Assessing activity onset time and efficacy for clinically effective antidepressant and antimanic drugs in animal models based on dominant–submissive relationships

Ewa Malatynska, Albert Pinhasov, Christopher J. Creighton, Jeffrey J. Crooke, Allen B. Reitz, Douglas E. Brenneman, Mariusz S. Lubomirski

Abstract

There is confusion in the literature on the measurement of the drug activity onset time (AOT) for both clinical and non-clinical studies of antidepressant and antimanic drugs. The questions asked are: How often and at which time points should drug effects be measured? At what level of a drug effect should AOT be determined? Is the placebo (control) effect important for consideration of drug AOT? This paper reviews approaches taken to answer these questions and to assess drug therapeutic AOT. The first part of the paper is devoted to a review of methods used in clinical trials with depression as an indication. The second part is focused on approaches taken in animal models of depression and how they could help in assessing drug AOT. Finally, a summary of pharmacological values on which the AOT depends is presented and a new statistical approach to data analysis method proposed. The allied experimental design for pre-clinical and clinical studies may help to characterize and differentiate AOT for available and new generation of antidepressants and antimanic drugs. Published by Elsevier Ltd.

Keywords: Animal models; Depression; Mania; Reduction of submissive behavior model; Reduction of dominant behavior model; Antidepressants; Antimanic drugs; Activity onset time; Clinical trials; Non-linear regression analysis; Levenberg–Marquardt algorithm

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*Corresponding author. Tel.: +1 215 628 5121; fax: +1 215 540 4666.
E-mail address: Emalatyn@PRDUS.jnj.com (E. Malatynska).

1Current address: Department of Molecular Biology, College of Judea and Samaria, Ariel, Israel.

2Current address: Trius Therapeutics, 6310 Nancy Ridge Dr. San Diego, CA 92121, USA.
1. Introduction: definition of drug activity onset time

The number of responders to treatment with antidepressant or anxiolytic drugs is estimated to be between 50% and 70% in typical patient populations (for review see Preskorn, 1984; Hirschfeld et al., 2002). For this reason, it is important for a clinician to know how much time needs to be allowed before a decision can be made that the patient is not a responder to the antidepressant drug applied and should be switched to another. Thus, the activity onset time (AOT) of an antidepressant drug needs to be assessed. During the last three decades, there have been substantial efforts made to establish the AOT for anxiolytic and especially antidepressant drugs. There is no consensus on the best model to determine AOT. The results presented are controversial with some papers arguing for an onset delay of antidepressant drug activity and others for almost immediate activity (for review see Katz et al., 1987, 1996, 2004a, b, 2006; Stassen et al., 1993, 1996; Derivan, 1995; Muller and Moller, 1998; Thompson, 2002). There is also no consensus of what onset of activity means. However, one definition was proposed by Katz et al. (1996), who stated that onset of activity is a “…time point at which significant, persistent improvement in the severity of the state of depression, or in one or more of its major clinical components is initiated.” Most often this time point was determined as the time of the first significant difference measured between placebo and drug-treated groups. Alternatively, the time point of the first measured statistically significant difference between two effective treatments was an indication of which was the faster acting drug. This approach is illustrated in Fig. 1a using data from Claghorn (1977). The significant difference in the Hamilton Depression Rating Score (HDRS) occurs between placebo and maprotiline-treated groups on the first measurement at day-7 of the treatment and for imipramine versus placebo at day-14. It is concluded that maprotiline has a faster onset of activity than imipramine. However, what this experimental design and analysis of the results is telling us is that the response to antidepressants was greater than the response to placebo on day-7 for maprotiline and on day-14 for imipramine. Does this mean that maprotiline has faster onset of activity than imipramine? From the data analysis shown in Fig. 1a, we cannot really answer this question. There are several disadvantages with this approach as accurately listed by Stassen et al. (1993) with one of them definitely being that the point in time at which the improvement begins cannot be accurately determined.

To know if one treatment effect onset is faster than another the time-course for different treatments needs to be compared at the same drug response level. With this principle in mind, data from Fig. 1a were subjected to non-linear regression analysis and fitted using GraphPad Prism software (GraphPad Software, Inc. San Diego, CA), to the hyperbolic $E_{\text{max}}$ equation

$$Y = E_{\text{max}}X^h(t)/(ED50^h + X^h(t)),$$

where $X(t)$ is the concentration at time $t$, $E_{\text{max}}$ is the maximum effect and $h$ is the Hill coefficient. This analysis enabled us to calculate the AOT at the 50% level for each treatment ($AOT_{50}$). The results are illustrated in Fig. 1b. With this analysis, we did not fully reach the equal efficacy requirement for the analyzed treatments but we got closer to this goal. In this specific example, the calculated $AOT_{50}$ values were in days $5.3 \pm 2.7$ (95% confidence interval (CI) = −6.4 to 17.1) for maprotiline; $9.7 \pm 2.4$ (CI = −0.6 to 20.0) for imipramine and $13.0 \pm 17.7$ (CI = −63.0 to 89.1) for placebo. The mean values were not significantly different. However, the statistical power of the analyzed data, in terms of a sample size and number of time points measured, was too low to detect significant differences. On the other hand, this example enabled us to establish grounds for the importance of including a requirement for the comparison of different treatments at equal response levels in the working definition for the calculation of the drug AOT. This analysis suggests several important

\[ E_{\text{max}} = \frac{Y}{X^h(t)/(ED50^h + X^h(t))}, \]

\[ h = \frac{\ln(c)}{\ln(X^h(t)/(ED50^h + X^h(t)))}. \]

Fig. 1. Improvement in Hamilton Depression Score during a 4-week double blind control clinical trial with maprotiline (MAP) imipramine (IMI) and placebo (PL) treated psychiatric outpatients. Plotted data are from Claghorn (1977). (a) Definition of the activity onset time as the first time point with a statistically significant difference between drug and placebo treated groups. (b) Activity onset time (AOT) determined at 50% of the effect using hyperbolic $E_{\text{max}}$ equation for non-linear analysis (see text for details).
variables for the measurement of AOT. If the time at which a significant difference between treatments is the endpoint, then the statistical power of the measurements is critical. Power, in this case, will depend on both subject number and the maximal difference between the starting and final responses achieved, assuming that the measurement error is the same for all treatments. Note that it is unclear in this case that the two drug treatments were capable of producing the maximum response possible for both drugs used. The use of a suboptimal dose regimen for one treatment would reduce the statistical power of its response determination relative to an optimal dose regimen for the other treatment and would likely increase its apparent AOT. However, it is interesting to note that the non-linear regression analysis applied showed that prediction of AOT may be independent, to a certain extent, of when the first measurement was taken and of the measurement intervals. However, it is necessary to collect enough data points for accurate non-linear regression analysis.

In this review, we will discuss briefly previous approaches to the determination of antidepressant AOT taken in clinical trials and for animal models of depression. Giving as an example data from dominant–submissive relationship (DSR)-based models, particularly the Reduction of Submissive behavior model (RSBM), we will use our working definition of the AOT to design experimental settings allowing the determination of AOT as a constant for a given drug.

2. Methods used to assess activity onset time for drugs in clinical trials of depression

At the beginning of the antidepressant era when imipramine was discovered, Khun (1958) in the report describing this event, stated that mood depression improved within a few days of treatment for a majority of patients. Early research on antidepressants that were mostly directed toward efficacy studies supported this view (for review see Katz et al., 1996). However, the necessity of comparing the effects of drug-treated and untreated groups obscured this early interpretation since placebo-treated groups show a level of antidepressant effect. Usually it was obscured this early interpretation since placebo-treated groups show a level of antidepressant effect. Usually it was presumed that drug activity onset in the population of patients was usually estimated as a ratio of responders to non-responders, or expressed as a decreased average in the depression rating score. In both cases, the level of non-responders to responders significantly influenced conclusions related to the effect level and consequently the calculation of AOT (for review see Derivan, 1995). In the 1990s’ and beyond, several researchers based the measurement of drug activity onset in the population of patients solely on responders arguing that if a true drug effect occurs it happens quickly and usually in a persistent manner. These approaches were based on studies done by Small et al. (1981), who separated patients into responder and non-responder groups. Quitkin et al. (1984a, b) and Quitkin and Stewart (1984) introduced a pattern analysis that identified persistent and non-persistent response and early and delayed onset of activity and the combination of these two, response level and onset patterns. Expectations of an early antidepressant effect from responder groups also changed the timing of data collection in clinical trials from weekly to 3 day or even more frequent intervals (for in-depth discussion of these approaches see reviews by Katz et al., 2002, 2004a). An analysis of the impact of effect measurement frequency on apparent drug AOT was conducted by Mallinckrodt et al. (2006) who used a categorical repeated measures approach and a traditional assessment schedule, and found frequent estimates beneficial for data evaluation.

The problem of responders and non-responders is not limited to the drug-treated groups, but also concerns placebo-treated groups of patients. Small et al. (1981) showed that selecting responders from all study groups (placebo, ECT, trazodone and imipramine treated) completely eliminated differences between treatment groups in contrast to data encompassing results obtained from the total number of patients participating in each group of the study. These data are also discussed in the review by Derivan (1995) and clearly indicate the necessity of including placebo groups in research of antidepressant efficacy and AOT. At present, it is impossible to determine how many drug responders would improve without treatment in the course of a study or to predict a priori who would be a non-responder in the placebo group. To solve this latter problem, recent clinical trials applied a prescreen for placebo response to trial-recruited patients and included only placebo non-responders into the treatment phase of the clinical trial (Walczak et al., 1996; Katz et al., 2004b; Brannan et al., 2005). Researchers discussing the ideal clinical trial also include an initial placebo phase as a necessity (Leon et al., 2001).

The division of the patient population between responders and non-responders resulted in a need for a precise definition of these groups. Generally, it is accepted in clinical practice that a patient who achieved a 50% improvement on the event rating score is a responder
(Small et al., 1981). The 50% effect level criterion requires the establishment of minimum and maximum effect levels that can be identified by the survival analytical approach (Kaplan and Meier, 1958) used by Stassen et al. (1993). Survival analysis that originally determined death rate among patients with cancer was used in the clinical trials for cancer treatment. Stassen et al. (1993) adapted this analysis to survival function of patients who respond to treatment. Using this analysis, he defined the threshold for improvement as being 15–25% with respect to the corresponding initial values in contrast to those used before at ≥50% level. In a review of methods used to establish the onset of antidepressant activity, Thompson (2002) proposed to distinguish three milestones in the improvement of depression symptoms: onset event, response event and remission event based on the survival analytical approach.

The response to drug treatment is usually dependent on the dosing schedule. A suboptimal dose resulting in a reduced response makes it difficult to measure AOT and will increase the apparent AOT value that is dependent on achieving a statistical difference. It is usually difficult to predict an efficacious dose capable of producing a significant effect in clinical trials. It is also common to titrate an initial starting dose at the beginning of treatment to avoid the emergence of unwanted effects. This is unfortunate in regard to AOT because dose is a factor that needs to be fixed to calculate, predict or compare AOT for different antidepressants. Gruwez et al. (2005) described a mathematical model designed to predict the onset of activity for combinations of pindolol and selective serotonin reuptake inhibitors (SSRIs) that depended on the pindolol dose in a simulated clinical trial. A dependence of AOT on drug dose is also concluded from experiments involving the RSBM described later in this paper.

In recent years, different statistical approaches have been proposed for data analysis to determine the onset of drug activity in clinical trials (for review see Leon, 2001; Thompson, 2002). In addition to the survival analytical approach mentioned earlier (Kaplan and Meier, 1958; Stassen et al., 1993), different mixed-effect linear regression models were also proposed (Gibbons et al., 1988, 1993; Hedeker et al., 1991; Hedeker and Gibbons, 1994). These models have several advantages over those previously proposed (e.g., they can address the rate of symptoms declining in two sets of parallel samples), for a brief review and discussion of these models see Leon, (2001).

3. Animal models: are responses to antidepressant treatment delayed?

Most animal models of mania and depression are based on a single measurement of drug effect. For example, mania models measuring locomotor activity as an end point or depression models like the Forced Swim Stress (FST) (Porsolt et al., 1977, 1978) or the Tail Suspension (TST) (Steru et al., 1985, 1987) tests cannot apply several consecutive measurements to the same group of animals because animal habituation would interfere with the drug effect measured. To determine differences between acute and chronic drug effects in such tests, drugs usually are applied to distinct groups of animals with experiments conducted after different treatment times (Detke et al., 1997; Cryan et al., 2005; West and Weiss, 2005; Kusmider et al., 2006). This is also true for the Learned Helplessness (LH) model (Overmier and Seligman, 1967; Seligman and Maier, 1967; Seligman et al., 1968, 1975, 1980; Seligman, 1972; Seligman and Beagley, 1975). In the LH test, most research done uses chronic administration of antidepressants followed by an acute effect measurement procedure (for review see Willner, 1990, 1995; Willner and Mitchell, 2002). Sometimes the procedure was extended to a few days of consecutive testing (Martin et al., 1992; Takamori et al., 2001; Millan et al., 2004).

In these tests the estimate of the relative onset of drug activity was based solely on the measurement of response at two or more different time points. A drug with a higher response at an earlier time point was concluded to have a faster onset of activity. For example, in the FST, Dremencov et al. (2004) measured immobility of genetically produced Flinders Sensitive Line (FSL) rats after 7- and 14-days of treatment with the antidepressants desipramine or nefazodone. FSL rats show depressive-like behaviors in various tests (Overstreet et al., 1995) and are considered to be a valuable model of depression (Willner and Mitchell, 2002). Immobility in this experiment was measured 24 h after last treatment. Nefazodone at 100 mg/kg decreased immobility after 7- and 14-days of treatment while desipramine at 5 mg/kg decreased immobility only after 14-days of treatment. It was concluded that nefazodone had a faster onset of activity than desipramine. However, what was really shown is that after 7-days of treatment nefazodone at 100 mg/kg was more efficacious than desipramine at 5 mg/kg, which was also true for day-14. It should be added that the authors discussed the use of these two different dosages as being the minimally effective dose for both drugs. This could justify their conclusion to a certain extent. Thus, it is still possible that nefazodone has a faster onset of activity, but a different experiment should be conducted that would address this question more directly. Such an experiment is discussed in Section 5 of this review. Generally, measurements of the effect at different time points in various groups of animals are subjected to more external variability. For this reason, models that can make repeated measures over a period of treatment are better suited to assess AOT.

There are only a few models of depression that use repeated data collection from the same group of animals. These include, the chronic mild stress (CMS) (Katz and Hersh, 1981; Katz, 1982; Willner et al., 1987, 1992; Willner, 1997; Willner and Papp, 1997; Willner and Mitchell, 2002), resident-intruder (RI) (Thurmond, 1975; Miczek and O’Donnell, 1978; Miczek et al., 1982; Olivier et al., 1984; Kudryavtseva et al., 1991; Mitchell et al., 1991; Mitchell...
and Fletcher, 1993) and RSBM (Malatynska and Kostowski, 1984, 1988; Mitchell and Redfern, 1992; Malatynska et al., 1995, 2002a, 2005; Malatynska and Knapp, 2005). These models produce a depression-like state using chronic uncontrollable (for the subject) stress. The CMS used physical stress, while the RI and RSBM used social stress to produce such a state. The measured chronic effects of the stressful procedure develop over time and they are reduced by chronic but not acute treatment with antidepressant drugs.

In the CMS model, animals were exposed to a CMS for 8 weeks as described by Papp et al. (1996, 2000, 2002, 2003). A parallel unstressed-group of rats was used as the control. Stressed rats, in contrast to controls, developed anhedonia as measured by a reduced preference for sucrose solution consumption. Chronic treatment with antidepressant drugs restored sucrose solution consumption in stressed rats. Measurements of sucrose solution consumption were made once a week and the potential to make even more frequent measurements exists. Thus, it is possible to assess AOT in this test. For example, it was shown that the effect of imipramine and fluoxetine at 10 mg/kg were significantly different from control after 3 weeks of treatment. Venlafaxine at 2.5 mg/kg was effective after 5 weeks of treatment and at 10 mg/kg after 2 weeks of treatment (Papp and Moryl, 1996; Millan et al., 2001, 2004; Papp et al., 2003); while fluoxetine and maprotiline at 5 mg/kg were effective after 9 weeks of treatment (Muscat et al., 1992). In these experiments, the AOT could be shown at equal dose levels. However, the efficacy evaluation for each drug at the dose applied for an accurate determination of AOT is missing.

The RI and RSBM are models that utilized different aspects of animal social structure (for review see Malatynska and Knapp, 2005). Different investigators used various endpoints to measure relative position of an animal in the society of conspecifics. First, by the communication of animals through expression of different body postures (Grant and Mackintosh, 1963; Blanchard and Blanchard, 1977; Blanchard et al., 1977). Second, by mutually accepted priority of access to different resources (Masur and Benedito, 1974; Malatynska and Kostowski, 1984; Zagrodzka et al., 1987; Fonberg, 1988; Gentsh et al., 1988b; Mitchell and Redfern, 1992). The third is based on animal territoriality such as the relationship between resident and intruder animals (Willner et al., 1981, 1995; Frischknecht et al., 1982; Siegfried et al., 1982; Kudryavtseva et al., 1991). All these approaches evaluate animal behavior for an extended duration and researchers can collect data at different time intervals. Chronic, sub-chronic and acute measurements of antidepressant activity were demonstrated in these models (Malatynska and Kostowski, 1984; Mitchell and Redfern, 1992, 2005; Mitchell and Fletcher, 1993; Malatynska et al., 2002a, 2005; Mitchell et al., 2003; Malatynska and Knapp, 2005).

The RI test, like the RDBM and RSBM is based on the DSR of animals. In a sense, it can also be considered as a competition test. The difference is in the object of competition. In the RI, it is a territory while in the RSBM/RDBM it is a palatable food. In both cases, animals are prone to competition. In the RI, this is done by social isolation and territory ownership (resident) or previous defeat (intruder) and introduction to the new territory already occupied by another animal (Mitchell, 2005), while in the RSBM/RDBM it is done by food restriction (Malatynska and Knapp, 2005). The RI test is polarized between resident and intruder animals as the RSBM/RDBM is polarized between dominant and submissive animals. In both tests, there are also polarized approaches to study drug effects. The RSBM/RDBM is clearly divided between a model of depression (RSBM) and a model of mania (RDBM). Antidepressant drug effects were primarily studied in the RSBM (Malatynska et al., 2002a, 2005; Malatynska and Knapp, 2005), while in the RDBM, antimanic drugs were studied and antidepressant drug effects only to a very limited extent (Malatynska et al., 1995).

Different investigators have used different measures of behavior in the RI test. Some researchers studied defeated intruder behavior as a model of depression (Kudryavtseva et al., 1989, 1991; Kudryavtseva, 1991, 1994; Lumley et al., 2000; Rygula et al., 2006a, b). Kudryavtseva et al. (1991) and Lumley et al. (2000) measured changes in the intruder behavior using an ethological approach, while Rygula et al. (2006a, b) measured anhedonia developed by the intruder and its reversal by antidepressant drugs.

Mitchell and coworkers (Mitchell and Redfern, 1992, 2005; Mitchell and Fletcher, 1993; Mitchell et al., 2003; Mitchell, 2005) studied the effect of antidepressant drugs on resident animal aggression. However, aggression, especially directed toward other members of the society, is more characteristic for mania (aggression-irritation) than depression where aggression is usually directed against self. It appears, that chronic treatment with antidepressant drugs increases aggressive (assertive) behaviors in resident and intruder animals. High predictive validity for antidepressants was demonstrated with this approach. Interestingly, antidepressant drug doses used in resident animals were much lower than those used in other animal models of depression.

Since data collection in the RI test can be assessed with different frequencies, it is a valuable model to study drug AOT. For a detailed discussion of different approaches to this subject, see the review by Mitchell (2005). In this review, he also discusses a possibility of measuring antidepressant AOT in a social test using competition for sucrose pellets in triads of rats (Gentsh et al., 1988a, b, 1990a, b; Mitchell and Redfern, 1992; Millard and Gentsh, 2006). In summary, in these tests data were collected and/or evaluated weekly or daily. In the RI tests where the resident rats were treated, the data were originally collected weekly and attention was given mostly to the response level of the studied drugs (Mitchell et al., 1991; Mitchell and Fletcher, 1993). In the RI tests where
the intruder rats were treated, data were also collected weekly. Antidepressant drugs were considered to be active when statistical differences between the treated and untreated groups were noted. In the later research, Mitchell (2005) collected data daily for a short period of time (1 week) and evaluated AOT using a minimal effective dose. The minimal effective dose was defined in a prior acute treatment experiment. Inclusion of dose consideration to the determination of AOT was an important step. Observation of the effect every day enabled the determination of antidepressant onset time in days rather than weeks, but the observation time was too short to know how persistent the observed effect was. In addition, with the assessment of AOT as the point of an emerging significant difference between control and treatment group, it is difficult to determine if differences found were statistically significant, unless one is applying non-linear regression to data analysis or performing the experiment on separate groups of animals. In the next section, we will use as an example our experiments with DSR-based models, especially RSBM to further such approaches in pre-clinical determination of drug AOT.

4. Methods used to establish activity onset time in the DSR-based models: RSBM and RDBM

The DSR established in pairs of rats competing for a food reward under well-defined conditions, including its division into RSBM and RDBM, was described in our earlier publications (Malatynska and Knapp, 2005; Malatynska et al., 2005; Pinhasov et al., 2005). Briefly, dominance and submissiveness were measured in these tests as the relative success of two food restricted rats to gain access to a feeder. The dominant rat spent significantly more time at the feeder than the paired submissive one in an established DSR after 2 weeks of testing (Fig. 2). We have shown that treatment of the submissive subject for 3 weeks with imipramine, desipramine, maprotiline or fluoxetine significantly and dose dependently reduced submissive behavior (Malatynska et al., 2002a; Pinhasov et al., 2005). The effect was attenuated after cessation of treatment with desipramine (Malatynska et al., 2002a). Treatments of submissive rats with the anxiolytic, diazepam or the psychostimulant, amphetamine, were ineffective (Malatynska et al., 2002b; Malatynska and Knapp, 2005). The treatment of the dominant rat with drugs commonly used to alleviate mania in the clinic such as lithium chloride, sodium valproate, carbamazepine and clonidine significantly reduced its competitive behavior (Malatynska and Kostowski, 1984; Malatynska et al., 2002b; Malatynska and Knapp, 2005). Thus, submissive behavior was sensitive to and selectively reduced by antidepressants and dominant behavior was sensitive to a range of drugs used to treat mania in humans. This provides two tests for drugs used to normalize bipolar disorder extremes: mania and depression. The onset of activity for all drugs tested was delayed relative to the initiation of treatment in the RDBM and the RSBM.

4.1. Efficacy and AOT\textsubscript{50} of fluoxetine alone and in combination with WAY100635 in the RSBM

Measurement of AOT in DSR-based tests is facilitated by the chronic nature of the test. In these tests, animals are housed separately and meet daily in the test apparatus for a 5min competition session to allow DSR development. Under this condition, it takes about 2 weeks for a DSR to be established. Daily recording (during a 5-day work week) of time spent on the feeder for each animal of the pair was necessary to select dominant–submissive pairs and only these pairs were continued in the treatment phase of experiment. Thus, the second week was defined as the selection week. In the post selection experimental period, it was also necessary for animals to interact and to record their feeder time daily to observe maintenance or changes in their relations. Daily or averaged weekly observation can be used to assess AOT of applied treatments.

As for all behavioral experiments, there is an appreciable amount of variability in the data collected from DSR tests. To decrease this variability, we initially used average weekly scores for data analysis. In such settings, the measurement timing was similar to the timing of data collection in clinical trials for antidepressant or antimanic drug activity. Two approaches for data analysis were possible to determine the observed drug AOT in the DSR.

For the demonstration of how these approaches led to the development of a new method for determining antidepressant AOT, we will use RSBM experiments done
with fluoxetine and the 5HT\textsubscript{1A} receptor antagonist WAY100635. It was shown in the clinic that pindolol shortened SSRI antidepressant AOT (for review see Blier and Bergeron, 1995, 1997, 1998; Blier, 2001, 2003; Brousse et al., 2003). Pindolol is a beta-adrenergic and 5-HT\textsubscript{1A} receptor antagonist. Consequently, it was shown by \textit{in vitro} studies that pindolol’s effect on SSRIs AOT is due to presynaptic 5-HT\textsubscript{1A} receptor inhibition leading to a more rapid increase of the serotonin level in the synaptic cleft of serotoninergic neurons (Blier et al., 1997; Blier and de Montigny, 1998; Dawson and Nguyen, 2000). \textit{In vivo} studies were conducted to show that a presynaptic 5-HT\textsubscript{1A}-receptor antagonist, in combination with an SSRI, would shorten the antidepressant-like activity lag time (Hogg and Dalvi, 2004; Tatarczynska et al., 2004; Mitchell, 2005). We have repeated these experiments using the RSBM of depression, with an emphasis on the assessment of AOT.

Our first approach (method I) was to calculate the time to achieve the loss of significance in the difference between feeding time of dominant rat (FTD) and feeding time of submissive rats (FTS). This is illustrated in Fig. 3 for fluoxetine (Sigma Aldrich, St. Louis, MO) at 10 mg/kg (i.p.) and in combination with WAY100635, 0.5 mg/kg (i.p.) given 30 min before fluoxetine. WAY100635 (Tocris Bioscience, Ellisville, Missouri) is a 5-HT\textsubscript{1A}-receptor antagonist (Critchley et al., 1994; Gozlan et al., 1995; Dawson and Nguyen, 1998). We have shown that the significant difference between FTD and FTS was lost after 3 weeks of administration of fluoxetine alone, and after 2 weeks when WAY100635 treatment was added (Fig. 3).

A second approach (method II) was to calculate the time of an emerging significant difference between dominance levels (DL) of control pairs (both animals treated with vehicle) and DL of pairs where submissive animals were treated with antidepressant while their dominant partners with vehicle. The DL is the difference between FTD and FTS; (for detailed explanations see Malatynska and Knapp, 2005; Malatynska et al., 2005; Pinhasov et al., 2005). This is illustrated for fluoxetine alone and the combination of WAY100635 and fluoxetine in Fig. 4. Similar results to those obtained using method I, were obtained for the combination treatment, but for fluoxetine alone only a statistical tendency of difference with control groups was observed in this experiment. Nevertheless, this second approach was superior because it took into account a comparison with vehicle-injected control pairs. This kind of comparison with an independent group of paired rats is stronger than an intra-pair comparison related to the

\begin{figure}[h]
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\caption{Estimate of different drug activity onset time (AOT) by method I. AOT is defined here as the time point for the loss of significant difference in the feeder time between competing dominant (DOM) and submissive (SUB) rats. Effects of different drugs on average weekly feeder time of submissive rats are shown: (a) no compound, vehicle; (b) WAY100635 at 0.5 mg/kg; (c) Fluoxetine at 10 mg/kg; (d) combination of WAY100635 at 0.5 mg/kg and fluoxetine 10 mg/kg Symbols marked * have values significantly different between dominant and submissive animals at $P<0.05$; marked as ** at $P<0.01$ and marked as *** at $P<0.001$. The statistical significance was determined by ANOVA, followed by a Bonferroni post-hoc test.}
\end{figure}
dominant partner because it takes into account natural fluctuation of behavior for different pairs. Vehicle-injected pairs of animals and the placebo-dosed patients in clinical trials are comparable groups. There are a certain number of responders in both cases. In the experiment illustrated in Fig. 4 the number of animal pairs in the control group was seven. Two rats were “responders” resulting in 28% of total pairs. There were 14%, 64%, and 80% responders in WAY100635, fluoxetine and combined WAY100635 plus fluoxetine groups, respectively. We defined as responders, pairs of rats having more than a 50% decrease in their DL in the last week of the study relatively to baseline (the DL for a pair during the selection week). This definition is similar to that used in clinical trials. The percentage of responders is very often used as an endpoint for quantification of the effect and this number for fluoxetine in our model was similar to that obtained in clinical trials (Atmaca et al., 2003; Leuchter et al., 2004).

In both approaches, the first evaluation of drug activity occurred after 1 week of treatment. However, we had collected data as early as after the third injection (Fig. 2). Thus, we could calculate AOT on a daily basis. Such calculations are illustrated for control (vehicle), fluoxetine, WAY100635 and the combination of fluoxetine with WAY100635 in Figs. 5 and 6 and in Table 1. In Fig. 5, daily changes in submissive rat behavior in relation to its dominant partner can be seen. The first day of consistent loss of the statistically significant difference between FTD and FTS is denoted as the AOT of 14 days for fluoxetine alone and 11 days for the combination of WAY100635 with fluoxetine. There is no such activity for vehicle in the control group, and there is also no activity in the group of rats treated with WAY100635 alone. This calculation, as previously demonstrated on the weekly basis, provides only a single number for the group of animals so it is difficult to compare different treatments. Thus, we have again used normalized DL for the calculation of the AOT. This is illustrated in Fig. 6. The experimental design consisted of parallel groups of animals assigned to either control or a series of treatment groups. In order to capture the dynamics of the treatment response, the data were collected daily starting on the third day after the commencement of the treatment regimen. A non-linear regression model was fitted to the data from each treatment group: DL% = (a + bx)e−dx + g, (Ratkowsky, 1990), where e represents base of natural logarithm, a, b, d, g are model parameters estimated using Levenberg–Marquardt algorithm (Lovenberg, 1944; Marquardt, 1963) and x (time) represents the independent variable. The fitted curve with the corresponding confidence intervals was used to read off AOT with its confidence intervals for each treatment group. The AOT was taken as the time necessary to obtain a 50% reduction in DL compared to its value at the beginning of treatment. The AOT values obtained for different groups could be directly compared to check for significant statistical differences. The AOT50 was based on the minimum (Emin) and maximum (Emax) response to drug determined from non-linear regression analysis. Two softwares were used to do calculations based on the above model, Matlab V7 (Mathworks MA), and GraphPad Software, Inc. (San Diego, CA).

Using this procedure, we were able to generate and compare AOT50 and response values for different treatments. Our analysis revealed that the maximum response for fluoxetine was 35.8±12.3% and the AOT50 was 14.2±1.5 days. The maximum response was 92.8±14.4% and the AOT50 was 9.8±0.9 days for the combination of fluoxetine with WAY100635. Both values were significantly different (P<0.05) to fluoxetine treatment alone with the combination having a faster onset of activity and higher level of response. These data are listed in Table 1.
Thus, our experiment confirmed the hypothesis that the combination of a 5-HT<sub>1A</sub>-receptor antagonist with an SSRI may reduce the AOT and augment the response of the latter.

4.2. Maximal response and AOT<sub>50</sub> of imipramine and maprotiline in the RSBM

In clinical trials, maprotiline was shown to have either an equal or faster onset of activity than tricyclic antidepressants including imipramine (for review see Muller and Moller, 1998). We have shown in our previous studies that maprotiline may have an equal onset of activity with imipramine (Pinhasov et al., 2005). FTS became equal to FTD (no statistically significant difference) after 3 weeks administration of either maprotiline or imipramine at 10 mg/kg or after 2 weeks at 20 mg/kg (Pinhasov et al., 2005). However, the tendency for maprotiline to be slightly better was seen in comparison with water-treated controls. The DL of pairs with submissive rats treated with maprotiline (10 mg/kg) was different from pairs treated with vehicle after 3 weeks, while the DL of pairs with submissive rats treated with imipramine was not different from the DL of control pairs for the treatment duration (5 weeks). At the 20 mg/kg dose, the DL of both maprotiline and imipramine treated pairs was different from the DL of control pairs after 2 weeks of administration. Thus, there was no difference in their respective onset of activity. It was interesting to note that the higher dose of both drugs had a faster onset of activity than the lower dose (Pinhasov et al., 2005).

To obtain a better comparison between different treatments, we have plotted daily DL values described in the previous paragraph and applied non-linear regression analysis to them as described earlier in this paper for fluoxetine. The analysis is shown in Fig. 7a and b, while AOT<sub>50</sub> and maximal response ($E_{\text{max}} - E_{\text{min}}$) are given in...
Table 1. At 10 mg/kg, the order of mean values for AOT$_{50}$ was maprotiline (12.8 days) < fluoxetine (14.2 days) < imipramine (31.9 days). The confidence interval was unbounded for maprotiline and imipramine thus in this particular data set it is not possible to conclude if mean values are significantly different or not. However, in this study there was a small sample size of 4–5 rats per group for imipramine and maprotiline, so there is a chance that with a larger pool of samples analyzed the difference between maprotiline and imipramine would be statistically significant. At 20 mg/kg, the AOT$_{50}$ for maprotiline was significantly shorter (4.9 days, CI $= 3.6–6.5$ days) than for imipramine (9.7 days, CI $= 5.6–10.7$). From this experiment, it is evident that the AOT$_{50}$ measured depends on the drug dose. In clinical trials, the dose usually was varied and very often patients were started on a lower dose that was increased during first 2 weeks of the trial. Clinical trials with maprotiline on two different dose regimens resulted in different estimates of maprotiline AOT. In the first clinical trial, the dose of maprotiline was set from the start at 70 mg/day and patients were maintained at the same dose until the end of the study at 4 weeks (Forrest, 1977). In this trial the onset of activity for maprotiline was estimated to occur after the first week of a trial when the improvement achieved was about 59% of the initial rating score (Forrest, 1977). We have re-plotted data from this trial and using non-linear regression analysis calculated the AOT$_{50}$ which was 8.2 ± 0.4 days (CI $= 8.06–8.4$) from the patients’ rating score or it was 9.1 ± 2.3 days (CI $= −0.6 to 19.0$) if reanalyzed from the physicians’ rating score (Fig. 8). There was no significant difference for AOT$_{50}$ calculated from two different scoring systems. In the second clinical trial (1984), patients were receiving maprotiline for 6 weeks with an evaluation after 1, 2, 4 and 6 weeks. The usual dose of maprotiline was 75 mg/day that varied between 12 and 150 mg/day between patients and was varied for different patients in different parts of the trial depending on the outcome and side effects. In this trial about 50% of tested patients achieved moderate improvement between 3 and 4 weeks of drug administration. It is obvious that dose regimen was only one difference among many between these two clinical trials in drug effect evaluation. However, in light of our experiments with the RSBM, the drug regimen is a possible factor influencing AOT of a given drug.

It is worth to note that during the second clinical trial, in the multicenter evaluation of maprotiline for treatment of depression (Anon, 1984), a worsening of the depression symptoms in 2–4% of the patients was noticed. It is not clear from the publication what regimen of drug treatment these patients followed. The phenomenon of worsening of submissive rats competitiveness was noticed with the lower doses of different antidepressant during initial treatment (Pinhasov et al., 2005). This effect was even more obvious with our current non-linear analysis of a daily data. Under the conditions of our experiment, a reduction of the DL response reflects drug activity (improvement of symptoms), since DL values are always reduced if the response to a drug is positive. In the case of negative response to a drug (worsening of symptoms), the DL values are increased. If the drug does not have such activity, the maximum of the response should not exceed 100%. Any maximal DL value
significantly higher than the control value (about 100%) indicates negative drug activity. The worsening of the rats’ DSR was significantly different from control for fluoxetine administered by itself but not in combination with WAY100635. The worsening of the rats’ DSR was significantly different from control for imipramine and maprotiline at 10 mg/kg but not for imipramine at 20 mg/kg. At a 20 mg/kg dose, maprotiline did not worsen the DSR at all. We have observed such increased DL for all three antidepressants studied here during the first days of treatment. Since the maximum overshoot occurred within 2–3 days, we could say that the acute effect of lower antidepressant doses was to increase submissiveness (decrease in assertive behavior), while chronic treatment resulted with the inhibition of a submissive behavior (increase in assertive behavior). The time for the return to baseline and the area under the curve for this transient increase in DL was shorter for maprotiline than for imipramine. Overshoot DL values are listed for all drugs in Table 1. The increase in submissiveness produced by antidepressants could correspond to reported examples of antidepressant exacerbation of depression in some patients on the beginning of treatment. We are proposing the method described above for the evaluation of drug-worsening effect.

5. Considerations for key experimental design necessary to compare activity onset time of different treatments

Our working hypothesis stated in the introduction section was: “To know if one treatment is faster than another, time-course for different treatments need to be compared at the same level of response to the drug”. Data
from clinical and pre-clinical research showed clearly that drug response level is a crucial factor determining drug AOT. It is clear from the pre-clinical work of various researchers that antidepressant AOT depends on the dose of the drug used relative to its potency (i.e. ED-50) (Muscat et al., 1992; Papp and Moryl, 1996; Millan et al., 2001, 2004; Knapp et al., 2002; Papp et al., 2003; Mitchell, 2005; Pinhasov et al., 2005).

Because potency and efficacy of the drug can influence the sensitivity of \( AOT_{50} \) measurement, it is important to know both of these pharmacological drug values for AOT determination. To determine ED50 values accurately, it is necessary to establish drug efficacy (maximal drug effect, \( E_{\text{max}} - E_{\text{min}} \)). This is done by generating dose-response curves, ideally at the time of maximal response (for detailed discussion of efficacy in different settings see Kenakin (1997). The generation of dose-response values for drugs with different efficacy is illustrated in Fig. 9a. To compare the AOT values for different drugs the activity measurements at different time points should be conducted at the same % level of maximum efficacy. This level was arbitrary defined above as 50% of the effect. However, it can be determined at any percent of the maximum effect that can be accurately measured. This question is similar to the one asked in the clinic for the level of onset event, response event and remission event (Thompson, 2002). Thus, with the method described, the question of which level of efficacy is adequate for defining AOT remains open. However, for the comparison of the AOT for different treatments this level can vary within the linear part of the curve. The key point is that these comparisons should be conducted at the same efficacy level for drugs under investigation. As it is illustrated in Fig. 9b the best determination of AOT may be conducted at a dose giving 75% of the maximum effect. This is an efficacy level that is in the linear part of the regression curve and it is close to the 100% efficacy level indicating that largest number of subjects treated with the drug should be affected. It was proposed by Mitchell (2005) in the RI model to determine the ID50 value (inhibition dose at 50% of the effect) for drug studied in the acute experiment prior to the chronic experiment. However, the determination of the ED50 should be conducted in the same kind of experiment as acute and chronic treatments may affect different targets resulting in different potency estimates for the drug studied. In the RSBM experiments presented in this review, AOT50 was compared at equal dose levels for each drug. However, for the precise measurement of the AOT50 determination of ED50 for each drug should be carried out. Such experiments are planned in the future.

The use of the non-linear regression model (Ratkowsky, 1990) presented in this manuscript is a novel way of analyzing this type of experimental data which obviates some of the drawbacks of commonly used methods (Kaplan and Meier, 1958; Huitfeldt and Montgomery, 1983; Quitkin et al., 1984b). Non-linear regression modeling is flexible and can accommodate data collected in a different ways as it does not require coincidental time points between parallel groups. It also optimizes noise rejection as all data points are fitted simultaneously and it is capable of fitting complex dynamics of the response such as overshoot following initial dosing in some compounds.

6. Summary and conclusions

This review identifies important pharmacological values that are necessary to establish reliable drug AOT. Various data indicated that ED50 and efficacy are needed for the comparison of AOT for different drugs. We also propose the use of non-linear regression for data analysis, as it has the best flexibility to accommodate different types of data to calculate AOT50 values.

From the clinician perspective, it is important to know when to expect a relevant drug effect. It was proposed to calculate the probability of a positive response from non-responder after median time to onset (Laska and Siegel, 1995). It would be important to determine this median time of onset as a reliable drug value in the clinical trials. Instead of measuring efficacy at one or three doses, pilot
clinical trials could be structured to first determine ED$_{75}$ values and then the AOT$_{50}$ should be measured at these ED$_{75}$ values. Chronic models of depression, where subject handling is similar to that in clinical trials but variations in individual responses are more restricted, could help in the relative characterization of AOT and efficacy. Similarly as for first-in-human dose determination, there is value in pre-clinical measurements of ED$_{75}$ and AOT$_{50}$ to guide selection of the dose range required to start with for the ED$_{75}$ and AOT$_{50}$ calculation in clinical trials. Determination of these parameters is expected to provide physicians with information as to when and on what level the drug effect should be expected.

References


