Antidepressant activity: contribution of brain microdialysis in knock-out mice to the understanding of BDNF/5-HT transporter/5-HT autoreceptor interactions

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INTRODUCTION

Most of the antidepressants such as selective serotonin reuptake inhibitors (SSRIs) act as indirect agonists of monoamine receptors. While SSRI drugs produce relatively rapid blockade of serotonin [5-hydroxytryptamine (5-HT)] transporters (SERTs) in vitro, the onset of clinical benefits usually takes several (4–6) weeks to occur (Blier et al., 1987). This gap in timing between SSRI near-immediate effect on neurotransmitter systems and the slow symptomatic recovery is a paradox that has not been completely solved yet. At pre-synaptic level, SSRIs-induced blockade of SERT results in a rapid suppression of the firing activity of 5-HT neurons in the brainstem (Blier, 2001): these results have been obtained by using an electrophysiological technique in anesthetized animals.

MICRODIALYSIS: PRINCIPLE AND METHODOLOGY IN MICE

The principle of microdialysis technique is based on the balance between the release of neurotransmitters (e.g., 5-HT) and reuptake by selective transporters [e.g., serotonin transporter for serotonin 5-hydroxytryptamine (5-HT)].

Microdialysis already provided important information about the brain mechanism of action of antidepressants first in anesthetized rats in the early 1990s, and since then in conscious wild-type or knock-out mice. The principle of ICM is based on the balance between release of neurotransmitters (e.g., monoamines) and reuptake by selective transporters [e.g., serotonin transporter for serotonin 5-hydroxytryptamine (5-HT)] and complements to electrophysiology. This technique reflects pre-synaptic monoamines release and intrasynaptic events corresponding to ≈80% of whole brain tissue content. The inhibitory role of serotonergic autoreceptors infers that they limit somatodendritic and nerve terminal 5-HT release. It has been proposed that activation of 5-HT1A and 5-HT1B receptor subtypes limits the antidepressant-like activity of SSRIs. This hypothesis is based partially on results obtained in ICM experiments performed in naïve, non-stressed rodents. The present review will first remind the principle and methodology of ICM performed in mice. The crucial need of developing animal models that display anxiety and depression-like behaviors, neurochemical and brain morphological phenotypes reminiscent of these mood disorders in humans, will be underlined. Recently developed genetic mouse models have been generated to independently manipulate 5-HT1A autoreceptors and ICM helped to clarify the role of the pre-synaptic component, i.e., by measuring extracellular levels of neurotransmitters in serotonergic nerve terminal regions and raphe nuclei. Finally, we will summarize main advantages of using ICM in mice through recent examples obtained in knock-outs (drug infusion through the ICM probe allows the search of a correlation between changes in extracellular neurotransmitter levels and antidepressant-like activity) or alternatives (infusion of a small-interfering RNA suppressing receptor functions in the mouse brain). We will also focus this review on post-synaptic components such as brain-derived neurotrophic factor in adult hippocampus that plays a crucial role in the neurogenic and anxiolytic antidepressant-like activity of chronic SSRI treatment. Limitations of ICM will also be considered.

Keywords: knock-out mice, antidepressants, autoreceptors, serotonin, BDNF, microdialysis
Conventional intracerebral in vivo microdialysis

Whole brain tissue measurements represent a mixture of the intracellular (≈20%) and extracellular (≈80%) content. To obtain a measurement more directly related to synaptic transmission, it is interesting to sample specifically the content of the extracellular space, which is the site of exchanges between neurons, glial cells, and blood vessels (Zetterström et al., 1983). It contains various monoamines, excitatory and inhibitory amino acids, neuropeptides and their metabolites as well as precursors of these neurotransmitters. In the mid-1980s, the development of very sensitive analytical techniques such as liquid chromatography and electrochemical detection (LC-ED) had made possible to perform in vivo microdialysis first in anesthetized rodents, then in awake, freely moving animals.

In vivo microdialysis technique, in anesthetized or awake animals, was developed by the group of Delgado et al. (1972) in monkeys and then improved in rats by the group of Ungerstedt (Zetterström et al., 1983) in the early 1980s. It is based on the law of passive diffusion of low molecular-weight compounds through a porous membrane from the compartment with the highest concentration of neurotransmitters (the synaptic extracellular space) to the less concentrated compartment (i.e., the dialysis probe perfused with a buffer solution at physiological pH that does not contain neurotransmitters; Figure 1).

This technique, now currently applied in our laboratory in awake, freely moving WT control or knock-out (KO) adult mice, allows the collection of samples (named “dialysates”) every 10 or 20 min with a flow rate from 0.5 to 1.5 µl/min depending on the experimental protocol and the brain region studied. These samples contain, among other molecules, serotonin, its major metabolite (5-HIAA) and noradrenaline (NE), dopamine (DA), and their metabolites. These molecules are then quantified by using high-performance LC coupled to an amperometric detector (e.g., 1049A, Hewlett-Packard, Les Ulis, France). The limit of sensitivity for 5-HT is ~0.5 fmol/sample (signal-to-noise ratio ≈ 2).

The concentrations of neurotransmitters reflect the physiological balance between the calcium-dependent neurotransmitter release and its reuptake by SERT located on the membrane of pre-synaptic neurons. A comprehensive study of intracerebral microdialysis has four phases: (1) surgical stereotaxic implantation of the probe under anesthesia, (2) the collection of dialysates (first to measure baseline value of extracellular neurotransmitter), (3) the collection of brains for the accurate verification of the implantation site of the microdialysis membrane, and (4) of chromatographic analysis of dialysate samples (see Malagiet al., 2001; Guiard et al., 2004 for details).

Drug administration by reverse microdialysis

A major advantage of the microdialysis technique is to infuse a drug locally into the brain to confirm central effects on dialysates first measured following a peripheral injection of the drug. Thus, drugs with a high molecular weight can be dissolved in artificial cerebrospinal fluid (aCSF) and administered locally, for example, into the ventral hippocampus via a silico catheter glued to the microdialysis probe (flow rate: 0.2 µl/min for 2 min), at the dose of 10–100 ng (Guiard et al., 2007; Deltheil et al., 2008). For each experiment, a control group must receive the appropriate vehicle.

Zero net flux method of quantitative* intracerebral microdialysis

The zero net flux method of quantitative microdialysis is used to quantify basal extracellular neurotransmitter concentrations and their extraction fraction (E0) of this neurotransmitter, which provides an index of the functional status of the neurotransmitter uptake in vivo. Usually, four samples are collected to determine basal hippocampal 5-HT levels (as in David et al., 2001 in NK1 receptor KO mice), before local perfusion of increasing concentrations of 5-HT (0, 5, 10, and 20 nM). The dialysate 5-HT concentrations (Cout) obtained during perfusion of the various concentrations of 5-HT (Cin) are used to construct a linear regression curve for each animal (Guiard et al., 2008). The net change in 5-HT (Cin – Cout) is plotted on the y-axis against Cin on the x-axis. Extracellular 5-HT levels ([5-HT]ex) and the extraction fraction of the probe (E0) are determined as described by Parsons et al. (1991). The concentration of 5-HT in the extracellular space is estimated from the concentration at which Cin = Cout = 0 and corresponds to a point at which there is no net diffusion of 5-HT across the dialysis membrane. The extraction fraction (E0) is the slope of the linear regression curve and has been shown to provide an estimate of changes in transporter-mediated 5-HT uptake (Parsons et al., 1991; Gardier et al., 2003).

As an example of the relevance of the zero net flux method of quantitative microdialysis, we have recently shown the critical impact of a neuropeptide, brain-derived neurotrophic factor (BDNF) on serotonin neurotransmission under basal conditions and following SSRI treatment. In a series of experiments, we examined the consequences of either a constrictive decrease (Guiard et al., 2008) or increase in brain BDNF protein levels (Bennamoun et al., 2008; Deltheil et al., 2008, 2009) on hippocampal extracellular levels of 5-HT in conscious mice. The no net flux method allows unveiling differences in basal extracellular 5-HT levels in heterozygous BDNF+/− mice (Guiard et al., 2008). Indeed, this neurotrophic factor is known to play a role in mood and memory.
disorders and the mechanism of action of antidepressant drugs. However, the relationship between BDNF and serotonergic signaling is poorly understood. BDNF is a growth factor that plays a role in the development and survival of neurons. Serotonergic signaling, on the other hand, is involved in a wide range of physiological and psychological processes, including mood regulation, learning, and memory. Interpreting data relevant to these factors is important for understanding the mechanisms of action of antidepressant drugs.

The interpretation of these data is more appropriate when performed on AUC values in dialysate. The pharmacological properties of a compound are better reflected in these values because statistical analysis on AUC values better reflects the kinetics. This is because AUC analysis takes into account the entire time course of drug effects, not just the peak or trough values.

When we need to express time course data in microdialysis experiments, we use AUC values. The area under the curve provides a more comprehensive measure of the drug's effect over time. Microdialysis data are usually expressed as means ± SEM. For conventional microdialysis experiments, we use the AUC to describe time courses in experiments with high, middle, and low doses of SSRIs in rats.

**Statistical analysis and expression of results of microdialysis experiments in KO mice**

Usually, microdialysis data are reported as means ± SEM. For conventional microdialysis experiments, we use the AUC to describe time courses in experiments with high, middle, and low doses of SSRIs in rats.

**First in Rats**

When it was first used in rat brain in the mid-1980s, this technique measured, for example, extracellular concentrations of monoamines such as serotonin (5-HT). This technique reflects pre-synaptic release of 5-HT and intrasynaptic events. With its coupling to very sensitive analytical techniques, it has provided much information regarding changes in the local pre-synaptic release of monoamines following acute drug administration. Thus, it has been possible to obtain two major arguments supporting the hypothesis that somatodendritic 5-HT1A autoreceptors located in the raphe nuclei play an important role in the mechanism of action of SSRIs in rats.

First, we have learned that microdialysis and behavioral experiments were carried out by the same laboratories. Information included in this chapter was drawn from our own experience in this field and relevant publications from other investigators.

**Intracerebral in vivo microdialysis in rodents**

Another technique that has provided complementary information about the mechanism of action of SSRIs is intracerebral in vivo microdialysis (ICM). Microdialysis performed in awake, freely moving animals offers advantages over in situ or in vitro autoradiography and synaptosome techniques in BDNF mice. As expected, intraventricular microdialysis results obtained in rats have then been extended to mice. See also in Table 1 and Figure 6 in Guilloux et al. (2011), in which double 5-HT1A/1B−/− mice display a higher basal 5-HT levels in the frontal cortex and dorsal raphe nucleus (DRN) compared to WT mice.

(2) when it is sometimes important to collect some pharmacokinetic information about the short-term or long-lasting effect of a new drug in rodents. The AUC analysis of microdialysis data disregards information about differences in Cmax and duration of the drug effects.

(3) when a gray line (Figure 3 in Guilloux et al., 2006; Figures 2 and 3 in Nguyen et al., 2013) indicates the duration time of the forced swim test (FST, i.e., 6 min), which was performed, in a separate group of animals, at the maximum effect of the antidepressant on cortical extracellular 5-HT levels in mice. It emphasizes that microdialysis and behavioral experiments were carried out by using the same experimental protocol.
measure SSRI-induced changes in DRN 5-HTtext in awake, freely moving KO mice (Bortolozzi et al., 2004; Guard et al., 2004).

Next, we have learned from microdialysis performed in rats that SSRIs cause a larger increase in 5-HTtext at nerve endings following an acute treatment versus a chronic one. As the treatment is prolonged, a robust and time-dependent downregulation of SERT was observed (Pineyro et al., 1994; Benmansour et al., 2002), while 5-HT1A autoreceptors gradually desensitize leading to a progressive recovery to normal of the firing rate of 5-HT neurons (Blier et al., 1986; Chaput et al., 1996; El Mansari et al., 2005). However, these molecular events seem to depend on 5-HT1A autoreceptor internalization (Popa et al., 2010). Indeed, we studied the function of the 5-HT system in the raphe nuclei and hippocampus by using repeated in vivo microdialysis sessions in awake, freely moving mice. We assessed the degree of 5-HT1A autoreceptor desensitization by using a local infusion of the 5-HT1A receptor antagonist, WAY 100635, in the raphe via reverse microdialysis. We found that the axonofugal-like effects of fluoxetine correlate in time and amplitude with 5-HT1A autoreceptor desensitization, but neither with the basal extracellular levels of 5-HT in the raphe nuclei, nor in the hippocampus. These results suggests that the beneficial axonofugal/antidepressant-like effects of chronic SSRI treatment depend on 5-HT1A autoreceptor internalization, but do not require a sustained increase in extracellular 5-HT levels in a territory of 5-HT projection such as hippocampus. Several studies of patients with depression appear to confirm these experimental results, suggesting that co-administration of a 5-HT1A autoreceptor antagonist (pindolol) and an SSRI accelerated the onset of the antidepressant effect (Portella et al., 2011). However, given the complex pharmacology of pindolol, new drug developments may help to discover either selective and silent 5-HT1A receptor antagonists to be prescribed in combination with SSRIs, or dual action agents (SSRI + 5-HT1A receptor antagonists; Artigas et al., 2006).

**NEXT IN WILD-TYPE AND KNOCK-OUT MICE**

The use of pharmacological tools in mice

Changes in the amount of neurotransmitters (mainly monoamines such as 5-HT, NE, and DA) in synapses can be viewed as near-immediate effects of SSRI on brain neurotransmitter systems. In vivo brain microdialysis allows to measure basal extracellular levels of these neurotransmitters giving an idea of neurochemical events occurring at nerve terminals in brain regions of awake, freely moving rodents. In our laboratory, we extensively applied this technique in genetic and pharmacological studies aimed at investigating the relationship between neurotransmitters and brain regions, or between neurochemical changes and animal behaviors (see examples below). Among the main interests of microdialysis application is the infusion of drugs through the microdialysis probe (reverse dialysis) in conscious KO mice as well as in WT mice used as controls in these pharmacological experiments (e.g., intra-raphe perfusion of substance P in Guard et al., 2007; BDNF in Delheil et al., 2009).

As already mentioned, most prescribed serotonergic antidepressants show limited efficacy and delayed onset of action, partly due to the activation of somatodendritic 5-HT1A autoreceptors by the excess extracellular 5-HT produced by SSRI in the raphe nuclei. A group of scientists in Spain recently addressed this problem using an original strategy. Bortolozzi et al. (2012) administered a small interfering RNA (siRNA) to suppress acutely 5-HT1A autoreceptor-mediated negative feedback mechanisms in the mouse brain. They developed a conjugated siRNA (C-1A siRNA) by covalently binding siRNA targeting 5-HT1A receptor mRNA with the SSRI sertraline in order to concentrate it in serotonin axons, rich in SERT sites. The intracerebroventricular (I.C.V.) infusion of C-1A-siRNA to mice resulted in its selective accumulation in serotonin neurons. This was associated with antidepressant-like effects in the forced swim and tail suspension tests, but did not affect anxiety-like behaviors in the elevated plus-maze. In addition, C-1A-siRNA administration markedly decreased 5-HT1A autoreceptor expression and suppressed 5-OH-DPAT [7-(dipropylamino)-5,6,7,8-tetrahydronaphthalen-1-ol]-induced hyperthermia (a pre-synaptic 5-HT1A receptor effect in mice) without affecting post-synaptic 5-HT1A receptor expression in the hippocampus and prefrontal cortex. Moreover, I.C.V. C-1A-siRNA infusion augmented the increase in cortical dialysate 5-HT levels induced by fluoxetine to the level measured in 5-HT1A receptor KO mice. Hence, C-1A-siRNA represents a new approach to treat mood disorders as monotherapy or in combination with SSRI.

To learn whether or not the in vitro affinity of SSRIs toward monoamine transporters can predict in vivo microdialysis data, we studied whether a single administration of a range of doses [1, 4, and 8 mg/kg, given intraperitoneally (i.p.)] of paroxetine, citalopram, or venlafaxine may simultaneously increase dialysate 5-HTtext and norepinephrine (NEtext) by using in vivo microdialysis in the frontal cortex of awake, freely moving mice (David et al., 2003). We found that citalopram and paroxetine have the highest potency to increase cortical 5-HTtext and NEtext, respectively. In addition, the rank of order of efficacy of these antidepressant drugs to increase cortical 5-HTtext in vivo in mice was as follows: venlafaxine > citalopram > paroxetine, while the efficacy to increase cortical NEtext in mice of paroxetine and citalopram is similar, and greater than that of venlafaxine. Thus, the highest doses of the very selective SSRI citalopram and the very potent SSRI paroxetine were able to increase cortical NEtext. Surprisingly, the serotonin-norepinephrine reuptake inhibitor (SNRI) venlafaxine increased cortical 5-HTtext to a greater extent than NEtext in the range of doses studied in mice.

We recently confirmed these data with escitalopram, the S(+)-enantiomer of citalopram. To analyze the mechanisms by which SSRIs activate noradrenergic transmission in the brain, we compared the effects of escitalopram on both 5-HTtext and NEtext in the frontal cortex of WT versus mutant mice lacking the 5-HT transporter (SERT+−; Nguyen et al., 2013). In particular, the possibilities that escitalopram enhances NEtext either by a direct mechanism involving the inhibition of the low- or high-affinity NE transporters or by an indirect mechanism promoted by 5-HTtext elevation were explored. The FST was used to investigate whether enhancing cortical 5-HTtext and/or NEtext affected the antidepressant-like activity of escitalopram. As expected, a single systemic administration of escitalopram increased cortical 5-HTtext and NEtext in WT mice. However, escitalopram failed to...
increase cortical 5-HT
 expression in SERT−/− mice, whereas its neurochemical effects on NHEX persisted in these mutants. In WT mice, these neurochemical changes induced by escitalopram were associated with increased swimming parameter in the FST. Finally, escitalopram, at relevant concentrations, failed to inhibit cortical NE and 5-HT uptake mediated by low-affinity monoamine transporters (i.e., organic cation transporters such as OCT1, 2, or 3). These experiments suggest that escitalopram enhances, although moderately, cortical NE in vitro by a direct mechanism involving the inhibition of the high-affinity NE transporter (NET). Such in vitro effects of SSRIs could not be predicted by measuring the in vivo affinity of SSRIs toward SERT and NET in brain synaptosomes.

These results are not surprising. Indeed, experimental conditions (rats versus mice; whole brain versus cortical membranes; cell bodies versus nerve terminal regions; etc.) highly influence the values of binding parameters of ligands to neurotransmitter receptors or transporters measured in vitro (e.g., Kᵢ, GTP-gamma5 binding, etc.). The potency and selectivity of SSRIs as determined in vitro do not take into account noradrenergic projections and others, which obviously interfere in vivo, but not in vitro. Thus, function of monoamines transporters is much more complex than previously thought. In vivo experiments help to depict this complexity when it is possible to measure correlation between neurochemical parameters and behavior paradigms.

The use of mutated mice

The mouse genome can be specifically manipulated to produce the targeted deletion, replacement of genes, or down-/over-expression of related proteins in the brain (Sotnikova and Gainetdinov, 2007). This was first obtained in embryonic stem (ES) cells, but more recently, temporal and spatial controls of gene expression were possible in adult mice. In the field of anxiety and depression, preclinical studies such as those described above, have been mostly performed in healthy, “not depressed” animals. In the mid-1990s, genetically manipulated mice became available. It complicated the experimental protocol because it was necessary to include littermates as WT control mice. Great hopes were placed in mutant lines, some of them being considered as putative animal models of anxiety or depression. Several lines of transgenic (Tg) mice (carrying a human gene) or KO mice (i.e., homogenous mice lacking the two copies of a gene coding for a receptor or transporter of neurotransmitter or neuropeptide) were generated between 1994 and 1998. The first KO mice were generated by homologous recombination. This technique allowed the creation of animal-related patterns of human brain pathologies. The genetic background is a fundamental parameter for analyzing the phenotype of KO mice. Historically, the mutant mice were established using ES line 129/Sv. However, creating new lines of mutant mice on a genetic background C57BL/6 is now preferred, although there are limits on the use of this strain in some behavioral tests (see Gardier, 2009 for a review).

At that time, the procedure of ICM needed to be quickly adapted to perform experiments in an animal model having a smaller brain size than rats. Microdialysis experiments were first performed in tyrosine hydroxylase Tg mice by Nakahara et al. (1991). Then, it was applied to 5-HT transporter KO mice (Saudou et al., 1994; Trillat et al., 1997), to DA transporter (DAT) KO mice (Gainetdinov et al., 1997), and so on. Of course, at the end of the experiments, the precise location of the microdialysis probe must be macroscopically verified according to the stereotaxic coordinates given by the mouse brain atlas (Paxinos and Franklin, 2001).

Regarding the pharmacological knowledge of antidepressants, the choice of KO mice as experimental models of anxiety-depression was remarkably appropriate because it is now well recognized that major depressive disorders result from a combination of genetic and environmental factors. In addition, knowing that anxiety and depression have a high co-morbidity (Goeman and Coplan, 1996; Leonardo and Hen, 2006), it is critical for basic research to develop animal models that present behavioral, neurochemical, and brain morphological phenotypes reminiscent of depression and anxiety. Some “serotonergic” KO mice display important changes in their basal phenotype. For example, constitutive 5-HT1A receptor KO mice were simultaneously described by three different laboratories as an animal model of anxiety-related disorder (Heisler et al., 1998; Parks et al., 1998; Rambou et al., 1998). They display decreased exploratory activity and increased fear of aversive environments and exhibited a decreased immobility in the FST, an effect commonly associated with antidepressant treatment. Brain microdialysis performed in 5-HT1A receptor KO mice have proven to be a valuable technique to address key questions regarding the mechanism of action of antidepressants.

One of the most interesting applications of microdialysis is to allow the study of basal extracellular levels of neurotransmitters, for example, in 5-HT1A receptor KO mice. While conventional microdialysis does not allow reliable measurements of these basal levels (see Conventional Intracerebral In Vivo Microdialysis), the no net flux (or zero net flux) method of quantitative microdialysis in mutants allows the direct and accurate determination of basal extracellular levels of neurotransmitters (see Zero Net Flux Method of Quantitative Intracerebral Microdialysis) the DRN is a brain region where 5-HT
 expression is known to regulate serotonergic transmission through activation of 5-HT1A autoreceptors.

When microdialysis was performed in the DRN, it was found that baseline DRN 5-HT
 expression did not differ between WT control and KO mice. This result suggests a lack of tonic control of 5-HT1A autoreceptors on DR 5-HT release (Bortolozzi et al., 2004; Guilloux et al., 2006).

Furthermore, microdialysis helped to decipher the brain region-dependent effects of antidepressants. Both a saline injection and handling for 3 min increased DRN 5-HT
 expression in 5-HT1A receptor KO mice, but not in control mice. Fluoxetine, a serotonergic antidepressant, induced a dose-dependent increase in DRN 5-HT
 in both genotypes, but this effect was markedly more pronounced in 5-HT1A KO mice. These results suggest that the increased responsiveness of dialysate 5-HT
 in the DRN of
5-HT_{1A} receptor KO mice at least in part explain the anxious phenotype of these mutants. Such information can help to define a better treatment of anxiety-related disorders.

The inhibitory 5-HT_{1A} receptor exists in two separate populations with distinct effects on serotonergic signaling, i.e., an autoreceptor that limits 5-HT release throughout the brain and a heteroreceptor that mediates inhibitory responses to release 5-HT. Traditional pharmacologic and Tg strategies have tried to separate the distinct roles of these two receptor populations. Recently, Richardson-Jones et al. (2011) developed a new strategy to manipulate pre-synaptic 5-HT_{1A} autoreceptors in serotonergic raphe neurons without affecting 5-HT_{1A} heteroreceptors, generating mice with higher (1A-High) or lower (1A-Low) autoreceptor levels. In this latter line, it was thus possible to examine the brain 5-HT system by partially turning off 5-HT_{1A} autoreceptors at a specific time point and to study correlations between changes in 5-HT transmission and antidepressant-like activity of SSRIs in various behavioral tests. This strategy robustly affects raphe firing rates, but has no effect on either basal extracellular 5-HT levels as measured by in vivo microdialysis in the frontal cortex and ventral hippocampus. Interestingly, following 8 days of fluoxetine treatment, a difference in 5-HT levels was found in the hippocampus, with higher levels in the 1A-Low mice. In addition, 1A-Low mice displayed a larger increase in 5-HT in response to an acute challenge of fluoxetine in both brain regions. Together with electrophysiology data showing an increased spontaneous neuronal activity in the dorsal raphe of 1A-Low mice under stressful conditions, the microdialysis results were consistent with an increased serotonergic tone in these animals in response to an SSRI. Compared to 1A-High mice, 1A-Low mice show a blunted physiological response to acute stress, increased behavioral despair, and no behavioral response to antidepressant, thus modeling what we can find in patients with the 5-HT_{1A} risk allele. Indeed, human studies implicate a polymorphism in the promoter of the 5-HT_{1A} receptor gene in increased susceptibility to depression and decreased treatment response (Lemond et al., 2003). These mice may thus be conceived as a human equivalent to SSRI response (1A-Low) and resistance (1A-High; Blier, 2010). These results establish a causal relationship between 5-HT_{1A} autoreceptor levels and response to antidepressants.

The same group of researchers used a recently developed genetic mouse system to independently manipulate 5-HT_{1A} autoreceptor and heteroreceptor populations. They found that 5-HT_{1A} autoreceptors affect anxiety-like behavior, while 5-HT_{1A} heteroreceptors affect responses to forced swim stress, with no effects on anxiety-like behavior (Richardson-Jones et al., 2011). These results establish distinct roles for the two receptors’ populations, providing evidence that signaling through endogenous 5-HT_{1A} autoreceptors is necessary and sufficient for the establishment of normal anxiety-like behavior. Taken together, these data obtained in KO mice brought a lot of information about the pathophysiology of psychiatric disorders and their treatments.

Thus, in 2012, we have at our disposal a large number of genetically engineered mice, some of them being interesting animal models of anxiety and depression. These mice are very helpful to discover the underlying pathological mechanisms that limit the effects of current treatments of major depressive episodes and to identify the nature of the molecular cascades leading to the installation of disorders such as anxiety and depression. In addition, KO mice help to study the effects of acute and chronic treatment with antidepressants.

Recent advances in experimental approaches using genetically manipulated mice have already been summarized in the literature (Sotnikova and Gainetdinov, 2007). Knowing the large number of KO mice generated to date, it is not possible to detail the findings of each putative model interesting in the anxiety and depression field of research (SERT−/− mice, Bengel et al., 1998; 5HT1 receptor KO mice, Froger et al., 2001; Guiard et al., 2004; β-arrestin 2 KO mice, Rouxieu et al., 2008). Therefore, the remainder of the present chapter will only describe some examples, which explain these statements.

**ADVANTAGES AND LIMITATIONS OF USING MICRODIALYSIS IN KO MICE**

Depressive disorders result from a combination of genetic and environmental factors. To date, several genes appear to have in humans and animals, a greater influence than the other and emerge from the literature. Among them, the presence of a polymorphism of either SERT (Bengel et al., 1998; Kuzkova et al., 2010), 5-HT_{1A} receptor (Lemond et al., 2003), the tryptophan hydroxylase type 2 (TPH-2; Invernizzi, 2007), or BDNF (Chen et al., 2006) is associated with the occurrence of depression related to stress, or to a response to behavioral tests predictive of the antidepressant-like activity of a molecule (Ponomol et al., 1977; Steru et al., 1985).

**ADVANTAGES**

In these KO mice, we can measure, for example, the paradigms of stress to predict the antidepressant potential of a molecule and the selectivity of behavioral responses in comparison with non-mutated control animals: if these responses are diminished or absent in KO mice deprived of a gene encoding a neurotransmitter receptor, we may conclude that this receptor plays a major part either in the antidepressant-like effect and/or of the molecule. Regarding microdialysis, changes in dialysate levels of neurotransmitters following acute (Malagé et al., 2001) or chronic (Gardier et al., 2003) SSRI treatment can highlight the mechanisms of action of these drugs.

Thus, we combined KO mice and receptor antagonist strategies to investigate the contribution of the 5-HT_{1B} receptor subtype in mediating the effects of an SSRI, paroxetine in mice (Malagé et al., 2001). Using microdialysis, we found that a single systemic administration of paroxetine (1 or 5 mg/kg by the i.p. route) increased 5-HT in the ventral hippocampus and frontal cortex of WT control and mutant mice. However, in the ventral hippocampus, the SSRI induced a larger increase in dialysate 5-HT levels in KO 5-HT_{1B} mice than in control mice. In addition, either the absence of the 5-HT_{1B} receptor (in KO 5-HT_{1B} mice) or its pharmacological blockade with the mixed 5-HT_{1B/D} receptor antagonist, GR 127935 (in WT mice) potentiated the effect of a single administration of paroxetine on extracellular 5-HT levels in the ventral hippocampus. Thus, these data underline several points:

- **fphar-04-00098** — 2013/8/6 — 19:55 — page 6 — #6
(a) complementary results were obtained by combining KO mice and receptor antagonist strategies.  
(b) there were already by BDNF (paroxetine as measured on swimming behavior was potentiated by serotoninergic nerve terminals and revealed the importance of a particular brain region, the ventral hippocampus. It is interesting to notice that recently, many experimental arguments have accumulated to suggest that antidepressants exert their behavioral activity in adult rodents, at least in part, by inducing of cellular and molecular changes in the adult hippocampus (David et al., 2010).

By using microdialysis, we can also study changes in dialysate 5-HT levels in the DRN (see Introduction). Data described above in 5-HT1A receptor KO mice illustrated this important contribution. This experiment can give further information when combined with measurements of the electrical activity of 5-HT neurons. Again, the comparison of results between a KO mice model and WT mice is very informative.

Neurochemical changes as measured by using microdialysis can have functional consequences since they correlated with behavioral data obtained, for example, in the FST. Three examples can illustrate these benefits.

Example 1, in WT mice: intra-hippocampal BDNF infusion correlated with behavioral data in this protocol, suggesting that a co-administration improved the antidepressant-like activity of the SSRI (Deltheil et al., 2008, 2009).

Example 2, in 5-HT1A receptor KO mice: as described in Guiloux et al. (2006), paroxetine (1 and 4 mg/kg) dose-dependently increased cortical 5-HT levels in both WT and KO genotypes, but the effects were greater in mutants (Figure 3A). Paroxetine administration also dose-dependently decreased the immobility time in both strains of mice, but the response was much greater in SERT−/− mice (Figure 3B). Overall these results suggest that the genetic inactivation of 5-HT1A receptors, abolished the inhibitory feedback control exerted by somatodendritic 5-HT1A autoreceptors, thus enhancing the response of mutant mice to stressful conditions such as the FST. Thus, following SSRI administration, an indirect activation of pre-synaptic 5-HT1A receptors by endogenous 5-HT may limit its antidepressant-like effects in the FST in WT mice.

Example 3, in SERT−/− mice: another interest of brain microdialysis is to allow the measurement of several neurotransmitters in the same sample. Thus, we recently examined the effects of the (S)-enantiomer of citalopram, escitalopram (ESC) on both [5-HT]ext and extracellular levels of [NE]ext in the frontal cortex (FCx) of freely moving WT and mutant mice lacking SERT−/− by using ICM (Nguyen et al., 2013). In WT mice, a single systemic administration of escitalopram produced a significant increase in cortical [5-HT]ext and [NE]ext (Figure 4A). As expected, escitalopram failed to increase cortical [5-HT]ext in SERT−/− mice, whereas its neurochemical effects on [NE]ext persisted in these mutants. In addition, in WT mice submitted to the FST, escitalopram increased swimming parameter without affecting climbing behavior (Nguyen et al., 2013).

FIGURE 2  
(A) Microdialysis data showing that an acute intra-hippocampal injection of BDNF (100 ng) potentiated the effects of the systemic administration of an SSRI, paroxetine (1 mg/kg) on dialysate 5-HT in the hippocampus of freely moving wild-type mice. Results are expressed as AUC values (mean ± SEM) calculated for the amount of 5-HT collected during the 0–120 min post-treatment period. 
(B) Antidepressant-like activity of paroxetine as measured on swimming behavior in the forced swim test (FST) was potentiated by BDNF. Thus, neurochemical changes correlated with behavioral data in this protocol, suggesting that a BDNF + SSRI combination may offer new alternatives to treat mood disorders (from Deltheil et al., 2008; *p < 0.05; **p < 0.01 when compared to the vehicle-treated group; §p < 0.05 when compared to the paroxetine/vehicle-treated group and paroxetine/BDNF-treated group; §§p < 0.01 when compared to the BDNF/vehicle-treated group and BDNF/paroxetine-treated group; two-way ANOVA, Fisher's LSD post hoc test).

![Image](https://example.com/image1.png)
Indeed, to study the direct consequences of alterations in the targeted gene, constitutive KO mice are very valuable tools because of compensatory processes that have taken place in reaction to life-long changes in gene expression (Groenink et al., 2003). The constitutive deletion of the NET, for example, induced an up-regulation of two other monoamine transporters DAT and SERT (Solich et al., 2011). An increase in the binding of [3H]paroxetine to the SERT and [3H]GBR-12935 to the DAT was observed in various brain regions of NET-KO mice, without alterations of mRNA encoding these transporters, as measured by in situ hybridization. This important finding obviously impacts the interpretation of previous data. Similarly, in SERT<sup>−/−</sup> mice, Zhou et al. (2002) reported that 5-HT was found in DA neurons of homozygous<sup>−/−</sup> mice. To verify the role of the DA transporter in these ectopic uptake, SERT<sup>−/−</sup> mice were treated with DA uptake blocker GBR-12935: ectopic 5-HT in DA neurons was disappeared. These data indicate that 5-HT can be taken into DA neurons in rodents when SERT is not functionally adequate to remove extracellular 5-HT levels, and (c) the DA transporter is responsible for the 5-HT uptake into DA neurons. Thus, cross neuronal type uptake exists and serves as a compensatory backup when a specific transporter is dysfunctional. Thus, when using mice lacking an important protein from the earliest period of their existence, one has to be aware that compensatory alterations may occur in the brain as well as at the periphery. This point must be considered when it comes to interpretation of the experimental results.

Table 1 summarizes the main advantages as well as some critical points of the intracerebral microdialysis technique.

### LIMITATIONS

There are also limits regarding the use of constitutive KO mice. Compensatory events may occur when mice are generated by homologous recombination (Gardier, 2009). For example, 5-HT<sub>1A</sub> receptor KO mice exhibit a higher efficacy of 8-OH-DPAT-induced hypothermia suggesting that an adaptive thermo regulatory process involving the functional activity of somatodendritic 5-HT<sub>1A</sub> receptors is altered in 5-HT<sub>1A</sub><sup>−/−</sup> mice (Gardier et al., 2001). By contrast, Bouthenet et al. (2002) found no indications for adaptive changes in pre-synaptic 5-HT<sub>1A</sub> receptor function in 5-HT<sub>1A</sub> knockout mice as measured telemetrically on body temperature and heart rate responses.
animals susceptibility genes and proteins involved in the patholog-
cal processes leading to anxiety and depression. These biological
markers could then be helpful to pose the diagnosis of the dis-
ease in human. They also give information on their functional
role, thus offering opportunities to develop new drug treatments.

When performed in KO mice, and together with other techniques,
brain microdialysis was very useful to define central monoaminer-
gic dysfunctions having behavioral consequences similar to those
associated with endogenous depression in humans. Some KO mice
with mutations of serotonin targets (e.g., the 5-HT transporter
SERT, 5-HT<sub>1B</sub>, 5-HT<sub>1A</sub>, and 5-HT4 receptors) display changes in
phenotypes similar to those induced by chronic treatment with
antidepressants in WT control mice.

Chronic antidepressant treatment may regulate the expres-
sion of neurotrophic factors such as BDNF and stimulate the
process of adult neurogenesis in the dentate gyrus of the hip-
pocampus in rats (Malberg et al., 2000) and adult mice (Santarelli
et al., 2003; David et al., 2009). Changes in adult neurogene-
sis are only seen after chronic, but not acute, antidepressant
treatment. Microdialysis studies in heterozygous mice for BDNF
(Szapacs et al., 2004; Deltheil et al., 2008, 2009; Guiard et al.,
2008) contributed to this knowledge by exploring the relationship

Table 1 | Summary of the main advantages and some critical points of the intracerebral microdialysis technique in freely moving mice.

<table>
<thead>
<tr>
<th>Main advantages of using microdialysis in WT and KO mice*</th>
<th>Some limitations of using microdialysis in WT and KO mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>- In vivo pre-synaptic test to study consequences of autoreceptor of transporter blockade on release and reuptake of neurotransmitters.</td>
<td>- Compared to electrophysiology, technique of reference.</td>
</tr>
<tr>
<td>- Direct access of exogenous molecules into the brain tissue, with minimal damage: an ideal approach to confirm brain effects observed following a systemic administration. Even more interesting when the drug does not cross easily the blood brain barrier (such as molecules with a high molecular weight: neurotrophic factors, e.g., BDNF).</td>
<td>- Large outer diameter of microdialysis probe (0.2 mm).</td>
</tr>
<tr>
<td>- Some KO mice brain microdialysis was very useful to define central monoaminer-gic dysfunctions having behavioral consequences similar to those associated with endogenous depression in humans. Some KO mice susceptibility genes and proteins involved in the pathologic processes leading to anxiety and depression.</td>
<td>- During microdialysis experiments, the samples are collected every 15–20 min (in the hippocampus and frontal cortex), every 10 min in raphe nucleus. This is due to the slow flow rate of the perfusion medium (≈1 µl/min), which leads to a poor temporal resolution compared to electrophysiology (400 µl/min).</td>
</tr>
<tr>
<td>- Some advantages of this technique are very interesting in KO mice knowing the difficulties to breed most of them.</td>
<td>- Time consuming:</td>
</tr>
<tr>
<td>- Possibility to implant two probes in the same mouse: a probe at the vicinity of cell bodies (e.g., raphe nuclei when studying the neuronal 5-HT system), and a probe at serotonergic nerve terminals (hippocampus, frontal cortex), thus evaluating a neural circuit.</td>
<td>- One experimenter, two mice, 1 day; 10–12 animals per group; delayed results (HPLC). Possible improvement with more sensitive analytical methods such as capillary electrophoresis coupled to a laser-induced fluorescence detection (Parrot et al., 2007; Danesoy et al., 2008), but it remains a very complex technique.</td>
</tr>
<tr>
<td>- Possibility of measuring several neurotransmitters in the same dialysate sample of WT and KO mice (Nguyen et al., 2013)</td>
<td>- 3–6 months to complete an experiment, i.e., to evaluate the effects of several doses of an agonist-antagonist compared to mice treated with the vehicle or in WT controls. Even longer when using Tg or KO mice (breeding, genotyping, selection of age, sex, and so on...).</td>
</tr>
<tr>
<td>- The same of WT or KO mouse can be studied for two consecutive days, e.g., on day 1 following administration of the vehicle in the control group, and on day 2 following the novel pharmacological treatment</td>
<td>- Delicate animal handling, to avoid effects of stress, thus requiring an experienced experimenter to perform in vivo microdialysis in freely moving mice.</td>
</tr>
<tr>
<td>- Chronic microdialysis: when using a guide cannula, it is possible to collect samples once a week for several weeks in the same WT or KO mouse (Popa et al., 2010)</td>
<td>- Absolute need to check the exact location of the probe, macroscopically on brain coronal sections at the end of the experiment. Especially in mice (Bart et al., 2004).</td>
</tr>
<tr>
<td>- When applied in awake, freely moving animals, functional consequences of SSR1-induced increases in extracellular neurotransmitter levels can be studied, e.g., correlation between changes in brain 5-HT&lt;sub&gt;1A&lt;/sub&gt; and behavioral data (the swimming time in the FST) for example (Deltheil et al., 2009, Nguyen et al., 2019).</td>
<td>- Poor prognostic value of basal extracellular concentrations of 5-HT, DA, and NA.</td>
</tr>
<tr>
<td>- Extracellular concentrations of metabolites in dialysates (e.g., 5-HIAA, the main metabolite of 5-HT): it reflects intracellular metabolism of 5-HT, it has little interest because dialysate 5-HIAA levels decrease, independently of the dose of the indi-cant 5-HT receptor antagonist administered. These changes are not related to the neuronal activity (Malagié et al., 1995; Rocher et al., 1998).</td>
<td></td>
</tr>
</tbody>
</table>

*Some advantages of this technique are very interesting in KO mice knowing the difficulties to breed most of them.
between the hippocampal 5-HT system (i.e., the function of its transporter, one of the main targets of antidepressants) and brain BDNF levels.

In the future, our efforts to understand the pathophysiology of mood disorders, especially anxiety/depression, will focus on the antidepressant responses, especially in non-stressed and stressed rodents. Microdialysis techniques will continue to decipher region-dependent relationships between brain neurotransmitters and circuits involved in the mechanism of action of an antidepressant drugs’ polytherapy, soon available on the market. Furthermore, original strategies are now available to rescue the expression of a particular receptor subtype in a tissue-specific and temporally controlled manner in mice. For example, it is well known that agonists of the 5-HT1A receptor such as buspirone have anxiolytic properties, and K0 mice lacking this receptor show increased anxiety-like behavior (as indicated above). However, the relevant brain regions involved in anxious phenotype have not been delineated. Using such a tissue-specific, conditional rescue strategy for the 5-HT1A receptor, Gross et al. (2002) engineered mice in which the expression of the 5-HT1A receptor gene was under the control of the antisense doxycycline. The gene of interest was switched off when the mice were fed with the antibiotic. They used autoradiography to demonstrate that high levels of post-synaptic 5-HT1A receptor expression in the hippocampus and cortex of the rescue mice, but the pre-synaptic 5-HT1A autoreceptor, was undetectable in the raphe nuclei. By using mice in which the 5-HT1A receptor can be knocked out at will, they show that the absence of the receptor in newborns lead to anxiety-like behavior, whereas its knock-out during adult life has no effect. In addition, they found that postsynaptic developmental processes help to establish adult anxiety-like behavior. Generating such a rescue mice is a long-lasting process, but each animal can be used as its own control.

Another strategy can be used to rescue a gene of interest, in which the K0 mice line previously generated was used as the control group. A gene of interest is re-expressed into the midbrain of K0 mice by stereotaxically injecting a lentiviral vector carrying this gene coding for a receptor to test for the selectivity of behavior. This strategy was recently applied to study the role of beta2-subunit of the nicotinic acetylcholine receptor (nAChR) Maskos et al., 2005 in mediating the reinforcement properties of nicotine. In this example, microdialysis experiments were performed to confirm the rescue of nicotine effects in the vectorized line of mice compared to WT and K0 lines. Regarding the serotonin field of research, global disruption of 5-HT2A receptor signaling in mice reduces inhibition in conflict anxiety paradigms without affecting depression-related behaviors. Selective rescue of 5-HT2A receptor in the cortex normalized conflict anxiety behaviors (Wüstefeld et al., 2006). These findings indicate a specific role for cortical 5-HT2A receptors in the modulation of anxiety. These techniques allow greater precision and flexibility to generate KO rodents for understanding neurotransmitter function. No doubt that such novel and powerful tools, together with techniques of knock-in or siRNA recently applied to the field of 5-HT receptors, will continue to give unexpected information on molecular and cellular mechanisms involved in mood disorders and their treatments.

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