CHAPTER 1

Introduction

Until approximately 15 years ago, pharmaceutical companies rarely considered the needs of children when developing medicines, even though the majority of active substances licensed by the European Agency for the Evaluation of Medicinal Products (EMA) were considered to be relevant for use in children. Thus, fewer than half of all licensed active substances considered relevant for use in children had a pediatric indication in at least a subset of the pediatric population[1]; moreover, the off-label or unlicensed use of drugs in pediatric pharmacotherapy was highly prevalent among many European countries[2–4]. Although off-label use does not necessarily reflect off-knowledge use[5], off-label use can be a frequent cause of adverse events among hospitalized children[6]. In addition, the availability of several classes of medicines with a pediatric indication was limited, as pediatric medicines may not be age-appropriate (for example, they may not be dose-applicable or come in a suitable formulation), even if their use is authorized[7]. These issues are pertinent to pediatric pharmacology in general[8], but they may be even more pertinent to children and adolescents with a psychiatric[9] or neurological disorder (such as epilepsy[10] or pain[11]).

DIFFERENCES BETWEEN CHILDREN AND ADULTS WITH CNS DISEASE

The pharmacological treatment of children and adolescents with psychiatric disorders, as well as several neurological disorders, has traditionally followed the development program in adults, without cross-age validation or recognizing the inherent differences between children and adults with respect to their neuropsychopathology and neuropsychopharmacology[12,13]. For example, unlike in adulthood, the manifestation of schizophrenia in childhood and adolescence carries a particularly poor prognosis, and this poor outcome may be due to the disease's effect during a highly susceptible time in the development and neurobiological maturation of children and adolescents[14]. Moreover, in contrast to adults who experience seizures, many newborn infants and children who experience seizures remain refractory to therapy[15]. Finally, some disorders such as certain epilepsy syndromes are specific to children and have therefore been largely neglected, particularly with respect to drug development.

In addition, because of development-specific differences in pharmacokinetics (PK) and/or pharmacodynamics (PD), the relationships between drug action and drug exposure in children cannot be understood fully by simply extrapolating information from adult patients. For example, PK can differ considerably between children and adults as a result of physiological differences, differences in the maturity of enzyme systems, and differences in clearance mechanisms[16]. Age-related differences in PK can lead to differences in the blood concentration of a drug even when the same dose is given to a child and an adult, or to a young child and an older child, as reported for methylphenidate[17]. The brain-to-serum concentration ratio of a drug can also vary with age, potentially leading to age-dependent differences in concentration at the drug's site of action. For example, children with bipolar disorder have been reported to have lower lithium brain-to-serum ratios than adults[18]. In addition, several aspects of drug therapy may be related to brain development. Neural development in childhood and adolescence involves a highly coordinated sequence of events that is characterized by both progressive and regressive processes. As a result of this sequence of events, the development of gray matter, white matter, total brain volume, and neuronal connectivity is age-dependent[19], and perturbations in these developmental patterns may play a central role in the pathogenesis of several childhood neuropsychiatric and neurodevelopmental disorders, including autism, ADHD, fragile X syndrome, 22q11 deletion syndrome, Williams syndrome, Down syndrome, and Turner syndrome[20]. On the other hand, far less is known regarding the changes in brain function that result from these age-dependent and disorder-related changes in brain structure. However, a growing body of data suggests that healthy children have differential trends in the development of task performance in distinct functional areas of the central nervous system (CNS)[21–24], and children with neuropsychiatric disorders may have impaired neuropsychological function in several domains, as reported for ADHD[25]. Brain development can also affect drug efficacy and pharmacoresistance[13]. For example, monoaminergic systems, the target for most CNS drugs, undergo considerable plasticity and rearrangement during childhood and

adolescence[26–28], and animal studies have revealed age-related differences in the drug responses of several psychostimulant drugs such as amphetamine[29], cocaine[30,31], and methylphenidate[32,33]. In addition, γ-aminobutyric acid (GABA)—the activity of which is affected in many neurological and neurodevelopmental disorders[34] – excites immature neurons but inhibits neurons in the normal adult brain[35]. As a result of these age-dependent differences in PK and PD, children may respond differently than adults to CNS drugs. Unfortunately, our understanding of age-dependent differences in PK and PD is based almost entirely on animal models, and remarkably few clinical trials in humans have focused on understanding the age-dependent differences in response to CNS drugs.

DRUG DEVELOPMENT FOR CHILDREN WITH CNS DISEASE

In addition, the registration of CNS drugs for use in children and adolescents has lagged behind new developments in adults[36]. In particular, the steps involved in recognizing, classifying, and treating psychiatric disorders generally proceed much more slowly in children and adolescents than in adults[12]. In fact, the field of clinical psychopharmacology has only recently begun to include children and adolescents on a relatively large scale. This delay is the result of a combination of factors, including concerns regarding drug safety and tolerance in this vulnerable population, diagnostic uncertainties, and the pharmaceutical industry's reluctance to seek pediatric registration and to perform labeling studies for diagnoses that are not traditionally considered to be predominant disorders of childhood and adolescence (for example schizophrenia and bipolar disorder)[37]. In terms of clinical research, several areas within the field of pediatric neuropsychopharmacology have been neglected, including—but not limited to—eating disorders, early-onset schizophrenia, mental retardation[38], and pediatric critical care settings[39]. With respect to epilepsy, the most prevalent severe neurological disease among children, extremely few trials have been performed in children compared to adults; moreover, most antiepileptic drug trials in children are performed only once, whereas the majority of adult trials are replicated[13]. The developmental aspects of judgment and decision-making in children and adolescents with neuropsychiatric disorders such as cognitive immaturity and impaired cognitive processing are also unique to pediatric neuropsychopharmacology research[40]. Unlike other fields within pediatric pharmacology (such as pediatric oncology), child and adolescent psychiatry and pediatric neurology have not been tightly integrated into psychopharmacological networks, and professional societies for child and adolescent psychiatry have only recently begun to establish their own networks for psychopharmacology research[41,42].

The situation has been complicated even further by a decrease in the number of new drug registrations for psychiatric and neurological indications in adults. Such research is generally considered to be too risky, as the subjective nature of endpoints in psychiatry and neurology makes it difficult to determine whether a drug is effective, even using large-scale trials. As a result, the major changes in CNS drugs over the years have focused primarily on pharmacological modifications (such as improving the drug's intrinsic efficacy, selectivity, and/or kinetic properties[43]) rather than developing novel treatments.

Despite the relative paucity of scientific data to support the safe and efficacious use of CNS drugs in children and adolescents, and despite a lack of novel treatments, the number of treated children and adolescents, as well as the duration of exposure to CNS drugs, has increased substantially over the past few decades[44–46]. To close this gap, researchers need to acquire evidence regarding the adequate dosing, efficacy, and safety of these treatments[47]. Recent European legislation (the EU Pediatric Regulation) will likely drive an increase in pediatric trials and specific label changes, dosing recommendations, and age-appropriate formulations. Unless a specific waiver or deferral is granted, the Regulation requires the industry to plan clinical trials in children early in the development of new medicines for treating adults or for line extensions for on-patent medications. Several challenges have emerged when working within the framework of this new legislation[48]. For example, performing randomized, controlled trials in children can raise specific technical, logistic, legal, and financial difficulties that are not usually associated with trials performed in adults. In addition, even studies that are performed

successfully in children are not necessarily maximally informative, as many studies measure PK only or perform a single post-dose PD measurement. Because of these challenges—even in fields with extensive pediatric research such as ADHD—it can be difficult to reach valuable and relevant conclusions regarding pharmacological profiles and/or dose-dependent effects based solely on currently available studies.

NEED FOR NON-INVASIVE MEASUREMENT METHODS

Therefore, there is an urgent need for validated assessment tools that are suitable for evaluating the efficacy and safety of CNS drugs in the pediatric population[49]. In addition, because clinical research in pediatric patients is generally hampered by interrelated logistic and ethical constraints (including the limited number, scope, and invasiveness of study-related interventions that can be performed if they fall outside the realm of routine clinical care), researchers should attempt to reduce the burden placed on participating children and adolescents by using non-invasive or minimally invasive measurement methods. Changes to these methods that are designed to reduce patient burden (for example, implementing a change in the sampling procedure) have been reported to increase patient enrollment in studies of rare pediatric diseases[48].

The most accessible and non-invasive means to measure drug activity in the brain is to measure drug-related CNS functional activity using methods that provide sufficient sensitivity and specificity[43]. Although validated biomarkers are rare in neurology and psychiatry, many neuropsychiatric drugs affect a variety of CNS functions in a dose-dependent and concentration-dependent manner, particularly drugs that affect neurotransmitter activity. This provides the opportunity to quantify CNS effects, even in situations in which the activity measured is not an essential step in the pathogenic cascade. The affected functions can often be roughly or partially linked to the specific pharmacological mechanism (for example, linking $GABA_A$-ergic activation to reduced saccadic peak velocity). Thus, these physiological functions can be useful biomarkers for measuring a pharmacological effect, even if they have no clear functional relationship with psychosis, anxiety, or depression. It is very likely that a clear concentration-dependent effect of a highly specific compound is mediated by the drug's mechanism of action, even if the functional relationship itself is not clear[43], thereby enabling researchers to compare different drug formulations and age-dependent differences in drug profiles.

To relate drug-related changes in CNS functional activity to changes in PK, drug concentrations must be measured. Traditional PK protocols—with multiple samples and indwelling catheters or multiple venipunctures—are undesirable in therapeutic pediatric drug research, and they are strictly unacceptable in non-therapeutic pediatric drug research. To overcome some of these limitations, other sample collection methods for determining drug concentration (for example, saliva sampling) have been developed and validated. Studies have reported that patients and parents prefer saliva sampling over venous blood drawing[50], and some drugs can be detected in the saliva relatively soon after administration, enabling the researcher to compile a concentration-time profile. This method has the added benefit of allowing on-site testing without the need for medical personnel or complicated post-collection sample processing, thereby further decreasing the burden placed on the children.

A drug's secretion and distribution in the saliva is dependent upon its physico-chemical properties. Lipophilicity, degree of ionization, and protein binding are the major determinants of the saliva:plasma concentration (S/P) ratio[51]. For example, drugs that are heavily protein bound and drugs that are extremely hydrophilic and positively charged at physiological pH (for example, aminoglycosides) can be undetectable in saliva. The transition of drugs to the CNS is favored by low molecular weight, a lack of ionization at physiological pH, and lipophilicity[52], all of which theoretically favor the drug's secretion into the saliva. In certain cases, saliva concentration may even be preferred over total plasma concentration, particularly for highly protein-bound drugs, as saliva concentration can reflect the free fraction of a non-ionized drug (and thus reflects the intracellular concentration in target tissues). For example, the concentration of phenytoin in cerebrospinal fluid correlates more closely

with the saliva concentration than the blood concentration[53]. Saliva sampling is also recommended over plasma sampling for the therapeutic monitoring of several other anti-epileptic drugs[54]. Unfortunately, however, the usefulness of determining the saliva concentration of several other drugs has been questioned because of variability in the S/P ratio. Interpreting the saliva drug concentration of neutral, weakly acidic (pK_a > 8.5), or weakly basic (pK_a < 5.5) drugs with negligible protein binding (such as ethanol, antipyrine, and paracetamol) is relatively easy, as the S/P ratio is approximately 1. In contrast, interpreting the saliva drug concentration of drugs with other properties can be challenging, as the S/P ratio can be low for acidic drugs (pK_a < 8.5) and drugs with high protein binding (such as caffeine and phenobarbital), and the concentration can be influenced by active transport mechanisms (such as lithium) or the occurrence of an alkaline reaction in aqueous solutions, for example in the case of basic drugs (pK_a > 5.5) with low protein binding (such as methylphenidate, procainamide, amphetamine, and lidocaine). Therefore, the use of saliva sampling for measuring concentration of several drug types has been limited. In addition, because many drugs are administered orally, buccal contamination can affect the S/P ratio at early time points after administration. Nevertheless, if the sources of variability in the S/P ratio can be minimized or quantified, measuring the saliva drug concentration might be a meaningful alternative to measuring plasma drug concentration, particularly for the aforementioned drug types.

SCOPE OF THIS THESIS

In this thesis, we explored non-invasive methods for monitoring the pharmacokinetics and pharmacodynamics of commonly used CNS stimulants (methylphenidate and caffeine) and depressants (ethanol and melatonin) in children and/or adolescents. Neuropsychological and neurophysiological functions were measured longitudinally using the NeuroCart, a battery of tests developed at the Centre for Human Drug Research that includes tests for alertness, visuomotor coordination, motor control, memory, and subjective drug effects. Drug concentrations in children and adolescents were measured non-invasively. We evaluated the feasibility of using saliva as an alternative to plasma for measuring drug concentration in two studies. For this purpose, caffeine was chosen as an example of a basic CNS drug that is primarily non-ionized in human saliva, and methylphenidate was chosen as a typical basic CNS drug that is primarily ionized in human saliva. This thesis concludes with the report of two clinical trials that were designed to develop age-appropriate formulations for potential use in children.

In **chapter 2**, the impact of the Pediatric Regulation on the development of pediatric drugs – including CNS drugs – is evaluated. In this study, we evaluated for which drug classes and therapeutic subgroups pediatric development was agreed by (or waived by) the Regulation. In addition, we evaluated whether the Regulation is likely to increase the development of drug classes for pediatric conditions for which there exists an unmet pediatric need based on usage and availability data.

The psychostimulant methylphenidate (MPH) is the most commonly prescribed medication for treating pediatric attention-deficit/hyperactivity disorder (ADHD). However, previously published studies investigating the effects of immediate-release methylphenidate (MPH-IR) have yielded contradictory results due to several sources of variability, including a lack of standardized biomarkers and drug-effect measurements. Despite its widespread use, and despite extensive research, researchers still lack useful, validated biomarkers for studying the effect of MPH in children with ADHD. In **chapter 3,** we identified generally applicable, non-invasive biomarkers for monitoring the acute effects of MPH-IR in children and adolescents with ADHD. The presence of unexplained variations in the S/P ratio during the time course of both MPH-IR and extended-release MPH can interfere with the further exploration of saliva as an alternative method for determining MPH concentration in children. Therefore, a liquid chromatography-tandem mass spectrometric method that uses a hydrophilic interaction liquid chromatography column (HILIC) was validated and is presented in **chapter 4**. This analytical tool provides an accurate and precise quantification of MPH in both plasma and saliva samples. **Chapter 5** describes a study in which we attempted to

identify sources of variability in MPH concentration and to determine the correlation between MPH concentration in the saliva and plasma in healthy adult volunteers using a population-based PK approach.

Chapter 6 describes a study of the effect profile of low-dose caffeine in healthy adolescents. Caffeine concentration was measured non-invasively in saliva samples. Because saliva can be contaminated by residual caffeine after drinking a caffeinated beverage, a second study was performed in young adult subjects in order to measure contamination after drinking a caffeinated beverage compared to swallowing a caffeine capsule. In addition, previous studies in adults related caffeine effects to changes in plasma concentration; therefore, simultaneous saliva and plasma samples were collected, and the S/P ratio of caffeine was measured. Based on the data obtained from this kinetics study, a population-based PK model was built in order to obtain estimated plasma drug levels in adolescents; this model could prove useful in the development of a caffeine pharmacokinetics-pharmacodynamics (PK/PD) model in adolescents.

Chapter 7 describes a study to measure the acute effects of low-dose alcohol on sensitive biomarkers for alcohol effects in healthy adolescents. To correlate the measured effects with concentration, alcohol concentration was measured non-invasively using end-expired breath sampling. A PK/PD model was then developed using data from this study with adolescents as well as data collected from previous alcohol studies performed in adults in order to characterize alcohol PK, the effects on objective and subjective biomarkers, and sources of variability in PK and PD, including age.

Sleep problems are highly prevalent among children with neurodevelopmental disorders and can have a substantial impact on the child and the child's family. Melatonin is effective for treating sleep problems in these children, and melatonin appears to have a favorable short-term and long-term risk profile, which has led to the increasingly widespread clinical use of melatonin as an off-label medicine. However, no commercially available, age-appropriate prolonged-release melatonin preparation is currently available. Melatonin was identified by the EMA as having a pediatric therapeutic need, including the need to develop an age-appropriate sustained-release formulation and the need to collect data regarding melatonin's PK, efficacy, and safety in children with autism and a sleep disorder. The study described in **chapter 8** is part of a Pediatric Investigation Plan (PIP) under the Pediatric Regulation and was a cross-over ascending dose study of Circadin (1 mg mini-tablets), a prolonged-release melatonin formulation. In this study, the PK profile, safety, and acceptability of Circadin mini-tablets were evaluated in 16 children and adolescents with autism and a sleep disorder. Whole-saliva samples were collected non-invasively from passive drool, and melatonin concentration was measured.

Because of its rapid onset and rapid recovery profile, midazolam is the medication of choice for providing conscious sedation and management of epileptic seizures. Nasal delivery of midazolam is a patient-friendly alternative to parenteral delivery routes. Nasal delivery offers several practical advantages; for example, it allows for direct, easy, needle-free administration, and it can be administered safely without professional assistance. However, previous formulations for delivering midazolam nasally have not been very successful due to the lack of solvents that can dissolve midazolam at therapeutic dosages without causing nasal mucosa damage. Therefore, in **chapter 9**, we evaluated the PK, efficacy, and tolerability of a new, highly concentrated aqueous midazolam formulation (Nazolam) in healthy adult volunteers. This new formulation has potential for use in providing conscious sedation and epilepsy management in children and adolescents.

In **chapter 10**, the feasibility and applicability of saliva sampling in pediatric populations will be discussed based on data obtained from pediatric clinical studies of caffeine, methylphenidate, and melatonin described in this thesis. In addition, the (potential of) pharmacological profiling of pharmacodynamics in children and adolescents will be discussed based on literature review and clinical studies of methylphenidate, caffeine, alcohol and midazolam. This chapter concludes with potential practical applications of this approach and suggestions for future directions.

REFERENCES

1 Ceci A, Felisi M, Baiardi P, Bonifazi F, Catapano M, Giaquinto C et al. Medicines for children licensed by the European Medicines Agency (EMEA): the balance after 10 years. *Eur J Clin Pharmacol* 2006; 62(11):947-952.

2 Conroy S, Choonara I, Impicciatore P, Mohn A, Arnell H, Rane A, et al. Survey of unlicensed and off label drug use in paediatric wards in European countries. European Network for Drug Investigation in Children. BMJ. 2000;320:79-82.

3 Pandolfini C, Bonati M. A literature review on off-label drug use in children. *Eur J Pediatr.* 2005;164:552-558.

4 Cuzzolin L, Atzei A, Fanos V. Off-label and unlicensed prescribing for newborns and children in different settings: a review of the literature and a consideration about drug safety. *Expert Opin Drug Saf.* 2006;5:703-718.

5 Rose K. European Union Pediatric Legislation Jeopardizes Worldwide, Timely Future Advances in the Care of Children With Cancer. *Clin Ther.* 2014;36:163-177.

6 Mason J, Pirmohamed M, Nunn T. Off-label and unlicensed medicine use and adverse drug reactions in children: a narrative review of the literature. *Eur J Clin Pharmacol.* 2012;68:21-28.

7 van Riet-Nales DA, de Jager KE, Schobben AF, Egberts TC, Rademaker CM. The availability and age-appropriateness of medicines authorized for children in The Netherlands. *Br J Clin Pharmacol.* 2011;72:465-473.

8 Roberts R, Rodriguez W, Murphy D, Crescenzi T. Pediatric drug labeling: improving the safety and efficacy of pediatric therapies. JAMA. 2003;290:905-911.

9 Radley DC, Finkelstein SN, Stafford RS. Off-label prescribing among office-based physicians. *Arch Intern Med.* 2006;166:1021-1026.

10 Chiron C, Kassai B, Dulac O, Pons G, Nabbout R. A revisited strategy for antiepileptic drug development in children: designing an initial exploratory step. CNS *Drugs.* 2013;27:185-195.

11 Davies EH, Ollivier CM, Saint RA. Paediatric investigation plans for pain: painfully slow! *Eur J Clin Pharmacol.* 2010;66:1091-1097.

12 Stoyanova-Beninska VV, Wohlfarth T, Isaac M, Kalverdijk LJ, van den Berg H, Gispen-de WC. The EU paediatric regulation: effects on paediatric psychopharmacology in Europe. *Eur Neuropsychopharmacol.* 2011;21:565-570.

13 Chiron C, Dulac O, Pons G. Antiepileptic drug development in children: considerations for a revisited strategy. *Drugs.* 2008;68:17-25.

14 Clemmensen L, Vernal DL, Steinhausen HC. A systematic review of the long-term outcome of early onset schizophrenia. BMC *Psychiatry.* 2012;12:150.

15 Noam Y, Raol YH, Holmes GL. Searching for new targets for treatment of pediatric epilepsy. *Epilepsy Behav.* 2013;26:253-260.

16 Ginsberg G, Hattis D, Sonawane B, Russ A, Banati P, Kozlak M, et al. Evaluation of child/adult pharmacokinetic differences from a database derived from the therapeutic drug literature. *Toxicol Sci.* 2002;66:185-200.

17 Wigal SB, Gupta S, Greenhill L, Posner K, Lerner M, Steinhoff K, et al. Pharmacokinetics of methylphenidate in preschoolers with attention-deficit/hyperactivity disorder. *J Child Adolesc Psychopharmacol.* 2007;17:153-164.

18 Moore CM, Demopulos CM, Henry ME, Steingard RJ, Zamvil L, Katic A, et al. Brain-to-serum lithium ratio and age: an in vivo magnetic resonance spectroscopy study. *Am J Psychiatry.* 2002;159:1240-1242.

19 Toga AW, Thompson PM, Sowell ER. Mapping brain maturation. *Trends Neurosci.* 2006;29:148-159.

20 Dennis EL, Thompson PM. Typical and atypical brain development: a review of neuroimaging studies. *Dialogues Clin Neurosci.* 2013;15:359-384.

21 Brocki KC, Bohlin G. Executive functions in children aged 6 to 13: a dimensional and developmental study. *Dev Neuropsychol.* 2004;26:571-593.

22 Luciana M, Nelson CA. The functional emergence of prefrontally-guided working memory systems in four- to eight-year-old children. *Neuropsychologia.* 1998;36:273-293.

23 van den Wildenberg WP, van der Molen MW. Developmental trends in simple and selective inhibition of compatible and incompatible responses. *J Exp Child Psychol.* 2004;87:201-220.

24 Karatekin C. Development of attentional allocation in the dual task paradigm. *Int J Psychophysiol.* 2004;52:7-21.

25 Chamberlain SR, Robbins TW, Winder-Rhodes S, Muller U, Sahakian BJ, Blackwell AD, et al. Translational approaches to frontostriatal dysfunction in attention-deficit/hyperactivity disorder using a computerized neuropsychological battery. *Biol Psychiatry.* 2011;69:1192-1203.

26 Gelbard HA, Teicher MH, Faedda G, Baldessarini RJ. Postnatal development of dopamine D1 and D2 receptor sites in rat striatum. *Brain Res Dev Brain Res.* 1989;49:123-130.

27 Court JA, Perry EK, Johnson M, Piggott MA, Kerwin JA, Perry RH, et al. Regional patterns of cholinergic and glutamate activity in the developing and aging human brain. *Brain Res Dev Brain Res.* 1993;74:73-82.

28 Chen YI, Choi JK, Xu H, Ren J, Andersen SL, Jenkins BG. Pharmacologic neuroimaging of the ontogeny of dopamine receptor function. *Dev Neurosci.* 2010;32:125-138.

29 Bolanos CA, Glatt SJ, Jackson D. Subsensitivity to dopaminergic drugs in periadolescent rats: a behavioral and neurochemical analysis. *Brain Res Dev Brain Res.* 1998;111:25-33.

30 Laviola G, Wood RD, Kuhn C, Francis R, Spear LP. Cocaine sensitization in periadolescent and adult rats. *J Pharmacol Exp Ther.* 1995;275:345-357.

31 Chen YI, Choi JK, Xu H, Ren J, Andersen SL, Jenkins BG. Pharmacologic neuroimaging of the ontogeny of dopamine receptor function. *Dev Neurosci.* 2010;32:125-138.

32 Levant B, Zarcone TJ, Davis PF, Ozias MK, Fowler SC. Differences in methylphenidate dose response between periadolescent and adult rats in the familiar arena-novel alcove task. *J Pharmacol Exp Ther.* 2011;337:83-91.

33 Chen YI, Choi JK, Xu H, Ren J, Andersen SL, Jenkins BG. Pharmacologic neuroimaging of the ontogeny of dopamine receptor function. *Dev Neurosci.* 2010;32:125-138.

34 Deidda G, Bozarth IF, Cancedda L. Modulation of GABAergic transmission in development and neurodevelopmental disorders: investigating physiology and pathology to gain therapeutic perspectives. *Front Cell Neurosci.* 2014;8:119.

35 Khazipov R, Khalilov I, Tyzio R, Morozova E, Ben-Ari Y, Holmes GL. Developmental changes in GABAergic actions and seizure susceptibility in the rat hippocampus. *Eur J Neurosci.* 2004;19:590-600.

36 Weissman MM, Klerman GL. Depression: current understanding and changing trends. *Annu Rev Public Health.* 1992;13:319-339.

37 Correll CU. Clinical psychopharmacology of pediatric mood stabilizer and antipsychotic treatment, part 1: challenges and developments. *J Clin Psychiatry.* 2007;68:1301-1302.

38 Tobi H, Scheers T, Netjes KA, Mulder EJ, de BA, Minderaa RB. Drug utilisation by children and adolescents with mental retardation: a population study. *Eur J Clin Pharmacol.* 2005;61:297-302.

39 Stoddard FJ, Usher CT, Abrams AN. Psychopharmacology in pediatric critical care. *Child Adolesc Psychiatr Clin N Am.* 2006;15:611-655.

40 Spetie L, Arnold LE. Ethical issues in child psychopharmacology research and practice: emphasis on preschoolers. *Psychopharmacology (Berl).* 2007;191:15-26.

41 Kolch M, Schnoor K, Fegert JM. The EU-regulation on medicinal products for paediatric use: impacts on child and

adolescent psychiatry and clinical research with minors. *Eur Child Adolesc Psychiatry.* 2007;16:229-235.
42 Vitiello B. Research in child and adolescent psychopharmacology: recent accomplishments and new challenges. *Psychopharmacology (Berl).* 2007;191:5-13.
43 van GJ, Cohen A. Vanishing clinical psychopharmacology. *Br J Clin Pharmacol.* 2011;72:1-5.
44 Kalverdijk LJ, Tobi H, van den Berg PB, Buiskool J, Wagenaar L, Minderaa RB, et al. Use of antipsychotic drugs among Dutch youths between 1997 and 2005. *Psychiatr Serv.* 2008;59:554-560.
45 Schirm E, Tobi H, Zito JM, de Jong-van den Berg LT. Psychotropic medication in children: a study from the Netherlands. *Pediatrics.* 2001;108:E25.
46 Vitiello B. Pharmacoepidemiology and pediatric psychopharmacology research. *J Child Adolesc Psychopharmacol.* 2005;15:10-11.
47 Wohlfarth T, Kalverdijk L, Rademaker C, Schothorst P, Minderaa R, Gispen-de WC. Psychopharmacology for children: from off label use to registration. *Eur Neuropsychopharmacol.* 2009;19:603-608.
48 Winzenburg G. More than 5 years of European Paediatric Regulation: Statistics and industrial experience. *Int J Pharm.* 2014.
49 Stoyanova-Beninska VV, Wohlfarth T, Isaac M, Kalverdijk LJ, van den Berg H, Gispen-de WC. The EU paediatric regulation: effects on paediatric psychopharmacology in Europe. *Eur Neuropsychopharmacol.* 2011;21:565-570.
50 Gorodischer R, Burtin P, Hwang P, Levine M, Koren G. Saliva versus blood sampling for therapeutic drug monitoring in children: patient and parental preferences and an economic analysis. *Ther Drug Monit.* 1994;16:437-443.
51 Haeckel R, Hanecke P. Application of saliva for drug monitoring. An in vivo model for transmembrane transport. *Eur J Clin Chem Clin Biochem.* 1996;34:171-191.
52 Pardridge WM. Recent advances in blood-brain barrier transport. *Annu Rev Pharmacol Toxicol.* 1988;28:25-39.
53 Troupin AS, Friel P. Anticonvulsant level in saliva, serum, and cerebrospinal fluid. *Epilepsia.* 1975;16:223-227.
54 Gorodischer R, Koren G. Salivary excretion of drugs in children: theoretical and practical issues in therapeutic drug monitoring. *Dev Pharmacol Ther.* 1992;19:161-177.