HT_{2A} receptors, SB-242,084 for 5-HT_{2C} receptors, idazoxan for α_2 -adrenoceptors, and prazosin for α_1 -adrenoceptors. Firstly, electrophysiological activity of a lower motor neuron of the FMN was induced with glutamate. The glutamate current was then reduced slightly past the point where activity of the neuron was as close as possible to none. Following a 100 sec period of stable activity, 5-HT or NE was locally ejected for 60 sec through iontophoresis and induced excitation of the neuron. The neuron was further recorded for

100 sec as the excitatory effect of 5-HT or NE gradually wore off. Thus, the total effect of either 5-HT or NE over 160 sec was used for the assessment of the effects of repeated ECS on the responsiveness of FMN neurons to these monoamine neurotransmitters. Subsequently, the antagonist for the receptor of interest was injected i.v. through tail vein into the animal. It was then ensured that the pharmacological agent reaches the brain by waiting 100 sec. This was followed by further excitation of the neuron using the exact same current of either 5-HT or NE, and same 160 sec time frame of excitation similar to that prior to administration of the selective antagonist.

5.3. Pharmacological agents

The selective 5-HT_{2A} receptor antagonist MDL-100,907 and selective 5-HT_{2C} antagonist SB-242,084 were generously provided as gifts by Servier Laboratories (Neuilly-sur-Seine, France). Sodium chloride, serotonin creatinine sulfate complex, (±)-norepinephrine (+)-bitartrate salt, L-glutamic acid monosodium salt hydrate, α_1 -adrenoceptor antagonist prazosin hydrochloride, and α_2 -adrenoceptor antagonist idazoxan hydrochloride were purchased from Sigma-Aldrich. Drugs were dissolved in distilled water, with the exception of SB-242,084 and MDL-100,907, which were dissolved in hydroxypropyl-beta-cyclodextrin solution (β -OH; 2 g per 100 ml distilled water).

6. Burst firing analysis

Firing activity is important because it is the main determinant that mediates neurotransmitter release, whereby firing rate of action potentials is directly proportional to the amount of neurotransmitters released. Further, for a given number of action potentials over the defined period of time, those that occur in rapid succession, also

known as burst mode, as opposed to single spike behaviour, enhance neurotransmitter release for 5-HT (Gartside *et al.* 2000), NE (Hardebo 1992, Florin-Lechner *et al.* 1996), and DA neurons (Gonon 1988, Garris *et al.* 1994). Therefore, the firing patterns of LC, VTA, and SNc neurons were of interest and were analyzed by comparing interspike interval (ISI) to distinguish high yield burst activity from single spike firing activity, based on criteria set by Grace and Bunney (1983). The onset of a burst is defined as the occurrence of two spikes with an ISI shorter than 0.08 sec. The termination of a burst occurs when the final ISI exceeds 0.16 sec.

7. Statistical analysis

Results are expressed as mean \pm standard error of mean (SEM). Statistical comparisons between spontaneous firing patterns, responsiveness of FMN neurons to 5-HT and NE, and identification of monoamine receptor subtypes were performed using one-way analysis of variances (ANOVAs; Tukey's multiple comparison test was used for identifying which groups were significantly different, if necessary), Student's t-Tests (two-sample assuming equal variances), and chi-square tests, using GraphPad Prism 5 (La Jolla, USA). Statistical significance is taken as $p < 0.05$.

RESULTS

1. Effect of 2-week ECS on NE neurons of the LC

There was no significant difference in the mean spontaneous firing rate, or number of action potentials per second (Figure 10A), of NE neurons, between naïve and sham animals (t-test: $p = 0.62$; naïve = 1.70 ± 0.12 Hz, $n = 56$; sham = 1.78 ± 0.12 Hz, $n = 41$). For all other measures there was no difference between either control group (i.e., naïve and sham) for all experiments (Figures 11A to 14A). Thus, data for naïve and sham animals, in experiments where both were assessed, were pooled and designated as the control group.

Spontaneous firing rate of LC neurons was not different between control animals and those receiving repeated ECS (Figure 10B; t-test: $p = 0.21$; control = 1.74 ± 0.09 Hz, $n = 97$; ECS = 1.92 ± 0.13 Hz, $n = 75$). Several measures of burst activity was also similar between control and treated animals, including no differences in burst rate (Figure 11B; t-test: $p = 0.94$; control = 0.05 ± 0.01 Hz, $n = 23$; ECS = 0.05 ± 0.01 Hz, $n = 29$), percent of spikes occurring in burst mode (Figure 12B; t-test: $p = 0.52$; control = $4 \pm 1\%$; ECS = $5 \pm 1\%$), and number of spikes per burst (Figure 13B; t-test: $p = 0.38$; control = 2.2 ± 0.1 ; ECS = 2.3 ± 0.2).

However, with repeated ECS, the number of neurons per track was decreased by 32% (Figure 14B; t-test: $p = 0.04$; control = 3.0 ± 0.4 , $n = 32$; ECS = 2.1 ± 0.2 , $n = 36$). Perhaps as a compensatory mechanism, the firing mode of LC neurons was significantly different in the ECS-treated animals and included more burst mode firing, as evidenced by a greater absolute number of bursting neurons relative to neurons only displaying

single spike activity (Figure 15A; chi-square test: $p = 0.03$), from 24% of control neurons exhibiting burst activity (burst $n = 23$, regular $n = 74$) to 42% in repeated ECS animals (burst $n = 29$, regular $n = 46$), and an increase in number of bursting neurons per track, which was enhanced by 88% (Figure 15B; t-test: $p = 0.04$; control = 20 ± 5 , $n = 32$; ECS = 38 ± 7 , $n = 36$). Given that the number of neurons per track was diminished while several measures of burst activity were enhanced, neurotransmission of NE in terms of presynaptic components is believed to be minimally altered or unchanged with repeated ECS.

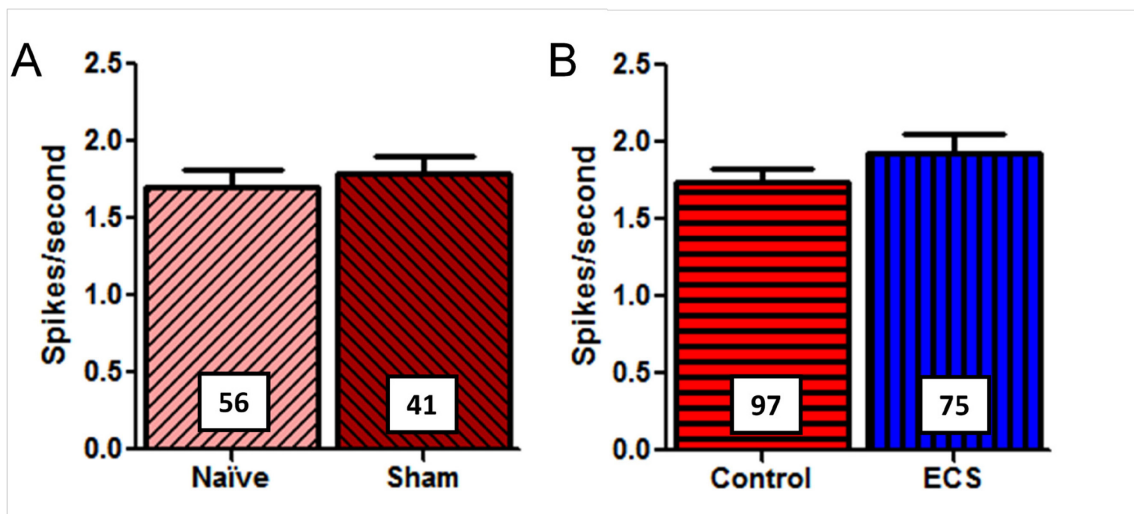


Figure 10. LC: Effects of repeated ECS on mean firing rate. Firing rate, in spikes per second, was not different between naïve ($n = 5$) and sham groups (A: $n = 3$; $p > 0.05$). Compared to control animals ($n = 8$), the firing rate in animals receiving repeated ECS ($n = 5$) was also unchanged (B: $p > 0.05$). Data are presented as mean rate \pm SEM. Numbers at the bottom of the columns indicate the number of neurons recorded, and n represents the number of rats.

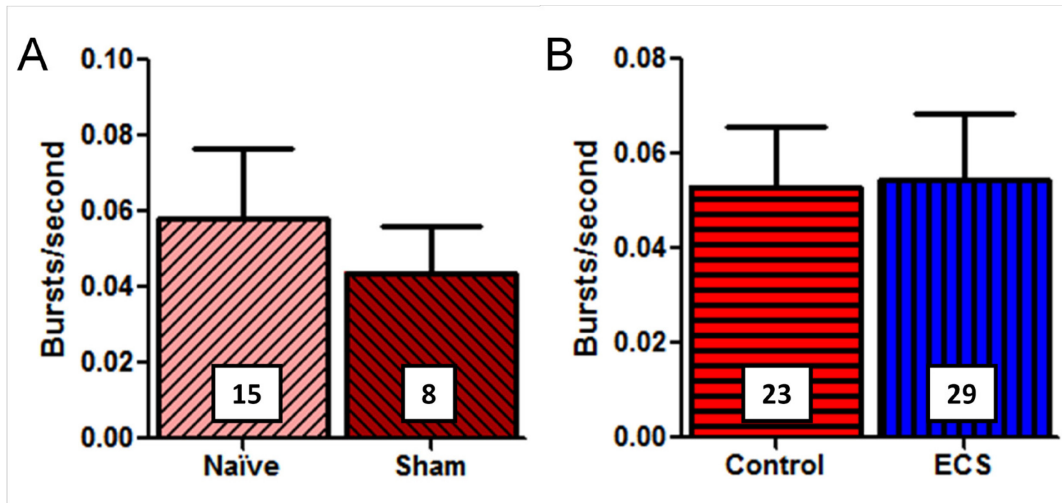


Figure 11. LC: Effects of repeated ECS on mean burst rate. Burst rate, in number of bursts per second, was not different between naïve (n = 5) and sham groups (A: n = 3; $p > 0.05$). Compared to control animals (n = 8), burst rate in animals receiving repeated ECS (n = 5) was also unchanged (B: $p > 0.05$). Data are presented as mean rate \pm SEM. Numbers at the bottom of the columns indicate the number of bursting neurons recorded, and n represents the number of rats.

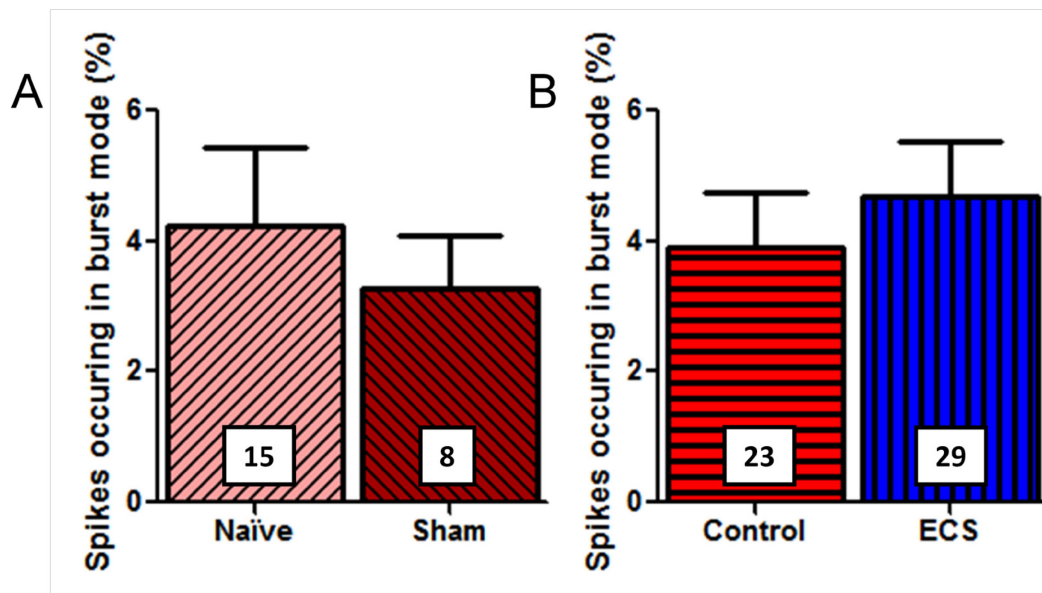


Figure 12. LC: Effects of repeated ECS on mean percentage of spikes in burst firing. Percentage of spikes in burst firing was not different between naïve (n = 5) and sham groups (A: n = 3; $p > 0.05$). Compared to control animals (n = 8), percentage of spikes in burst firing in animals receiving repeated ECS (n = 5) was also unchanged (B: $p > 0.05$). Data are presented as mean percentage \pm SEM. Numbers at the bottom of the columns indicate the number of bursting neurons recorded, and n represents the number of rats.

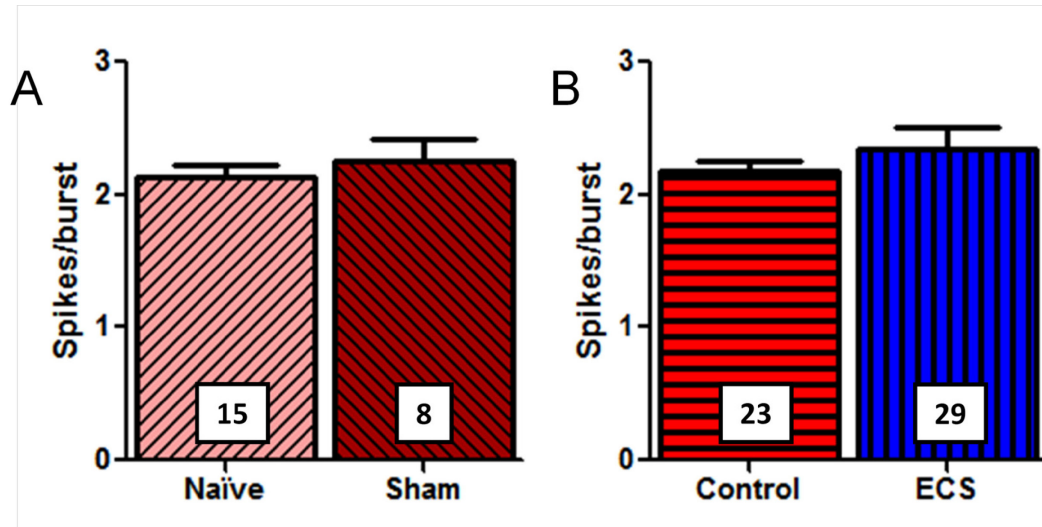


Figure 13. LC: Effects of repeated ECS on mean number of spikes per burst. Number of spikes per burst was not different between naïve (n = 5) and sham groups (A: n = 3; $p > 0.05$). Compared to control animals (n = 8), number of spikes per burst in animals receiving repeated ECS (n = 5) was also unchanged (B: $p > 0.05$). Data are presented as mean number \pm SEM. Numbers at the bottom of the columns indicate the number of bursting neurons recorded, and n represents the number of rats.

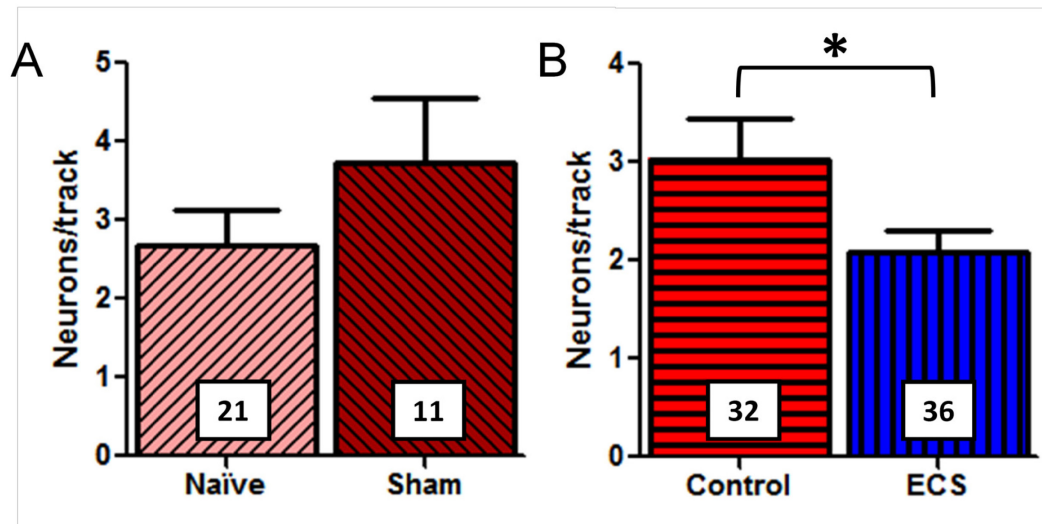


Figure 14. LC: Effects of repeated ECS on mean number of neurons per track. Number of neurons per track was not different between naïve (n = 5) and sham groups (A: n = 3; $p > 0.05$). Compared to control animals (n = 8), number of neurons per track in animals receiving repeated ECS (n = 5) was diminished (B: * $p < 0.05$). Data are presented as mean number \pm SEM. Numbers at the bottom of the columns indicate the number of tracks recorded, and n represents the number of rats.

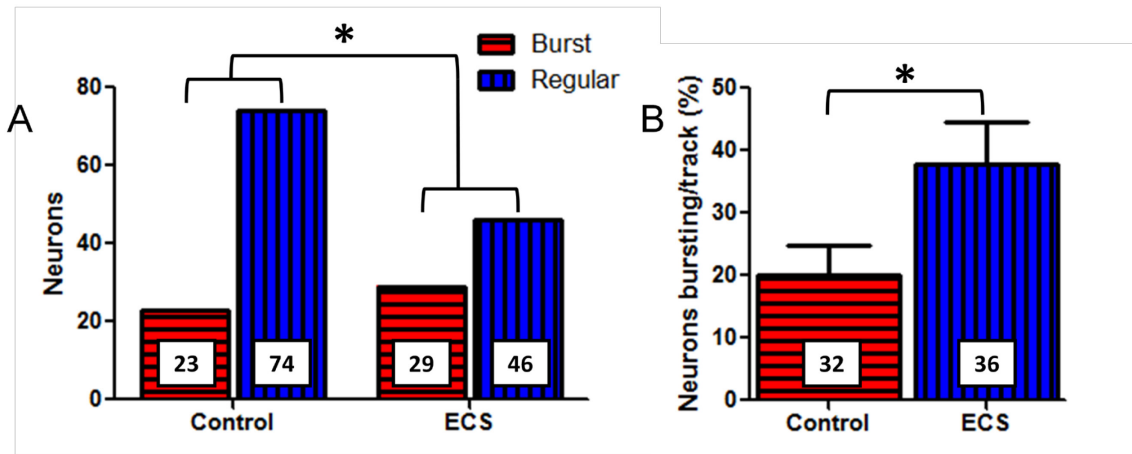


Figure 15. LC: Effects of repeated ECS on ratio of neurons that exhibited burst firing to single spike firing, and percentage of neurons that were found to be bursting per track. Ratio of burst-to-regular firing pattern of neurons was increased with repeated ECS ($n = 5$) compared to control (A: $n = 8$; $* p < 0.05$). Percentage of neurons that burst per track was also increased with repeated ECS compared to control (B). Data are presented as mean number or percent \pm SEM. Numbers at the bottom of the columns indicate the number of neurons recorded, and n represents the number of rats.

2. Effect of 2-week ECS on DA neurons of the VTA

Mean firing rate of VTA DA neurons was not different between control animals, animals receiving repeated ECS, animals receiving repeated subconvulsive stimuli (S30V), and animals 14 days after a single ECS (S+14D), but was significantly decreased by 21% in animals 2 days after a single ECS (S+2D) compared to control animals (Figure 16A; ANOVA: $p = 0.003$; control = 4.05 ± 0.12 Hz, $n = 134$; ECS = 3.49 ± 0.16 Hz, $n = 60$; S30V = 3.88 ± 0.24 Hz, $n = 45$; S+14D = 3.84 ± 0.23 Hz, $n = 57$; S+2D = 3.18 ± 0.16 Hz, $n = 53$). Attenuation of firing rate 2 days following a single ECS may be attributed to the 20% decrease in burst size, defined as the number of spikes in a burst event, as no other group was significantly different in this regard to one another or to the control group (Figure 17B; ANOVA: $p = 0.02$; control = 3.13 ± 0.14 , $n = 117$; ECS = 2.68 ± 0.10 , $n = 44$; S30V = 2.66 ± 0.14 , $n = 35$; S+14D = 2.88 ± 0.15 , $n = 51$; S+2D = 2.49 ± 0.11 , $n = 39$). However, this difference was not present in other measures of burst activity between the various groups (Figures 16B and 17A). Burst rate amongst the groups was

unchanged (ANOVA: $p = 0.11$; control = 0.39 ± 0.03 Hz; ECS = 0.28 ± 0.04 Hz; S30V = 0.38 ± 0.07 Hz; S+14D = 0.37 ± 0.06 Hz; S+2D = 0.25 ± 0.04 Hz), nor was the percentage of spikes occurring in burst mode (ANOVA: $p = 0.08$; control = $30 \pm 2\%$; ECS = $21 \pm 3\%$; S30V = $25 \pm 4\%$; S+14D = $26 \pm 4\%$; S+2D = $18 \pm 3\%$).

Therefore, two days after receiving a single ECS, each burst contained fewer action potentials, accounting for the lower frequency of action potentials and overall diminished activity in this region. However, this effect was no longer present 14 days after the single convulsive stimuli nor was it present after the full ECS treatment over 2 weeks. In other words, there was an eventual recovery from this diminished activity after the first ECS.

Another method of administering ECS in rats, via the use of ear-clips to administer the seizure-inducing stimuli rather than subcutaneously-placed needles, was tested to determine if either method exhibits differences in electrophysiological activity. Behaviourally, the convulsions induced by the electrical stimuli in either group were very similar, except for visibly stronger grand mal-associated thrashing in the ear-clip animals. The reason for this may be that these animals were not given anaesthesia or a muscle relaxant prior to receiving ECS. Given that certain anaesthetic agents such as isoflurane interfere with glutamate and GABA transmission (Westphalen and Hemmings 2003), and is not required in the ear-clip technique, this method of inducing seizures appears preferential for studies that assess measures that are influenced by these neurotransmitters (Zafra *et al.* 1991, Lu *et al.* 2006). Anaesthesia and muscle relaxants are used in the clinical practice of ECT and it would be ideal to replicate this in animal studies, but these agents are not necessary in producing the antidepressant effect. The ear-clip method of administering ECS was no different to repeated ECS using subcutaneously-placed

needles, in regards to measures including firing rate (Figure not shown; t-test: $p = 0.53$; ear-clip = 3.67 ± 0.20 Hz, $n = 22$), burst rate (t-test: $p = 0.43$; ear-clip = 0.21 ± 0.08 Hz, $n = 16$), percentage of spikes in burst mode (t-test: $p = 0.25$; ear-clip = $14 \pm 5\%$), and spikes per burst (t-test: $p = 0.06$; ear-clip = 2.3 ± 0.2). Given the behavioural and electrophysiological similarities in animals that received either ear-clip or subcutaneous needle administration of ECS, it is possible that either method would render the same effect in other regions of the brain.

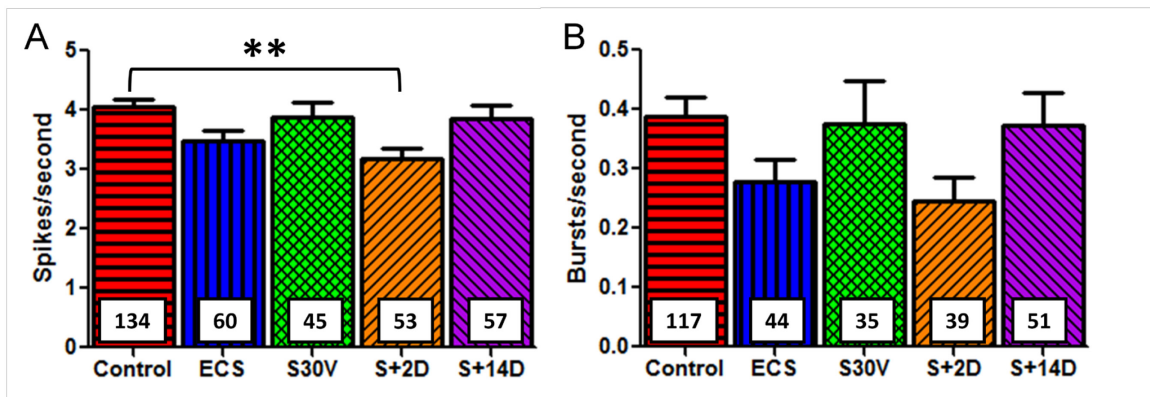


Figure 16. VTA: Effects of repeated ECS on mean firing and burst rate. Between control ($n = 12$), ECS ($n = 7$), subconvulsive (S30V; $n = 6$), two days after single ECS (S+2D; $n = 4$), and fourteen days after single ECS (S+14D; $n = 6$) groups, the only difference in firing rate was a decrease in S+2D animals compared to control animals (A: ** $p < 0.01$). Burst rate, in number of bursts per second was unchanged between groups (B: $p > 0.05$). Data are presented as mean rate \pm SEM. Numbers at the bottom of the columns indicate the number of neurons, and n represents the number of rats.

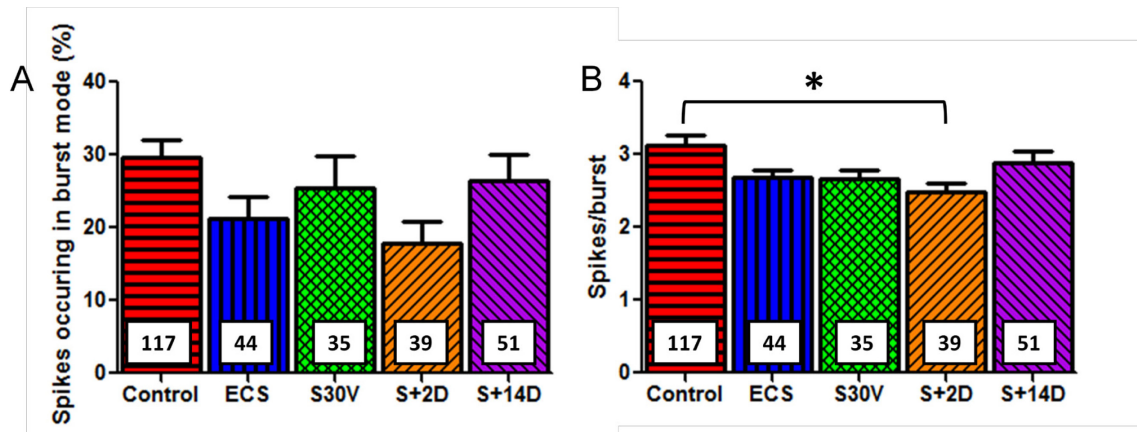


Figure 17. VTA: Effects of repeated ECS on mean percentage of spikes in burst firing and mean number of spikes per burst. Between control (n = 12), ECS (n = 7), S30V (n = 6), S+2D (n = 4), and S+14D (n = 6) groups, there was no difference in percentage of spikes in burst firing (A: $p > 0.05$). The number of spikes per burst, however, was decreased in S+2D animals compared to control animals (B: * $p < 0.05$). Data are presented as mean percentage or number \pm SEM. Numbers at the bottom of the columns indicate the number of bursting neurons, and n represents the number of rats.

3. Effect of 2-week ECS on DA neurons of the SNc

DA neurons of the nigrostriatal pathway influence motricity and may be impaired in depressed individuals because they often present with psychomotor retardation. Compared to sham animals, there was no change in firing (t-test: $p = 0.39$; sham = 3.48 ± 0.26 Hz, n = 39; ECS = 3.76 ± 0.21 Hz, n = 43) and burst rate (t-test: $p = 0.23$; sham = 0.14 ± 0.03 Hz, n = 18; ECS = 0.09 ± 0.02 Hz, n = 20) in ECS-treated animals (Figure 18). Additional measures of burst activity, including the percentage of spikes occurring in burst mode (t-test: $p = 0.11$; sham = $9 \pm 3\%$; ECS = $5 \pm 1\%$) and spikes per burst (t-test: $p = 0.77$; sham = 2.3 ± 0.1 ; ECS = 2.4 ± 0.2) were also unchanged following repeated ECS (Figure 19). However, the percentage of successful tracks in which at least a single DA neuron was detected and recorded, compared to unsuccessful tracks, was increased (chi-square test: $p = 0.01$) from 44% in control animals (successful n = 22; unsuccessful n = 28) to 74% in treated animals (successful n = 20; unsuccessful n = 7), indicating that it became easier to locate and record neurons after treatment (Figure 20). Accordingly, the

mean number of neurons per track was also increased in the treated animals (t-test: $p = 0.0053$; sham = 0.8 ± 0.2 , $n = 50$; ECS = 1.6 ± 0.3 , $n = 27$). Given that it took fewer attempts to successfully identify and record a DA neuron in the SNc following repeated ECS, it was only necessary to perform about half as many tracks in the experiments involving treated animal (sham $n = 50$; ECS $n = 27$). Overall, in the animals receiving repeated ECS, the firing and burst rates were unchanged but more neurons were recorded per descent through the nucleus. This suggests that repeated ECS induces an activation of a reserve pool of quiescent dopaminergic neurons in the SNc and perhaps increases neurotransmission of nigrostriatal DA.

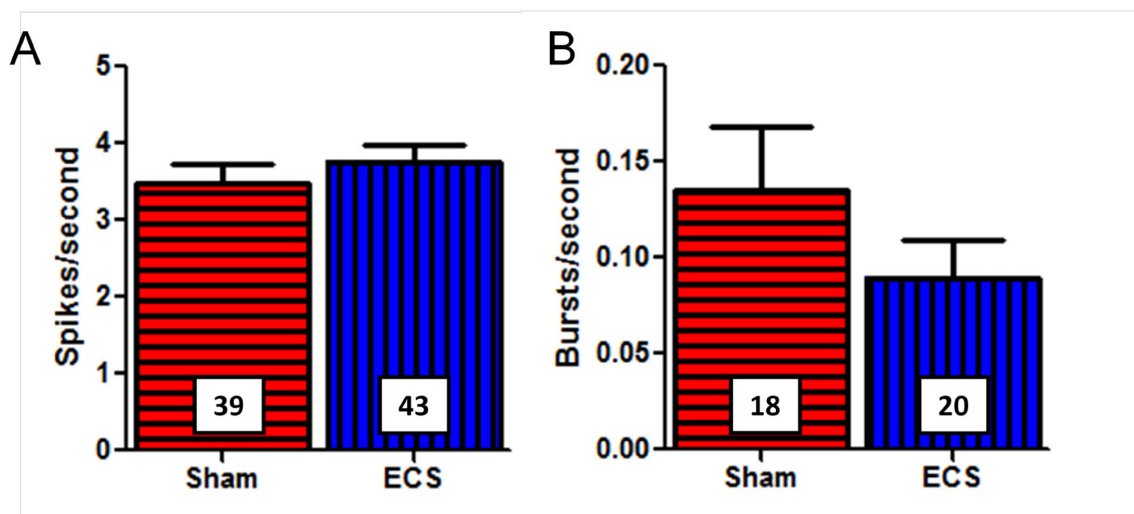


Figure 18. SNc: Effects of repeated ECS on firing and burst rate. Firing rate, in spikes per second, was not different between sham ($n = 5$) and ECS groups (A: $n = 4$; $p > 0.05$). Burst rate, in number of bursts per second, between sham and ECS animals was also unchanged (B: $p > 0.05$). Data are presented as mean rate \pm SEM. Numbers at the bottom of the columns indicate the number of neurons, and n represents the number of rats.

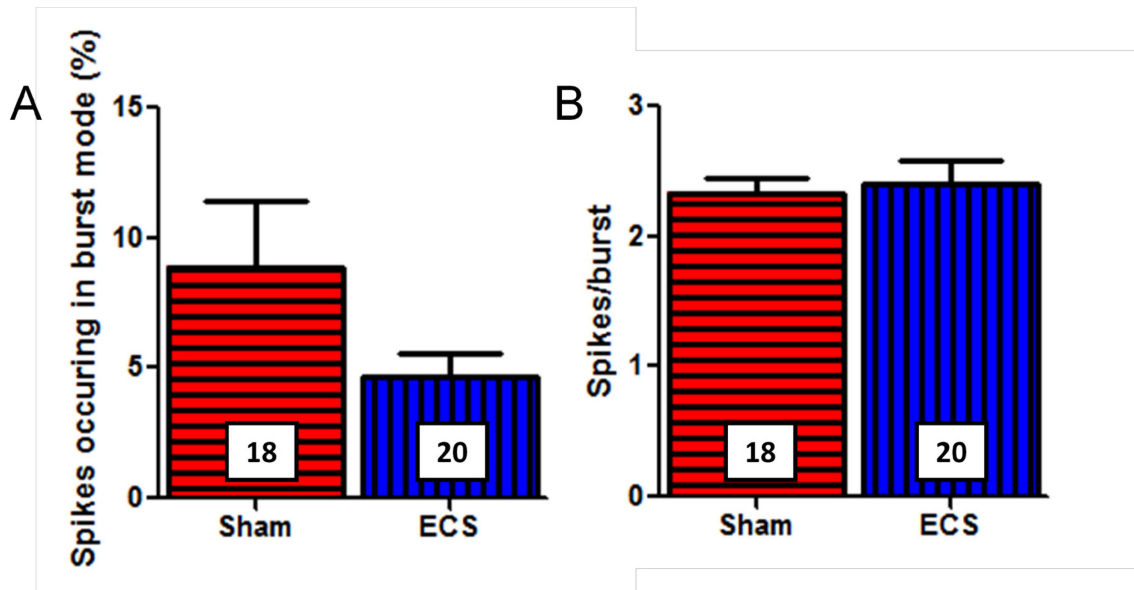


Figure 19. SNc: Effects of repeated ECS on mean percentage of spikes in burst firing and mean number of spikes per burst. Percentage of spikes in burst firing was not different between sham ($n = 5$) and ECS groups (A: $n = 4$; $p > 0.05$). Number of spikes per burst between sham and ECS animals was also unchanged (B: $p > 0.05$). Data are presented as mean percentage or number of spikes per burst \pm SEM. Numbers at the bottom of the columns indicate the number of bursting neurons, and n represents the number of rats.

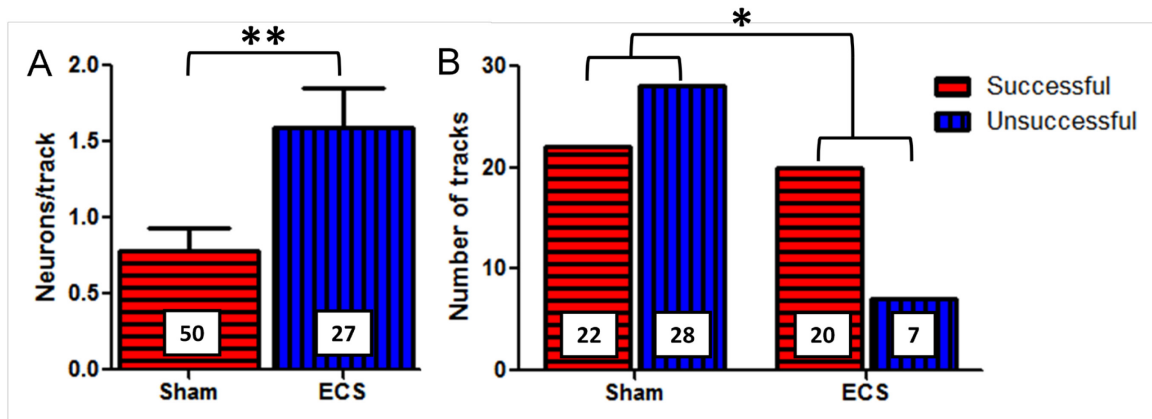


Figure 20. SNc: Effects of repeated ECS on mean number of neurons per track and ratio of successful-to-unsuccesful tracks. Compared to sham ($n = 5$), the mean number of neurons per track, including tracks that yielded no neuron, was increased in ECS animals (A: $n = 4$; ** $p < 0.01$). Ratio of successful-to-unsuccesful tracks, between sham and ECS animals was also increased (B: * $p < 0.05$). A successful track is defined as a descent through the region of interest which yielded as least one neuron. Data are presented as mean or total number \pm SEM. Numbers at the bottom of the columns indicate the number of tracks, and n represents the number of rats.

4. Characterization of 5-HT and NE receptors in the FMN

The excitatory monoamine receptors of the FMN were characterized by blocking 5-HT- and NE-induced facilitation of motor neuron electrophysiological activity by comparing the number of spikes induced by 5-HT or NE neurotransmitter before and after i.v. injections of selective antagonists. The selective 5-HT_{2A} receptor antagonist MDL-100,907 did not block 5-HT-induced excitation at doses of 0.2 mg/kg, 0.4 mg/kg, or 0.6 mg/kg (Figure 21; ANOVA: $p = 0.24$, $n = 10, 6, 3$). In contrast, the selective 5-HT_{2C} antagonist SB-242,084 blocked 5-HT-induced excitation in a dose-dependent manner at doses of 0.5 mg/kg and 1.0 mg/kg (ANOVA: $p = 0.004$, $n = 15, 5$). NE-induced excitation of FMN neurons was blocked by the selective α_1 -adrenoceptor antagonist prazosin at doses of 0.1 mg/kg, 0.2 mg/kg, and 0.3 mg/kg (Figure 22; ANOVA: $p < 0.0001$, $n = 11, 3, 9$), but not by the selective α_2 -adrenoceptor antagonist idazoxan at doses of 1.0 mg/kg or 3.0 mg/kg (ANOVA: $p = 0.25$, $n = 6, 3$). Therefore, the 5-HT_{2C} receptor and α_1 -adrenoceptor are the most important in mediating monoamine transmission in the FMN.

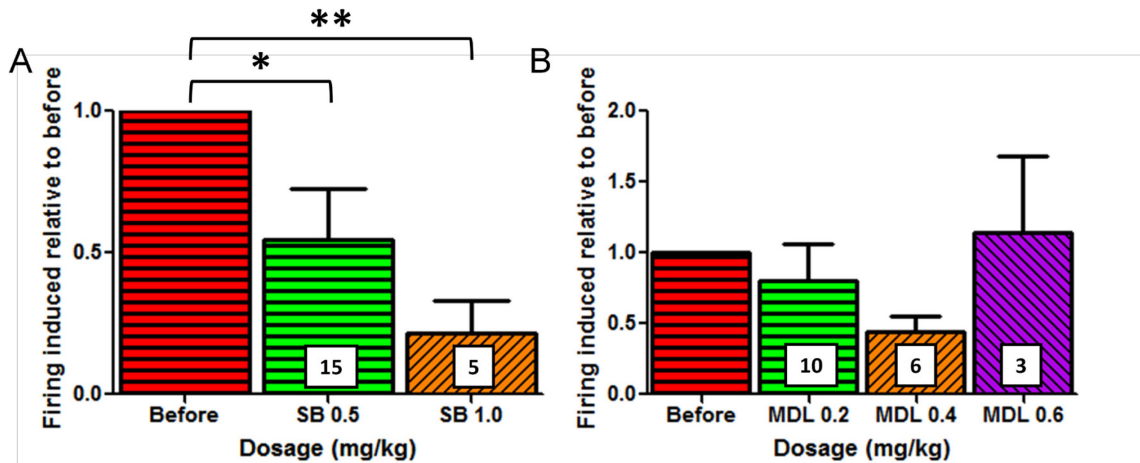


Figure 21. FMN: Characterization of 5-HT receptors. SB-242,084, at doses of 0.5 mg/kg and 1.0 mg/kg, inhibited 5-HT-induced facilitation of FMN activity, at iontophoretic 5-HT currents of 20 nA or 40 nA (A: ANOVA: * $p < 0.05$, ** $p < 0.01$). MDL-100,907, at doses of 0.2 mg/kg, 0.4 mg/kg, and 0.6 mg/kg, did not inhibit 5-HT-induced facilitation of FMN activity, at iontophoretic 5-HT currents of 20 nA or 40 nA (B: ANOVA: $p > 0.05$). Data are presented as mean number of spikes induced relative to before antagonist administration \pm SEM. Numbers at the bottom of the columns indicate number of neurons.

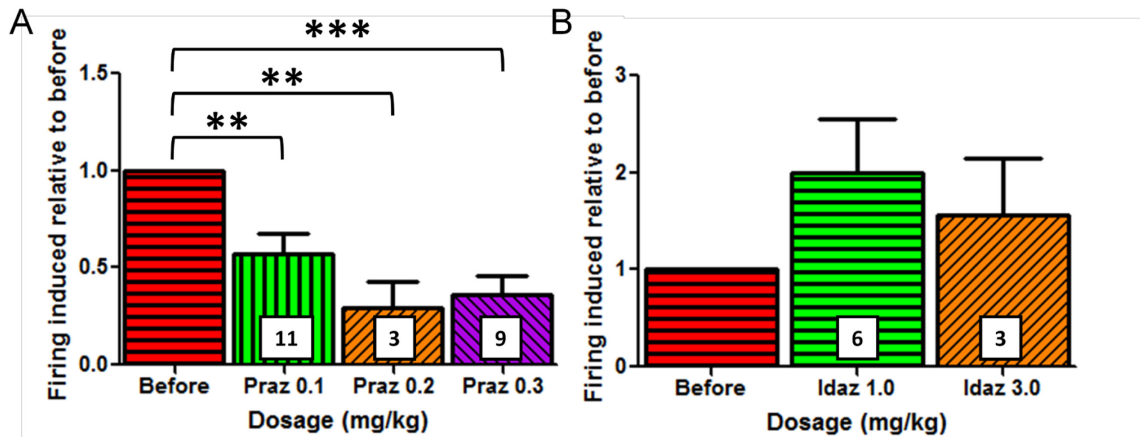


Figure 22. FMN: Characterization of NE receptors. Prazosin, at doses of 0.1 mg/kg, 0.2 mg/kg, and 0.3 mg/kg, inhibited NE-induced facilitation of FMN activity, at iontophoretic NE currents of 20 nA or 40 nA (A: ANOVA: ** $p < 0.01$, *** $p < 0.0001$). Idazoxan, at doses of 1.0 mg/kg and 3.0 mg/kg did not inhibit NE-induced facilitation of FMN activity, at iontophoretic NE currents of 20 nA or 40 nA (B: ANOVA: $p > 0.05$). Data are presented as mean number of spikes induced relative to before antagonist administration \pm SEM. Numbers at the bottom of the columns indicate number of neurons.

5. Effect of 2-week ECS on motor neurons of the FMN

FMN neurons can be activated through various means. A method that is physiologically relevant is activation with the use of iontophoretically-applied glutamate, given that the FMN naturally receives glutamatergic projections originating from the

cortex. In a comparison between animals receiving repeated ECS, S+2D, and sham animals, the responsiveness of neurons to glutamate was unchanged as indicated by similar glutamate ejection currents required to induce minimal firing activity when testing excitation of the neurons induced by 5-HT (Figure 23; ANOVA: $p = 0.70$; sham = -23.4 ± 2.7 nA, $n = 23$; ECS = -20.3 ± 3.1 nA, $n = 21$; S+2D = -22.9 ± 2.6 nA, $n = 22$) and NE (ANOVA: $p = 0.71$; sham = -20.1 ± 2.2 nA, $n = 22$; ECS = -21.6 ± 2.4 nA, $n = 20$; S+2D = -18.9 ± 2.0 nA, $n = 20$).

Although 5-HT and NE do not directly excite lower motor neurons of the FMN, they act as modulators and markedly enhance the excitability of motor neurons (McCall and Aghajanian 1979). To observe the effects of ECS on the responsiveness of FMN neurons to monoaminergic innervation, 5-HT and NE were locally applied using microiontophoresis alongside glutamate application. To better compare the effects of 5-HT and NE on firing activity, the glutamate current was lowered by 2-3 nA below the threshold current necessary to induce firing, such that basal firing activity was close to completely abolished. Once basal firing was maintained for at least 100 sec, 5-HT or NE was ejected using 20 nA and 40 nA currents for 60 sec. The ejection currents gradually facilitated the induction of action potentials, followed by a period of peak activity, which was represented by the highest volume of spikes in a 10 sec timeframe, and which typically occurred immediately after the ejection of the monoamine was halted (Figures 24 and 25). Within 100 sec of ejection stoppage, neuronal activity progressively returned to the basal firing rate. Although firing activity was increased throughout monoamine ejection, this increase was more evident and consistent across all recordings during the period of peak activity. However, in comparison to sham animals, the peak activity for

the rats treated with repeated ECS or single ECS induced by 5-HT (Figure 26; 20 nA, ANOVA: $p = 0.30$; 40 nA, ANOVA: $p = 0.17$; $n = 23, 21, 22$) and NE (Figure 27; 20 nA, ANOVA: $p = 0.12$; 40 nA, ANOVA: $p = 0.34$; $n = 23, 20, 20$) was not significantly increased.

The area under the firing activity curve represents the total number of spikes induced by 5-HT or NE (Figure 24 and 25). As expected, both 5-HT and NE facilitated motor activation in a dose-dependent manner, with more excitation induced when currents used to eject either compound was set to 40 nA compared to 20 nA. 5-HT receptor sensitivity was not changed compared to sham animals in either repeated ECS or single shock groups for currents of 20 nA (Figure 28; ANOVA: $p = 0.25$) and 40 nA (ANOVA: $p = 0.09$). On the other hand, FMN neuron sensitivity to NE was increased by 106% following repeated ECS when the ejection current was set to 20 nA (Figure 29; t-test: $p = 0.03$) and was increased by 75% for a current of 40 nA (t-test: $p = 0.03$). However, rats that received a single ECS and were recorded two days following the treatment had NE-induced activation intermediate to that of sham and repeated ECS animals, such that it was not significantly different to either group at ejection currents of 20 nA (ANOVA: $p = 0.06$) and 40 nA (ANOVA: $p = 0.08$). Overall, repeated ECS increased the mediatory role of only NE on FMN neurons by selectively increasing the responsiveness of neurons in the FMN to locally administered NE, and could represent the mechanism behind the alleviation of facial motricity deficits with ECT treatment.

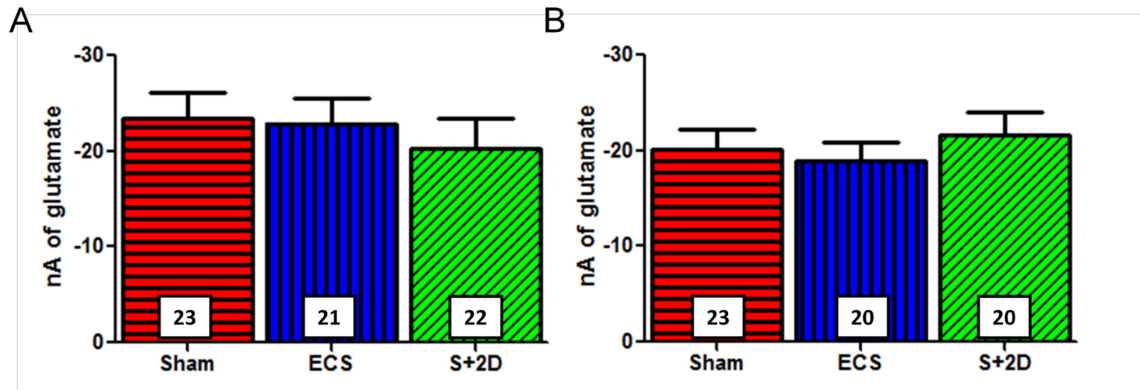


Figure 23. FMN: Effects of repeated ECS on responsiveness to glutamate. For the assessment of FMN neurons to 5-HT, there was no difference in the responsiveness of neurons to locally administered glutamate between sham (n = 15), ECS (n = 10), and S+2D groups (A: n = 13; p > 0.05). There was also no difference between sham (n = 18), ECS (n = 13), and S+2D groups (n = 12) for assessment of responsiveness to NE (B: p > 0.05). Data are presented as mean current of glutamate required to induce minimal firing of FMN neurons \pm SEM. Numbers at the bottom of the columns indicate the number of neurons, and n represents the number of rats.

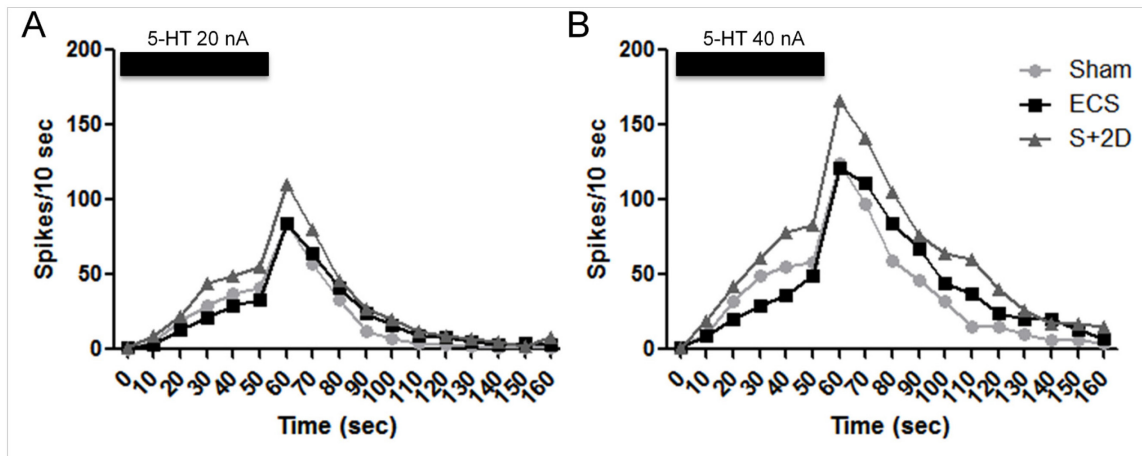


Figure 24. FMN: Effects of repeated ECS on responsiveness to 5-HT. Via iontophoresis, 5-HT was locally administered into the FMN using 20 nA (A) and 40 nA (B) currents in sham (n = 15), ECS (n = 10), and S+2D (n = 13) groups. Action potentials were immediately detected following 5-HT ejection, followed by a gradual increase in further activity. Peak facilitation of electrophysiological activity was measured to be in the 10 sec block immediately after 5-HT ejection was discontinued at 60 sec. 5-HT-induced facilitation of FMN activity returned to basal levels within 100 sec of ejection stoppage. Duration of 5-HT ejection is indicated by the black bar, and n represents the number of rats.

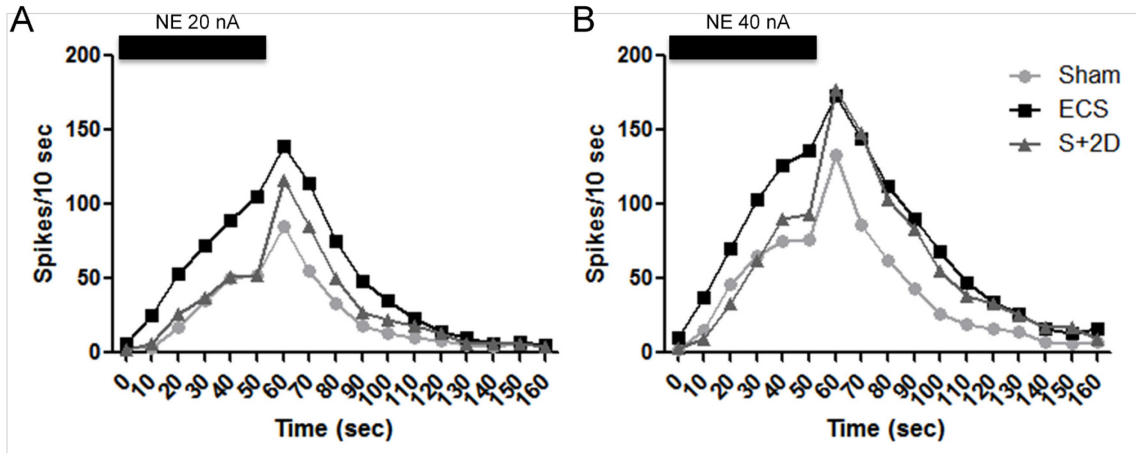


Figure 25. FMN: Effects of repeated ECS on responsiveness to NE. Via iontophoresis, NE was locally administered into the FMN using 20 nA (A) and 40 nA (B) currents in sham (n = 18), ECS (n = 13), and S+2D (n = 12) groups. Action potentials were immediately detected following NE ejection, followed by a gradual increase in further activity. Peak facilitation of electrophysiological activity was measured to be in the 10 sec block immediately after NE ejection was discontinued at 60 sec. NE-induced facilitation of FMN activity returned to basal levels within 100 sec of ejection stoppage. Duration of NE ejection is indicated by the black bar, and n represents the number of rats.

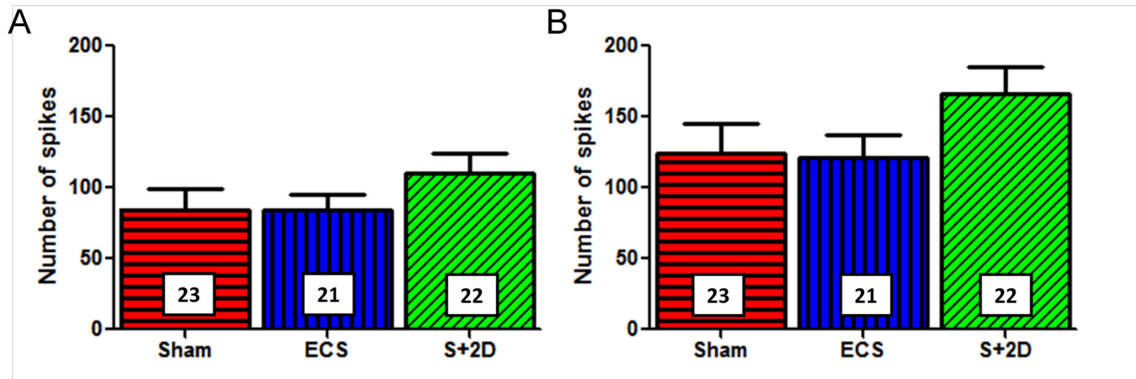


Figure 26. FMN: Effects of repeated ECS on peak activity induced by 5-HT. Peak number of spikes induced (during 60-70 sec time point) by locally administered 5-HT at iontophoretic currents of 20 nA (A: $p > 0.05$) and 40 nA (B: $p > 0.05$), was not different between sham (n = 15), ECS (n = 10), and S+2D (n = 13) groups. Data are presented as mean number of spikes induced \pm SEM. Numbers at the bottom of the columns indicate the number of neurons, and n represents the number of rats.

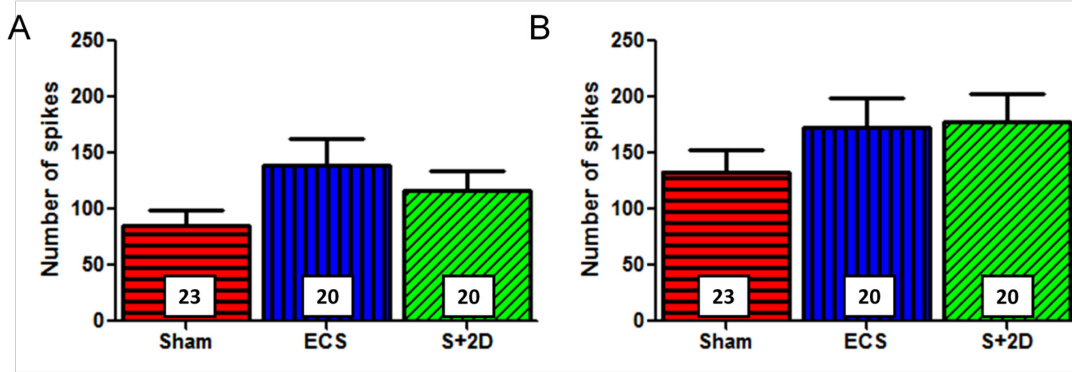


Figure 27. FMN: Effects of repeated ECS on peak activity induced by NE. Peak number of spikes induced (during 60-70 sec time point) by locally administered NE at iontophoretic currents of 20 nA (A: $p > 0.05$) and 40 nA (B: $p > 0.05$), was not different between sham ($n = 18$), ECS ($n = 13$), and S+2D ($n = 12$) groups. Data are presented as mean number of spikes induced \pm SEM. Numbers at the bottom of the columns indicate the number of neurons, and n represents the number of rats.

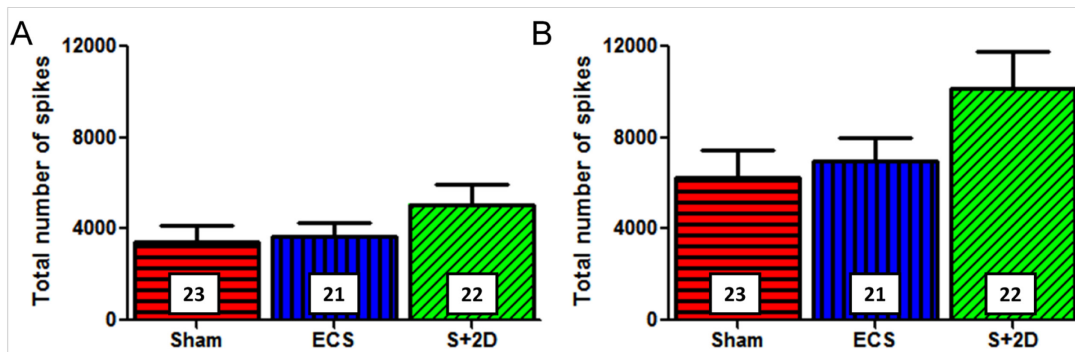


Figure 28. FMN: Effects of repeated ECS on overall activity induced by 5-HT. Responsiveness of FMN neurons to 5-HT, measured by the total number of spikes induced by locally administered 5-HT at iontophoretic currents of 20 nA (A: $p > 0.05$) and 40 nA (B: $p > 0.05$), was not different between sham ($n = 15$), ECS ($n = 10$), and S+2D ($n = 13$) groups. Data are presented as mean number of spikes induced \pm SEM. Numbers at the bottom of the columns indicate the number of neurons, and n represents the number of rats.

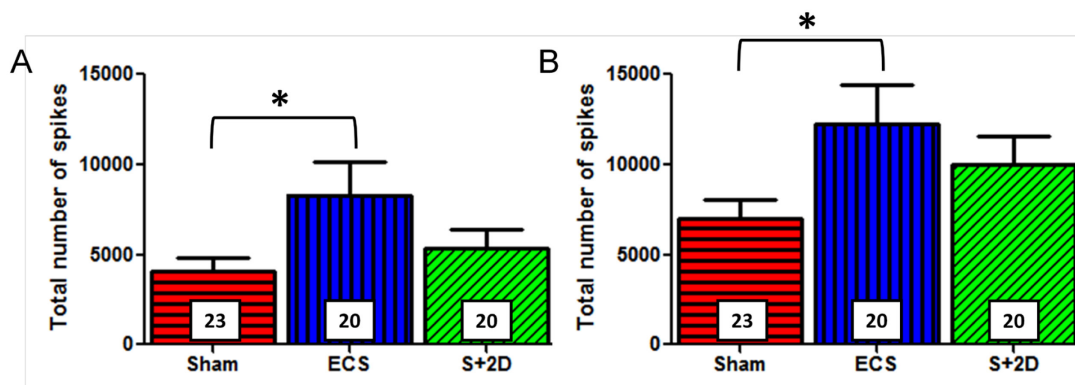


Figure 29. FMN: Effects of repeated ECS on overall activity induced by NE. Responsiveness of FMN neurons to NE, measured by the total number of spikes induced by locally administered NE at iontophoretic currents of 20 nA (A: * $p < 0.05$) and 40 nA (B), was increased in ECS animals ($n = 13$) compared to sham animals ($n = 18$). Responsiveness to NE between ECS and S+2D groups ($n = 12$) was not different at either ejection current ($p > 0.05$). Data are presented as mean number of spikes induced \pm SEM. Numbers at the bottom of the columns indicate the number of neurons, and n represents the number of rats.

DISCUSSION

Electroconvulsive therapy remains the “gold standard” in the treatment of depression, but its precise effects on the central nervous system have not been fully elucidated. In the present study, repeated ECS showed to have no overall effect on presynaptic components of neurotransmission of LC NE and VTA DA neurons (Figure 30). Although firing and burst rate was also unchanged in DA neurons of the SNc, there were more neurons per track in this region, suggesting that repeated administration of ECS elicits more spontaneously active neurons in the nigrostriatal pathway. Facilitation of electrophysiological activity of FMN neurons by serotonin and norepinephrine was found to be mediated by 5-HT_{2C} and α_1 -adrenergic receptors, respectively. Furthermore, the responsiveness of FMN neurons to locally-administered NE, but not 5-HT, was enhanced with repeated ECS, indicating that selective postsynaptic components of monoamine transmission is elevated in particular regions of the brain.

Due to the reciprocal interactions between the 5-HT, NE, and DA neurotransmitter systems, a change in a particular monoamine system may ultimately result in altered function and neurotransmission of multiple monoamine systems (Guiard *et al.* 2008). The effects of repeated ECS have been assessed in several aspects of these systems. Firstly, there is no change in 5-HT firing activity or 5-HT release, and no alteration in presynaptic 5-HT_{1A} and 5-HT_{1B} autoreceptor sensitivity in the dorsal raphe nucleus (DRN; Blier and Bouchard 1992). However, it should be emphasized that postsynaptic changes may also contribute to net neurotransmission. Studies have shown that 5-HT neurotransmission is increased postsynaptically in some regions with repeated ECS, despite no change in function presynaptically on serotonergic neurons at the cell body or

nerve terminal. In the hippocampus, neurotransmission increases following repeated ECS due to sensitization of postsynaptic 5-HT_{1A} and 5-HT₃ receptors (de Montigny 1984, Chaput *et al.* 1991, Mongeau *et al.* 1994, Piñeyro *et al.* 1995, Haddjeri *et al.* 1998, Dong *et al.* 1999). It is shown in the present report that responsiveness to NE, through the α_1 -adrenoceptor, but not 5-HT, which is mediated by the 5-HT_{2C} receptor, was significantly increased in the FMN.

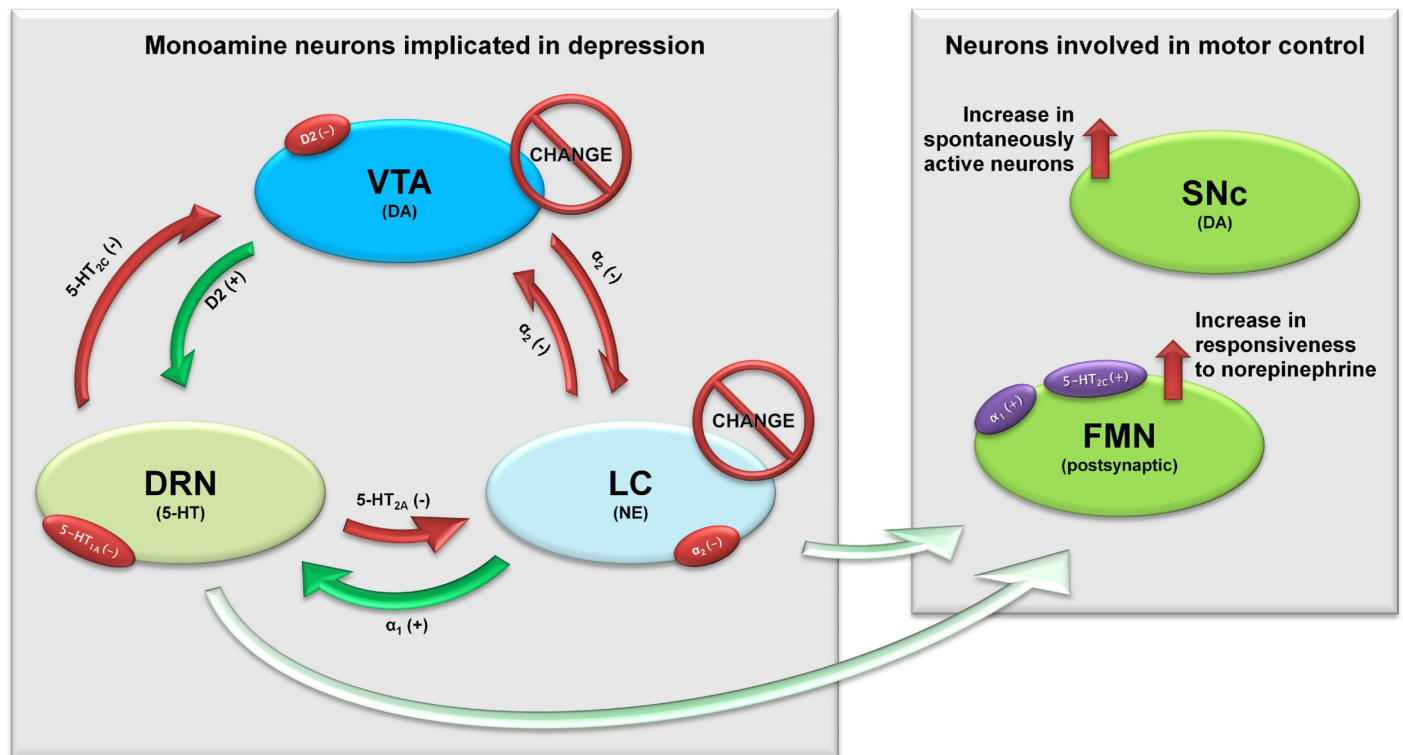


Figure 30. Summary of current findings. With repeated ECS, there was no overall change in the electrophysiological activity of NE neurons of the LC and DA neurons of the VTA. There were more neurons per track in the SNc, suggesting an increase in spontaneously active nigrostriatal DA neurons. Facilitation of electrophysiological activity of FMN neurons by serotonin and norepinephrine was found to be mediated by 5-HT_{2C} and α_1 -adrenergic receptors, respectively. Furthermore, the responsiveness of FMN neurons to locally-administered NE, but not 5-HT, was enhanced with repeated ECS.

Several antidepressant medications increase NE neurotransmission, as evidenced for instance by the efficacy and action of NRIs. However, SSRIs, SNRIs, and TCAs such as fluoxetine, reboxetine, and desipramine, respectively, all decrease LC firing rate which may be partly responsible for the noradrenergic side effects associated with particular

antidepressant treatments (Szabo *et al.* 2000, Grant and Weiss 2001, Szabo and Blier 2001, Seager *et al.* 2005). From the present findings, the overall firing activity of active NE neurons in the LC appear unchanged with repeated ECS, with more neurons changing firing mode to include burst activity, and a reduction in spontaneously active neurons, depicted by a decrease in neurons per track. Other measures of firing and burst activity were unchanged with repeated ECS in animals. This suggests perhaps little to no change in overall NE release. The firing rate of LC NE neurons is also unchanged 8 days after a single ECS (Tepper *et al.* 1982). It is plausible that this lack of impairment to noradrenergic transmission, other than the greater incidence of silent neurons, is partly responsible for the greater efficacy of ECT in relation to SSRIs, SNRIs, and TCAs for the treatment of major depression. Furthermore, from our present report, repeated ECS elevated postsynaptic sensitivity of FMN neurons to NE. Although NE receptor sensitivity is unchanged in the hippocampus (de Montigny 1984), it can be suggested that ECS in animals, and perhaps ECT in people, increase NE neurotransmission in other brain regions, including the FMN, due in part to sensitization of postsynaptic adrenoceptors, perhaps in particular to the α_1 -adrenoceptor.

Grant and Weiss (2001) reported that several AD treatments, including repeated ECS, impair the spontaneous firing activity of NE neurons. However, in their assessment of the effects of ECS after 1 day, only 10 neurons were recorded from a total of 4 animals. In their assessment of the effects of ECS after 5 days, 8 neurons were collected from only 2 animals, from which they concluded that there is a significant decrease in NE firing rate. Their main argument for such a low number of neurons was that the LC was difficult to isolate in some of the animals that had received antidepressant treatment. Also, it was

indicated that because significance was not initially observed, all “non-ECS” data was pooled with the control data, and it is unclear whether or not this included observations from other AD treatment groups. Therefore, the validity of the findings from the study by Grant and Weiss (2001) that suggests that repeated ECS reduces noreadrenergic electrophysiological activity is questionable.

The role of DA in depression has been generally overlooked in comparison to 5-HT and NE. However, the mesolimbic reward pathway, originating in the VTA, is impaired in depression, such that treatments that target the DA system have emerged. In the treatment of depression, DA-acting agents should be considered not only as augmentation agents, but perhaps also as monotherapy strategies, as evidenced by the efficacy of pramipexole (Corrigan *et al.* 2000). Given the extensive reciprocal interactions between the monoamine systems involved in MDD, the effects of a treatment on pathways other than those directly involved should not be overlooked. In the present study it was found that the overall electrophysiological activity of DA neurons in the VTA, including firing rate, burst rate, and neurons per track, was not affected by repeated ECS. This is not surprising given that the firing activity of 5-HT and NE neurons is also unchanged with repeated ECS. While a two week regimen of six suprathreshold convulsive stimuli did not have any effect in the VTA, firing rate was significantly reduced by 21% two days following a single ECS. The reason for this attenuated activity was not due to a change in burst rate, but rather to a diminished burst size, represented by 20% fewer spikes per burst event.

Given that DA neuron burst activity has been positively linked to higher DA concentrations in postsynaptic regions, such as the olfactory tuberculum and nucleus

accumbens (Gonon 1988, Garris *et al.* 1994), it can be suggested from the data in the present study that a single ECS leads to an abrupt decrease in extracellular DA levels in postsynaptic regions. Indeed, Brannan and colleagues (1993) demonstrated that the level of extracellular DA in the corpus striatum is decreased by 20% in animals receiving repeated ECS compared to sham animals. While the striatum is a key projection region of the mesostriatal DA pathway, projections from this pathway originate from both the VTA and substantia nigra and are directed at the anteromedial region of the striatum. Sustained SSRI treatment exhibits a similar decrease of VTA DA neuron firing activity (Dremencov *et al.* 2009), despite the efficacy of SSRIs as antidepressants. However, in the present study, attenuation of both firing rate and burst size was found to be restored to levels comparable to that of control animals after 14 days, as was shown with rats that were recorded 14 days following a single ECS, and with rats that were administered a full regimen of six ECS over a 2 week period. As one session of ECT is only rarely effective in the treatment of MDD in people, it is not plausible that the abrupt attenuation of spontaneous firing activity in the VTA is correlated with the alleviation of depressive symptoms. Despite the idea that the aim of antidepressant treatments is to increase monoamine neurotransmission, the therapeutic efficacy of ECT does not appear to be attributed to an elevated function of VTA DA neurons.

A recent study by West and Weiss (2010), in direct contrast to the present findings, indicates that repeated ECS, as well as other antidepressant treatments, including bupropion and several SSRIs (i.e., fluoxetine, paroxetine, and sertraline), but not phenelzine, all significantly increase the firing rate of VTA DA neurons in rats. However, it has been suggested that SSRIs, in particular escitalopram, robustly decreases DA

neuron firing rate (Dremencov *et al.* 2009). The decrease in DA firing activity can be attributed to over-activation of 5-HT_{2C} receptors on DA neurons in the VTA, that normally inhibit firing once activated, due to SSRI-induced elevation of 5-HT neurotransmission. The findings by West and Weiss (2010) also stand in contrast with those of previous observations, in which bupropion treatment was shown to have no effect on DA firing rate (El Mansari *et al.* 2008). The mean basal firing rate of VTA DA neurons recorded by West and Weiss (2010) was around 2.5 Hz for vehicle and 2.0 Hz for ECS sham animals. Rats receiving antidepressant treatments that have been found to be significantly different from control rats have firing rates between 3.0 Hz and 4.5 Hz, which is in line with the mean basal firing rate of VTA DA neurons in control animals in the present study, and others (Prisco *et al.* 1994, El Mansari *et al.* 2008, Dremencov *et al.* 2009, Valenti and Grace 2010). It may be envisaged that the results brought forth by West and Weiss (2010) are due to collection of data from non-DA neurons. For instance, GABA neurons in the VTA exhibit patterns of bursting that may appear similar to that of DA neurons but have a larger range of firing rates and shorter action potential durations (Guyenet and Aghajanian 1978). Unlike with other investigators, they included in their data neurons with firing rates as low as 0.1 Hz rather than 0.5 Hz as the minimum. Their low values in the control animals may also be attributable to their use of larger and perhaps older animals, in which Sprague-Dawley rats were already 550-700 g at the onset of treatment administration, in comparison to around 300 g at the time of electrophysiological recordings, as was the case in the present study and other studies that have assessed VTA DA firing activity (Prisco *et al.* 1994, El Mansari *et al.* 2008, Dremencov *et al.* 2009, Valenti and Grace 2010). Our lab recently attempted to replicate

the findings by West and Weiss (2010) by recording electrophysiological activity of VTA DA neurons in similarly sized Sprague-Dawley rats. The activity of DA neurons in these larger animals was similar between control and bupropion treatment, with the firing rate being similar to that of DA neurons in 300 g rats (i.e., 3.5-4.0 Hz; unpublished data). Therefore, the current findings indicating that there is no change in VTA DA firing activity with repeated ECS may be more representative of the true, or lack of, effect of ECT. Combination or augmentation strategies that enhance VTA DA transmission, using agents such as ketamine, could optimize the antidepressant response of ECT (Loo *et al.* 2010). Ketamine, an anaesthetic agent occasionally used with ECT, appears to act synergistically with ECT in improving depressive symptoms both more rapidly and effectively (Goforth and Holsinger 2007, Okamoto *et al.* 2010, Ibrahim *et al.* 2011). It remains to be seen whether or not treatments that increase VTA transmission indeed elevate DA function with ECT or ECS, and if clinical efficacy with these agents is superior.

In contrast to the apparent lack of significant change in presynaptic components of mesolimbic DA neurotransmission, it was observed in the present study that DA neurons of the nigrostriatal pathway originating from the SNc were more active following 2-week ECS, although there was no change in firing rate or measures of burst activity in these neurons. Although it was previously suggested by Tepper and colleagues (1982) that the firing rate of SNc DA neurons is not different between sham, repeated ECS, and single ECS animals, the number of DA units used for their analysis was not indicated. In the present study, the number of neurons per track was increased by 100% in the ECS animals and the ratio of successful-to-unsuccessful tracks was increased from 44% in

sham to 74% in ECS animals, both of which could be attributed to an enhanced recruitment of quiescent DA neurons, given that spontaneously inactive DA neurons are normally present in the SNc (Grace and Bunney 1984). Furthermore, it appears that ECS has neuroprotective action on the nigrostriatal pathway, as evidenced by the prevention of 6-hydroxydopamine-induced DA cell loss and motor impairment in rats (6-OHDA; Anastasia *et al.* 2007). Higher numbers of active neurons in the SNc could represent enhanced facilitation of motor control, which may contribute to alleviation of particular depressive symptoms, such as psychomotor retardation.

ECT and TCA treatments exert a common sensitization of dorsal hippocampus CA3 neurons to 5-HT, in which the hippocampus is a brain structure that plays an important role in the antidepressant response (Blier *et al.* 1987). This increase in response is due to sensitization of postsynaptic 5-HT_{1A} receptors (de Montigny and Aghajanian 1978, de Montigny 1984). Moreover, the symptoms of ECT responders parallel those of individuals who respond to TCA treatment. For example, depressed patients who achieve remission with ECT also often respond to TCAs. In addition, failed response to TCAs is a factor associated with high relapse rates with ECT treatment (Greenberg and Kellner 2005). Another effect of TCAs includes an increase of neuronal responsiveness to 5-HT and NE in the FMN, amygdala, and lateral geniculate body (Menkes *et al.* 1980, Wang and Aghajanian 1980, Blier *et al.* 1987). Therefore, these regions may be important targets related to the antidepressant response. However, there is a lack of studies on overall improvement in facial expressions following TCA monotherapy. Browning and Cowen (1986) were able to show that with ECT treatment, overall motor performance is improved, although there have been contradictory findings (Scovorn and Kilmann 1980).

In a review summarizing the effects of ECT on movement disorders in which patients were also depressed, 3 studies show mostly an improvement in measures of motor performance, while 2 studies show no change (Kennedy *et al.* 2003). Given that repeated ECS in rats augmented responsiveness to NE in the FMN in the present study, and also elevated neuronal activation in the SNc, it appears that neurons that facilitate motor activity are more active and more responsive with repeated ECS. Together, results suggest that these changes may contribute to an overall alleviation of facial motor problems in depression. However, ECS-induced alleviation of MDD specific motor deficits may not be limited to facial regions. By restoring the function of lower motor neurons in the spinal cord, other aspects of psychomotor retardation, including posture and gait, could also be improved. It is plausible that motor-related depressive symptoms could be diminished by ECT given that motor neurons of the FMN and spinal cord are similarly modulated by 5-HT and NE as part of the neuronal control of a limb by activation of these motor neurons (White and Neuman 1980, Commissiong 1981).

The monoamine systems are the targets of most antidepressants. However, the exact mechanisms of particular antidepressant treatments, including ECT, are not well characterized. In animals, repeated ECS increases net 5-HT and NE transmission by selectively sensitizing postsynaptic serotonergic and noradrenergic receptors, respectively, but does not significantly affect presynaptic components of these systems. Repeated ECS increases the number of active DA neurons in the SNc, thereby possibly increasing DA neurotransmission in the nigrostriatal pathway, but has no effect on VTA DA neuron firing. Although ECT has a high success rate in the treatment of depression, about 50% experience relapse of a major depressive episode 6-12 months post-ECT.

Given that administration of repeated ECS increases responsiveness to 5-HT and NE in selective postsynaptic regions and has no effect on DA neuron activity in the VTA, combination and augmentation strategies for ECT candidates and non-responders should be oriented towards therapies that enhance mesolimbic DA neurotransmission.

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