

Cognitive impact of cytotoxic agents in mice

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Received: 19 December 2013 / Accepted: 5 May 2014 / Published online: 4 June 2014
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Abstract

Rationale and objectives Adjuvant chemotherapy is associated with changes in cognition in a subgroup of cancer patients. Chemotherapy is generally given as a combination of cytotoxic agents, which makes it hard to define the agent responsible for these observed changes. Literature on animal experiments has been difficult to interpret due to variance in experimental setup.

Methods We examined the effects of cytotoxic agents administered separately on various cognitive measures in a standardized animal model. Male C57Bl/6 mice received cyclophosphamide, docetaxel, doxorubicin, 5-fluorouracil, methotrexate, or topotecan. These agents represent different compound classes based on their working mechanism and are frequently prescribed in the clinic. A control group received saline. Behavioral testing started 2 or 15 weeks after treatment and included testing general

measures of behavior and cognitive task performance: spontaneous behavior in an automated home cage, open field, novel location recognition (NLR), novel object recognition (NOR), Barnes maze, contextual fear conditioning, and a simple choice reaction time task (SCRIT).

Results Cyclophosphamide, docetaxel, and doxorubicin administration affected spontaneous activity in the automated home cage. All cytotoxic agents affected memory (NLR and/or NOR). Spatial memory measured in the Barnes maze was affected after administration with doxorubicin, 5-fluorouracil, and topotecan. Decreased inhibition in the SCRIT was observed after treatment with cyclophosphamide, docetaxel, and topotecan.

Conclusions Our data show that, in mice, a single treatment with a cytotoxic agent causes cognitive impairment. Not all cytotoxic agents affected the same cognitive domains, which might be explained by differences in working mechanisms of the various agents.

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Keywords Mice · Chemotherapy · Cytotoxic agent · Behavior · Cognition

Introduction

An increasing number of clinical studies describing the incidence and nature of cognitive impairment after adjuvant chemotherapy for non-CNS tumors are being published. These studies have shown that chemotherapy may impair memory, speed of processing, and executive functioning in a subgroup of patients (Ahles and Saykin 2007). These negative effects on cognitive performance can already be noticed during treatment and may persist years after completion. The impact of these changes in cognition varies between patients, ranging from mild to more severe (Wefel and Schagen 2012). Although

progress has been made in unraveling the mechanisms behind this phenomenon (Ahles and Saykin 2007), many questions have remained unanswered.

Since chemotherapy is generally given as a combination of two or more cytotoxic agents, from clinical studies alone it is hard to determine which agents are responsible for the changes in cognition and which cognitive domains are affected. To elucidate this, animal studies exploring chemotherapy-induced cognitive impairment and its mechanism(s) are necessary and a first step in developing neuro-protective strategies that might improve quality of life of cancer survivors. Current data from animal studies show that the frequently used cytotoxic agents, such as methotrexate (MTX) (Briones and Woods 2011; Foley et al. 2008; Li et al. 2010a, b; Lyons et al. 2011b; Madhyastha et al. 2002; Seigers et al. 2008, 2009; Sieklucka-Dziuba et al. 1998; Yang et al. 2011, 2012; Yanovski et al. 1989), 5-fluorouracil (5-FU) (Briones and Woods 2011; ElBeltagy et al. 2010; Fardell et al. 2010; Foley et al. 2008; Lyons et al. 2012; Mustafa et al. 2008), and cyclophosphamide (Briones and Woods 2011; Christie et al. 2012; Lyons et al. 2011a; Pukhalsky et al. 2012; Reiriz et al. 2006; Yang et al. 2010), can induce cognitive impairment when given by intraperitoneal or intravenous injections to animals in clinically relevant dosages. The majority of animal studies used hippocampal-dependent learning tasks, such as the Morris water maze and novel location recognition (Seigers and Fardell 2011).

Despite this substantial set of studies, conclusions from these are hard to draw. There exists a large variation in the experimental design between animal studies, first with respect to the species, strain, gender, and age of the animals used, and second in factors such as cytotoxic agents, dosages, number of injections, administration route, time between administration and testing, and the behavioral tasks explored. For example, comparing the cognitive effects of 5-FU, Lyons et al. showed that administration with five intraperitoneal injections of 25 mg/kg 5-FU induced cognitive impairment as measured in the novel object recognition task 4 weeks after administration in male Lister-Hooded rats (Lyons et al. 2012). A similar effect was seen in a study of Fardell et al., in which male Hooded Wistar rats were injected intraperitoneal once with 75 mg/kg 5-FU alone or in combination with oxaliplatin and tested 2 weeks after administration (Fardell et al. 2011). However, Fremouw et al. reported that female C57Bl/6 mice treated with three weekly intraperitoneal 5-FU (100 mg/kg) injections were not impaired in the novel object recognition task 15 days after the last injection (Fremouw et al. 2012). Typically, the existence of many non-comparable data sets limits drawing firm conclusions and clearly calls for a more comprehensive analysis.

Here we studied the cognitive effects of a spectrum of chemotherapeutic agents at a single genetic background using highly standardized tests to minimize

environmental confounders. We chose to perform this comprehensive analysis in the C57BL/6 mouse strain as it is often used in studies examining cognition because of its intermediate phenotype in spontaneous behavior (Loos et al. 2013) and its good learning skills (Brooks et al. 2005). Cytotoxic agents are generally divided into several classes (DeVita et al. 2005), based on their specific working mechanisms, although they all impair the ability of a (cancerous) cell to divide and/or induce apoptosis. From each of these classes, we selected an important, often prescribed, cytotoxic agent for our study: cyclophosphamide (alkylating agent), docetaxel (microtubule agent), doxorubicin (anthracycline), 5-FU (antimetabolite), MTX (antimetabolite), and topotecan (topoisomerase I inhibitor). Mice were treated with a single injection of a single cytotoxic agent. Although patients generally receive a combination of several agents in multiple treatment cycles, multiple injections can increase stress levels and/or chemotherapy-associated sickness, and both can negatively affect cognitive impairment (Borcel et al. 2008). After injection with one of these cytotoxic agents, the animals were subjected to a battery of different behavioral tasks (Table 1). The tasks were selected to represent a wide range of cognitive domains, including memory (novel location recognition and novel object recognition), spatial memory (Barnes maze), contextual- and cued-associative memory (fear conditioning), and inhibitory control/attention (simple choice reaction time task). Spontaneous behavior (automated home cage) and anxiety (open field) were also explored. This high-throughput/high-content behavior testing started either 2 or 15 weeks after injection, i.e., in separate groups of animals, to examine cognition short- and long-term after administration. For all cytotoxic agents, specific cognitive impairments were found.

Material and methods

Animals

Adult (11 weeks of age) male C57BL/6J mice (Charles River, France), average body weight at the start of the experiment 26.2 ± 0.09 g SEM, were housed individually in clear Plexiglas cage ($26 \times 20 \times 14$ cm) on a layer of wood shavings with a fixed 12:12 h light/dark cycle (lights on at 07.00AM) and food and water ad libitum. Experiments started weeks after arrival of the animals according to the protocol described below. Body weight of the animals was measured on a regular basis, and body weight gain was calculated as absolute body weight/absolute body weight on the day of the injection $\times 100$ %. For day 0 (the day of the injection), this will give

Table 1 Overview of the different behavioral tasks and the domains measured

Task	Domain
Automated home cage	Spontaneous behavior
Open field	Anxiety
Novel location recognition	Hippocampal dependent memory
Novel object recognition	Peri-postrhinal cortex-dependent memory
Barnes maze	Spatial memory
Fear conditioning	Context- and cued-associative memory
Simple choice reaction time task	Inhibitory control/attention

a relative body weight of 100 %; body weight gain is determined as the relative body weight being more than 100 %. Behavioral testing was carried out 2–12 weeks after drug administration (short-term) of 15–25 weeks after drug administration (long-term) according to the schedules in Tables 2 and 3, which circumvents the potential acute effects of the cytotoxic on cognitive measures. A power analysis was conducted to calculate the cohort size. Because of the additional dosages, the control group (both short- and long-term) consisted of 16 mice; all other groups (also both short- and long-term) consisted of 12 mice. All experiments were approved by the Animal Experimentation Committee of the VU University Amsterdam. The principles of laboratory animal care were followed as well as the guidelines of the current version of the Dutch law and the guidelines for the care and use of mammals in Neuroscience and Behavioral Research.

Cytotoxic agents

The dosages of the cytotoxic agents used were based on previous results of our own lab and/or the animal literature. Cyclophosphamide was delivered by an intraperitoneal injection of 150 mg/kg (1,000 mg powder, dissolved in saline, Baxter BV, Utrecht, The Netherlands) (Janelsins et al. 2010). Docetaxel was delivered by an intraperitoneal injection of 33 mg/kg (10 mg/ml solution, Hospira Benelux BVBA, Brussels, Belgium) (Kemper et al. 2004).

Doxorubicin was given first in a concentration of 10 mg/kg intravenously (2 mg/ml solution, Pharmachemie BV, Haarlem, The Netherlands) (Sieklicka-Dziuba et al. 1998) and in an additional group receiving 5 mg/kg doxorubicin. 5-FU was injected intraperitoneal in a dosage of 75 mg/kg (50 mg/ml solution, Pharmachemie BV, Haarlem, The Netherlands) (Foley et al. 2008; Fremouw et al. 2012; Gandal et al. 2008; Walker et al. 2011; Winocur et al. 2006, 2011, 2012). MTX was delivered by an intravenous injection of 250 mg/kg (Seigers et al. 2008, 2009). An additional group of animals received 500 mg/kg MTX. The animals treated with MTX also received repeated intra-peritoneal injections of the tetrahydrofolate calcium leucovorin (10 mg/ml solution, Sandoz BV, Almere, The Netherlands), using a protocol similar to application in patients. Eighteen hours after the injection of MTX, leucovorin was administered in a concentration that was 8 % of the injected MTX dose; after 26, 42, and 50 h, the administered concentration was reduced to 4 %. Topotecan was given intraperitoneally in a dosage of 25 mg/kg (4 mg powder, dissolved in 5 % glucose, GlaxoSmithKline, Zeist, The Netherlands). Because no behavioral studies previously published explored the effect of topotecan, the dose was based on our own preliminary studies. Control animals received 0.3 ml saline intraperitoneally. All cytotoxic agents and saline were administered under a short-lasting (<3 min) mild isoflurane anesthesia, to control for any potential effect of the short-lasting isoflurane anesthesia on behavior.

Automated home cage

Measurements in a home cage environment (PhenoTyper model 3000, Noldus Information Technology, Wageningen, The Netherlands) were performed as previously described (Loos et al. 2013; Maroteaux et al. 2012) and started 13 days (short-term) of 104 days (long-term) after drug administration. Mice were housed in the automated home cage for 7 days, and the first 3 days were used to analyze spontaneous home cage behavior. The cage (30×30×35 cm) consisted of clear Perspex walls with an opaque Perspex floor with cellulose-based bedding. Water and food were distributed ad libitum from two adjacent walls. In the corner of the opposite walls, a

Table 2 Time schedule of the short-term experiment

Day	–14	0	13–20	22	23	26–30	35–37	40–82
Activity	Arrival	Treatment	Automated home cage	Open field	NLR/NOR	Barnes maze	Fear conditioning	SCRTT

Day 0 is the day of the injection

NLR novel location recognition, NOR novel object recognition, SCRTT simple choice reaction time task

Table 3 Time schedule of the long-term experiment

Day	-14	0	104–111	113	114	117–121	126–128	131–173
Activity	Arrival	Treatment	Automated home cage	Open field	NLR/NOR	Barnes maze	Fear conditioning	SCRIT

Day 0 is the day of the injection

NLR novel location recognition, NOR novel object recognition, SCRIT simple choice reaction time task

triangular shelter (10 cm high from non-transparent material) with two entrances was fixed to the walls. The top of the cage contained infrared LEDs as well as an infrared camera for video tracking. For the detection of spontaneous behavior, an analysis method was applied (Loos et al. 2013) that distinguishes six groups of parameters: kinematic parameters of move and arrest segments, shelter segments, habituation effects across days, the effect of light/dark phase, anticipation of, and response to light-dark phase transitions, and activity bout characteristics.

From these parameters, 20 key spontaneous behavior parameters were chosen as shown in Table 4.

Open field

The open field measures anxiety (Denenberg 1969) and was executed 22 days (short-term) or 113 days (long-term) after administration. The animal was placed in a white polyvinylchloride box (50×50×50 cm), illuminated with white fluorescent light from above (60 Lux) and was

Table 4 Key spontaneous behavior parameters of the automated home cage

Behavior parameters	
Kinematic parameters of move	
Mean long arrest duration–light	Mean duration per long arrest during the light phase
Long movement max. velocity	Average velocity of the 95th percentile fastest long movement segments
Long arrest threshold	Cut-off value to separate short and long arrests
Long movement threshold	Cut-off value to separate short and long movements
Shelter segments	
Long shelter visit duration–dark	Cumulative duration of long shelter visits during the dark phase
Long shelter visit fraction of total visits	The fraction of shelter visits with duration longer than long shelter visit threshold
Long shelter visit threshold	Cut-off value to separate intermediate and long shelter visits
Short shelter visit threshold	Cut-off value to separate short and intermediate shelter visits
Habituation effects across days	
Activity duration–habituation ratio dark	Habituation effect: change in cumulative activity duration during the dark phase of day 3 compared with the dark phase of day 1
The effect of light/dark phase	
Activity duration–darklight index	Effect of light regime: index describing the difference in cumulative activity duration between light and dark phases.
Activity duration–habituation ratio light	Habituation effect: change in cumulative activity duration during the light phase of day 3 compared with the light phase of day 1
Anticipation of, and response to, light	
Activity change in anticipation of dark	Change in activity during the last 5 h of the light phase in anticipation of the upcoming dark phase
Activity change in anticipation of light	Change in activity during the last 5 h of the dark phase in anticipation of the upcoming light phase
Activity change in response to dark	Change in activity during the first 2 h of the dark phase in response to the onset of the dark phase
Activity change in response to light	Change in activity during the first 2 h of the light phase in response to the onset of the light phase
Activity bout characteristics	
Activity duration–dark	Cumulative duration of activity during the dark phase
Activity duration–light	Cumulative duration of activity during the light phase
Mean activity duration–dark	Mean duration per activity bout during the dark phase
Mean activity duration–light	Mean duration per activity bout during the light phase
OnShelter zone number–dark	Cumulative number of visits to OnShelter zone during the dark phase

allowed to explore the arena freely for 10 min. The box was cleaned with 70 % ethanol in between animals. The box was divided into two zones, an outer zone and an inner zone (25×25 cm). Time spent in the inner zone (seconds) was analyzed with Biobserve (Biobserve GmbH). For each cytotoxic group, the time spent in the inner zone was compared with the control animals with a significant difference indicating that the cytotoxic agent induces anxiety.

Novel location recognition (NLR) and novel object recognition (NOR)

The NLR and NOR tasks were given 23 days (short-term) or 114 days (long-term) after administration and were executed in the open field box to explore recollection-like object memory (Dere et al. 2007) (Fig. 1). First the animal received an acquisition trial, in which two equal objects (LEGO, cube 3.2×3.2×3.2 cm or pyramid 4.8×3.2×3.2 cm, counterbalanced among the groups) were placed in opposite corners of the box. After an inter-trial time of 2 h, the animal was retested as in session 1 with one of the objects relocated to a novel location (NLR), followed by an inter-trial time of 2 h. During the third session, the object that was moved in session 2 was replaced by a novel object with an equal color but different shape (NOR). The mouse was allowed to freely explore the box and the objects for 5 min in all sessions, after which it returned to its home cage. All objects were firmly taped to the floor of the box, and the box was cleaned with 70 % ethanol in between animals. For all trials, the amount of time spent exploring the objects was analyzed with Biobserve (Biobserve GmbH). A discrimination index was calculated via the following formula: $[\text{time spent exploring the changed object}] / [\text{time spent exploring the changed object} + \text{time spent exploring the unchanged object}]$. The discrimination index was compared with a corrected chance level of 0.5 by subtracting the average of the control group from the discrimination index of each individual control animal hence providing a variance of chance level. We compared the discrimination index of each cytotoxic treated group to this fictive chance level group (with a mean discrimination index of 0.5) (Akkerman et al. 2012).

Barnes maze

The Barnes maze explores spatial learning (Paylor et al. 2001). It consists of a large round platform (122 cm diameter, 80 cm above the floor) with 24 holes (9 cm from the edge of the maze). Round cylinders (8×4.5 cm diameter) were placed underneath all holes except for one, which was designated as “escape hole” and had an escape box hanging underneath (15×5.5×5.5 cm). The room was illuminated with 1,000 Lux, and external cues were located on the wall. Barnes maze training consisted of two sessions per day, for 5 days and started 26 days (short-term) or 117 days (long-term) after drug administration. The mouse was placed in a cylinder, which was located in the center of the Barnes maze for 30 s, after which it was lifted via a pulley system. The mouse was allowed to explore the Barnes maze freely for a maximum of 5 min or until it found the escape hole. If it did not find the escape hole within these 5 min, it was guided by hand. During the first two trials on day 1, nesting material from the home cage of the animal was placed in the escape hole. The escape hole was removed for the last session on day 5 and replaced by a cylinder equal to the other cylinders, and this probe trial lasted for 5 min. The Barnes maze was cleaned with 70 % ethanol in between the testing of animals and was rotated 90° after each trial with the escape hole placed in the same spatial location. All sessions were analyzed with Biobserve (Biobserve GmbH). The following parameters that represent different aspects of learning behavior were analyzed during the learning phase: escape latency, learning strategy, errors, perservative errors, and serial errors. For the probe trial, the Barnes maze was divided into nine zones (a center zone and eight pie-shaped zones consisting of three holes each, Fig. 2). Time spent (in seconds) in the zone where the escape hole used to be was analyzed providing information on how well the animals had learned to spatially locate the escape hole.

Fear conditioning

With the fear conditioning task, cued- and context-associative memory, depending on both the hippocampus and amygdale, is tested (Phillips and LeDoux 1992). The fear conditioning protocol started 35 days (short-term) or 126 days (long-term)

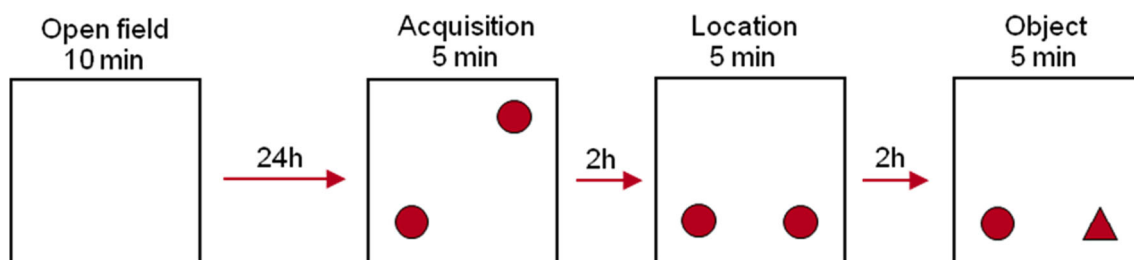


Fig. 1 Experimental setup of the open field, novel location task, and novel object recognition task

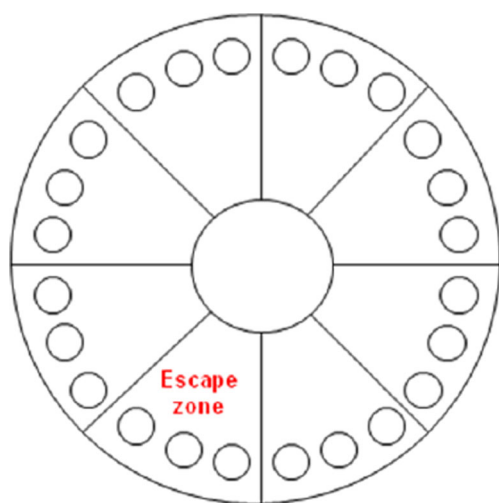


Fig. 2 Schematic representation of the zone division in the Barnes maze during the probe trial

after injection and lasted for 3 days. Freezing behavior in the fear conditioning system (TSE systems, Bad Homburg, Germany) was measured by a photo beam detection system (10 Hz detection rate, resolution of 1.3×2.5 cm). Fear conditioning was performed in a Plexiglas chamber ($36 \times 21 \times 21$ cm) with a stainless grid floor placed in a gray box (box A). Box A had continuous lighting (200 Lux) and background noise (white noise, 68 dB). On the first day, the animal was brought into the experiment room (lights switched off) and was placed in box A for 3 min, after which a tone (200 ms pulsed, 30 s) was sounded, followed by a foot shock (0.7 mA, 3 s). Thirty seconds after the foot shock, the animal was removed from the box and placed in its home cage. The box was cleaned with 70 % ethanol in between animals. Twenty-four hours later, contextual fear memory was tested by placing the animal in box A for 3 min. Again, the box was cleaned with 70 % ethanol in between testing of animals. Another 24 h later, generalized fear and cued fear were tested by bringing the mice into the experiment room (lights switched on) and placing the animals in a novel Plexiglas box providing a different context (box B). This box had a smooth floor, no continuous lighting, no surrounding grey box, and had a different smell (acetic acid). The animal was first placed in this box for 3 min (generalized fear), after which the tone was sounded for 3 min (cued fear). After each animal, the box was cleaned with 1 % acetic acid. Freezing was defined as no movement (velocity < 1 cm/s) for at least 3 s.

Simple choice reaction time task (SCRRT)

The simple choice reaction time task measures inhibitory control and attention in a similar manner as the five-choice reaction time task (Loos et al. 2010). The simple-choice reaction time task was executed in an operant

conditioning box ($25 \times 20 \times 18$ cm) and started 40 days (short-term) or 131 days (long-term) after drug administration and was based on an individually paced schedule. The animals were kept on approximately 85 % of their body weight for the duration of the task and were tested 30 min per day, 5 days per week. The designated start-stimulus was assigned to the middle of five holes (hole 3). The go-stimulus was assigned to the cue light in the hole immediately to the left or right (hole 2 or 4, randomly assigned) of the start-stimulus. The trial started with the illumination of the start-response in a variable 3 ratio. The animal had to make a response in the start-stimulus, which turned off the start-light followed by the illumination of the go-stimulus. A response in the go-stimulus switched off the go-light and was followed by a reward (14 mg sucrose pellet) in the magazine. Five seconds after the reward was given, the next trial started. Premature start- and go-responses in holes that were not illuminated, and perseverative start-responses after the go-stimulus was illuminated, resulted in a time out of 5 s, in which the house light and stimulus light were both switched off. The go-stimulus was switch on for an individually titrated limited hold (LH) period, which was set based on the response time to the go-stimulus during the previous trial. If the animal did not respond to the go-stimulus within the LH, a time-out was given of 5 s. The percentage of these omissions was calculated as: $100 \times [\text{omissions of go-response} / (\text{omissions of go-response} + \text{correct go-responses} + \text{perseverative start-responses})]$. For each next trial, the percentage of omissions was set to 30 % by decreasing the LH 0.7-fold if the percentage of omissions was lower than 25 % and increasing the LH by 1.25-fold if the percentage of omissions was higher than 35. The number of perseverative start-pokes was analyzed, and more perseverative start-pokes in a treated group compared with the control animals indicate decreased inhibition. Furthermore, impulsivity, which was defined as the percentage of premature go-responses ($[\text{number of trials with premature go-response} / \text{number of started trials}] \times 100$) and the latencies between the onset of the go-stimulus and a go-response (Go-RT), were calculated.

Statistics

Body weight and the learning phase of the Barnes maze were analyzed using repeated-measure ANOVA, followed by an independent sample *t*test to compare each cytotoxic-treated group with the control group. The behavioral tasks were analyzed using independent sample *t*tests to compare each cytotoxic-treated group with the control group. The discrimination index of the NLR and NOR of each cytotoxic group was compared with a fictive control group with a mean discrimination index of 0.5 using a one-sample *t*test according to (Akkerman et al. 2012).

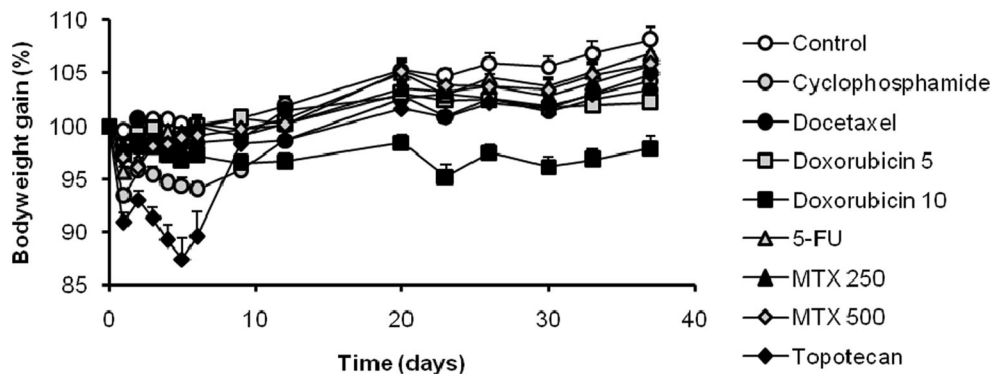


Fig. 3 Body weight gain short-term after treatment. *Open circle*: control, *grey circle*: cyclophosphamide, *black circle*: docetaxel, *grey square*: doxorubicin 5 mg/kg, *black square*: doxorubicin 10 mg/kg, *grey pyramid*: 5-FU, *black pyramid*: MTX 250 mg/kg, *grey diamond*: MTX 500 mg/kg, *black diamond*: topotecan. Body weight before the injection (given on day 0) is expressed as 100 %; error bars represent standard error of the mean. The difference in body weight gain between the different treatments was significant, $F_{8,102}=9.673$, $p<0.001$. The difference between the control animals and the animals treated with cyclophosphamide is

significant from days 1 through 12 ($p<0.05$), and from days 26 through 37 ($p<0.05$). Docetaxel is significant from days 26 through 37 ($p<0.05$). Doxorubicin 5 mg/kg is significant from days 26 through 37 ($p<0.05$). Doxorubicin 10 mg/kg is significant from days 1 through 37 ($p<0.01$). 5-FU is significant from days 1 through 3 ($p<0.01$). MTX 250 mg/kg is significant from days 2 through 4 ($p<0.05$). MTX 500 mg/kg is significant from days 1 through 4 ($p<0.05$). Topotecan is significant from days 1 through 37 ($p<0.05$)

Because the aim of the study was to compare the effects of the cytotoxic agents only to the control group and not with each other, correction for multiple comparisons was not conducted. For all statistical tests, a probability value less than 0.05 is considered to be statistically significant.

Results

Body weight

All cytotoxic agents induced a decrease in body weight gain compared with administration of saline during both the short-term experiment ($F_{8,102}=9.673$, $p<0.001$, Fig. 3)

and the long-term experiment $F_{8,102}=8.732$, $p<0.001$, Fig. 4). Most agents affected body weight gain only directly after treatment (as summarized in Table 5). However, doxorubicin 10 mg/kg (short- and long-term) and topotecan (short-term) decreased body weight gain during the entire experiment, which suggests that these animals still could have been ill during the behavioral tasks. Therefore, the effects of these substances on spontaneous behavior should be taken into account when drawing conclusions on the cognitive effects.

Spontaneous behavior in an automated home cage

Spontaneous behavior was measured in an automated home cage (PhenoTyper model 3000, Noldus Information

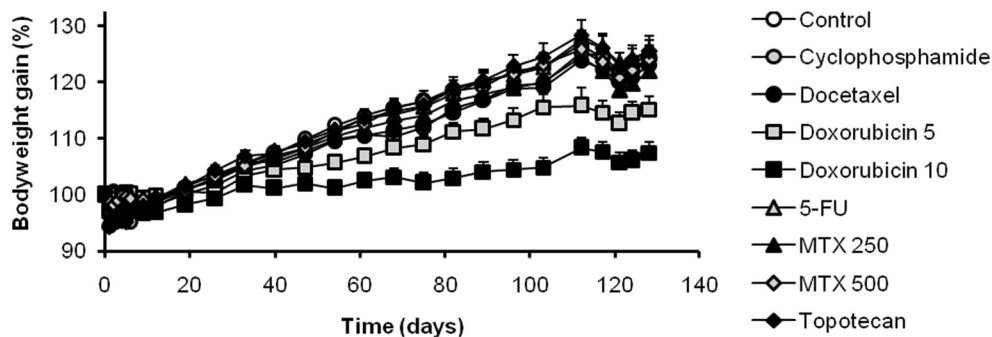


Fig. 4 Body weight gain long-term after treatment. *Open circle*: control, *grey circle*: cyclophosphamide, *black circle*: docetaxel, *grey square*: doxorubicin 5 mg/kg, *black square*: doxorubicin 10 mg/kg, *grey pyramid*: 5-FU, *black pyramid*: MTX 250 mg/kg, *grey diamond*: MTX 500 mg/kg, *black diamond*: topotecan. Body weight before the injection (given on day 0) is expressed as 100 %; error bars represent standard error of the mean. The difference in body weight gain between the different treatments was significant, $F_{8,102}=8.732$, $p<0.001$. The difference between

the control animals and the animals treated with cyclophosphamide is significant from days 1 through 9 ($p<0.05$). Doxorubicin 5 mg/kg is significant from days 40 through 128 ($p<0.05$). Doxorubicin 10 mg/kg is significant from days 12 through 128 ($p<0.05$). 5-FU is significant from days 1 through 2 ($p<0.01$). Effect of MTX 250 mg/kg is significant from days 1 through 4 ($p<0.05$). MTX 500 mg/kg is significant on days 1 and 2 ($p<0.05$). Effect of topotecan is significant from days 1 through 4 ($p<0.05$)

Table 5 Significant difference in body weight gain of all cytotoxic agents compared with control animals, both short- and long-term, after treatment

	ST		LT	
	Days	<i>P</i> value	Days	<i>P</i> value
Cyclophosphamide	1–12 26–37	<0.05 <0.05	1–9	<0.05
Docetaxel	26–37	<0.05		
Doxorubicin 5 mg/kg	26–37	<0.05	40–128	<0.05
Doxorubicin 10 mg/kg	1–37	<0.01	12–128	<0.05
5-FU	1–3	<0.01	1–2	<0.01
MTX 250 mg/kg	2–4	<0.05	1–4	<0.05
MTX 500 mg/kg	1–4	<0.05	1–2	<0.05
Topotecan	1–37	<0.05	1–4	<0.05

Indicated are days after administration at which significant differences in body weight of treated animals versus controls was observed

Technology, Wageningen, The Netherlands) during days 13 through 20 (short-term) or days 104 through 111 (long-term) after drug administration. Spontaneous behavior was analyzed with respect to six groups of behaviors (Table 6, Appendix Figs. 14, 15, 16, 17, 18, 19, 20, 21, 22 and 23) (Loos et al. 2013). Most effects on spontaneous behavior were seen after treatment with docetaxel and doxorubicin. From the kinematic parameters, it was seen that these cytotoxic agents increased the long arrests (Appendix Fig. 14), decreased velocity (Appendix Fig. 15),

and altered the difference between short and long arrests and movements (Appendix Figs. 16 and 17). From the sheltering parameters, it was seen that these agents affected both the duration and the number of long shelter visits (Appendix Figs. 18, 19, and 20). Furthermore, these cytotoxic agents also decreased several activity parameters (Appendix Figs. 21, 22, and 23), which was also seen after treatment with 5-FU, MTX (500 mg/kg), and topotecan. No effects were seen during habituation, at the light/dark phase transition, or on light/dark phase anticipation parameters (data not shown).

Open field

Time in the inner zone during the open field was analyzed with Biobserve (Biobserve GmbH) at day 22 (short-term) and at day 113 (long-term) after cytotoxic administration. For each cytotoxic agent, treated mice were compared with control animals for the time spent in the inner zone, and a significant difference indicates that the cytotoxic agent induces anxiety and/or alters exploration behavior. Short-term after administration, the animals treated with 5-FU spent less time in the inner zone compared with control animals ($p < 0.05$), indicative of enhanced anxiety-related behavior (Fig. 5). This effect was not present long-term after administration (Fig. 5). No other cytotoxic agent caused a significant effect on the time spent in the inner zone of the open field compared with control treatment.

Table 6 Overview of significant differences with respect to the six different types of behavior as represented by plus signs

		Kinematics	Sheltering	Habituation	Light/dark phase	Anticipation pattern	Activity
CYC	ST	+					+
	LT		+				+
DOC	ST	+	+				+
	LT						
DOX 5	ST	+	+				+
	LT						
DOX 10	ST	+	+				+
	LT						
5-FU	ST	+					
	LT						
MTX 250	ST						
	LT						
MTX 500	ST	+					
	LT						
Top	ST	+					
	LT	+					

ST short-term, LT long-term, CYC cyclophosphamide, DOC docetaxel, DOX 5 doxorubicin 5 mg/kg, DOX 10 doxorubicin 10 mg/kg, 5-FU 5-fluorouracil, MTX 250 methotrexate 250 mg/kg, MTX 500 methotrexate 500 mg/kg, TOP topotecan

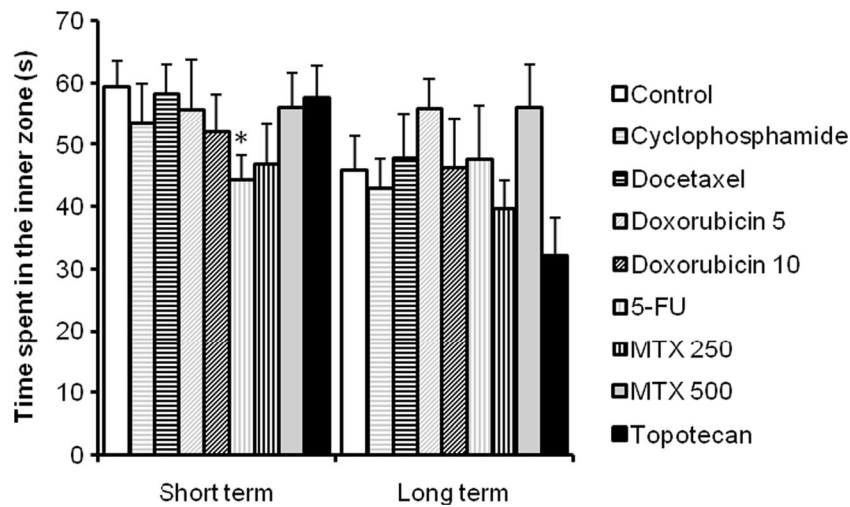


Fig. 5 Total time spent in the inner zone of the open field short- and long-term after treatment; error bars represent standard error of the mean. *Open bar*: control, *grey horizontal striped bar*: cyclophosphamide, *black horizontal striped bar*: docetaxel, *grey diagonal striped bar*: doxorubicin 5 mg/kg, *black diagonal striped bar*: doxorubicin 10 mg/kg, *grey vertical*

striped bar: 5-FU, *black vertical striped bar*: MTX 250 mg/kg, *grey bar*: MTX 500 mg/kg, *black bar*: topotecan. Compared with control animals, 5-FU-treated animals spent significantly less time in the inner zone ($p < 0.05$)

Novel location recognition (NLR) and novel object recognition (NOR)

The NLR and NOR were performed 23 days (short-term) or 114 days (long-term) after administration. The novel location recognition task explores hippocampal-dependent memory (Mumby et al. 2002), and the novel object recognition task explores peri-postrhinal cortex-dependent memory (Winters et al. 2004). During the NLR, when compared with a fictive control group with a mean

discrimination index of 0.5 (meaning no difference in time spent exploring the changed or the unchanged object), control ($p < 0.05$), doxorubicin- (5 mg/kg, $p < 0.005$), and MTX-treated (250 mg/kg, $p < 0.05$; 500 mg/kg, $p < 0.005$) animals spent significantly more time exploring the object on the novel location than the object on the familiar location short-term after administration (Fig. 6). In contrast, animals treated with cyclophosphamide, docetaxel, doxorubicin (10 mg/kg), 5-FU, and topotecan did not show novel location preference (represented as no

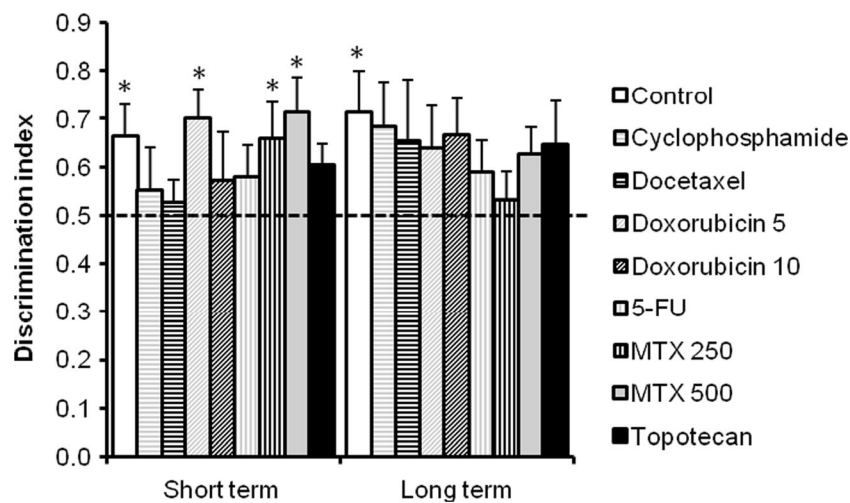


Fig. 6 Discrimination index in the novel location recognition task; error bars represent standard error of the mean. *Open bar*: control, *grey horizontal striped bar*: cyclophosphamide, *black horizontal striped bar*: docetaxel, *grey diagonal striped bar*: doxorubicin 5 mg/kg, *black diagonal striped bar*: doxorubicin 10 mg/kg, *grey vertical striped bar*: 5-FU, *black vertical striped bar*: MTX 250 mg/kg, *grey bar*: MTX 500 mg/kg, *black bar*: topotecan. Short-term after treatment control ($p < 0.05$),

doxorubicin- (5 mg/kg, $p < 0.005$), and MTX-treated (250 mg/kg, $p < 0.05$; 500 mg/kg, $p < 0.005$) animals spent significantly more time exploring the object on the novel location than the object on the familiar location. Long-term after treatment, control animals ($p < 0.05$) spent more time exploring the object on the novel location than the object on the familiar location

significant difference compared with chance level), indicating decreased hippocampal memory short-term after treatment. Long-term after treatment, only control animals spent significantly ($p < 0.05$) more time exploring the object on the novel location than the object on the familiar location (Fig. 6). This indicates that treatment with all cytotoxic agents decreased memory in the hippocampus long-term after treatment (again, represented as no significant difference compared to chance level). However, this may be due to a lack of power in the treated groups.

During the NOR, when compared with a fictive control group with a mean discrimination index of 0.5 (meaning no difference in time spent exploring the changed or the unchanged object), control ($p < 0.05$), cyclophosphamide ($p < 0.005$), docetaxel- ($p = 0.001$), doxorubicin- (5 mg/kg, $p < 0.005$), and MTX-treated (250 mg/kg, $p = 0.005$; 500 mg/kg, $p < 0.005$) animals spent significantly more time exploring the novel object than the familiar object short-term after treatment (Fig. 7). This indicates that treatment with doxorubicin (10 mg/kg), 5-FU, and topotecan decreased memory in the peri-postrhinal cortex short-term after treatment. In the long-term experiment, no effects were seen in exploration of the objects when compared with a fictive control group with a mean discrimination index of 0.5 for any cytotoxic treatment, but also the control animals did not show a preference for the novel object (Fig. 7).

Barnes maze

The Barnes maze was performed at days 26 through 30 (short-term) or days 117 through 121 (long-term) after

treatment, and it measures spatial memory. The animals first received nine learning trials in which escape latency, errors, preservative errors, and serial errors were analyzed. The daily escape latency short-term after treatment is shown in Fig. 8. All animals showed a significant learning curve: control $F_{4,60} = 8.071$, $p < 0.001$; cyclophosphamide $F_{4,44} = 5.283$, $p = 0.001$; docetaxel $F_{4,44} = 17.092$, $p < 0.001$; doxorubicin 5 mg/kg $F_{4,44} = 7.556$, $p < 0.001$; doxorubicin 10 mg/kg $F_{4,44} = 6.648$, $p < 0.001$; 5-FU $F_{4,44} = 14.054$, $p < 0.001$; MTX 250 mg/kg $F_{4,44} = 5.478$, $p = 0.001$; MTX 500 mg/kg $F_{4,44} = 23.642$, $p < 0.001$; and topotecan $F_{4,44} = 4.515$, $p < 0.005$. No treatment effect or treatment by day interaction effect was seen in the number of errors, perseverative visits, serial visits or serial errors, nor did any cytotoxic-treated group differ in learning curve compared with control treatment.

The daily average escape latency long-term after treatment is shown in Fig. 9. All animals showed a significant learning curve after treatment: control $F_{4,60} = 7.828$, $p < 0.001$; cyclophosphamide $F_{4,44} = 7.626$, $p < 0.001$; docetaxel $F_{4,44} = 10.549$, $p < 0.001$; doxorubicin 5 mg/kg $F_{4,44} = 19.572$, $p < 0.001$; doxorubicin 10 mg/kg $F_{4,44} = 4.074$, $p < 0.01$; 5-FU $F_{4,44} = 4.783$, $p < 0.005$; MTX 250 mg/kg $F_{4,44} = 5.707$, $p = 0.001$; MTX 500 mg/kg $F_{4,44} = 17.036$, $p < 0.005$; and topotecan $F_{4,44} = 5.174$, $p < 0.005$. No treatment effect or treatment by day interaction effect was seen in any of the measures, nor did any cytotoxic treated group differ in learning curve compared with control treatment.

For the probe trial, the Barnes maze was divided into nine zones (a center zone and eight pie-shaped zones consisting of three holes each, Fig. 2), and the time spent in the zone where

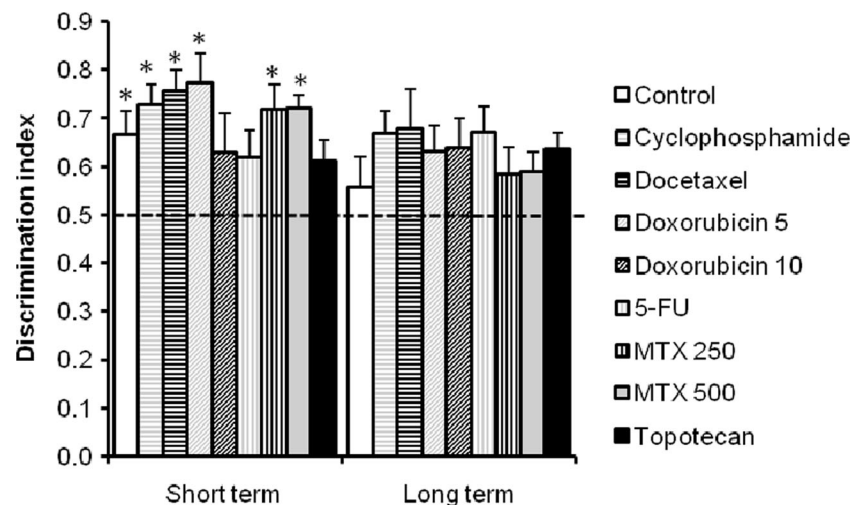


Fig. 7 Discrimination index in the novel object recognition task; error bars represent standard error of the mean. *Open bar*: control, *grey horizontal striped bar*: cyclophosphamide, *black horizontal striped bar*: docetaxel, *grey diagonal striped bar*: doxorubicin 5 mg/kg, *black diagonal striped bar*: doxorubicin 10 mg/kg, *grey vertical striped bar*: 5-FU, *black vertical striped bar*: MTX 250 mg/kg, *grey bar*: MTX 500 mg/kg,

black bar: topotecan. Short-term after treatment control ($p < 0.05$), cyclophosphamide- ($p < 0.005$), docetaxel- ($p = 0.001$), doxorubicin- (5 mg/kg, $p < 0.005$), and MTX-treated (250 mg/kg, $p = 0.005$; 500 mg/kg, $p < 0.005$) animals spent significantly more time exploring the novel object than the familiar object. No effects were seen in exploration of the objects long-term after treatment

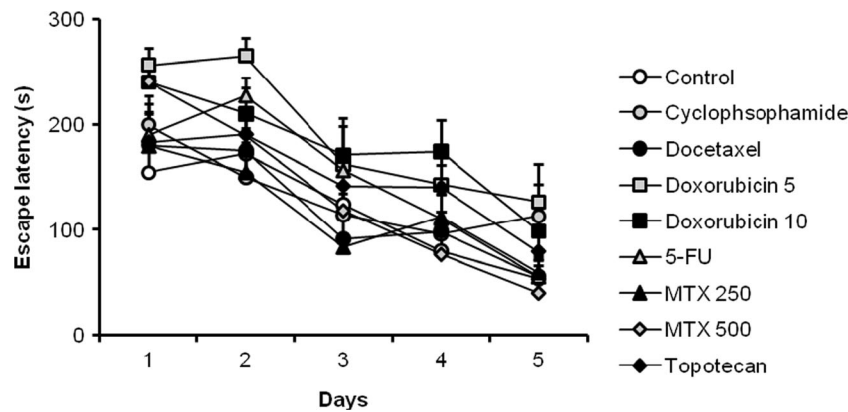


Fig. 8 Daily average escape latency during the Barnes maze short-term after treatment; error bars represent standard error of the mean. *Open circle*: control, *grey circle*: cyclophosphamide, *black circle*: docetaxel, *grey square*: doxorubicin 5 mg/kg, *black square*: doxorubicin 10 mg/kg, *grey pyramid*: 5-FU, *black pyramid*: MTX 250 mg/kg, *grey diamond*: MTX 500 mg/kg, *black diamond*: topotecan. All animals showed a

significant learning curve after treatment, control $F_{4,60}=8.071$, $p<0.001$; cyclophosphamide $F_{4,44}=5.283$, $p=0.001$; docetaxel $F_{4,44}=17.092$, $p<0.001$; doxorubicin 5 mg/kg $F_{4,44}=7.556$, $p<0.001$; doxorubicin 10 mg/kg $F_{4,44}=6.648$, $p<0.001$; 5-FU $F_{4,44}=14.054$, $p<0.001$; MTX 250 mg/kg $F_{4,44}=5.478$, $p=0.001$; MTX 500 mg/kg $F_{4,44}=23.642$, $p<0.001$; topotecan $F_{4,44}=4.515$, $p<0.005$

the escape hole was located during the learning phase during the first 4 min of the probe trial was analyzed with Biobserve (Biobserve GmbH). When compared with control animals, animals treated with doxorubicin 10 mg/kg, 5-FU, and topotecan spent significantly less time in the escape zone short-term after treatment ($p<0.005$, $p<0.05$, and $p<0.05$, respectively), indicating an impairment in spatial memory. No significant treatment effects were found during the probe trial long-term after treatment (Fig. 10).

10 mg/kg increased the percentage of time spent freezing short-term after treatment for both contextual- and cued-fear conditioning (both $p<0.05$, Fig. 11) and long-term after treatment for cued fear conditioning ($p<0.05$, Fig. 12), compared with control animals, indicating that treatment with a high-dosage doxorubicin increased associative memory. No other cytotoxic agent affected associative memory.

Fear conditioning

Fear conditioning, which measures contextual- and cued-associative memory, was executed at day 35 (short-term) or at day 126 (long-term) after treatment. Doxorubicin

Simple choice reaction time task (SCR TT)

The SCR TT started at day 40 (short-term) or day 131 (long-term) after treatment and measures aspects of inhibitory control and attentional performance. No effect was seen on task acquisition, neither short- nor long-term after

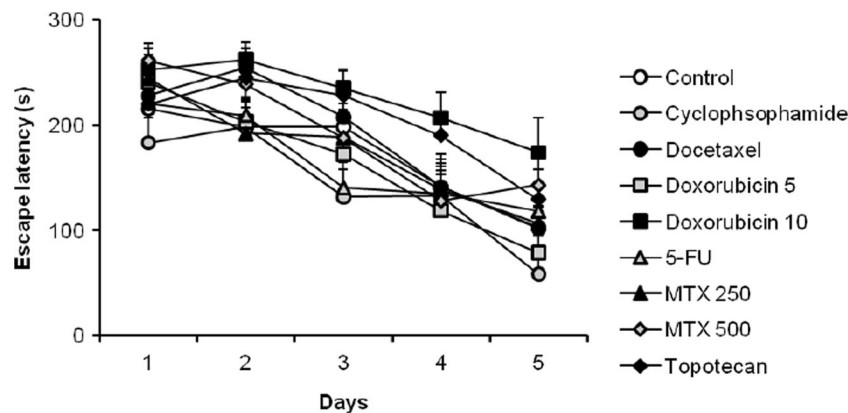


Fig. 9 Daily average escape latency during the Barnes maze long-term after treatment; error bars represent standard error of the mean. *Open circle*: control, *grey circle*: cyclophosphamide, *black circle*: docetaxel, *grey square*: doxorubicin 5 mg/kg, *black square*: doxorubicin 10 mg/kg, *grey pyramid*: 5-FU, *black pyramid*: MTX 250 mg/kg, *grey diamond*: MTX 500 mg/kg, *black diamond*: topotecan. All animals showed a

significant learning curve after treatment, control $F_{4,60}=7.828$, $p<0.001$; cyclophosphamide $F_{4,44}=7.626$, $p<0.001$; docetaxel $F_{4,44}=10.549$, $p<0.001$; doxorubicin 5 mg/kg $F_{4,44}=19.572$, $p<0.001$; doxorubicin 10 mg/kg $F_{4,44}=4.074$, $p<0.01$; 5-FU $F_{4,44}=4.783$, $p<0.005$; MTX 250 mg/kg $F_{4,44}=5.707$, $p=0.001$; MTX 500 mg/kg $F_{4,44}=17.036$, $p<0.005$; topotecan $F_{4,44}=5.174$, $p<0.005$

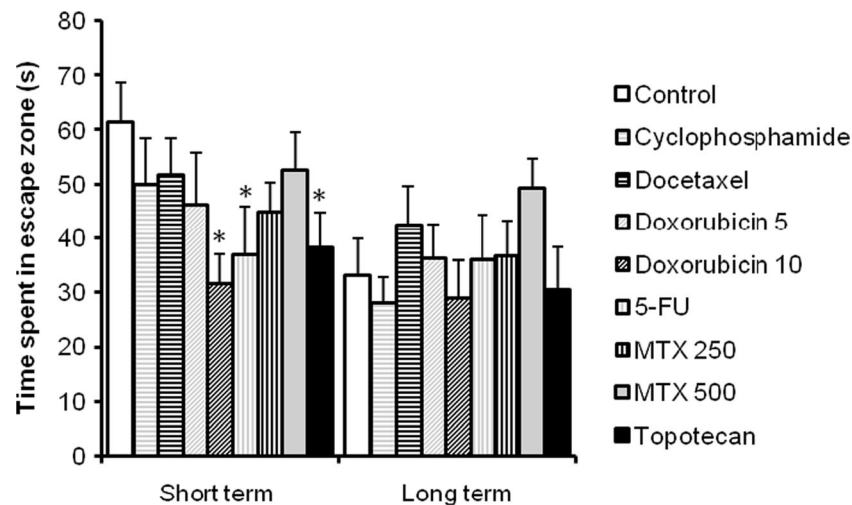


Fig. 10 Time spent in the escape zone during the probe trial of the Barnes maze short- and long-term after treatment; error bars represent standard error of the mean. *Open bar*: control, *grey horizontal striped bar*: cyclophosphamide, *black horizontal striped bar*: docetaxel, *grey diagonal striped bar*: doxorubicin 5 mg/kg, *black diagonal striped bar*: doxorubicin 10 mg/kg, *grey vertical striped bar*: 5-FU, *black vertical striped bar*:

MTX 250 mg/kg, *grey bar*: MTX 500 mg/kg, *black bar*: topotecan. Animals treated with doxorubicin 1 mg/kg, 5-FU, and topotecan spent significantly less time in the escape zone short-term after treatment compared with control animals ($p < 0.005$, $p < 0.05$, and $p < 0.05$, respectively). No significant treatment effects were found long-term after treatment

administration, meaning that all animals were able to learn the task. Cytotoxic treatment did not affect the number of initiated trials, indicating no gross differences in motivation to execute the task. Compared with control treatment, an increase in inappropriate perseverative start-pokes during Go was seen short-term after treatment with cyclophosphamide ($p = 0.001$), docetaxel ($p < 0.05$), and topotecan ($p = 0.001$), indicating that treatment with these agents decreased this aspect of inhibitory control. This effect was not present long-term after treatment (Fig. 13). No short- or long-term treatment effect was

seen on other parameters of inhibitory control or attentional performance.

Discussion

Summary

There is an inconsistency in the animal literature in terms of cognitive effects of cytotoxic agents, which might be explained by the large variation in the experimental setup (e.g.,

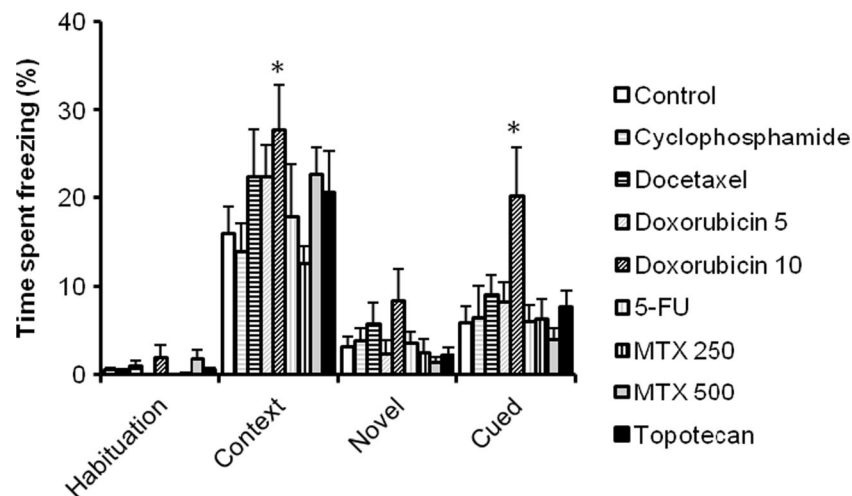


Fig. 11 Time spent freezing in the fear conditioning task short-term after treatment; error bars represent standard error of the mean. *Open bar*: control, *grey horizontal striped bar*: cyclophosphamide, *black horizontal striped bar*: docetaxel, *grey diagonal striped bar*: doxorubicin 5 mg/kg, *black diagonal striped bar*: doxorubicin 10 mg/kg, *grey vertical striped bar*:

bar: 5-FU, *black vertical striped bar*: MTX 250 mg/kg, *grey bar*: MTX 500 mg/kg, *black bar*: topotecan. Compared with control animals, doxorubicin 10 mg/kg increased the percentage of time spent freezing for contextual- and cued-fear conditioning (both $p < 0.05$)

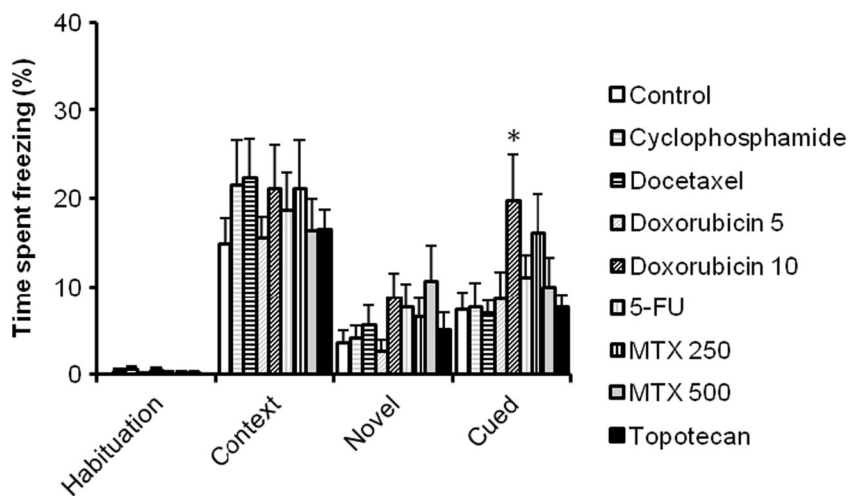


Fig. 12 Time spent freezing in the fear conditioning task long-term after treatment; error bars represent standard error of the mean. *Open bar:* control, *grey horizontal striped bar:* cyclophosphamide, *black horizontal striped bar:* docetaxel, *grey diagonal striped bar:* doxorubicin 5 mg/kg, *black diagonal striped bar:* doxorubicin 10 mg/kg, *grey vertical striped*

bar: 5-FU, *black vertical striped bar:* MTX 250 mg/kg, *grey bar:* MTX 500 mg/kg, *black bar:* topotecan. Compared with control animals, doxorubicin 10 mg/kg increased the percentage of time spent on freezing for cued-fear conditioning ($p < 0.05$)

animal model, cytotoxic agent explored, administration route, number of injections, dosage, and time between treatment and testing). This inconsistency called for a comprehensive systemic analysis of the cognitive effects of frequently used cytotoxic agents in a standardized animal model evaluating both short- and long-term after drug administration which is provided in this study. To fully explore the complexity of chemotherapy-induced cognitive impairment, a number of cognitive domains were explored including various forms of memory (e.g., general memory, spatial memory, and context- and cued-associative memory) and executive functions (inhibitory control and attentional performance) (Table 1).

Furthermore, spontaneous behavior and anxiety were also assessed, since these variables may affect the measurement of cognition. In brief, cyclophosphamide affected spontaneous behavior, memory (NLR), and inhibitory control in the SCRTT. Docetaxel affected spontaneous behavior and impaired recognition memory (NLR) and inhibitory control (SCRTT). Doxorubicin affected spontaneous behavior, memory (NLR and NOR), spatial memory (BM), and both contextual- and cued-dependent associative memory. 5-FU impaired memory (NLR and NOR) and spatial memory (BM). MTX impaired memory (NLR), and topotecan affected memory (NLR, NOR), spatial memory (BM), and inhibitory

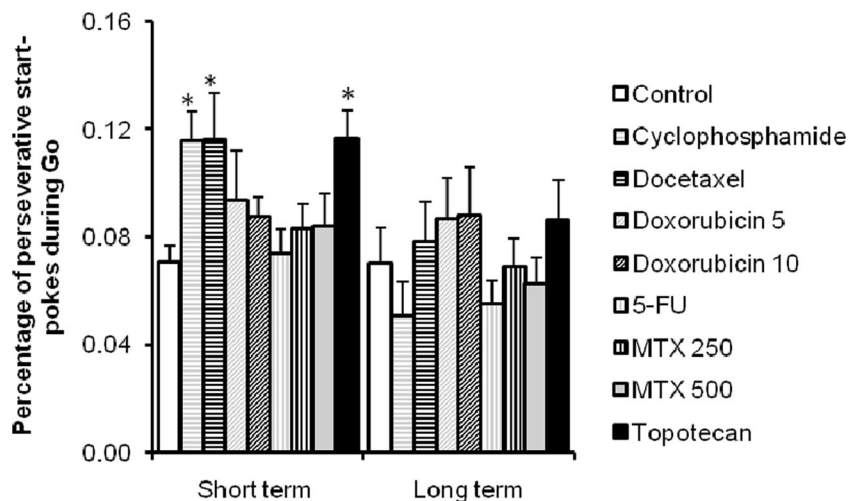


Fig. 13 Percentage of perseverative start-pokes during the last four sessions of the simple choice reaction time task; error bars represent standard error of the mean. *Open bar:* control, *grey horizontal striped bar:* cyclophosphamide, *black horizontal striped bar:* docetaxel, *grey diagonal striped bar:* doxorubicin 5 mg/kg, *black diagonal striped bar:* doxorubicin 10 mg/kg, *grey vertical striped bar:* 5-FU, *black vertical*

striped bar: MTX 250 mg/kg, *grey bar:* MTX 500 mg/kg, *black bar:* topotecan. Compared with control treatment, an increase in perseverative start-pokes during Go was seen short-term after treatment with cyclophosphamide ($p = 0.001$), docetaxel ($p < 0.005$), and topotecan ($p = 0.001$). No effect was seen long-term after treatment

Table 7 Overview of significant differences of the cytotoxic agents compared with control animals as represented by plus signs; minus signs indicate absence of appropriate control

		OF	NLR	NOR	BM	FC	SCRTT
CYC	ST		+				+
	LT		+	-	-		
DOC	ST		+				+
	LT		+	-	-		
DOX 5	ST						
	LT		+	-	-		
DOX 10	ST		+	+	+	+	
	LT		+	-	-	+	
5-FU	ST	+	+	+	+		
	LT		+	-	-		
MTX 250	ST						
	LT		+	-	-		
MTX 500	ST						
	LT		+	-	-		
Top	ST		+		+		+
	LT		+	-	-		

ST short-term, LT Long-term, CYC cyclophosphamide, DOC docetaxel, DOX 5 doxorubicin 5 mg/kg, DOX 10 doxorubicin 10 mg/kg, 5-FU 5-fluorouracil, MTX 250 methotrexate 250 mg/kg, MTX 500 methotrexate 500 mg/kg, TOP topotecan, OF open field, NLR novel location recognition, NOR novel object recognition, BM Barnes maze, FC fear conditioning, SCRTT simple choice reaction time task

control (SCRTT) (summarized in Table 7). We showed that all cytotoxic agents tested in our study cause cognitive impairment, with different agents affecting different cognitive domains. This may be due to the differences in their mechanism of action and warrants further mechanistic studies.

Cytotoxic agents acting on different cognitive domains

We showed that not all cytotoxic agents affected the same cognitive domains. Given the fact that we used highly standardized protocols for testing on a single genetic background, these differences are likely explained by the difference in mechanism of the agents. All cytotoxic agents explored in this study have a specific working mechanism, which defines the different classes of cytotoxics. It has been suggested that cytotoxic agents from these classes do not only affect dividing cells, e.g., progenitor cells, but also act on non-dividing cells in a class-specific manner (Dietrich et al. 2006; Dietrich 2010). Also, agents from the same class may induce different damage. For example, the antimetabolites MTX and 5-FU induce well-defined, different neurotoxic syndromes and do so by different modes of action (Boogerd 1995). Importantly, vulnerability of neurons to cytotoxic agents appears related to specific areas in the brain (Rzeski et al. 2004). The effect of

the difference in working mechanism of the several cytotoxic agents on the brain needs exploration in follow-up studies.

Short- versus long-term effects

Our study was designed to incorporate the assessment of short- versus long-term effects of the cytotoxic agents. We observed robust cognitive phenotypes on the short-term. For the long-term, we observed impaired learning behavior in the long-term control animals in the NOR and Barnes maze. However, this impairment may be due to a decrease in activity. Barreto et al. reported that, even though old (18 months) C57Bl/6 mice were able to learn the Barnes maze, they needed more time to do so compared with young (2 months old) animals due to lower velocity (Barreto et al. 2010). Our aged control animals (>27 weeks at the time of testing) also showed a longer escape latency during the learning phase and lower velocity during the probe trial in the Barnes maze compared with the young animals. Furthermore, during the probe trial, the control animals did not spend more time in the correct octant compared with chance level, indicating that they had not learned the task. Moreover, that activity is affected by age can be seen in old animals that were also less active in the automated home cage when compared with the young animals. This means that, for the two tasks in which the old control animals did not show learning behavior (NOR and BM), no conclusions can be drawn on the long-term effect of the cytotoxic agents on these cognitive domains. Thus, this leaves all effects on cognition found on the short-term.

In the animal literature, almost all studies explored cognition short-term after cytotoxic treatment: within days up to 1 month after administration. But even though not many long-term experiments are performed, there is evidence that the effects of chemotherapy are long-lasting. For example, a decrease in BrdU cells was seen up to 6 weeks after repeated treatment with carmustine, cisplatin (Dietrich et al. 2006), or 5-FU (ElBeltagy et al. 2012). Han et al. (2008) even described a decrease in BrdU cells that lasted up to 6 months after treatment with 5-FU (Han et al. 2008). But, also, a single injection can have long-lasting effects on behavior: Fardell et al. (2010) showed that 120 days after one MTX injection rats still suffered from cognitive impairment (Fardell et al. 2010). This suggests that cognitive impairment after chemotherapy is a long-term effect, but this could not be visualized in our experiment due to the lack of a proper control group. This is supported by clinical data: Even though most clinical studies also focused on short-term cognitive changes after chemotherapy (up to 2 years after treatment ended), a recent review shows that there are a number of studies describing long-term chemotherapy-induced cognitive changes (from 5 to 21 years after treatment). These cognitive changes are possibly caused by the observed long-term reduction in both grey and white matter and altered brain activation (Koppelmans et al. 2013). As mentioned in the "Introduction", chemotherapy is

generally given as a combination of two or more cytotoxic agents, making it hard to determine which agents cause cognitive impairment in patients. However, a number of cytotoxic agents are frequently mentioned in relation to cognitive impairment both short- and long-term after treatment, including cyclophosphamide, methotrexate, 5-FU, and doxorubicin (Ahles et al. 2012; Koppelmans et al. 2013).

Compound-specific effects

We explored two dosages of doxorubicin and MTX. The dose of 5 mg/kg doxorubicin was used to minimize side-effects; the dose of 500 mg/kg MTX was used to also explore cognitive effects at a higher concentration. In previous papers, we have shown that 250 mg/kg MTX dosage causes cognitive impairment in novel object recognition, Morris water maze learning, and fear conditioning in rats (Seigers et al. 2008, 2009). In the current study, modest cognitive effects were seen with this agent. This discrepancy may be caused by inter-species (rat vs. mouse) differences in pharmacokinetics and sensitivity (Kitamura et al. 2008; Lobo and Balthasar 2003).

Doxorubicin is known to cause cardiotoxicity in both humans (DeVita et al. 2005) and animals (Bernard et al. 2011; Riad et al. 2009; van Acker et al. 1996). One indication for the presence of physical problems after doxorubicin administration in our study is the retardation in body weight gain, which was seen within a few days after administration and lasted for the full duration of the experiment. Furthermore, animals treated with 10 mg/kg doxorubicin were less active in the automated home cage, Barnes maze, and fear conditioning than animals treated with 5 mg/kg doxorubicin. This suggests that the cognitive effects of the high doxorubicin dosage might be confounded, e.g., by cardiotoxicity.

Affecting hippocampus-dependent learning

The novel location recognition task is a hippocampal-dependent learning task (Mumby et al. 2002). The novel object recognition task, however, can either be hippocampal-dependent (when spatial cues are present) or peri-postrhinal cortex-dependent, depending on the design. In a paper of Winters and colleagues (Winters et al. 2004), rats with either peri-postrhinal cortex or hippocampal lesions were subjected to a spatial radial maze and object recognition task. Animals with a hippocampal lesion performed poorly in the spatial radial maze task but well on the object recognition task. A reverse effect was seen in animals with a peri-postrhinal cortex lesion. Our experimental setup for the object recognition task prevented the animals from being able to use spatial cues, resulting in a non-spatial learning task. This suggests that treatment with cyclophosphamide, docetaxel, and MTX only affected hippocampal memory, whereas treatment with

doxorubicin, 5-FU, and topotecan affected both hippocampal and peri-postrhinal memory.

Comparison to clinic studies

This experiment was performed in young adult, male mice, even though the majority of clinical studies exploring the effect of chemotherapy on cognition is performed in middle-aged women. We cannot rule out specific effects that might relate to age of testing. The use of male mice was chosen as it rules out the effect of circadian changes in estrogen levels in females that are known to impact on cognitive function, in both humans (Hampson 1990) and rodents (Bimonte-Nelson et al. 2003).

Our mice were treated with a single cytotoxic agent which is not comparable to the clinical situation in which patients receive a chemotherapy cocktail. This setup was chosen because we wanted to pinpoint which agents cause cognitive impairment and the domains affected, which is still impossible when combination treatment is given. Furthermore, the administration of multiple injections can lead to additional stress and/or increased chemotherapy-associated sickness which are both known to have a negative impact on cognition (Borcel et al. 2008). Because we wanted to keep confounding factors as small as possible, we decided to give a single injection at a dose level that was high but tolerable for a mouse. Behavioral testing was performed after the animals had recovered from the injection in terms of body weight gain and were not showing signs of sickness. But these differences between the clinical situation and our experiment should be taken into account when interpreting the data.

Conclusion

This study demonstrates that different cytotoxic agents used in chemotherapy all affect cognition in C57Bl/6J mice on the short-term but act at different cognitive aspects. This insight provides handles for follow-up studies that are aimed to further explore the underlying mechanism of cognitive impairment in general, and to investigate how agents from different classes affect the brain in distinct manners. However, our conclusion may be limited by the animal model and experimental setup such as age of the animal, dosages, time between treatment and testing, the sensitivity of the behavioral tasks, and cohort size.

Acknowledgments The authors declare that the experiments performed in this manuscript are in compliance with the current laws of The Netherlands.

Funding This research was funded by the Dutch Cancer Society, grant number NKI 2010-4829.

Conflict of interest The authors declare that they have no conflict of interest.

Appendices

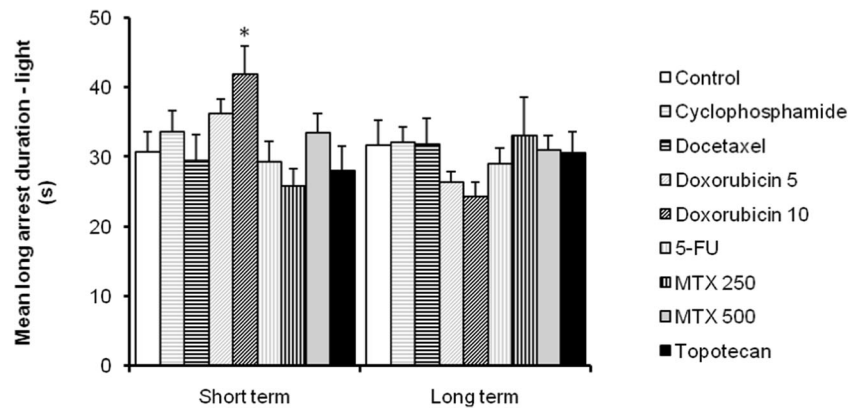


Fig. 14 Mean duration per long arrest during the light phase in the automated home cage short- and long-term after treatment; error bars represent standard error of the mean. *Open bar*: control, *grey horizontal striped bar*: cyclophosphamide, *black horizontal striped bar*: docetaxel, *grey diagonal striped bar*: doxorubicin 5 mg/kg, *black diagonal striped bar*: doxorubicin 10 mg/kg, *grey vertical striped bar*: 5-FU, *black vertical*

striped bar: MTX 250 mg/kg, *grey bar*: MTX 500 mg/kg, *black bar*: topotecan. Doxorubicin 10 mg/kg increased the mean duration per long arrest during the light phase compared with control animals short-term after treatment ($p < 0.05$). These effects were not present long-term after treatment

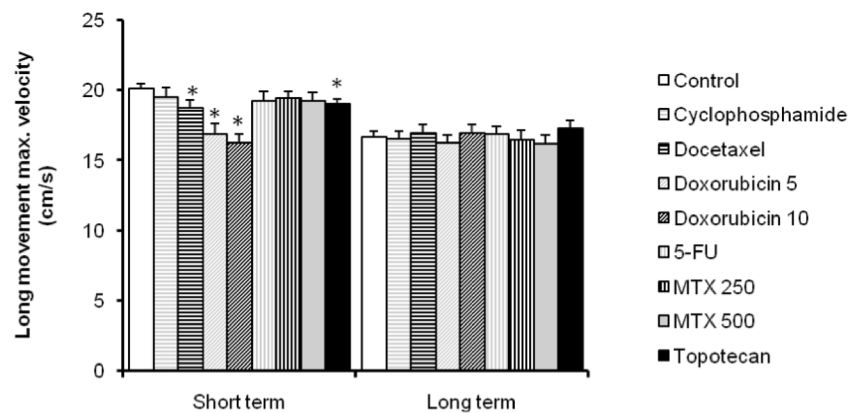


Fig. 15 Average velocity of the 95th percentile fastest long movement segments in the automated home cage short- and long-term after treatment; error bars represent standard error of the mean. *Open bar*: control, *grey horizontal striped bar*: cyclophosphamide, *black horizontal striped bar*: docetaxel, *grey diagonal striped bar*: doxorubicin 5 mg/kg, *black diagonal striped bar*: doxorubicin 10 mg/kg, *grey vertical striped bar*: 5-

FU, *black vertical striped bar*: MTX 250 mg/kg, *grey bar*: MTX 500 mg/kg, *black bar*: topotecan. Docetaxel ($p < 0.05$), doxorubicin (both dosages $p < 0.001$), and topotecan ($p < 0.05$) decreased the average velocity of the 95th percentile fastest long movement segments compared with control animals short-term after treatment. These effects were not present long-term after treatment

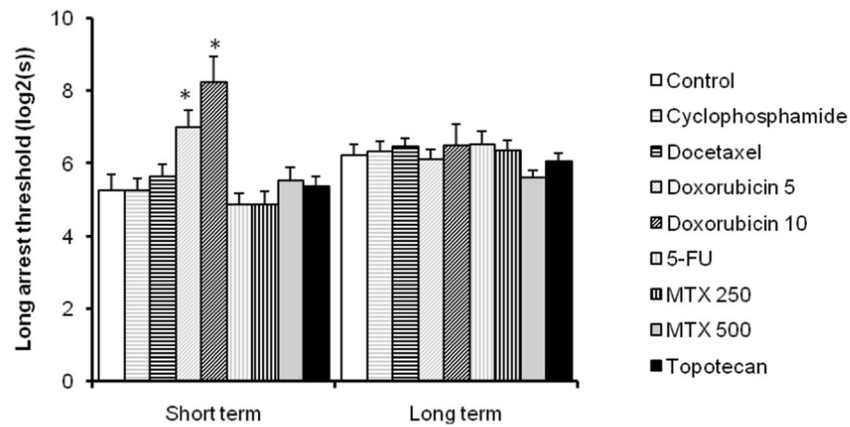


Fig. 16 Cut-off value to separate short and long arrests in the automated home cage short- and long-term after treatment; error bars represent standard error of the mean. *Open bar*: control, *grey horizontal striped bar*: cyclophosphamide, *black horizontal striped bar*: docetaxel, *grey diagonal striped bar*: doxorubicin 5 mg/kg, *black diagonal striped bar*: doxorubicin 10 mg/kg, *grey vertical striped bar*: 5-FU, *black vertical*

striped bar: MTX 250 mg/kg, *grey bar*: MTX 500 mg/kg, *black bar*: topotecan. Both dosages of doxorubicin increased the cut-off value compared with control animals (5 mg/kg $p < 0.05$, 10 mg/kg $p < 0.001$) short-term after treatment. These effects were not present long-term after treatment

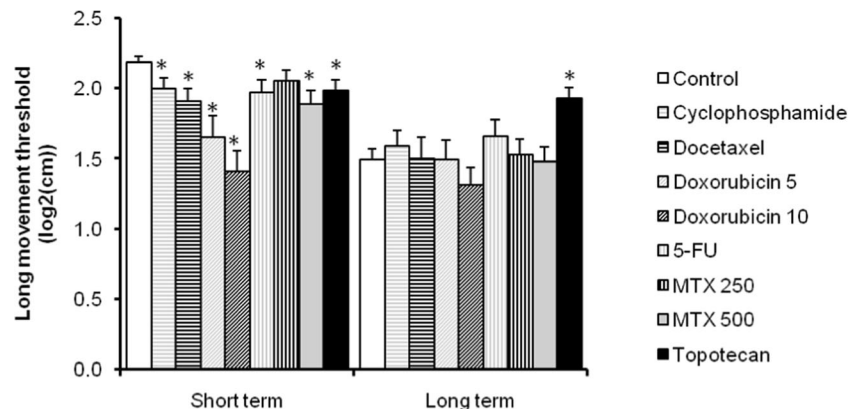


Fig. 17 Cut-off value to separate short and long movements in the automated home cage short- and long-term after treatment; error bars represent standard error of the mean. *Open bar*: control, *grey horizontal striped bar*: cyclophosphamide, *black horizontal striped bar*: docetaxel, *grey diagonal striped bar*: doxorubicin 5 mg/kg, *black diagonal striped bar*: doxorubicin 10 mg/kg, *grey vertical striped bar*: 5-FU, *black vertical striped bar*: MTX 250 mg/kg, *grey bar*: MTX 500 mg/kg, *black bar*:

topotecan. Cyclophosphamide ($p < 0.05$), docetaxel ($p = 0.005$), doxorubicin (both dosages $p < 0.001$), 5-FU ($p < 0.05$), MTX (500 mg/kg $p < 0.01$), and topotecan ($p < 0.05$) decreased the cut-off value to separate short and long movements compared with control animals short-term after treatment. This effect was reversed long-term after treatment with topotecan ($p < 0.001$)

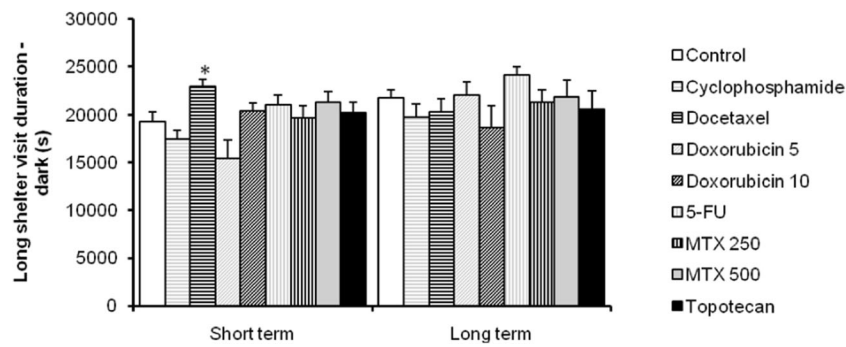


Fig. 18 Cumulative duration of long shelter visits during the dark phase in the automated home cage short- and long-term after treatment; error bars represent standard error of the mean. *Open bar*: control, *grey horizontal striped bar*: cyclophosphamide, *black horizontal striped bar*: docetaxel, *grey diagonal striped bar*: doxorubicin 5 mg/kg, *black diagonal striped bar*: doxorubicin 10 mg/kg, *grey vertical striped bar*: 5-FU,

black vertical striped bar: MTX 250 mg/kg, *grey bar*: MTX 500 mg/kg, *black bar*: topotecan. Docetaxel increased the cumulative duration of long shelter visits compared with control animals ($p < 0.05$) short-term after treatment. These effects were not present long-term after treatment

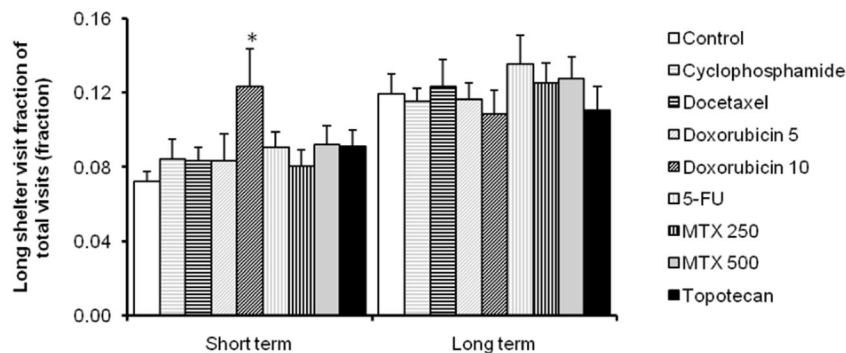


Fig. 19 The fraction of shelter visits with duration longer than long shelter visit threshold in the automated home cage short- and long-term after treatment; error bars represent standard error of the mean. *Open bar*: control, *grey horizontal striped bar*: cyclophosphamide, *black horizontal striped bar*: docetaxel, *grey diagonal striped bar*: doxorubicin 5 mg/kg, *black diagonal striped bar*: doxorubicin 10 mg/kg, *grey vertical striped bar*: 5-FU,

black vertical striped bar: MTX 250 mg/kg, *grey bar*: MTX 500 mg/kg, *black bar*: topotecan. Doxorubicin (10 mg/kg) increased the fraction of long shelter visits compared with control animals ($p < 0.01$) short-term after treatment. These effects were not present long-term after treatment

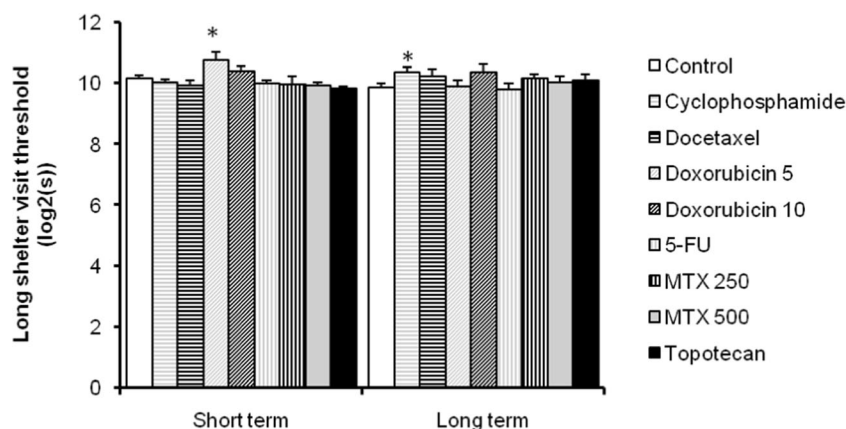


Fig. 20 Cut-off value to separate intermediate and long shelter visits in the automated home cage short- and long-term after treatment; error bars represent standard error of the mean. *Open bar*: control, *grey horizontal striped bar*: cyclophosphamide, *black horizontal striped bar*: docetaxel, *grey diagonal striped bar*: doxorubicin 5 mg/kg, *black diagonal striped bar*: doxorubicin 10 mg/kg, *grey vertical striped bar*: 5-FU, *black vertical striped bar*: MTX 250 mg/kg, *grey bar*: MTX 500 mg/kg, *black bar*: topotecan. Doxorubicin (5 mg/kg, $p < 0.05$) increased the cut-off value to separate intermediate and long shelter visits compared with control animals short-term after treatment. Long-term after treatment, cyclophosphamide increased the cut-off value to separate intermediate and long shelter visits compared with control animals ($p < 0.05$)

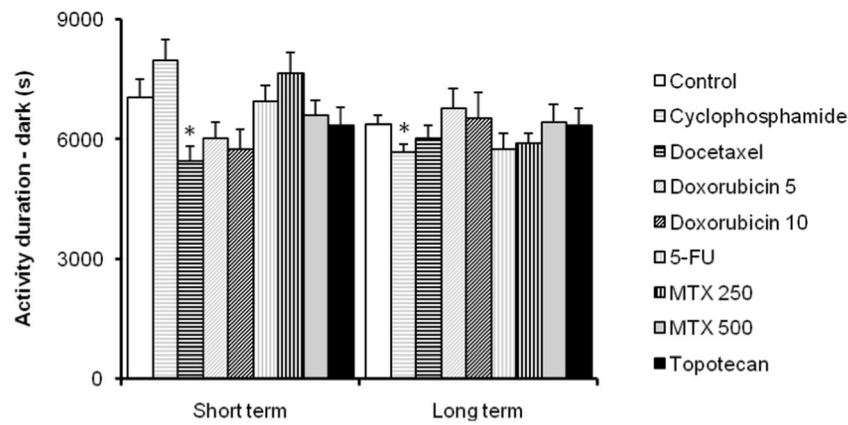


Fig. 21 Cumulative duration of activity (seconds) during the dark phase in the automated home cage short- and long-term after treatment; error bars represent standard error of the mean. *Open bar*: control, *grey horizontal striped bar*: cyclophosphamide, *black horizontal striped bar*: docetaxel, *grey diagonal striped bar*: doxorubicin 5 mg/kg, *black diagonal striped bar*: doxorubicin 10 mg/kg, *grey vertical striped bar*: 5-FU,

black vertical striped bar: MTX 250 mg/kg, *grey bar*: MTX 500 mg/kg, *black bar*: topotecan. Docetaxel treatment decreased the cumulative duration of activity compared with control animals ($p < 0.05$) short-term after treatment. Long-term after treatment, cyclophosphamide treatment decreased the cumulative duration of activity compared with control animals ($p < 0.05$)

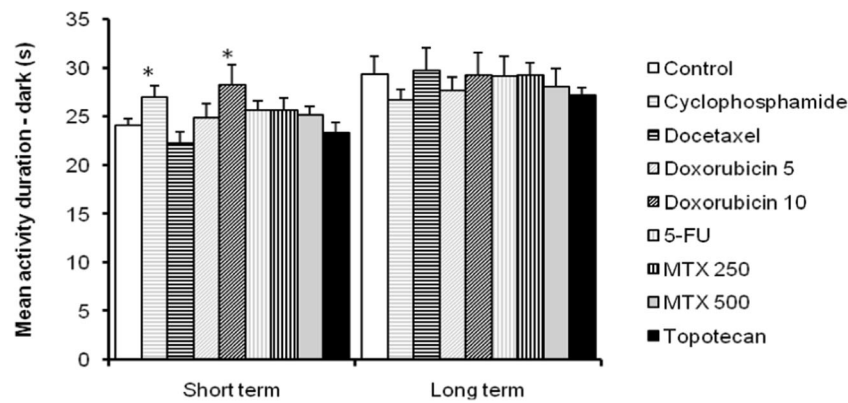


Fig. 22 Mean duration per activity bout (seconds) during the dark phase in the automated home cage short- and long-term after treatment; error bars represent standard error of the mean. *Open bar*: control, *grey horizontal striped bar*: cyclophosphamide, *black horizontal striped bar*: docetaxel, *grey diagonal striped bar*: doxorubicin 5 mg/kg, *black diagonal striped bar*: doxorubicin 10 mg/kg, *grey vertical striped bar*: 5-FU,

black vertical striped bar: MTX 250 mg/kg, *grey bar*: MTX 500 mg/kg, *black bar*: topotecan. Both cyclophosphamide and doxorubicin (10 mg/kg) treatment increased the mean duration per activity bout compared with control animals (both $p < 0.05$) short-term after treatment. These effects were not present long-term after treatment

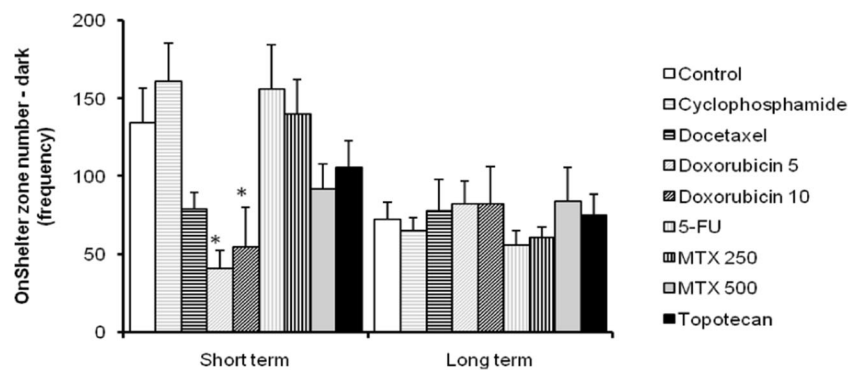


Fig. 23 Cumulative number of visits to OnShelter zone (frequency) during the dark phase in the automated home cage short- and long-term after treatment; error bars represent standard error of the mean. *Open bar*: control, *grey horizontal striped bar*: cyclophosphamide, *black horizontal striped bar*: docetaxel, *grey diagonal striped bar*: doxorubicin 5 mg/kg, *black diagonal striped bar*: doxorubicin 10 mg/kg, *grey vertical striped bar*

bar: 5-FU, *black vertical striped bar*: MTX 250 mg/kg, *grey bar*: MTX 500 mg/kg, *black bar*: topotecan. Both dosages of doxorubicin decreased the cumulative number of visits to the OnShelter zone compared with control animals (5 mg/kg, $p = 0.005$; 10 mg/kg, $p < 0.05$) short-term after treatment. These effects were not present long-term after treatment

References

- Ahles TA, Saykin AJ (2007) Candidate mechanisms for chemotherapy-induced cognitive changes. *Nat Rev Cancer* 7:192–201
- Ahles TA, Root JC, Ryan EL (2012) Cancer- and cancer treatment-associated cognitive change: an update on the state of the science. *J Clin Oncol* 30:3675–3686
- Akkerman S, Prickaerts J, Steinbusch HW, Blokland A (2012) Object recognition testing: statistical considerations. *Behav Brain Res* 232:317–322
- Barreto G, Huang TT, Giffard RG (2010) Age-related defects in sensorimotor activity, spatial learning, and memory in C57BL/6 mice. *J Neurosurg Anesthesiol* 22:214–219
- Bernard Y, Ribeiro N, Thuaud F, Turkeri G, Dirr R, Boulberdaa M, Nebigil CG, Desaubry L (2011) Flavaglines alleviate doxorubicin cardiotoxicity: implication of Hsp27. *PLoS One* 6:e25302
- Bimonte-Nelson HA, Singleton RS, Hunter CL, Price KL, Moore AB, Granholm AC (2003) Ovarian hormones and cognition in the aged female rat: I. Long-term, but not short-term, ovariectomy enhances spatial performance. *Behav Neurosci* 117:1395–1406
- Boogerd W (1995) Neurological complications of chemotherapy. In: de Wolff FA (ed) *Handbook of clinical neurology*, vol 21. Intoxications of the nervous system, part II. Elsevier Science, Amsterdam, pp 527–546
- Borcel E, Perez-Alvarez L, Herrero AI, Brionne T, Varea E, Berezin V, Bock E, Sandi C, Venero C (2008) Chronic stress in adulthood followed by intermittent stress impairs spatial memory and the survival of newborn hippocampal cells in aging animals: prevention by FGL, a peptide mimetic of neural cell adhesion molecule. *Behav Pharmacol* 19:41–49
- Briones TL, Woods J (2011) Chemotherapy-induced cognitive impairment is associated with decreases in cell proliferation and histone modifications. *BMC Neurosci* 12:124
- Brooks SP, Pask T, Jones L, Dunnett SB (2005) Behavioural profiles of inbred mouse strains used as transgenic backgrounds. II: cognitive tests. *Genes Brain Behav* 4:307–317
- Christie LA, Acharya MM, Parihar VK, Nguyen A, Martirosian V, Limoli CL (2012) Impaired cognitive function and hippocampal neurogenesis following cancer chemotherapy. *Clin Cancer Res* 18:1954–1965
- Denenberg VH (1969) Open-field behavior in the rat: what does it mean? *Ann N Y Acad Sci* 159:852–859
- Dere E, Huston JP, Souza Silva MA (2007) The pharmacology, neuroanatomy and neurogenetics of one-trial object recognition in rodents. *Neurosci Biobehav Rev* 31:673–704
- DeVita VT, Hellman S, Rosenberg SA (2005) *Cancer: principles & practice of oncology*, 7th edn. Williams & Wilkins, Lippincott, pp 332–422
- Dietrich J (2010) Chemotherapy associated central nervous system damage. *Adv Exp Med Biol* 678:77–85
- Dietrich J, Han R, Yang Y, Mayer-Proschel M, Noble M (2006) CNS progenitor cells and oligodendrocytes are targets of chemotherapeutic agents in vitro and in vivo. *J Biol* 5:22
- ElBeltagy M, Mustafa S, Umka J, Lyons L, Salman A, Chur-yoe GT, Bhalla N, Bennett G, Wigmore PM (2010) Fluoxetine improves the memory deficits caused by the chemotherapy agent 5-fluorouracil. *Behav Brain Res* 208:112–117
- ElBeltagy M, Mustafa S, Umka J, Lyons L, Salman A, Dormon K, Allcock C, Bennett G, Wigmore P (2012) The effect of 5-fluorouracil on the long term survival and proliferation of cells in the rat hippocampus. *Brain Res Bull* 88:514–518
- Fardell JE, Vardy J, Logge W, Johnston I (2010) Single high dose treatment with methotrexate causes long-lasting cognitive dysfunction in laboratory rodents. *Pharmacol Biochem Behav* 97:333–339
- Fardell JE, Vardy J, Shah JD, Johnston IN (2011) Cognitive impairments caused by oxaliplatin and 5-fluorouracil chemotherapy are ameliorated by physical activity. *Psychopharmacology (Berlin)* 220:183–193
- Foley JJ, Raffa RB, Walker EA (2008) Effects of chemotherapeutic agents 5-fluorouracil and methotrexate alone and combined in a mouse model of learning and memory. *Psychopharmacology (Berlin)* 199:527–538
- Fremouw T, Fessler CL, Ferguson RJ, Burguete Y (2012) Preserved learning and memory in mice following chemotherapy: 5-fluorouracil and doxorubicin single agent treatment, doxorubicin-cyclophosphamide combination treatment. *Behav Brain Res* 226:154–162
- Gandal MJ, Ehrlichman RS, Rudnick ND, Siegel SJ (2008) A novel electrophysiological model of chemotherapy-induced cognitive impairments in mice. *Neuroscience* 157:95–104
- Hampson E (1990) Estrogen-related variations in human spatial and articulatory-motor skills. *Psychoneuroendocrinology* 15:97–111
- Han R, Yang YM, Dietrich J, Luebke A, Mayer-Proschel M, Noble M (2008) Systemic 5-fluorouracil treatment causes a syndrome of delayed myelin destruction in the central nervous system. *J Biol* 7:12
- Janelins MC, Roscoe JA, Berg MJ, Thompson BD, Gallagher MJ, Morrow GR, Heckler CE, Jean-Pierre P, Opanashuk LA, Gross RA (2010) IGF-1 partially restores chemotherapy-induced reductions in neural cell proliferation in adult C57BL/6 mice. *Cancer Invest* 28:544–553
- Kemper EM, Verheij M, Boogerd W, Beijnen JH, van Tellingen O (2004) Improved penetration of docetaxel into the brain by co-administration of inhibitors of P-glycoprotein. *Eur J Cancer* 40:1269–1274
- Kitamura Y, Hirouchi M, Kusuhara H, Schuetz JD, Sugiyama Y (2008) Increasing systemic exposure of methotrexate by active efflux mediated by multidrug resistance-associated protein 3 (mrp3/abcc3). *J Pharmacol Exp Ther* 327:465–473
- Koppelman V, Breteler MM, Boogerd W, Seynaeve C, Schagen SB (2013) Late effects of adjuvant chemotherapy for adult onset non-CNS cancer; cognitive impairment, brain structure and risk of dementia. *Crit Rev Oncol Hematol* 88:87–101
- Li Y, Vijayanathan V, Gulino M, Cole PD (2010a) Intrathecal methotrexate induces focal cognitive deficits and increases cerebrospinal fluid homocysteine. *Pharmacol Biochem Behav* 95:428–433
- Li Y, Vijayanathan V, Gulino ME, Cole PD (2010b) Systemic methotrexate induces spatial memory deficits and depletes cerebrospinal fluid folate in rats. *Pharmacol Biochem Behav* 94:454–463
- Lobo ED, Balthasar JP (2003) Pharmacokinetic-pharmacodynamic modeling of methotrexate-induced toxicity in mice. *J Pharm Sci* 92:1654–1664
- Loos M, Staal J, Schoffemeer AN, Smit AB, Spijker S, Pattij T (2010) Inhibitory control and response latency differences between C57BL/6J and DBA/2J mice in a Go/No-Go and 5-choice serial reaction time task and strain-specific responsivity to amphetamine. *Behav Brain Res* 214:216–224
- Loos M, Koopmans B, Aarts E, Maroteaux G, van der Sluis S, NeuroBSIK Mouse Consortium, Verhage M, Smit AB (2013) High throughput phenotyping of spontaneous behavior: variation within and across 11 inbred mouse strains. *Genes Brain Behav*
- Lyons L, ElBeltagy M, Bennett G, Wigmore P (2011a) The effects of cyclophosphamide on hippocampal cell proliferation and spatial working memory in rat. *PLoS One* 6:e21445
- Lyons L, ElBeltagy M, Umka J, Markwick R, Startin C, Bennett G, Wigmore P (2011b) Fluoxetine reverses the memory impairment and reduction in proliferation and survival of hippocampal cells caused by methotrexate chemotherapy. *Psychopharmacology (Berlin)* 215:105–115
- Lyons L, ElBeltagy M, Bennett G, Wigmore P (2012) Fluoxetine counteracts the cognitive and cellular effects of 5-fluorouracil in the rat hippocampus by a mechanism of prevention rather than recovery. *PLoS One* 7:e30010

- Madhyastha S, Somayaji SN, Rao MS, Nalini K, Bairy KL (2002) Hippocampal brain amines in methotrexate-induced learning and memory deficit. *Can J Physiol Pharmacol* 80:1076–1084
- Maroteaux G, Loos M, van der Sluis S, Koopmans B, Aarts E, van Gassen K, Geurts A, Largaespada DA, Spruijt BM, Stiedl O, Smit AB, Verhage M (2012) High-throughput phenotyping of avoidance learning in mice discriminates different genotypes and identifies a novel gene. *Genes Brain Behav* 11:772–784
- Mumby DG, Gaskin S, Glenn MJ, Schramek TE, Lehmann H (2002) Hippocampal damage and exploratory preferences in rats: memory for objects, places, and contexts. *Learn Mem* 9:49–57
- Mustafa S, Walker A, Bennett G, Wigmore PM (2008) 5-Fluorouracil chemotherapy affects spatial working memory and newborn neurons in the adult rat hippocampus. *Eur J Neurosci* 28:323–330
- Paylor R, Zhao Y, Libbey M, Westphal H, Crawley JN (2001) Learning impairments and motor dysfunctions in adult Lhx5-deficient mice displaying hippocampal disorganization. *Physiol Behav* 73:781–792
- Phillips RG, LeDoux JE (1992) Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behav Neurosci* 106:274–285
- Pukhalsky A, Shmarina G, Alioshkin V (2012) Cognitive disorders in mice: cytokine signaling pathways as therapeutic targets. *OMICS* 16:71–77
- Reiriz AB, Reolon GK, Preissler T, Rosado JO, Henriques JA, Roesler R, Schwartzmann G (2006) Cancer chemotherapy and cognitive function in rodent models: memory impairment induced by cyclophosphamide in mice. *Clin Cancer Res* 12:5000–5001
- Riad A, Bien S, Westermann D, Becher PM, Loya K, Landmesser U, Kroemer HK, Schultheiss HP, Tschope C (2009) Pretreatment with statin attenuates the cardiotoxicity of doxorubicin in mice. *Cancer Res* 69:695–699
- Rzeski W, Pruskil S, Macke A, Felderhoff-Mueser U, Reiher AK, Hoerster F, Jansma C, Jarosz B, Stefovskaja V, Bittigau P, Ikonomidou C (2004) Anticancer agents are potent neurotoxins in vitro and in vivo. *Ann Neurol* 56:351–360
- Seigers R, Fardell JE (2011) Neurobiological basis of chemotherapy-induced cognitive impairment: a review of rodent research. *Neurosci Biobehav Rev* 35:729–741
- Seigers R, Schagen SB, Beerling W, Boogerd W, van Tellingen O, van Dam FS, Koolhaas JM, Buwalda B (2008) Long-lasting suppression of hippocampal cell proliferation and impaired cognitive performance by methotrexate in the rat. *Behav Brain Res* 186:168–175
- Seigers R, Schagen SB, Coppens CM, van der Most PJ, van Dam FS, Koolhaas JM, Buwalda B (2009) Methotrexate decreases hippocampal cell proliferation and induces memory deficits in rats. *Behav Brain Res* 2:279–284
- Sieklucka-Dziuba M, Saczonek J, Dziuba J, Kleinrok Z (1998) Central action of some cytostatics—methotrexate (MTX) and doxorubicin (DXR). II. The influence on the seizure activity and the learning and memory processes in mice. *Ann Univ Mariae Curie Sklodowska [Med]* 53:81–88
- van Acker SA, Kramer K, Voest EE, Grimbergen JA, Zhang J, van der Vijgh WJ, Bast A (1996) Doxorubicin-induced cardiotoxicity monitored by ECG in freely moving mice. A new model to test potential protectors. *Cancer Chemother Pharmacol* 38:95–101
- Walker EA, Foley JJ, Clark-Vetri R, Raffa RB (2011) Effects of repeated administration of chemotherapeutic agents tamoxifen, methotrexate, and 5-fluorouracil on the acquisition and retention of a learned response in mice. *Psychopharmacology (Berlin)* 217:539–548
- Wefel JS, Schagen SB (2012) Chemotherapy-related cognitive dysfunction. *Curr Neurol Neurosci Rep* 12:267–275
- Winocur G, Vardy J, Binns MA, Kerr L, Tannock I (2006) The effects of the anti-cancer drugs, methotrexate and 5-fluorouracil, on cognitive function in mice. *Pharmacol Biochem Behav* 85:66–75
- Winocur G, Binns MA, Tannock I (2011) Donepezil reduces cognitive impairment associated with anti-cancer drugs in a mouse model. *Neuropharmacology* 61:1222–1228
- Winocur G, Henkelman M, Wojtowicz JM, Zhang H, Binns MA, Tannock IF (2012) The effects of chemotherapy on cognitive function in a mouse model: a prospective study. *Clin Cancer Res* 18:3112–3121
- Winters BD, Forwood SE, Cowell RA, Saksida LM, Bussey TJ (2004) Double dissociation between the effects of peri-posterior cortex and hippocampal lesions on tests of object recognition and spatial memory: heterogeneity of function within the temporal lobe. *J Neurosci* 24:5901–5908
- Yang M, Kim JS, Song MS, Kim SH, Kang SS, Bae CS, Kim JC, Wang H, Shin T, Moon C (2010) Cyclophosphamide impairs hippocampus-dependent learning and memory in adult mice: possible involvement of hippocampal neurogenesis in chemotherapy-induced memory deficits. *Neurobiol Learn Mem* 93:487–494
- Yang M, Kim JS, Kim J, Kim SH, Kim JC, Kim J, Wang H, Shin T, Moon C (2011) Neurotoxicity of methotrexate to hippocampal cells in vivo and in vitro. *Biochem Pharmacol* 82:72–80
- Yang M, Kim JS, Kim J, Jang S, Kim SH, Kim JC, Shin T, Wang H, Moon C (2012) Acute treatment with methotrexate induces hippocampal dysfunction in a mouse model of breast cancer. *Brain Res Bull* 89:50–56
- Yanovski JA, Packer RJ, Levine JD, Davidson TL, Micalizzi M, D'Angio G (1989) An animal model to detect the neuropsychological toxicity of anticancer agents. *Med Pediatr Oncol* 17:216–221