Optimization and robustness of the inhibitory avoidance test for pharmacological screening on long-term memory

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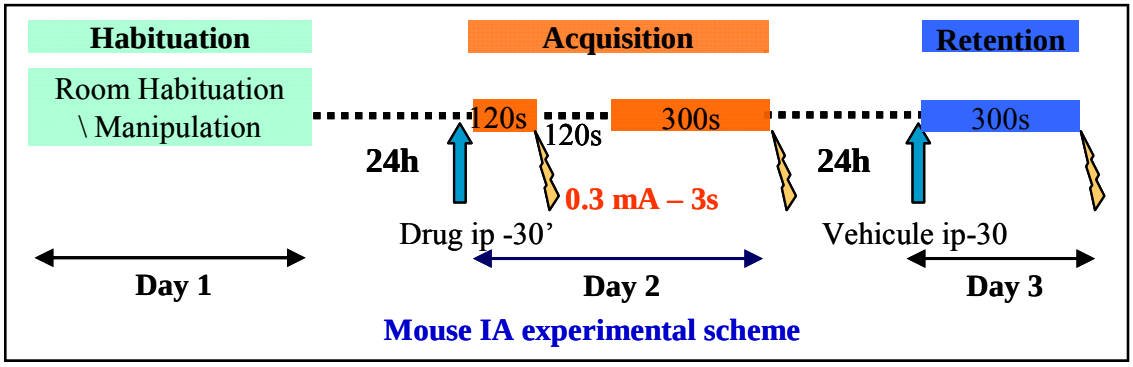
One of the challenges in pharmacological screening is to maximize the throughput of tests in order to evaluate the potential efficacy of a large number of compounds in a minimal amount of time. The inhibitory avoidance test, also called passive avoidance, has long been used as a screening test to evaluate drug effects on the memory in rodents [1,2]. The test is based on the natural photophobia of mice or rats, and evaluates the long-term memory of animals. The apparatus (Ugo Basile, see picture below) consists of a box divided into two compartments of equal sizes (18x10x16 cm) and equipped with a grid floor. One compartment is made of white panels, and illuminated with a lamp placed on the top of the chamber (≈ 350 lux). The other compartment is made of black panels (≈4 lux). The two compartments are separated by a guillotine door. In a typical trial (acquisition trial), the animal is placed in the bright compartment and readily enters the dark compartment. At that moment, the door separating the two compartments automatically closes, and the animal receives a brief mild electric shock (0.3mA-3s). During a subsequent trial (retention trial), the latency to enter the dark compartment is recorded as an index of memory consolidation. The longer the latency to enter the dark compartment, the better the animal is supposed to remember it received an electric shock during a previous trial [3].

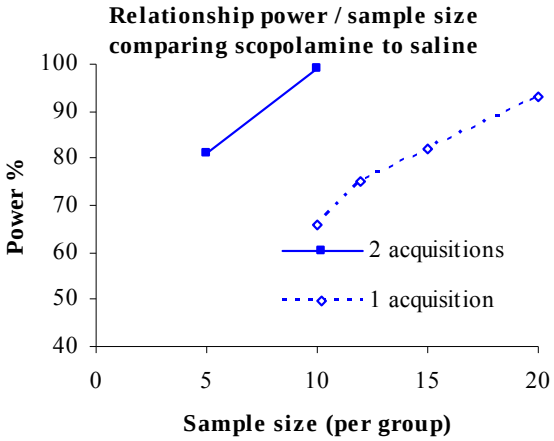
The experimental schemes found in the literature for the inhibitory avoidance paradigm substantially vary in terms of shock intensity, duration and number of trials [4]. Increasing the number of trials or increasing the sample size obviously results in a decreased throughput. The shortest protocol for an inhibitory avoidance test therefore comprises two trials: a single acquisition trial followed by a single retention trial run 24h later. The downside of this protocol is the large inter-individual variability observed in the responses, which implies that a large sample size is usually required to obtain statistically meaningful information [5,6].

In our laboratory, we optimized the 2-trial inhibitory avoidance protocol (one acquisition trial and one retention trial at 24h later) in order to test the effects of drugs against scopolamine-induced memory deficit. Scopolamine is a muscarinic receptor antagonist, which induces a cholinergic deficiency modeling to some extent the memory deficits observed in Alzheimer's disease [7]. All experiments used 8- to 9-week old male C57Black6J mice (25-30g) purchased from Charles River– France, and housed in groups of 5-6 in polypropylene cages under standard conditions (20°C, light/dark cycle 12h/12h, water and food ad libitum). They were habituated to these housing conditions for 1.5 weeks prior to experimentation. All experiments were carried out according to the European guideline 86/609/CEE and 2003/65/CE and to the Belgian legislation from August 14th, 1986 and its amendments.

We aimed at improving throughput by trying to find out a balance between a) minimizing the number of trials, and (b) maximizing the scopolamine-induced memory deficit in order to increase the window available for modulating the deficit. The 2-trial protocol was improved by adding an acquisition trial run shortly (2 minutes) after the first acquisition trial. Upon stepping through into the dark side mice received a non-escapable mild electric shock (3mA-3s). The cut-off times were 120 sec for the first acquisition trial, and 300s for the second acquisition trial as well as for the retention trial. The retention was run 24 h later as in the 2-trial protocol (see experimental scheme). This procedure was preceded 24h earlier by a session where mice were handled and habituated to the experimental room. Vehicle, test compounds and scopolamine (0.3 mg/kg) were administered i.p. 30 min. prior to the first acquisition trial. Only vehicle was administered 30 min prior to the retention trial.

The results indicated that the addition of a second acquisition trial increased the differences in latencies between vehicle and scopolamine-treated groups. In consequence, the statistical power [8], which is the probability of statistically detecting true differences between groups, was largely increased (see chart) so that the sample size needed to detect a statistically significant difference between saline and scopolamine-treated





groups was divided by three. For example, to reach a power of 80%, the sample size with a single acquisition was 15, whereas the same power was reached with only 5 mice in the two-trial acquisition protocol.

Using a 3-trial protocol, we also tested several reference drugs for their potential to reverse the scopolamine-induced memory deficit. These drugs were tacrine and donepezil, two acetylcholine esterase inhibitors, and thioperamide, an H3 receptor antagonist. The comparison of the efficacy over time showed (a) that the retention latencies for vehicle and scopolamine-treated groups were pretty stable across the year, and (b) that tacrine reversal of the scopolamine-induced memory deficit was reproducible throughout the year.

Altogether, our results indicate that the addition of a second acquisition trial shortly after the first acquisition trial increased the amplitude of the scopolamine-induced deficit, which improved the statistical power, and consequently decreased the sample size needed to show clear cut results. This protocol appears therefore to be an interesting 'compromise' between time investment (number of trials) and gain in statistical power without being forced to increase the dose of scopolamine to induce larger deficits. It also fits with the ethic's rule for animal experimentation proposing to design protocols for decreasing the number of animals used in laboratory tests.

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