Translational potential of the touchscreen-based methodology to assess cognitive abilities in mice

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Translational potential of the touchscreen-based methodology to assess cognitive abilities in mice

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To my family, especially my grandmother Solange,

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Publications

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Posters

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Oral communication

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Table of contents

List of tables

List of figures

List of abbreviations

^{11}C-PIB	Pittsburgh Compound B (radioactive tracer)
^{18}F-FDG	2-deoxy-2-[^{18}F]-fluoro-D-glucose (radioactive tracer)
5-CSRTT	5-Choice Serial Reaction Time Task
5-CCPT	5-Choice Continuous Performance Task
Aß	Amyloid-beta peptide
ACN	Anterior Cingulate Cortex
AD	Alzheimer's Disease
ADAS-Cog	Alzheimer's Disease Assessment Scale of Cognitive functions
ADDLs	Amyloid-Derived Diffusible Ligands
AICD	APP IntraCellular Domain
ApoE	Apolipoprotein E
APP	Amyloid Precursor Protein
BM	Barnes Maze
BOLD	Blood-Oxygen Level Dependent (signal)
CA 1-3	Cornu Ammonis (fields 1-3)
CANTAB	Cambridge Automated Neuropsychological Task Battery
CDR	Clinical Dementia Rating
CFC	Conditional Fear Conditioning
CNS	Central Nervous System
CNTRICS	Cognitive Neuroscience Treatment Research to Improve Cognition in Schizophrenia
CPu	Caudate Putamen
CR	Conditioned Response
CS	Conditioned Stimulus
CSF	Cerebrospinal Fluid
CTL	Correct Touch Latency
DBB	Diagonal Band of Broca
DLS	Dorsolateral Striatum
DMS	Dorsomedial Striatum
DNA	Deoxyribonucleic Acid
DNMTS	Delayed Non-Matching To Sample
DS	Dorsal Striatum
EOFAD	Early Onset Familial Alzheimer's Disease
fMRI	functional Magnetic Resonance Imaging

FTD	Fronto-Temporal Dementia
GWAS	Genome Wide Association Study
HD	Huntington's Disease
HPC	Hippocampus
IT	Initial Touch
ITI	Inter-Trial Interval
ITL	Incorrect Touch Latency
LBD	Lewy Bodies' Dementia
LD	Location Discrimination
LHT	Limited Holding Time
LOAD	Late Onset Alzheimer's Disease
LTP	Long-Term Potentiation
MATRICS	Measurement And Treatment Research to Improve Cognition in Schizophrenia
MAP	Microtubule-Associated Protein
MCI	Mild Cognitive Impairment
MI	Must Initiate
ML	Magazine Latency
MMSE	Mini-Mental State Examination
MS	Medial Septum
MT	Must Touch
MWM	Morris Water Maze
NBM	Nucleus Basalis Magnocellularis
NFT	Neurofibrillary Tangle
NHP	Non-Human Primate
NINCDS-ADRDA	National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association
NMDA (-R)	N-Methyl-D-Aspartate (Receptor)
NORT	Novel Object Recognition Task
NS	Neutral Stimulus
OCD	Obsessive Compulsive Disorder
PAL	Paired Associates Learning (d for different, s for similar stimuli)
PCN	Precuneus Cortex
PCR	Polymerase Chain Reaction
PDE	Phosphodiesterase
PET	Positron Emission Tomography
PFC	Prefrontal Cortex

PHF	Paired Helicoidal Filament
PI	Punish Incorrect
PPA	Primary Progressive Aphasia
PPF	Paired Pulse Facilitation
PS 1/2	Presenilins 1/2
PT	Pre-Training
P-Tau	Hyperphosphorylated Tau
PVD	Pairwise Visual Discrimination
RAM	Radial Arm Maze
rd	Retinal degeneration mutation
R-O	Response-Outcome (association)
ROC	Receiver Operating Characteristics
RRTTS	Rewarded Response To The Screen
RSRTTS	Rewarded Selective Response To The Screen
SAB	Spontaneous Alternation Behavior
SD	Semantic Dementia
S-R	Stimulus-Response (association, learning)
TG	Transgenic animals
TGA	Transient Global Amnesia
t-Tau	Total Tau
TUNL	Trial Unique Non matching to Location
UR	Unconditioned Response
US	Unconditioned Stimulus
VD	Vascular Dementia
VMCL	Visuo-Motor Conditional Learning
VS	Ventral Striatum
WT	Wild-Type animals

Scientific background

Chapter 1: Memory Systems – Historical Review

From their birth to death, human beings lay up and take advantage of an infinite number of informations that are both fundamental for the construction of their own identity and their connection and understanding of the surrounding world. This would not be possible in the absence of certain critical abilities, the so-called learning and memory processes. Nowadays, learning is commonly viewed as "the process by which persistent and measurable behavioral changes occur as a result of a particular experience", while memory rather refers to "the recording of our past experiences acquired through one or several learning episodes" (Gluck et al., 2007). However, these definitions have not suddenly arisen and represent the fruit of centuries of intensive theoretical and empirical researches, recently amplified by the multiplication of investigation techniques and biological models that allow studying the brain in great detail. In this first part, we wanted to relate some findings that have deeply influenced our current understanding and knowledge of learning and memory processes. These aspects were accompanied by a brief reminder of the hippocampal and striatal anatomies along with some of their most recognized functions in rodents and Humans.

I. The debut of experimental psychology

Since the Antiquity, many philosophers and psychologists have speculated about the deep nature and localization of memory. In particular, two main streams, the nativism and the empiricism, have argued over centuries in order to determine whether our knowledge directly resulted from our inherited qualities or was rather the outcome of our past experiences (also known as the nature *vs* nurture debate). However, all approaches remained theoretical as the depository structure of memory had not been identified. Contrasting with that period, the second half of the nineteenth century has then suddenly started lifting the veil on these long-standing questions. First, the convergence of different clinical studies has allowed highlighting the role of our brain in various mental processes such as language and memory. Thus, Paul Broca, a French surgeon and Karl Wernicke, a German anatomist, early described the case of two patients that presented aphasia (*i.e.* the loss of ability to speak and/or understand oral instructions despite other preserved cognitive functions) after restricted brain damages (Broca, 1865; Wernicke, 1874). In parallel, Théodule Ribot, a French philosopher, observed that the mnesic deficits that followed a brain injury usually

rather concerned recent than remote memories, a law now known as Ribot's gradient (Ribot, 1882). Finally, Sergei Korsakoff, a Russian neuropsychiatrist and Alois Alzheimer, a German psychiatrist, hypothesized a causal link between the memory impairments developed by their respective patients and the global atrophy of their brains observed during the autopsies (Korsakoff, 1887; Alzheimer, 1907).

These initial findings gave weight to the hypothesis promoting the requirement of the human brain during the accomplishment of daily memory tasks. Nonetheless, they did not permit to accurately pinpoint which brain region was responsible for mnesic deficits and more importantly, did not bring new informations about the way learning and memory processes should be considered and studied in human subjects. Remarkably, at the same time, a German psychologist, Hermann Ebbinghaus, began to answer that question as he published the first experimental study of human memory (Ebbinghaus, 1885). Being his own subject, he used lists of small artificial words to empirically demonstrate the existence of two modalities that influenced the maintenance or forgetting of information. Whilst the repeated presentation of a similar material contributed to facilitate its encoding and posterior retrieval, he observed that a memory faded away when the delay of retention preceding the retrieval phase increased. This pioneering methodological work deeply modified the way both clinicians and researchers thereafter comprehended the notion of memory. Furthermore, the different types of recall (notably free and cued recalls) proposed by Ebbinghaus inspired the development of neuropsychological tests designed to diagnose pathological conditions in patients with memory deficits. Eventually, in association with further studies on consolidation theory pursued by Georg Müller and Alfons Pilzecker (Lechner et al., 1999), two German psychologists, it also paved the way for the elaboration of a structural definition of the memory. Indeed, memory is often referred as the sum of different processes that are used to acquire, store and retrieve given information. These three major operating processes, named encoding, storage and retrieval, successively intervene throughout the consolidation of a specific learning and ultimately result in memory. It is noteworthy, however, that memory can then be regularly updated during reconsolidation (Martin and Clark, 2007; Nader and Einarsson, 2010; McKenzie and Eichenbaum, 2011).

II. The birth of behaviorism

At the dawn of the twentieth century, experimental psychologists perfectly understood that the exploration of learning and memory processes would be much easier in animals than

Humans. Yet, two questions remained to solve: which animals should be selected to perform such studies? And above all, how could researchers quantitatively measure the existence of such learning in those species?

The first to provide a solution to this last, crucial point was Ivan Pavlov, a Russian physiologist. In the framework of his field of research, he studied the gastric functions in dogs after food delivery. Upon one of his experiments, he realized that the saliva production was systematically increased by the onset of an external stimulus: the arrival of his assistant in charge of the food distribution. To control if dogs learned to produce more saliva in reaction to the repetitive exposure to certain environmental stimuli associated with the food intake, he conceived a famous study during which the food delivery always followed the noise of a doorbell (Pavlov, 1927). Over the successive matchings between these two distinct stimuli, he stated that dogs salivated as soon as the bell rang, even before the food onset, and therefore verified that the increasing amount of salivation corresponded to a progressively learned association in these animals (**Figure 1**). The form of learning Pavlov had discovered was named classical or Pavlovian conditioning.

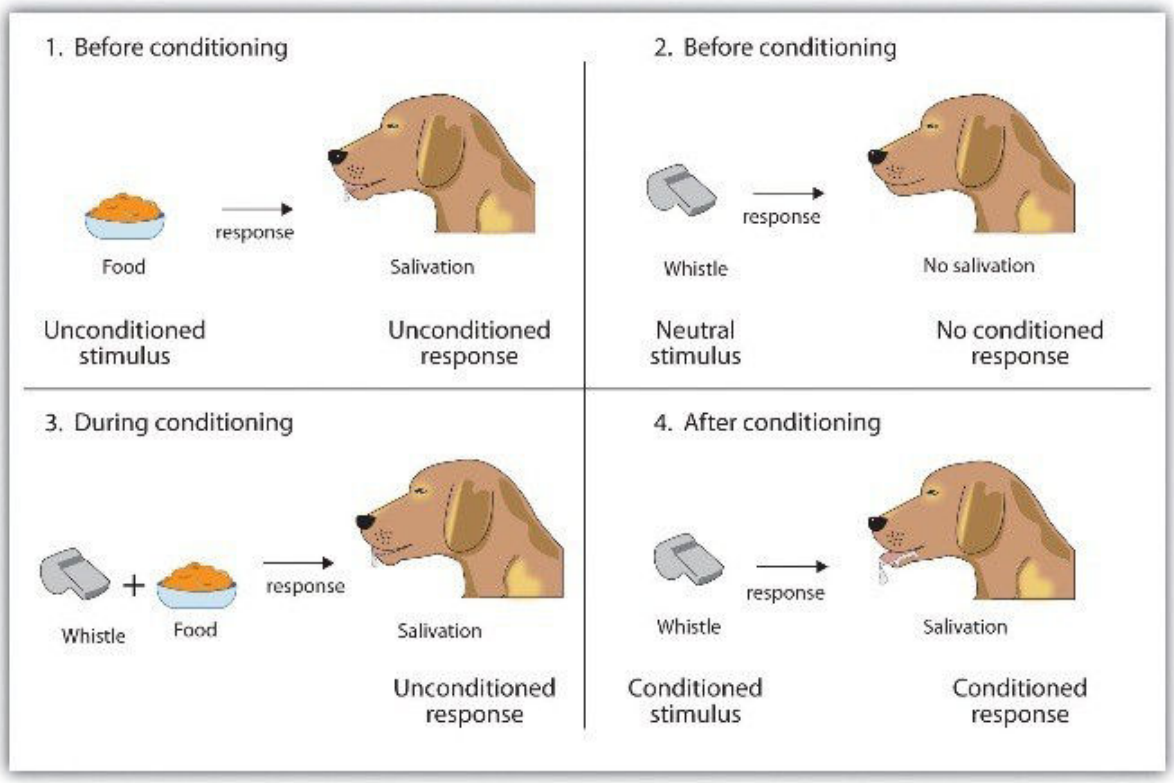


Figure 1. Description of the concept of classical conditioning as exemplified by Pavlov's experiments. At the start, an unconditioned response (UR) is produced by the dog as a specific consequence of the presentation of an unconditioned stimulus (US). A neutral stimulus (NS) does not elicit this US.

However, after the association of the US and the NS, this latter becomes a conditioned stimulus (CS) able to elicit the conditioned response (CR) in the absence of US. Picture from Stangor (2010).

Around the same time, Edward Thorndike, an American psychologist, took a bold step forward by laying the foundations of another form of learning in animals currently known as operant or instrumental conditioning. During his thesis, he trained cats locked in puzzle boxes to learn a defined rule (e.g. pulling a lever) in order to escape that unpleasant environment. After a few trials, animals were quicker to execute the required response, which incited Thorndike to express that the consequences of a specific learning strongly shaped the future behavioral responses of an animal (Thorndike, 1927). That law of effect easily spread among the scientific community as it was in agreement with the basic principles of our own behavior and survival across evolution.

The major advances made by Pavlov and Thorndike revealed the possibility to study how interactions of an individual with its environment could yield observable behavioral changes leading to learning over time. That new approach gave rise to a popular research stream, the behaviorism. Burrhus Skinner, an American psychologist, rapidly became the leader of this school of thought. On one hand, completing the work of Thorndike, he sought to determine to what extent the consequence of a behavioral response could influence its later occurrence. He described two categories of consequence, reinforcement and punishment that respectively increased or decreased the prospective probability of reproduction of a certain behavior (Skinner, 1951). On the other hand, he developed one of the first tools to study learning and memory processes in animals: within Skinner's boxes, learning abilities of rodents or pigeons were assessed via the progressive acquisition of a response implicating the activation of a certain *manipulandum* (lever press or response key) positively reinforced with the delivery of a food/water reward. In the same line, also notable were the efforts of Clark Hull, another American psychologist. Convinced that a mechanistic model could explain the laws governing our behavior and hence predict our future responses, he leaned on experimental studies led in operant chambers with animals to propose a mathematical theory of learning (Hull, 1943).

Globally, the work of behaviorists turned out very useful for the emphasis of a certain number of phenomena defining modern psychology, among which generalization, discrimination or extinction processes (Tavris et al., 1999). Yet, it also showed some important limitations. First, without noticing, partisans of the behaviorism totally focused their attention on a specific form of memory, now defined as habit, and gradually rendered possible by the association of stimuli and learned responses (S-R associations). Second, most of them

overshadowed the putative roles played by our “internal states” (such as consciousness, thought and emotions) during a simple learning and its consolidation. Third, apart from Karl Lashley, they did not bring any information on the nature of brain regions operating the acquisition of learning and maintenance of a memory. Lashley, an American psychologist, investigated in rats the effects of cortical lesions on acquisition or expression of learning executed in a maze but failed to localize the physical trace of memory (engram). In accordance with his own results (Lashley, 1931), he indicated that learning performance was conversely correlated with the size of the cortical damage – the mass action law – and suggested that the engram was impossible to confine because the whole cortex shared similar functional properties that permitted compensating after the destruction of a particular region and maintaining the information – the equipotentiality law. That last principle was of course false, but Lashley had at least the virtue of exploring the neural substrates of memory by hinging on an adequate scientific methodology.

III. Challenging the initial thought: the story of patient H.M.

It is often acknowledged that the modern era of memory research has actually begun approximately sixty years ago (Squire, 2004; Nadel and Hardt, 2011). In the fifties, surgeons were looking for new solutions to prevent the occurrence of chronic, devastating seizures in patients suffering from epilepsy. As pharmacological treatments were sometimes insufficient to keep intact patient’s quality of life, clinicians wanted to test new options based on resectional surgeries. In 1953, William Scoville, an American surgeon, bilaterally removed the medial temporal lobe of one of his patients, Henry Molaison (subsequently known as the famous patient H.M.). After the operation, he confirmed the success of the resection as epileptic seizures had clearly disappeared but unexpectedly noticed the appearance of serious memory issues in H.M. Four years later, Brenda Milner, a Canadian neuropsychologist, started to regularly visit H.M. so as to shed light on the nature of his memory defects. During her initial psychological examinations (Scoville and Milner, 1957), she found a striking contrast between his intellectual and mnesic capacities: whilst he displayed preserved perception, reasoning or abstract thinking, he was seemingly unable to achieve tasks where he had to retain verbal or visual material more than one or two minutes. In addition to that anterograde amnesia, he presented a partial retrograde amnesia but could without any problem remember and give numerous details about events of his early life. Altogether, these remarkable outcomes taught us more about memory than almost a century of experimental research. They challenged some of our initial thoughts like the fact that

memory and intellectual faculties were stored in a similar brain area and clearly showed the existence of different forms of memory, some of which were selectively affected in patient H.M. after his surgery.

The case of patient H.M. first helped to discriminate between short- and long-term memories (**Figure 2**). Interestingly, some authors had already suggested this idea in the past, notably William James, an American psychologist, who already attached great importance to the fine distinction between primary and secondary memories (James, 1890). Moreover, further studies later contributed to the improvement of the long-term memory profile of the patient after his evaluation in various tests like the mirror-tracing or rotary pursuit tasks revealed spared “motor skills” (Milner, 1968; Corkin, 2002).

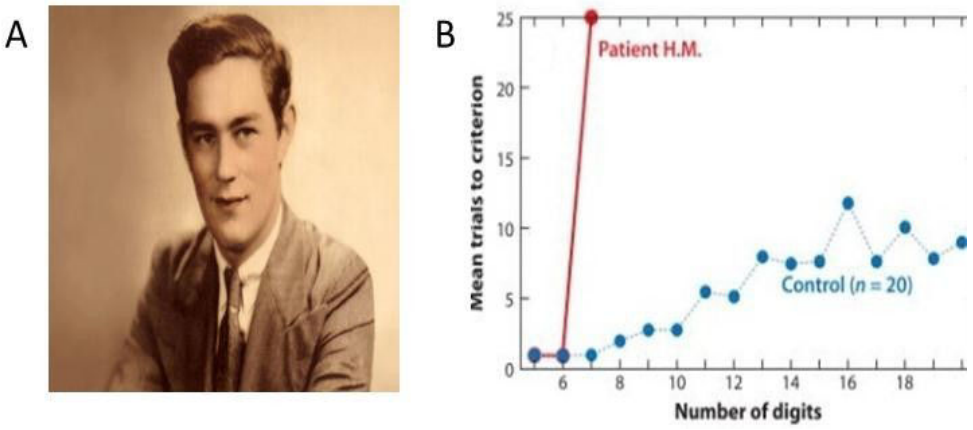


Figure 2. Patient H.M. (panel A); in a task testing his immediate memory span (Drachman and Arbit, 1966), he performed similarly to control subjects if the digit string did not exceed 6 digits, but was unable to transform this transient information and forgot almost instantly a list of 7 or more digits, even after their successive repetitions (panel B, picture from Squire and Wixted, 2011). These results inspired the posterior development of models of short-term or working memories (Atkinson and Shiffrin, 1971; Baddeley, 2000).

As certain long-term memories seemed preserved while others were severely disabled, the neuropsychological profile of H.M. immediately intrigued many scientists and encouraged numerous experiments and hypotheses over the next two decades. However, early behavioral studies conducted in non-human primates and rats with lesions comparable to H.M. failed to reproduce such mnesic deficits (Orbach et al., 1960) and it soon came into sight that further works would be necessary to dissect neuroanatomically the functional role(s) of all components of the medial temporal lobe. Within this ensemble, the

hippocampus was eventually identified as a key structure implicated in certain forms of learning, which gave birth to the proposal of new long-term memory dichotomies: semantic vs episodic memory (Tulving, 1972); associative *vs* recognition memory (Gaffan, 1974); contextual retrieval *vs* habit (Hirsh, 1974); locale *vs* taxon memory (O'Keefe and Nadel, 1978); memory *vs* habit (Mishkin and Petri, 1984). Nevertheless, in the late seventies, despite the common gut feeling exhibited by most of opinion leaders, there was still no experimental evidence supporting these theories.

IV. Dissociation studies: genesis of the Multiple Memory Systems Theory

The first proof of a long-term memory dissociation following the lesions of distinct brain regions in rats finally took place a decade later. As aforementioned, the effects of hippocampal lesions in rats had been broadly described in many behavioral paradigms during the seventies. In memory tasks, animals were in some cases profoundly impaired (Gaffan, 1972; Walker and Means, 1973; O'Keefe and Nadel, 1978; Winocur and Gilbert, 1984) or presented no deficit (Harley, 1972; Samuels, 1972). Nevertheless, in parallel, another subcortical structure started drawing the attention. The striatum, or caudate nucleus in rats, belongs to the basal ganglia and was originally assumed to be responsible for motoric functions. However, it was observed that as for the hippocampus, striatal lesions could sometimes lead to memory disturbances in cognitive paradigms (Winocur, 1974; Whishaw et al., 1987). At that time, it was fully understood that if a single apparatus allowed discriminating the effects of hippocampal or striatal lesions in function of the chosen environmental conditions, it would represent a powerful tool for subsequent comparisons. In this way, the first seminal dissociation study emerged (Packard et al., 1989). In an eight-arm Radial Maze, rats with fimbria-fornix (equivalent to hippocampal) or striatal lesions were assessed in one of two different protocols: the Win Stay or Win Shift variants (**Figure 3**).

In the Win Stay, four out of the eight arms were baited and animals had to specifically visit these arms by associating them with the presence of a visual stimulus located at their entrance. As in Skinner’s boxes, the repetitive pairings of the intra-maze sensorial stimulus (here, the light) and the response were supposed to favor the appearance of a habitual form of learning. In the Win Shift, all arms were baited and animals had to use environmental visual cues to elaborate a spatial mapping of the maze (Tolman, 1948; O'Keefe and Nadel, 1978). In both paradigms, control animals reduced their number of errors over sessions. Rats

with striatal lesions learned the Win Shift paradigm as controls but showed a strong deficit in the Win Stay task; by contrast, rats with fimbria-fornix lesions had better performances than control animals in the Win Stay task but were severely affected in the Win Shift paradigm. To conclude, the hippocampus and striatum were indicated to be respectively involved in spatial and habitual forms of long-term memory via the use of allocentric and egocentric strategies.

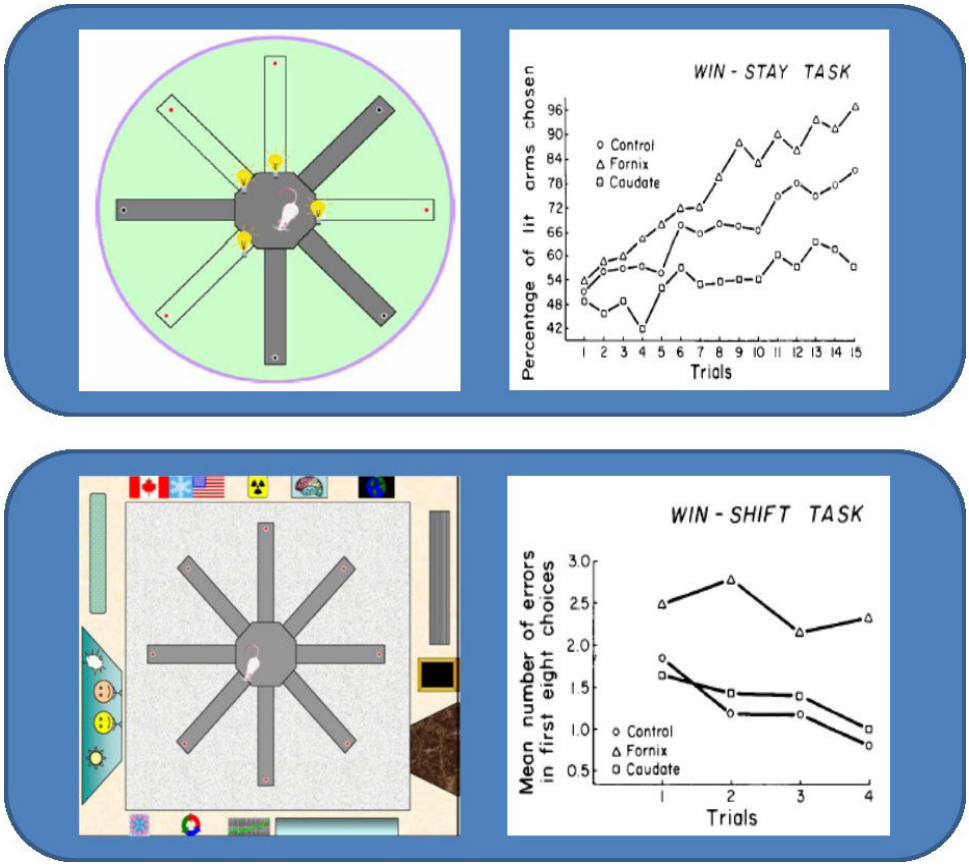


Figure 3. Representation of the two protocols used in the eight-arm Radial Maze, the Win-Stay (top panel) and Win-Shift (bottom panel), and corresponding results in fimbria-fornix *vs* caudate lesioned animals (original pictures from Packard et al. (1989) and McDonald et al. (2004b).

The existence of these two forms of long-term memory was later confirmed in rodents in the Morris Water Maze (Packard and McGaugh, 1992) and human patients with Alzheimer's or Parkinson's disease (Knowlton et al., 1996). Since then, other dissociation studies implicating rodents (McCormick et al., 1982; McDonald and White, 1993) or Humans (Adolphs et al., 2005; Gerwig et al., 2005) have again extended the frontiers of our knowledge beyond the simple existence of two memory systems: henceforth, we talk about multiple memory systems in mammals (Squire and Zola, 1996; White and McDonald, 2002).

V. The current classification and definition of long-term memories

The global organization of long-term memory systems has been latterly summarized (Squire, 2004). In this review, putting aside the notion of short-term memory, Squire takes into account the bulk of past human and animal experimental studies to distinguish no less than seven long-term memory systems necessarily falling into one of two memory categories, declarative and non-declarative memories (Cohen and Squire, 1980; Squire, 1992). The major differences between declarative and non-declarative memories are that in the former case, information must be processed and retrieved consciously (but for a different approach, see Henke, 2010) and through the use of verbal language, while in the latter case, none of these conditions is a pre-requisite to guarantee the success of the encoding and recollection of the material. Declarative memory is composed of two long-term memory systems, whereas non-declarative memory encompasses the five remainders (**Figure 4**).

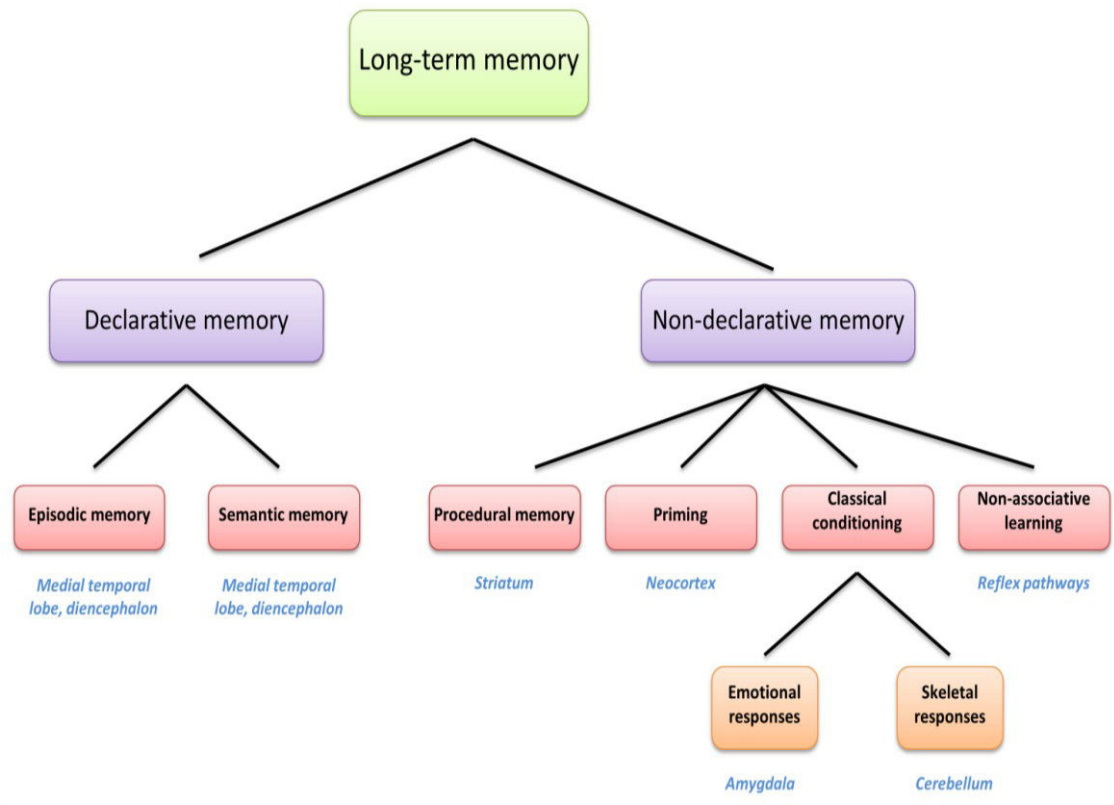


Figure 4. Current classification of memory systems in the mammalian brain. Long-term memory, which differs from short-term memory, embraces declarative and non-declarative memories, which can themselves be cleaved in different forms of memories. Adapted from Squire (2004).

In an effort to simplify their presentation and comprehension, we will define each form of long-term memory, cite the related brain region(s) whose association allows the use of the nomenclature “system” and give a practical example in Humans:

- Episodic memory (Tulving, 1972; Tulving, 2002) refers to the autobiographical memory of an individual, and can be perceived as the conscious mental recollection of a unique past event that occurred in a meaningful spatio-temporal context. The integrity of the medial temporal lobe and diencephalon is pivotal for its correct functioning. For instance, try to remember a significant past event of your own life that means a lot to you (the obtention of your diploma, your wedding…) and an unexpectedly high amount of details will surface.

- Semantic memory (Tulving, 1985) concerns the conscious access to our factual knowledge. As for episodic memory, it can be orally communicated and the integrity of the medial temporal lobe and diencephalon is pivotal for its correct functioning. A basic example would consist in asking someone the answer to a question of a general nature: What is the capital of Germany?

- Procedural memory denotes to a form of memory appearing from operant learnings that are progressively refined until they become finely tuned and automatic (habits). Importantly, motor or cognitive skills remain unconsciously accessible during our whole life. The integrity of the striatum is pivotal for its correct functioning. Learning how to ride a bike or to play piano definitely requires this form of memory.

- Priming effect (Kolb and Whishaw, 2003) designates the subsequent quicker perception of a stimulus in an individual who has been previously exposed to the same or a comparable material. The integrity of the neocortex is pivotal for its correct functioning. A common example concerns the recognition of a picture: after its first, yet rapid exposition to a subject: in these conditions, it seems that subjects need less time to identify the image than unexposed controls.

- Classical associative conditioning has been partly tackled with Pavlov’s experiments. There are two different kinds of conditioned responses. Conditioned emotional responses, driven by the amygdala, result from the association of a previously neutral stimulus (NS) with an aversive event (US, electric shock) that induces fear (UR). As a consequence, the posterior

occurrence of this stimulus (CS) directly induces fear (CR), which could explain, for example, the appearance of anxiety disorders like phobias and post-traumatic stress disorders in Humans (Maren, 2001; Maren et al., 2013). From their part, conditioned skeletal responses ensue from more basic associations that always lead to motor changes via a cerebellar activation. In Humans and animals, the eyeblink conditioning (Vogel et al., 2002; Rampello et al., 2011) illustrates this last explanation: at the beginning, an air puff (US) provokes the eyeblink of a subject (UR). But after repeatedly matching this air puff with a tone (NS), this previously neutral stimulus becomes a CS and can in turn elicit the eyeblink reflex (CR).

- Finally, non-associative conditioning can be divided into two antagonistic classes: habituation and sensitization. These phenomena, first studied in a marine snail called *Aplysia* (Pinsker et al., 1973; Castellucci et al., 1978), refer to the likelihood of a behavioral response to decrease or increase after the repeated exposure to a stimulus. In all mammals, the integrity of reflex pathways is pivotal for its correct functioning. One typical example that witnesses to our daily habituation is our progressive acclimation to environmental changes. During a stay in a hotel, one is likely to have difficulties to fall asleep the first night; however, this problem rapidly disappears. In contrast, if your neighbor organizes a first party that prevents you from sleeping, you will react resolutely but far more vigorously if, for instance, he celebrates his birthday the week after.

At this stage, two complementary pieces of information should be drawn to the attention of the reader. As a matter of fact, although this taxonomy is supposed to be valid in all mammals, it is worth mentioning that the existence of certain memories remains unproven in lower species, especially episodic memory as further detailed (Clayton and Dickinson, 1998; Morris, 2001). In addition, these memory systems, initially viewed as parallel and independent, have recently been showed to obey to more complex laws. In certain cases, according to the nature and context of learning, they can either collaborate (cooperation) or face each other (competition). These discoveries have motivated the launch of a new strand of research targeting the understanding of psychopathological conditions within the framework of multiple, interacting memory systems (McDonald et al., 2004a; McDonald et al., 2004b; Packard, 2009; Packard and Goodman, 2012). In the next section, we will individually address the neuroanatomy and most common functional roles of two subcortical structures, the hippocampus and striatum, which actively participate to learning and memory processes in rodents and Humans. These elements will be succinctly completed by experimental data

that have confirmed competition / cooperation between these two brain regions during spatial memory tasks in rodents.

VI. Focus on two structures: the hippocampus and striatum

1. Neuroanatomy of the hippocampus

Whatever the species considered, it is always striking to note that the brain architecture has been so highly conserved in Vertebrates. The medial temporal lobe consists of four main structures: the hippocampal region and surrounding hippocampal perirhinal, entorhinal and parahippocampal cortices (Squire and Zola-Morgan, 1991).

In Humans, monkeys and even rodents, the hippocampal formation is similarly composed of five portions: the hippocampus, subiculum, presubiculum, parasubiculum and entorhinal cortex (Amaral and Lavenex, 2007). Among these different areas, the hippocampus corresponds to a seahorse-shaped bilateral structure. It is made of two distinct parts: the dentate gyrus and the Ammon's horn, also named *Cornu Ammonis* (often divided in three subregions, named CA1 to CA3 fields). As early described by Ramon y Cajal (Cajal, 1911), these two lamellar regions are embedded and stratified in a characteristic manner (**Figure 5**).

Hippocampal afferences mainly consist of the entorhinal cortex, the contralateral hippocampus, the basal forebrain (septal nuclei) and the brain stem. Among these inputs, the perforant path refers to the multiple connections linking the entorhinal cortex and all hippocampal subregions (dentate gyrus, CA3 field, CA1 field, and even the subiculum). Within the hippocampus, apart from an auto-associative network present in CA3 field, unidirectional pathways globally convey information through a trisynaptic excitatory circuit. Starting from the dentate gyrus, mossy fibers project to the CA3 field, that in turn project to the CA1 field via Schaffer's collaterals. Eventually, CA1 neurons project to the adjacent subiculum, from which efferent neurons spread towards the fornix, a bundle of fibers that enables the diffusion of information to numerous other brain regions. This first output allows explaining why fimbria-fornix lesions were used to mimic hippocampal lesions in animals: some, but not all functions were significantly altered following the suppression of that essential output signal (Walker and Olton, 1984). Remarkably, neurons from the subiculum also actively target the entorhinal cortex, which constitutes both a major afference and efference of the hippocampus.

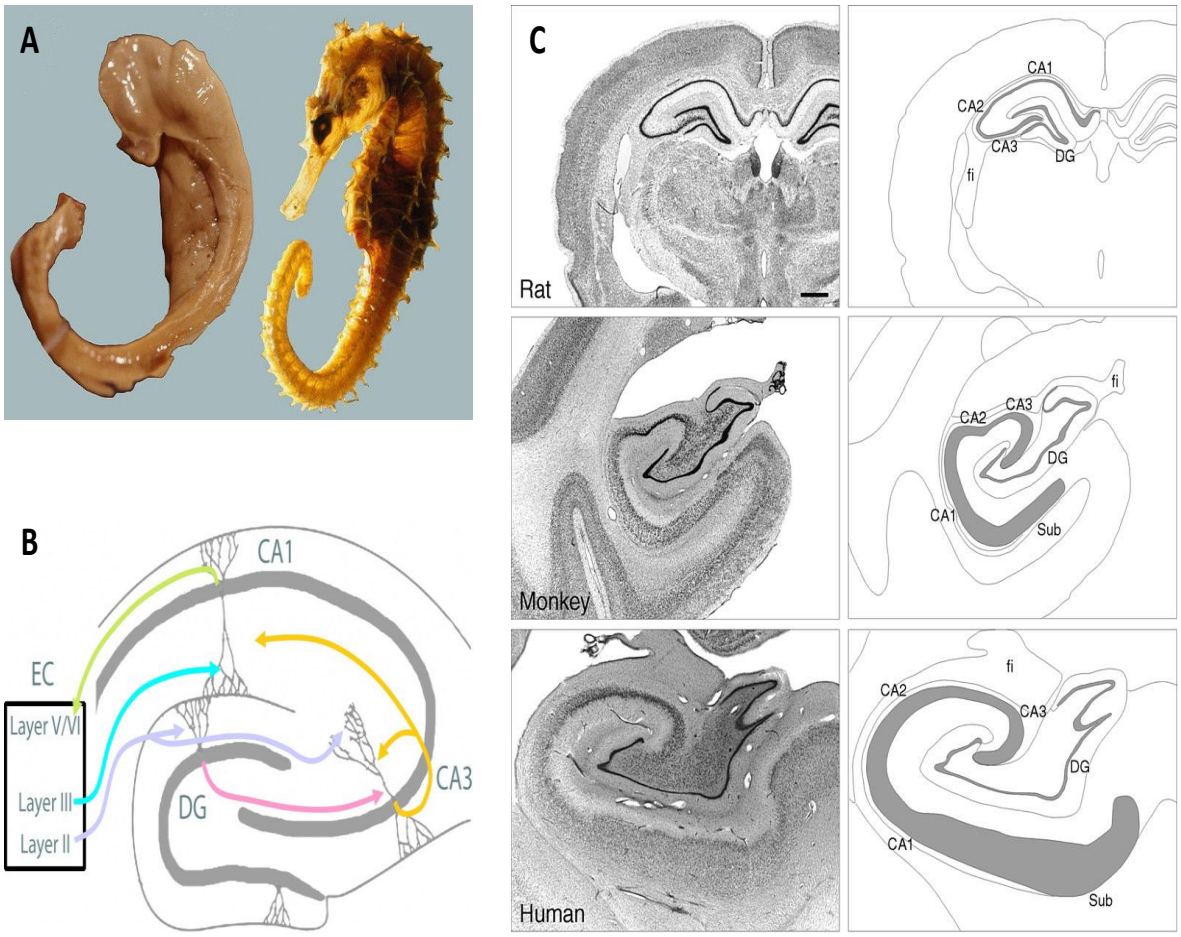


Figure 5. The hippocampus, its connectivity and conserved structure across species. This brain region is so called because of its likeness with a marine fish, the seahorse (panel A); the entorhinal cortex is a momentous partner as it provides afferences to the different areas of the hippocampus and gets back efferent projections in external layers, as indicated by the different arrows (panel B); the architecture of the hippocampal formation in Nissl-stained slices from rats, monkeys and Humans (panel C, from top to bottom). Panels A and C retrieved from Andersen et al. (2007). Panel B: picture from K. Diba's lab website (University of Wisconsin, Milwaukee).

2. Functional roles of the hippocampus in learning and memory

Since its anatomical description, many cognitive and non-cognitive functions have been attributed to the hippocampus, among which putative roles in emotion (Papez, 1937), attention (Kaada et al., 1953; Adey, 1967), behavioral inhibition (Douglas, 1967; Kimble, 1968), anxiety (Gray, 1983; Bannerman et al., 2004; Barkus et al., 2010) and learning and memory processes (Schmajuk and Isaacson, 1984; Good, 2002). Much has been published on this last aspect and we will voluntarily restrict our review to the three following key ideas.

a) Spatial navigation and memory

Because of its hierarchized organization, the hippocampus has long been utilized within the scope of neurophysiological studies in animals (Best and White, 1999). Various experimental techniques using this model have afforded innovative concepts to emerge, as suggested for example by the phenomenon of long-term potentiation (LTP) that is now known to reflect the consolidation of a memory at a cellular level (Bliss and Lomo, 1973; Cooke and Bliss, 2006) and will be deeper detailed in the next chapter. In many respects, another method has revolutionized our initial understanding of hippocampal function: the recording of neuronal activity in freely moving animals. *In vivo* electrophysiological studies in vigil rats have showed that certain hippocampal cells, named place cells, were able to fire individually when animals explored the environment (O'Keefe and Dostrovsky, 1971; O'Keefe and Conway, 1978). Interestingly, all place cells were not activated at the same time. Rather, it appeared that the expression of a specific firing pattern corresponded to a determined spatial location of the animal (Poucet et al., 2000). This finding had a tremendous impact as it indicated that rodents were capable to establish relationships between external cues to build a spatial cognitive map, as hypothesized earlier by Tolman. Further behavioral works later came to the same conclusion, chiefly after hippocampectomized rodents were showed to be markedly impaired in navigational learning tasks that included a strong spatial memory component (O'Keefe et al., 1975; Olton and Papas, 1979; Morris et al., 1982).

Place cells have been more recently identified in the hippocampus of Humans (Ekstrom et al., 2003). Moreover, the parallel development of imaging techniques (PET, fMRI) and neuropsychological tasks appealing virtual environments (taxi driver game or navigation in a virtual town/maze, **Figure 6**) has allowed correlating the volume and/or activation of the hippocampus with spatial memory performance both in normal and physiopathological conditions such as amnesia or Alzheimer's disease (Maguire et al., 2000; Astur et al., 2002; Burgess et al., 2002; Lithfous et al., 2013).

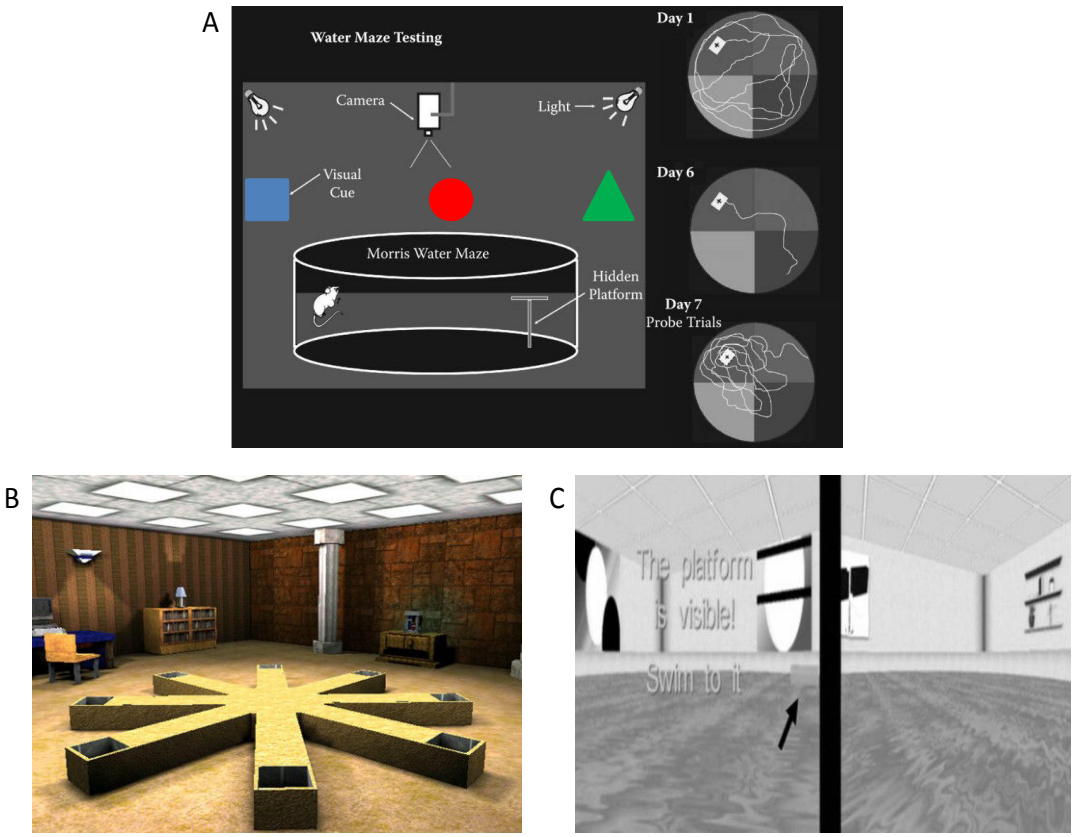


Figure 6. Examples of paradigms testing spatial navigation and learning in rodents or Humans. In the most common protocol of the Morris Water Maze (MWM) task, a rodent must use visual distant cues (allocentric strategy) to find a submerged platform. Learning can be verified over time, ultimately with a probe trial during which the experimenter measures the time the animal spends in the quadrant of the pool that previously contained the platform (panel A). Virtual Radial (panel B) and Morris Water (panel C) Mazes for Humans. In the last paradigm, two versions of the task allow assessing egocentric (left part, visible platform) or allocentric (right part, extra-mazes cues) strategies. Pictures from Buccafusco (2009), Astur et al. (2005) and Cornwell et al. (2008), respectively.

b) Relational memory

As previously discussed, animal studies based on hippocampal lesions have historically revealed deficits in many different behavioral tasks. However, the involvement of the hippocampus in spatial memory only accounted for some of these deficits (Compton et al., 1994). In the contextual fear conditioning, for example, hippocampal lesioned rats froze significantly less than controls after re-exposure to the context in which they had been prior punished with a foot shock (Phillips and LeDoux, 1992; Frankland et al., 1998). This brought a new hypothesis which emphasized the hippocampus as a neural mediator of relational

memory (Eichenbaum and Cohen, 2001). By and large, this theory somehow generalized the role of the hippocampus in spatial memory by assuming that it could process all manners of relationships between stimuli of various natures (objects, faces, places, contexts, events…) by forming flexible representations that would remain permanently accessible to recall. In rodents, that approach was relevant with the fact that hippocampal lesioned animals were unable to acquire or express certain forms of associative, non-spatial learning such as successive odor paired-associates or transverse patterning problems (Bunsey and Eichenbaum, 1996; Dusek and Eichenbaum, 1998).

In Humans, it also strengthened that performance of amnesic patients (like patient H.M.) was not only heavily disturbed in spatial memory tasks, but also in more complex, associative memory paradigms (Corkin, 2002; Giovanello et al., 2003; Konkel et al., 2008). Nonetheless, researchers who endeavored to compare the involvement of the hippocampus in spatial and relational memory tasks tested in a same subject were sometimes more likely to find a connection in the former case (Kumaran and Maguire, 2005; Ryan et al., 2009).

c) Episodic memory

We have earlier given a short, yet reasonable definition of episodic memory for mammals. However, we cannot resist quoting (Tulving, 2002) about episodic memory in Humans: *"Episodic memory is a recently evolved, late-developing, and early-deteriorating past-oriented memory system, more vulnerable than other memory systems to neuronal dysfunction, and probably unique to Humans. It makes possible mental time travel through subjective time, from the present to the past, thus allowing one to re-experience, through autonoetic awareness, one's own previous experiences."*

Episodic memory is often pointed out to possess three main characteristics in Humans (Clayton et al., 2003). First, the content of that memory includes the association of several elements belonging to different informational dimensions within a single episode: here, the event (What?), the context spatial (Where?) and the lapse of time (When?) are considered as all essential to construct an appropriate framework (What-where-when?). Second, this newly integrated representation needs to be flexible, so that the individual can access to the encoded information at any time. Note that these features are quite reminiscent of the relational theory; however, in the case of episodic memory, this retrieval is experienced as a conscious mental time travel. Compelling evidence now indicates a central role of the hippocampus in numerous studies combining neuropsychological tasks assessing episodic memory and functional neuroimaging (Vargha-Khadem et al., 1997; Squire and Zola, 1998;

Cabeza and St, 2007), although additional structures have been more recently identified (Addis et al., 2007; Allen and Fortin, 2013).

The notion of episodic memory has long been problematic when discussing behavioral results in non-human animals. Indeed, their ability to encode and retrieve a past, complex, multi-dimensional representation - like Humans do - has been called into question and is still currently open to debate. For that matter, some authors do not hesitate talking about *episodic-like memory* in lower animals (Clayton and Dickinson, 1998; Morris, 2001; Crystal, 2010).

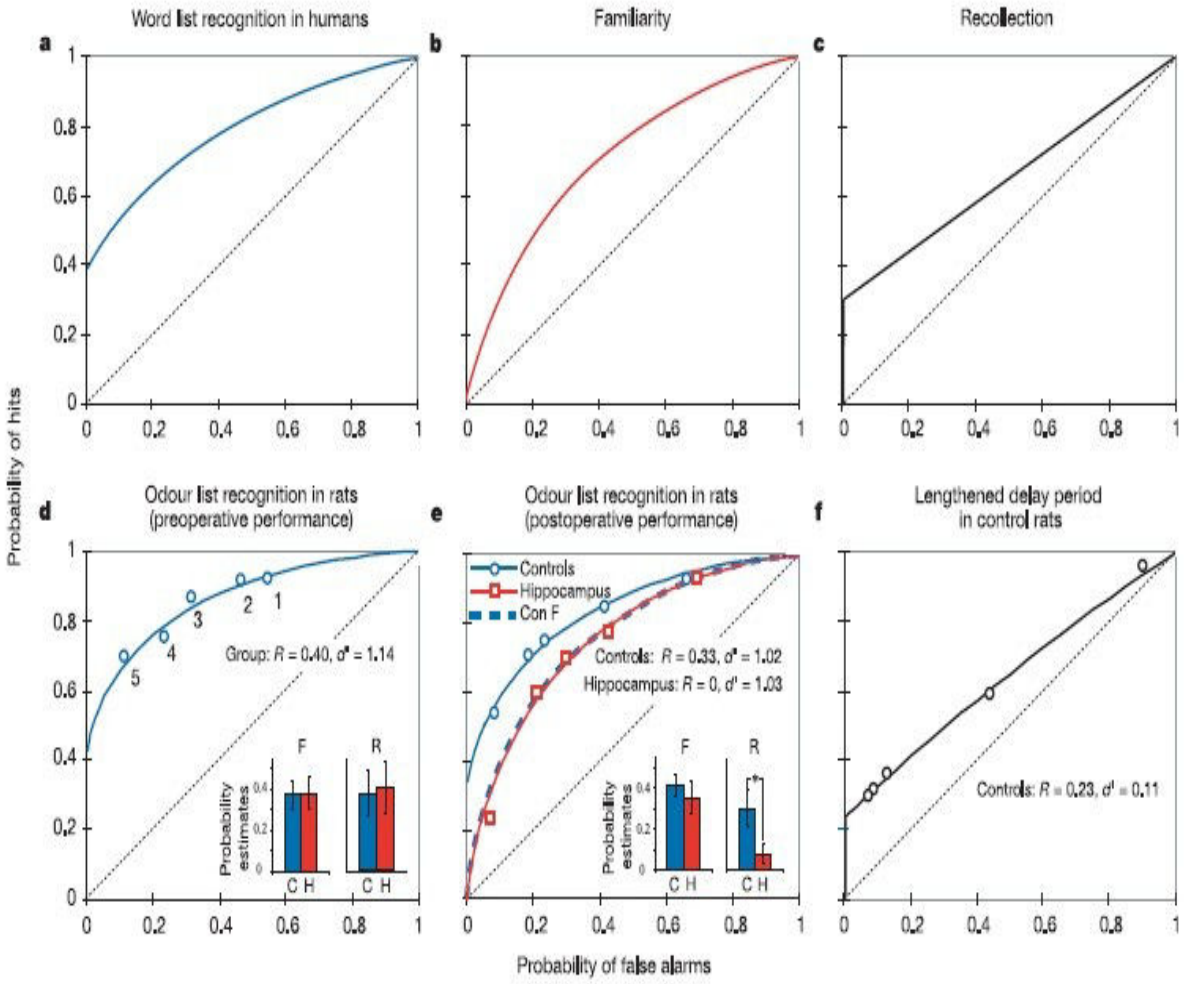


Figure 7. Representation of receiver operating characteristic (ROC) curves in Humans in a word recognition task (a-c) or in rats in an odor recognition task (d-f). Each learning can be broken up into two distinct processes, familiarity and recollection. Nevertheless, only recollection process is sensitively impaired after irreversible damage to the rat hippocampus. Picture from Fortin et al. (2004).

Learning What - Preliminary works have quickly showed that both rodents and monkeys were capable of integrating visual information and later discriminating new from previously encountered stimuli. Object recognition memory was therefore evaluable in cognitive paradigms like Delayed Non-Matching To Sample (DNMTS) or Novel Object Recognition (NORT) tasks (Gaffan et al., 1984; Ennaceur and Delacour, 1988). These assays had been initially developed to assess working memory, but manipulating the retention delay offered new perspectives regarding the evaluation of long-term memory. Importantly, hippocampal lesions led to conflicting experimental results in rats, with some studies reporting a pervasive deficit (Clark et al., 2000; Prusky et al., 2004) whilst others did not (Mumby, 2001; Forwood et al., 2005). In parallel, the integrity of other regions of the medial temporal lobe, like the perirhinal cortex (Winters and Bussey, 2005; Warburton and Brown, 2010; Winters et al., 2010b), was highlighted during a comparable object recognition task. However, the sensorial modality of the item to remember strongly influenced the nature of regions processing a piece of information. Thus, in a subsequent study using receiver operating characteristic (ROC) curves, (Fortin et al., 2004) found that as previously demonstrated in Humans learning in a word recognition task (Yonelinas, 2001), rats could retrieve the material in an odor recognition task according to two distinct processes: conscious recollection and familiarity. Besides, in both species, only conscious recollection was sensitive to hippocampal damage, promoting a functional role of this region in recognition memory (**Figure 7**).

Learning What and Where – In as much as animals could form recognition and spatial memories by assimilating individually items or places, it was very likely that they would manage to deal with cognitive tasks binding them together. This associative form of learning, called paired associates learning, was investigated by pairing different sorts of items (object, odor or food flavor) with spatial locations (Gilbert and Kesner, 2002; Day et al., 2003; Langston et al., 2010). In rodents, partial or complete hippocampal dysfunction resulted in a seeming decrease of the performance in these tasks, reproducing the severe deficit observed in amnesic patients evaluated in analogous associative tasks (Crane and Milner, 2005; Goodrich-Hunsaker et al., 2009).

Learning What, Where and When – A prominent work has concluded that integrating the three components of a unique, episodic memory was possible in birds (Clayton et al., 2001; Salwiczek et al., 2010). During a first session, scrub jays were trained to transport and hide different sorts of food (worms or peanuts). Later, they not only remembered the presence of the food in specific caches they had chosen, but also recollected when they had hidden it, as suggested by their inclination to consume uppermost perishable worms after short delays (4

hours) and non-perishable peanuts after long delays (5 days). All features of episodic memory can also be associated within a single representation and recalled later in rodents, as observed in recent works (Eacott et al., 2005; Babb and Crystal, 2006; Kart-Teke et al., 2006; Zhou and Crystal, 2011). Yet, so far, mental time travel seems to be out of reach for animals other than Humans (Roberts et al., 2000), which limits the translation across species.

3. Neuroanatomy of the striatum

Basal ganglia are described as a set of bilateral subcortical nuclei including the striatum, the globus pallidus, the subthalamic nucleus, the nucleus accumbens, the olfactory tubercle and the substantia nigra. Among these different structures, the striatum can itself be separated into two principal regions, the caudate and putamen. The frontier between these two regions is not obvious in all species: in Humans, cats, dogs or primates, the internal capsule forms a visible tract of white matter fibers giving birth to a physical boundary line between caudate nucleus and putamen while in rodents, both regions are undifferentiated and have been therefore renamed caudoputamen (CPu) complex (Paxinos and Franklin, 2001).

A common distinction concerns the notion of dorsal *vs* ventral striatum. In this respect, despite initial criticism (Nauta, 1979), most of researchers have now widely accepted that in rodents, the dorsal striatum (DS) corresponds to the upper part of the caudoputamen complex and the ventral striatum (VS) to its lower part including the ventromedial part, the nucleus accumbens (core and shell) and olfactory tubercle. Furthermore, a recent dissociation has been proposed to consider differences of connectivity exhibited by subparts of the dorsal striatum. Consequently, the dorsomedial (DMS) and dorsolateral (DLS) regions are often evoked separately (Balleine et al., 2007; Bornstein and Daw, 2011; Penner and Mizumori, 2012).

At first blush, the striatum seemed to be a heterogeneous anatomical structure with regard to the high degree of organization depicted by the hippocampus. On one hand, the existence of two biochemical compartments (striosomes and matrix) revealed important differences in the local distribution of neurotransmitters and neuropeptides in neurons (Graybiel and Ragsdale, Jr., 1978; Gerfen, 1989; Prensa et al., 1999). Thus, compared with the matrix, neurochemical markers were generally poorly stained in striosomes (also called striatal bodies or patches). On the other hand, even though the striatum receives multiple cortical afferences (not to mention other brain areas), neocortical, allocortical and mesocortical projections were

disseminated toward different subregions (Faull et al., 1986; McGeorge and Faull, 1989; Groenewegen et al., 1990), exacerbating this idea of a multiconnected but chaotic area. Actually, it turned out that the morphological striatal mosaic was closely related to the nature of the pattern of connectivity expressed (Gerfen, 1989; Gerfen, 1992). In addition, these two aspects made possible the exercise and maintenance of various cognitive and non-cognitive functions (Graybiel, 1997) after the passage of information via non-overlapping striatal territories.

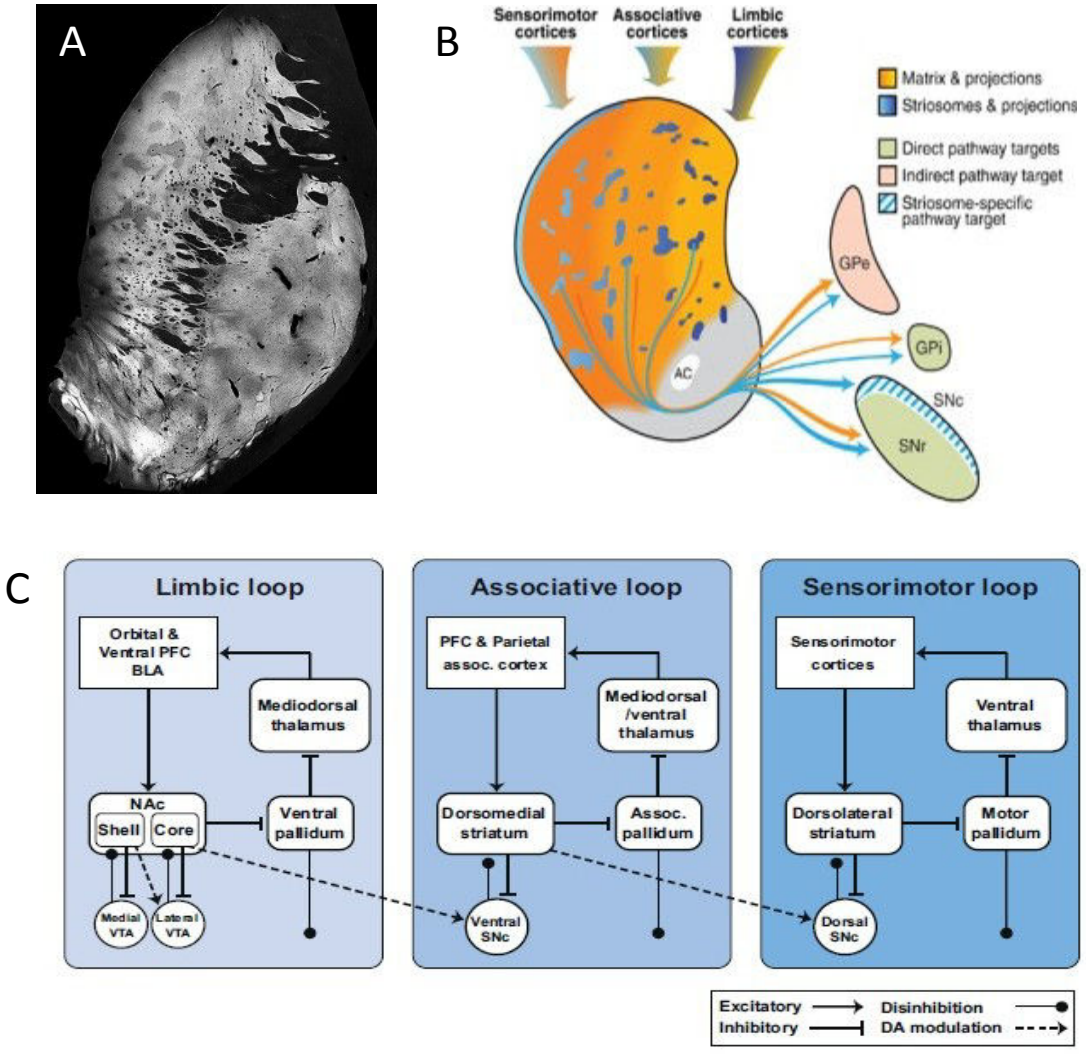


Figure 8. The striatum: neurochemical compartmentalization and heterogeneous connectivity. A slice of human striatum stained with a cholinergic marker reveals the existence of striosomes (dark grey) within the matrix (panel A); schematic representation of afferent and efferent striatal projections (panel B) and corresponding neuro-circuitries (panel C). Pictures from the McGovern Institute website, Crittenden and Graybiel (2011), Penner and Mizumori (2012).

Retrograde and anterograde tract-tracing studies have gradually afforded delineating the nature of afferent and efferent projections of the striatum (Parent and Hazrati, 1993; Kobbert et al., 2000). Parallel segregated circuits implicating cortical, striatal, thalamic regions as well as other nuclei belonging to the basal ganglia have been therefore refined in monkeys (Alexander et al., 1986), rats (Groenewegen et al., 1990) and Humans (Lehericy et al., 2004). Notwithstanding the initial statement of five parallel cortical-ganglio-thalamo-cortical loops, three distinct circuits (**Figure 8**) are now validated across species (Devan et al., 2011; Penner and Mizumori, 2012):

- The limbic loop connects ventral and orbital prefrontal along with entorhinal, piriform cortices and limbic structures like the hippocampus and the basolateral amygdala to the ventral striatum, particularly the nucleus accumbens. Feedback is mediated by neuronal efferent projections successively reaching the ventral pallidum, mediodorsal thalamus and the aforementioned regions;

- The associative loop connects prefrontal and parietal associative cortices to the dorsomedial striatum. Feedback is in that case mediated by neuronal efferent projections successively reaching the associative pallidum, the mediodorsal or ventral parts of the thalamus and the original cortical areas;

- Finally, the sensorimotor loop connects sensorimotor cortices to the dorsolateral striatum. Feedback is then mediated by neuronal efferent projections successively reaching the motor pallidum, the ventral thalamus and inaugural somatosensory and motor cortices.

4. Functional roles of the dorsal striatum in learning & memory

Specific memory functions have been allocated to each of these different loops. For the sake of brevity, we will mainly restrict our description to the dorsal striatum but excellent reviews have also handled global striatal functions (see Grahn et al., 2009; Pennartz et al., 2011; Everitt and Robbins, 2013).

a) Early studies of the striatum in animals

First lesion studies in animals targeted the whole caudate nucleus without distinguishing its different subregions. As a result, learning impairments were early indexed into a wide range of behavioral tasks assessing for example delayed alternation (Rosvold et al., 1958; Divac et

al., 1967), spatial delayed response (Divac, 1968; Potegal, 1969), passive avoidance (Neill and Grossman, 1970; Allen and Davison, 1973) or reversal learning (Divac, 1971). Among these paradigms, spatial deficits stood out of the crowd as they contrasted with those previously described in hippocampal lesioned animals.

As formerly outlined, the hippocampus can process spatial information through the integrative mapping of relationships between distal sensorial cues within a given environment. This form of learning, called place learning is possible by using an allocentric strategy. There is, however, an alternative strategy during navigational tasks. Response learning is selected through an egocentric strategy when the striatum appeals to internal characteristics of the animal such as its position and spatial orientation, *i.e.* idiothetic cues (Restle, 1957). We have already alluded to the first dissociation study which emphasized the respective effects of hippocampal or striatal lesions in two variants of a spatial paradigm requiring one of these two strategies (Packard et al., 1989). Numerous other lesion or pharmacological studies have followed (Packard and White, 1991; Packard and McGaugh, 1992; McDonald and White, 1993; Devan et al., 1996).

In a modified cross-maze task, an elegant study has corroborated the fact that spatial learning could be guided by distinct strategies mediated by the hippocampus or the striatum (Packard and McGaugh, 1996). From the same starting box, animals were first trained to enter an invariably baited arm every day (**Figure 9**). In parallel, at early and late stages of learning, they were introduced in the opposite starting box during probe trials to assess which strategy they used preferentially. Control animals early selected an allocentric strategy that resulted in place learning but later based their behavior on an egocentric strategy ending in response learning. In contrast, animals receiving intra-hippocampal or intra-striatal administrations of lidocaine (an inhibitor of neuronal activity) before probe trials exhibited specific behavioral patterns: in the formers, early place learning was severely impaired but late response learning was comparable to controls; in the latters, early place learning was similar to controls but late response learning was replaced by place learning. These results primarily showed that in rats, the hippocampal activity was essential during early stages of the paradigm while the striatal activity subsequently directed the progressive acquisition of response learning. Note however that the initial hippocampal-dependent strategy was still accessible but masked by the striatal-dependent one at the end of the task.

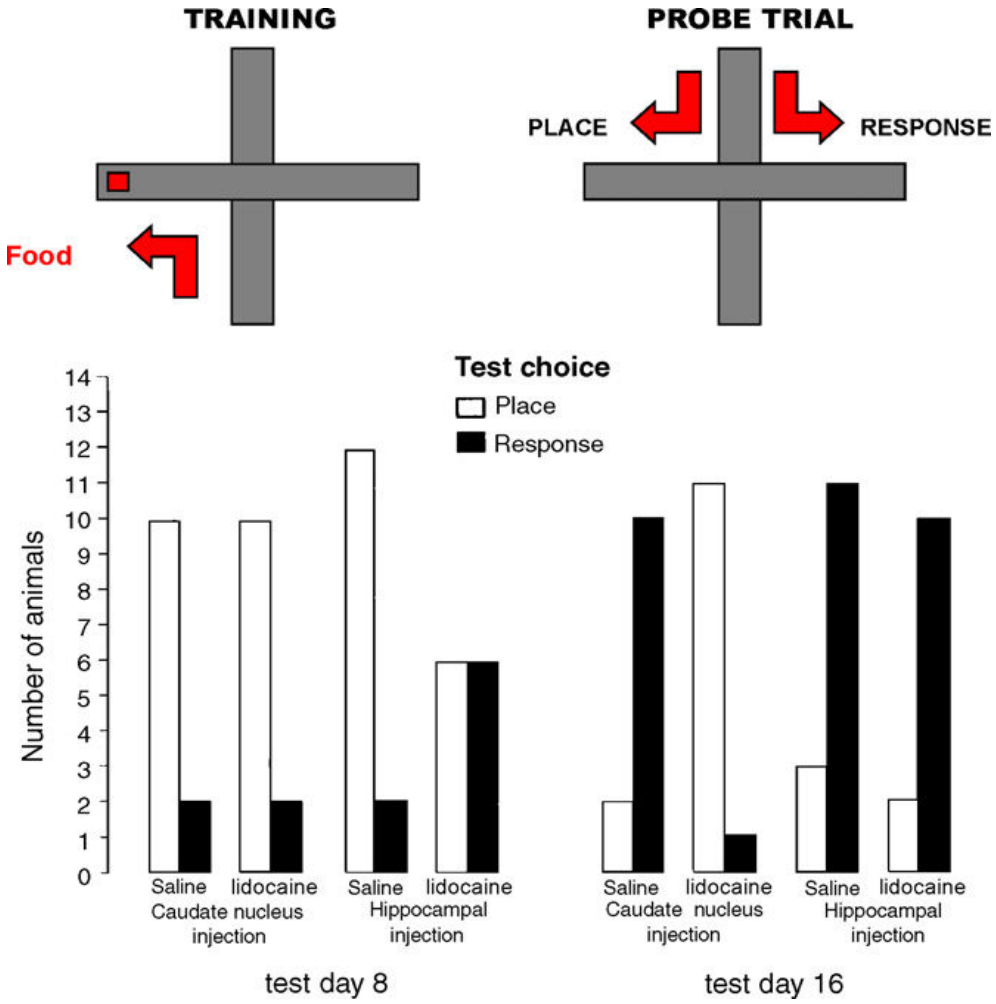


Figure 9. Dual-solution procedure in the modified cross-maze for rats. After training, animals can select a place or response strategy. When injected intra-hippocampally, lidocaine provokes the loss of place learning at early stages. Conversely, at late stages, intra-striatal lidocaine administration results in the extinction of response learning to the detriment of previously acquired place learning. Data from Packard and McGaugh (1996).

Additionally, caudate nucleus lesions were also known to disrupt the acquisition of tasks assessing the formation of motor or visual stimulus-response (S-R) associations in operant paradigms in rats (Dunnett and Iversen, 1982; Mitchell and Hall, 1988; Viaud and White, 1989; Reading et al., 1991).

b) Dorsolateral vs dorsomedial striatum: habitual vs goal-directed action in rodents

Given the growing body of evidence promoting the heterogeneous nature of cortico-striatal topographical connections, further studies in rats have explored whether lesions limited to

the dorsomedial (DMS) or dorsolateral (DLS) parts of the striatum resulted in observable behavioral differences. These works proved conclusively that the dorsal striatum could be dissociated in two different functional regions: the DLS involved in S-R associations and the DMS implicated in certain forms of spatial learning (Whishaw et al., 1987; Brown and Robbins, 1989; Devan et al., 1999).

More recently, the functional role of the DMS has been somehow extended after instrumental conditioning experiments were performed in rodents. Encoding the simplest instrumental choice (e.g. pressing a lever or pulling a chain to get different rewards) is behaviorally distinguishable from a temporal point of view in animals (Balleine and Dickinson, 1998; Balleine and O'Doherty, 2010). More precisely, the association between a behavioral response and the occurrence of an outcome (R-O) is very sensitive to certain changes during early learning. Thus, outcome devaluation and contingency degradation considerably decrease the probability that the animal later reproduces a similar response. However, if the animal is trained for a longer time in the same operant task, it becomes insensitive to such manipulations, demonstrating that the development of stimulus-responses (S-R) associations renders the behavior more rigid and independent of the outcome value (**Table 1**). Such early reflexive and late reflective actions are now often referred as goal-directed and habitual actions, respectively, and differentially intervene within decision-making process (Dolan and Dayan, 2013).

As expected, habitual actions quantified in instrumental tasks are also specifically impaired after DLS lesions in rodents (Yin et al., 2004; Featherstone and McDonald, 2004; Featherstone and McDonald, 2005). More interestingly, flexible goal-directed actions are sensitive to excitotoxic lesions, muscimol inactivation or pharmacological blockade of the DMS in rats learning a task stressing an action-outcome association (Yin et al., 2005a; Yin et al., 2005b). Furthermore, electrophysiological data equally support these findings (Jog et al., 1999; Barnes et al., 2005; Kimchi and Laubach, 2009). It has notably been found that DMS and DLS neurons present distinct patterns of activity during training in a two-modality version of the T-Maze task (Thorn et al., 2010). While the phasic activity of DMS neurons progressively vanishes as the number of learning sessions increases, DLS neurons fire more and more intensively and then remain activated even after the two tasks have been mastered by the animal. This suggests an early involvement of the dorsomedial part of the striatum during the formation of R-O associations, progressively completed and eventually replaced by the dorsolateral part of the striatum at the end of learning when S-R associations prevail.

	Goal-directed action	Habit action
Other denomination	Response – Outcome (R-O)	Stimulus – Response (S-R)
Cognitive demand	High	Low
Behavioral flexibility	High	Low
Sensitivity to changes	Important when - devaluating the outcome - degrading the contingency	None for all these changes
Neural substrates (rodents)	Dorsomedial striatum Prefrontal and parietal cortices	Dorsolateral striatum Sensorimotor cortices
Neural substrates (Humans)	Caudate nucleus Ventromedial PFC, dorsal cingulate cortex	Posterior putamen Premotor cortex

Table 1. Comparison of essential features of the two possible types of instrumental learning. Adapted from Schwabe and Wolf (2011).

c) Dorso-striatal functions in Humans

Over the last decade, various learning and memory functions ascribed to the dorsal striatum in rodents have been examined in healthy volunteers. For instance, in virtual environments, experimental studies have emphasized the existence of egocentric and allocentric strategies in human subjects learning a spatial task. Consistent with animal literature, the selection of one of these two strategies was correlated with an increased activity of the caudate nucleus or right hippocampus (Hartley et al., 2003; Iaria et al., 2003; Etchamendy and Bohbot, 2007; Schmitzer-Torbert, 2007). Besides, similarly to rodents, neuroimaging studies have showed the respective implications of the anterior caudate or posterior putamen in goal-directed and habitual responding in healthy subjects (Tricomi et al., 2004; Tricomi et al., 2009). Currently, researchers endeavor to accurately define to what extent the physiological balance within the caudate and putamen regions is disrupted in miscellaneous neurodegenerative and

neuropsychiatric disorders like obsessive compulsive disorder (OCD), stress or drug abuse (Ghiglieri et al., 2011; Schwabe et al., 2011; Belin et al., 2013; Gillan et al., 2014).

VII. Interactions between memory systems in rodents

Kim and Baxter (2001) upheld the hypothesis that in the mammalian brain, memory systems are not always independent modules but may also sometimes interdependently interact. Collectively, their computational data (**Figure 10**) supported three possible classes of interactions between memory systems (Jaffard and Meunier, 1993; White and McDonald, 2002). As initially thought, two memory systems could of course be independent. However, they could also act cooperatively (cooperation) or competitively (competition).

Examples of competition between memory systems have already been illustrated in this chapter. In the Win-Stay protocol of the eight-arm Radial Maze, rats with hippocampal lesions better performed than controls while dorsal striatal lesions led to a clear deficit (Packard et al., 1989). In other words, this indicated that hippocampal damage could facilitate the acquisition of a procedural task which normally resulted from a conflicting relationship between the hippocampus and striatum to gain control over behavior. In the cross-maze (Packard and McGaugh, 1996), another competition is detectable between these two regions during late stages of the task. Caudate nucleus injection of lidocaine transiently extinguishes the expression of response learning and gives a glimpse of the initial but still present hippocampo-dependent place learning. Other studies have since confirmed competitive interactions between these two structures in specific learning situations (Chang and Gold, 2003; Martel et al., 2007; Lee et al., 2008).

Cooperation between memory systems has been less reported in the animal literature. Nevertheless, in a modified version of the Morris Water Maze designed to assess spatial and cued learning in parallel, (Devan et al., 1999) have showed that both hippocampal and dorsomedial lesions disturbed spatial learning, suggesting a cooperation between memory systems. Moreover, complementary works have confirmed this observation (Devan et al., 1996; Devan and White, 1999).

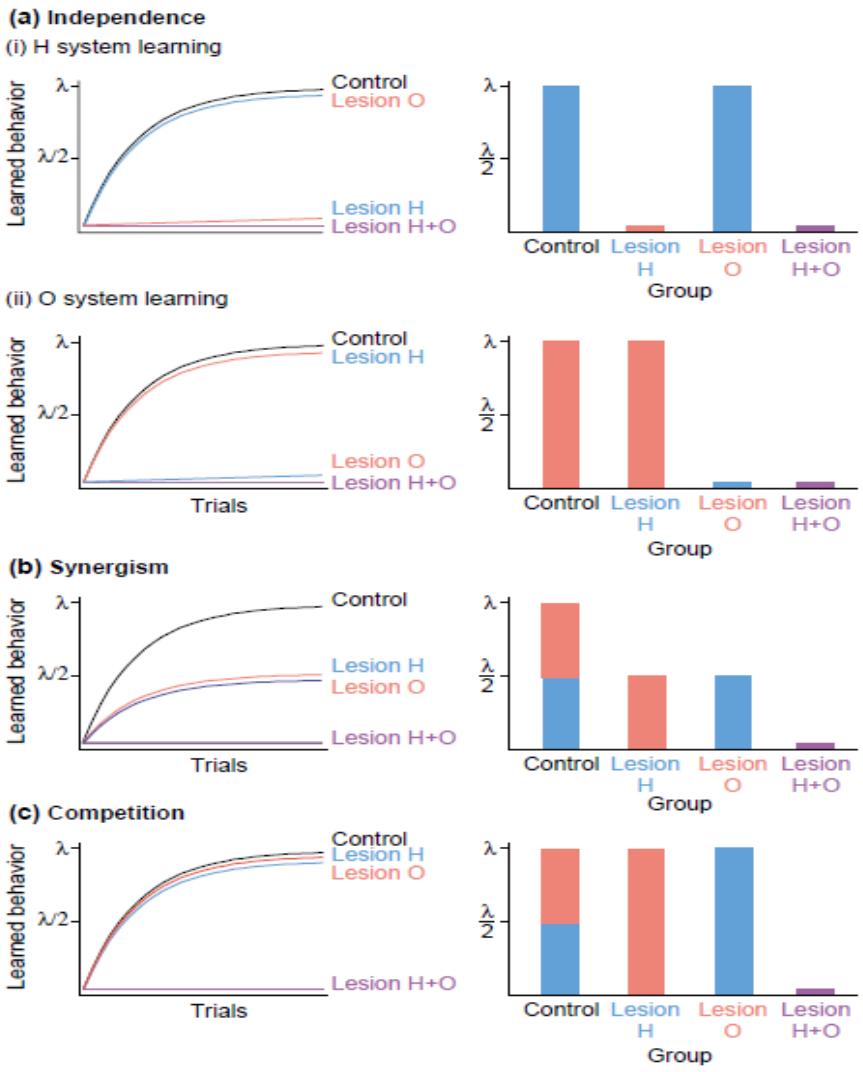


Figure 10. The three classes of memory system interactions. Two memory systems can be independent; in that case, lesion H will affect H system-dependent learning only whereas lesion O will disturb O system-dependent learning (panel a). Systems H and O can either cooperate (panel b) or compete (panel c): in the first situation, lesions H or O will individually reduce learning whilst in the second case, individual lesions H or O have no effect (note, however, that in reality, releasing an interference between 2 systems can induce a facilitation of learning). For all types of interactions described, combined lesions of H and O lead to a complete loss of function. Picture obtained from Kim and Baxter (2001).

In summary, we have reviewed in this first chapter most of experimental data that have historically contributed to the major discovery of multiple, interacting long-term memory systems. As early suggested by animal lesion and inactivation studies, memory systems are associated with the integrity of specific brain regions, whatever

the species considered. For instance, the medial temporal lobe, and more particularly the hippocampus, is generally requested during spatial, relational or episodic (-like) memory tasks whereas the striatum, especially its dorsal part, is rather engaged, although not exclusively, in procedural memory tasks. The relatively recent developments in neuroimaging techniques offer new perspectives to decipher how the human brain operates under normal or pathological conditions.

Chapter 2: Alzheimer's disease

Since the middle of the twentieth century, outstanding medical progresses have afforded the improvement of our living standards. Despite huge discrepancies between poorest and developed countries, a positive worldwide tendency has been observed as our life expectancy has averagely grown by 20 years over the last 50 years. Although appreciable, these results are unfortunately fraught with consequences. Population aging is indeed accompanied by a disquieting rise of neurodegenerative pathological conditions which constitute an economic burden and severely impact our societies.

I. Epidemiology of dementia

Among devastating aging-associated diseases, dementias point out clinical syndromes characterized by a substantial cognitive decline as well as a progressive loss of daily functioning. Diverse brain injuries are thought to cause these symptoms; therefore, different categories of dementia have been distinguished, among which frontotemporal dementia (FTD), semantic dementia (SD), Lewy bodies' dementia (LBD), vascular dementia (VD) following a stroke, primary progressive aphasia (PPA) or Alzheimer's disease (AD). In two recent publications, global prevalence of dementias and costs linked to their prevention and management have been estimated at the world level. Notwithstanding regional variations, results suggested that 5-7 % of persons over 60 years of age - equivalent to 35.6 million people - were affected by dementia in 2010 (Prince et al., 2013). Indirect and direct costs of dementia exceeded $600 billion in 2010 (Wimo et al., 2013). Furthermore, this public health issue is now amplified by prospective studies showing that this propensity is likely to consolidate and even expand in low-income countries (Reitz et al., 2011). According to the Delphi study, in the absence of appropriate treatments, dementia could reach by 2040 more than 80 million people all around the world (Ferri et al., 2005).

II. Genetic, risk and preventive factors of Alzheimer's Disease

As confirmed by different studies (Barker et al., 2002; Reitz and Mayeux, 2014), Alzheimer's disease is the most common form of dementia and represents 65-70 % of the cases. To date, two main forms of the pathology have been identified that however lead to the same clinical and histopathological picture (Bekris et al., 2010).

Early-Onset Familial Alzheimer's Disease (EOFAD) account for less than 5 % of the cases (Janssen et al., 2003). It usually occurs in patients aged fewer than 65 and who possess a family history of the pathology. In these individuals, genetic factors are directly linked to the development of the disease due to autosomal, dominant mutations that target one or several specific causative genes (Goate et al., 1991; Schellenberg et al., 1992; Sherrington et al., 1995; Ertekin-Taner, 2007): the amyloid precursor protein (APP) or presenilins 1 and 2 (PSEN1 and PSEN2) genes. Remarkably, products of these genes intervene in the regulation of amyloid peptide (Aß) production, as further detailed.

In comparison, Late-Onset Alzheimer's Disease (LOAD) corresponds to the sporadic form of the pathology. It generally appears in patients aged more than 65 and is thought to arise as the result of complex environmental and/or genetic interactions. Strengthening this hypothesis, both components have been somehow involved in the aetiology of the disease. For instance, the apolipoprotein E (APOE) gene, and particularly its allel Ɛ4, has been shown to significantly increase the risk of an individual to develop a LOAD (Corder et al., 1993; Saunders et al., 1993; Reitz et al., 2011). Interestingly, the isoform Ɛ4 of the apolipoprotein E has a lower proteolytic activity than others. Therefore, it hampers the clearance of soluble Aß peptide and promotes its aggregation (Jiang et al., 2008; Kim et al., 2009). Moreover, Genome Wide Association Studies (GWAS) have latterly highlighted the potential role of new genes in the pathology (Bertram and Tanzi, 2009; Lambert et al., 2013), although their precise functions remain to be determined. In the literature, a certain number of environmental factors have been otherwise showed to influence the likelihood of the LOAD emergence in Humans. Age is likely to be the highest risk factor: about one tenth of 65 years old people present a sporadic AD against one third of those older than 85 years (von Strauss et al., 1999; Corrada et al., 2008). Other risk factors include various pathological disorders/events like cerebrovascular disease, type II diabetes, metabolic syndrome or traumatic brain injury (Minati et al., 2009; Reitz and Mayeux, 2014). In contrast, protective factors have also been proposed, such as physical or intellectual exercise, education or diet (Kawas and Corrada, 2006; Povova et al., 2012).

III. Clinical features of Alzheimer's Disease

The pathology takes its name from Alois Alzheimer, a German psychiatrist who originally depicted most of symptoms in one of his patients, Auguste Deter (Alzheimer, 1907). Between 1901 and 1906, Alzheimer interviewed her many times and extracted from their successive

conversations valuable pieces of information about the disease. Thus, he noticed prominent memory and language problems. His patient was generally confused and unable to answer really simple questions about her own identity or spatio-temporal environment. She also showed sudden mood changes with aggressive episodes after long periods of vegetative state.

In line with these findings and one century of clinical investigation, (Lindner et al., 2008) have proposed to organize cardinal features of AD according to three main conceptual domains: cognitive symptoms, daily functioning and neuropsychiatric issues. In essence, cognitive functions comprise memory, language, orientation, attention, visuospatial and executive functions. Daily functioning symptoms refer to the progressive alteration of the whole of our basic and instrumental activities. Finally, neuropsychiatric symptoms denote potential behavioral features usually encountered in other psychopathological conditions: apathy, wandering, agitation, hallucinations, delusions, depression (Lyketsos et al., 2002; Steinberg et al., 2004).

Obviously, all symptoms do not arise simultaneously. Rather, they appear insidiously, which delays the establishment of a diagnosis in AD patients. This is all the more true that normal aging is generally accompanied by a small decline of specific cognitive functions (Ronnlund et al., 2005; McAvinue et al., 2012). It follows that normal and pathological states cannot be distinguished during a preclinical, asymptomatic stage of AD (Albert, 2011; Reiman et al., 2011). The next stage, also named Mild Cognitive Impairment (MCI), corresponds to a transitional phase between normal aging and early dementia for most patients categorized in this stage (Petersen et al., 1999; Petersen and Negash, 2008; Geda, 2012). First signs of cognitive disruption consensually appear in episodic memory, visuospatial and executive functioning tasks (Perry and Hodges, 2000; Blackwell et al., 2004; Iachini et al., 2009; Johnson et al., 2009; Albert, 2011; Saunders and Summers, 2011) but individuals remain able to live independently and to perform their day-to-day activities. During the mild stage of AD dementia (2-5 years duration), the progression of the pathology coincides with the onset of new deficits chiefly targeting language and face/object recognition (Locascio et al., 1995; Guarch et al., 2004; Spoletini et al., 2008). In parallel, memory impairments already present become more pronounced (**Table 2**) and the patient needs some support as his reasoning and problem-solving capacities start to dwindle.

Throughout the moderate stage of AD dementia (2-4 years duration), cognitive deficits persist or even worsen (Forstl and Kurz, 1999; Choe et al., 2008) whereas the patient

definitely loses his judgment and independency, requiring the assistance of a caregiver for his daily actions.

Disease	Episodic Memory	Semantic Memory	Classical Conditioning	Procedural Memory	Priming	Working Memory
AD	+++	++	+	-	±	++
FTD	++	++	n/d	-	n/d	+++
SD	+	+++	n/d	n/d	n/d	-
LBD	++	n/d	n/d	n/d	n/d	++
VD	+	+	±	+	±	++
PD	+	+	-	+++	-	++
HD	+	+	-	+++	-	+++
TGA	+++	±	n/d	-	-	-

Table 2. Selective memory systems disruption in certain neurological disorders. AD: Alzheimer's Disease; FTD: Fronto-Temporal Dementia; SD: Semantic Dementia; LBD: Lewy Bodies' Dementia; VD: Vascular Dementia; PD: Parkinson's Disease; HD: Huntington's Disease; TGA: Transient Global Amnesia. +++ indicates early and severe impairment; ++, a moderate impairment; +, a mild impairment; ±, conflictual results obtained in different studies; -, no impairment; n/d, not determined. Note that in spite of the difficulty to verify this assumption, some authors consider that procedural memory is affected in late stages of AD dementia. Adapted from Budson (2009).

This phase is also synonymous with a massive increase of neuropsychiatric symptoms, which constitute a psychological ordeal for relatives and a risk for the patient and his caretaker. Eventually, the severe stage of AD dementia (2-3 years) is characterized by the definitive loss of communication and emotional blunting. Placed in an adapted institution, the debilitated patient generally stays prostrate and mute. Because of his rare activity, he rapidly displays a muscular atrophy and loses weight. In an usual range of 7-10 years after the diagnostic (Holtzman et al., 2011), he eventually dies, not directly from AD dementia but rather from a secondary infection.

IV. Early diagnosis: neuropsychological tasks and biomarkers

Historically, neuropsychological tasks have long been high-class tools for clinicians to diagnose patients with a "probable" AD dementia from its mild stage (McKhann et al., 1984). At this stage, composite measures such as the Mini-Mental Examination State (MMSE; Folstein et al., 1975), Alzheimer's Disease Assessment Scale of Cognitive functions (ADAS-Cog; Rosen et al., 1984) or Clinical Dementia Rating (CDR; Morris, 1993) are sufficient enough to quantify a shift in cognitive performance. Similarly, daily functioning can also be followed up (Mathuranath et al., 2005). However, there are two issues linked to the use of these scales. First, as they aim to evaluate different cognitive domains at the same time, they do not allow discriminating precisely between different dementias (**Table 2**). Second, they are not accurate enough to detect subtle cognitive deficits as those seen in MCI patients (**Figure 11**). If specific tests have been developed to address the first problem (Kramer et al., 2003; Braaten et al., 2006; Weintraub et al., 2012), the solution to the second issue has actually come from the optimization of exploratory tools over the last two decades.

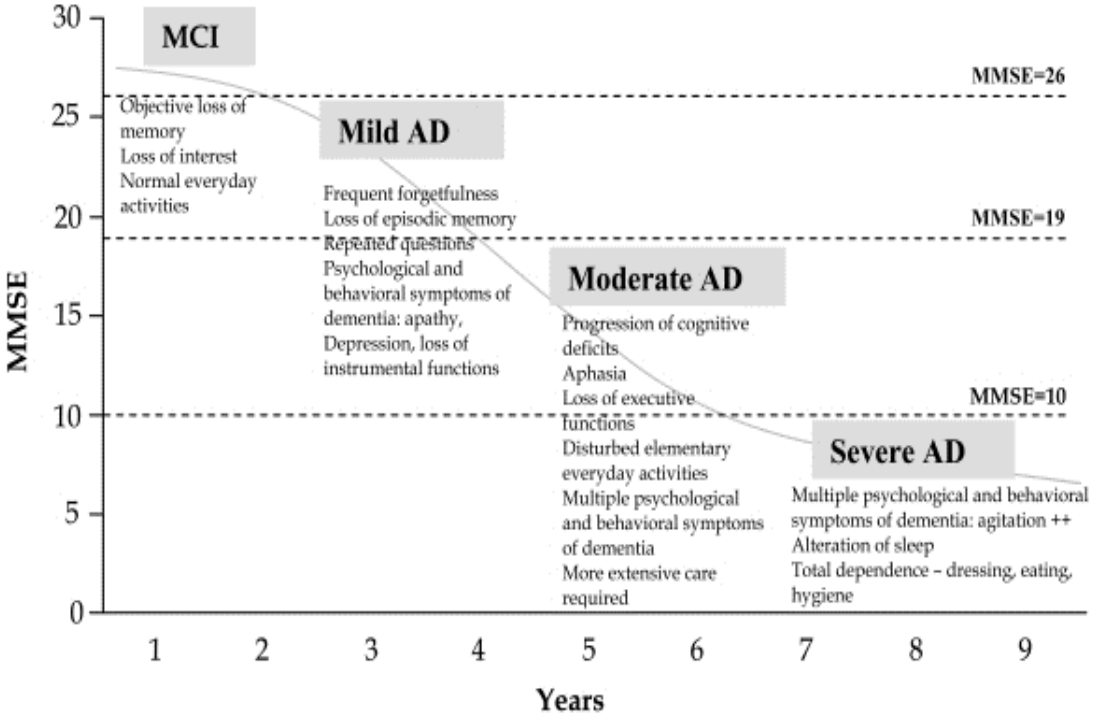


Figure 11. Deterioration of the cognitive performance of AD patients through the course of the pathology. Mini-Mental State Examination (MMSE) performance is stable in normal elderly people, with scores comprised in a mean range of 25-30 points. Figure from Feldman and Woodward (2005).

Supplementary diagnosis biomarkers have recently emerged, some of which are really promising as they allow distinguishing between normal and pathological aging as early as the MCI stage and even during the preclinical, cognitively asymptomatic stage of AD (Sperling et al., 2011). MCI patients early develop a characteristic amyloidosis now detectable thanks to neuroimaging or biochemical methods (Reiman and Jagust, 2012; Rosen et al., 2013). Specific radioligands used during Positron Emission Tomography (PET) have therefore stressed a higher fibrillar amyloid distribution and a decreased metabolic activity in certain brain regions (**Figure 12**) of MCI and AD patients when compared with healthy controls (Klunk et al., 2004; Mosconi et al., 2009; Devanand et al., 2010). Besides, taking advantage of the Blood-Oxygen Level Dependent (BOLD) signal, functional Magnetic Resonance Imaging (fMRI) studies have highlighted volumetric reductions of the hippocampus and entorhinal cortex in incipient and early stages of AD dementia, later spreading to posterior temporal and parietal lobes (den Heijer et al., 2010; Bernard et al., 2014). Important changes of biomarker measurements such as $Aß_{42}$ isoforms or total (t-Tau) and hyperphosphorylated Tau (P-Tau) proteins have also been observed in the cerebrospinal fluid (CSF) of patients over the course of the disease. While $Aß_{42}$ CSF levels consensually drops, t-Tau and P-tau CSF levels gradually increase as the disease progresses (Blennow and Hampel, 2003; Hampel et al., 2008). These innovative resources have increased both sensitivity and specificity of the "probable" AD dementia diagnosis and authors have consequently suggested to take them into account as supplement evidences in addition to the initial NINCDS-ADRDA guidelines (Dubois et al., 2007; McKhann et al., 2011). Nonetheless, it is noteworthy that the validation of a "definite" AD dementia is still conditioned by the presence of histopathological hallmarks verifiable only after the death of the patient.

V. Diagnosis confirmation: histopathological hallmarks of Alzheimer's Disease

After the death of Auguste Deter, Alzheimer carried out her autopsy. He immediately stated an extensive brain thinning. In addition, after collection and silver staining of brain slices, he observed two distinct families of abnormal inclusions distributed over the cortex and in some subcortical structures: amyloid plaques and neurofibrillary tangles (NFTs). Even now, the detection of these two hallmarks constitutes a unique histopathological signature essential for the post-mortem diagnostic of AD dementia.

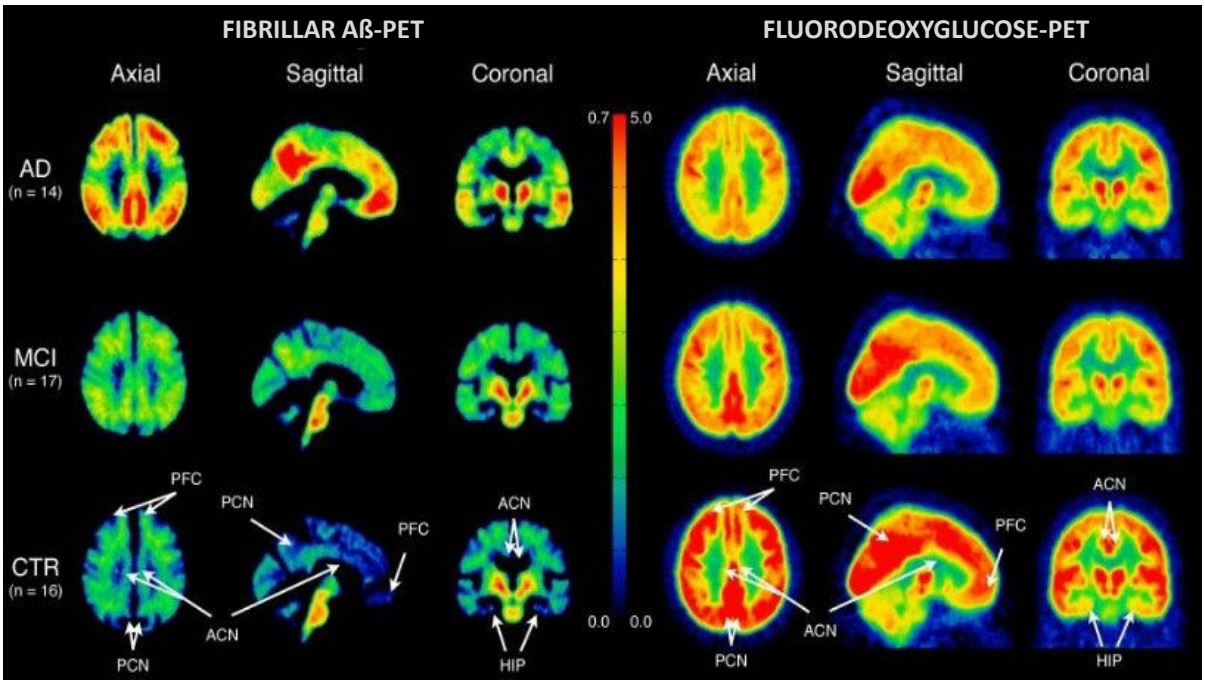


Figure 12. Positron Emission Tomography (PET) as an efficient neuroimaging technique to compare the formation of fibrillar amyloid (left part, fAß-PET) or brain glucose consumption (right part, FDG-PET) in AD, MCI or healthy subjects. The use of adapted radioactive tracers (^{11}C-PIB or ^{18}F-FDG) clearly demonstrates a higher fibrillar amyloid load associated to a hypometabolism in AD patients compared with controls. In both cases, MCI patients present an intermediary pattern. These changes concern selective brain regions: ACN = anterior cingulate cortex; PCN = precuneus cortex; PFC = prefrontal cortex; HPC = hippocampus. Picture adapted from Devanand et al. (2010).

Amyloid plaques are extracellular clusters resulting from the accumulative aggregation of different neuronal and glial elements (Dickson, 2001; Mott and Hulette, 2005). Their core is predominantly composed of a deposit of 4-kD ß-amyloid (Aß) polypeptides. The precise function of Aß remains so far undetermined. Present under several endogenous isoforms ($Aß_{39}$ to $Aß_{43}$), its name comes from its capacity to pleat into a stable ß-sheet conformation when assembled with other monomers in fibrillar proteinaceous forms (Serpell, 2000). There are different sorts of plaques (Wisniewski et al., 1979; Yamaguchi et al., 1989; Perl, 2010). Senile or neuritic plaques refer to plaques which have a well-defined delineated central amyloid nucleus surrounded by a corona of diverse dystrophic neurites while diffuse plaques designate smaller non-fibrillar aggregates that do not possess such a ring. Eventually, burned-out plaques appear as vestigial senile plaques as they have a fibrillar core resulting from the jumble of fibrillar amyloid with proteins such as apolipoprotein E or heparan sulfate

glycoproteins (Snow et al., 1996; Castillo et al., 1997; Nishiyama et al., 1997) but do not display any more the typical corona.

In contrast, neurofibrillary tangles (NFTs) and neuropil threads are intracellular fibrous inclusions that are primarily found in pyramidal neurons. They originate in the fibrillar aggregation of Tau, a neuronal Microtubule-Associated Protein (MAP) of 45-62 kD that assembles and stabilizes microtubules in the cytoskeleton (Weingarten et al., 1975). In pathological conditions such as AD, hyperphosphorylation of Tau leads in a first time to the formation of fibrils by oligomerization processes, then to a subsequent reorganization in paired helical filaments (PHF) mostly occurring in perykaria and dendrites (Kidd, 1963; Wisniewski et al., 1979). Consolidation of NFTs is also enabled by the complementary agglomeration of proteins available in the intracellular compartment among which ubiquitins or cholinesterases (Perry et al., 1987; Mesulam et al., 1987).

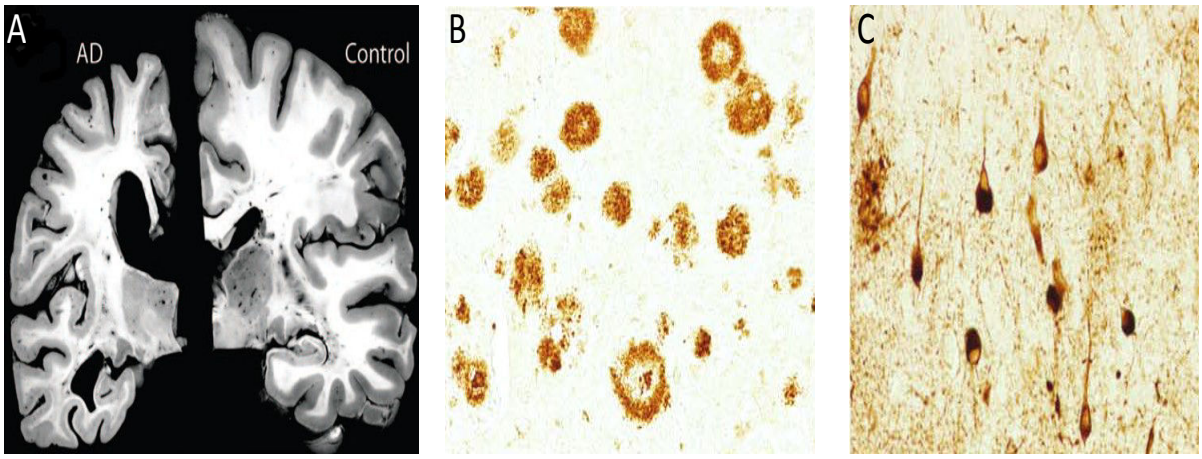


Figure 13. Macroscopic and microscopic brain alterations in AD patients. Post-mortem analysis of brain sections indicates an important atrophy (panel A) in AD dementia, notably confirmed by a characteristic ventricle enlargement in relation to healthy condition. In AD patients, amyloid dense-core plaques (panel B) and NFTs (panel C) can be discerned in vast numbers after appropriate immunostainings (here, Aß and AT8 antibodies respectively; optical microscopy X 400 magnification). Photos retrieved from Holtzman et al. (2011) and Minati et al. (2009).

Notwithstanding the efficacy of initial coloration techniques, amyloid plaques and neurofibrillary tangles are now more subtly revealed by immunohistochemistry. Highly-specific stainings result from the use of Aß or P-Tau antibodies, respectively (**Figure 13**). The huge number of human brains that have been examined post-mortem has showed that amyloid plaques preferentially pop up in the medial temporal (including the hippocampal

formation) and occipital lobes and only later spread to sensory, visual and motor cortical areas (Hyman et al., 1984; Braak and Braak, 1991). Brain temporal distribution of NFTs obeys to a more hierarchical mode defined through 6 stages (Braak and Braak, 1995): initially restricted to a single layer of the transentorhinal region (stages I-II), NFTs first extend their presence to the entorhinal and transentorhinal layers (stages III-IV) before reaching the neocortex during last stages of the pathology (stages V-VI). Interestingly, studies have found clinicopathological correlations between the distribution of NFTs and the severity of cognitive deficits displayed by the patient just before his death (Arriagada et al., 1992; Giannakopoulos et al., 2003).

VI. Involvement of Aß: the amyloid cascade hypothesis

Although the main constituents of amyloid plaques and NFTs, Aß and Tau respectively, had been biochemically characterized at the same time (Glenner and Wong, 1984; Brion et al., 1985; Grundke-Iqbal et al., 1986), two reasons have urged researchers to consider amyloid plaques as the causative agent of AD over the last 30 years. First, NFTs are not only expressed in AD patients, but also in various other disorders such as FTD, progressive supra-nuclear palsy or cortico-basal degeneration (Wisniewski et al., 1979; Goedert, 2004; Vandrovcova et al., 2010) while amyloid plaques are rather representative of AD dementia (but see about Down syndrome and normal aging: (Lott and Head, 2001; Rodrigue et al., 2009). Second, progresses in molecular biology have allowed identifying the presence of mutations directly localized on the precursor of Aß peptide (APP) and associated with a massive increase of Aß in certain EOFAD patients (Levy et al., 1990; Goate et al., 1991). Furthermore, subsequent studies have showed that PS1 or PS2 mutations encountered in 70 % of EOFAD cases (Rocchi et al., 2003) disrupt the balance of Aß production, notably by favoring the accumulation of the $Aß_{42}$ isoform (Lemere et al., 1996; Tomita et al., 1997).

Following these findings, many efforts have thus been made to understand how Aß is differentially generated in physiological or pathological conditions. As a matter of fact, it is now widely accepted that the proteolysis of APP can take place according to two distinct processes (for a review, see (Thinakaran and Koo, 2008; Zhang et al., 2012). To make it brief, in the non-amyloidogenic pathway, the ubiquitously expressed glycoprotein APP is successively cleaved by an α-secretase in the lumen and a γ-secretase present in the membrane. To that end, the action of the α-secretase is determinant as its unique site of cleavage prevents the production of Aß isoforms. Proteolytic products then consist in an APP

IntraCellular Domain (AICD) and in two extracellular particles, a secreted extracellular APP domain (s-APPα) and a P3 peptide, all of which are rapidly degraded. In the alternative amyloidogenic pathway, the original APP is first cleaved by a β-secretase, then by the γ-secretase as aforementioned. Both secretases possess more than one cleavage site, which permits on one hand the extracellular release of different Aß variants accompanied on the other hand by a different extracellular APP domain (s-APPß) and an APP IntraCellular Domain (AICD).

The exacerbated use of the amyloidogenic pathway leads to the quick overproduction of neurotoxic Aß isoforms in EOFAD patients but does not account for the late onset in sporadic forms of the pathology. However, it clearly and unequivocally implicates the amyloid peptide as the starting point of a serie of events resulting in AD dementia. Proposed in 1992, the amyloid cascade hypothesis assumes that neuronal loss, vascular damage and NFTs formation all appear as a consequence of Aß brain seeding and aggregation in AD patients (Hardy and Higgins, 1992). In particular, Aß peptide might interfere with neuronal changes by disrupting calcium signaling (Barger et al., 1993; Bojarski et al., 2008).

If the amyloid cascade hypothesis is still topical (**Figure 14**), important adjustments have since then been made to encompass progressive breakthroughs which have progressively marked out the field of AD (Lovestone, 2000; Haass and Selkoe, 2007; Karran et al., 2011). For example, among the range of Aß peptides abundantly produced by the differential cleavages of ß- and γ-secretases, $Aß_{42}$ is now recognized as the paramount monomeric species at the root of the cascade of events due to its inclination to oligomerize, fold and aggregate (Selkoe and Wolfe, 2007). Moreover, transient states have been described *in vitro* between the initial biosynthesis of Aß peptide and its final plaque aggregation: intermediary soluble oligomers, protofibrils and insoluble amyloid fibrils (Pike et al., 1991; Lambert et al., 1998). Among these, multimeric assemblies have attracted considerable attention since it has been showed that they correlate with the severity of cognitive faculties in AD patients (McLean et al., 1999; Mc Donald et al., 2010) whereas amyloid plaques do not (Nagy et al., 1995; Perrin et al., 2009). Data from transgenic animal models of AD have confirmed this finding as memory impairments and alterations of synaptic transmission are observed before the amyloid plaque deposition (see below). Hence, soluble oligomers would act as primary culprits and contribute to the advent of a constellation of cellular and synaptic changes inexorably resulting in neuronal dysfunction and cell death (Selkoe, 2008; Shankar et al., 2008; Benilova et al., 2012).

The amyloid cascade hypothesis is probably the best documented for Alzheimer's disease but some discrepant elements are not to its advantage (Korczyn, 2008). Mounting evidence suggests, for example, that the Aß peptide also accumulates in the brain of elderly people that do not develop the pathology (Katzman et al., 1988; Price and Morris, 1999; Esparza et al., 2013). Some authors have consequently proposed that amyloid plaques would represent a neuroprotective adaptation in response to the loss of physiological balance (Lee et al., 2004). This would be in agreement with the neurotrophic effects of low concentrated Aß monomers seen in cellular cultures (Yankner et al., 1990).

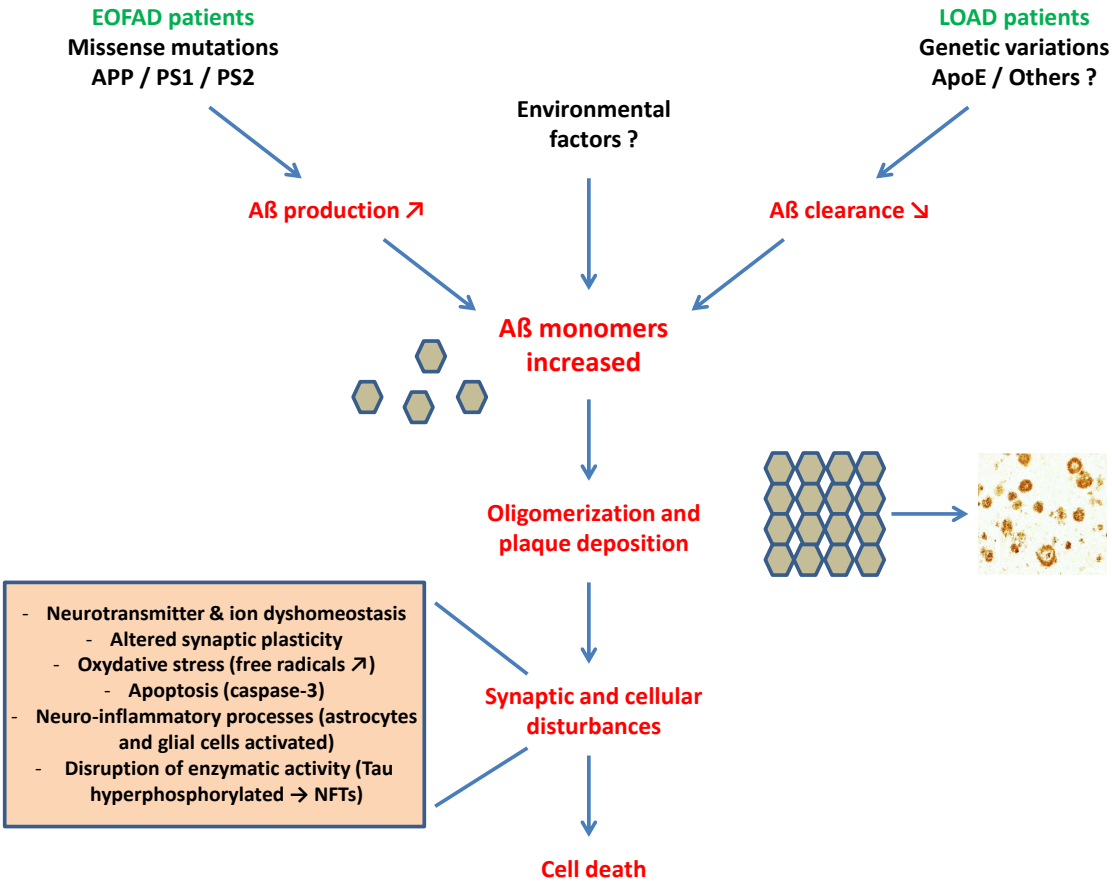


Figure 14. An updated version of the amyloid cascade hypothesis. Alzheimer's Disease appears following a sequence of events that occurs due to the influence of genetic mutations and/or environmental factors. Extracellular concentration of Aß monomers augments, notably $Aß_{42}$ which is more prone to oligomerization and aggregation. After a critical threshold has been reached, oligomers and amyloid plaques are heavily produced and impact cellular and synaptic functions through multiple deleterious mechanisms. Altogether, these disturbances lead to abnormal cellular death in the brain of AD patients. Adapted from Lambert and Amouyel (2011).

Additionally, other hypotheses have recently thrived, in turns incriminating AD dementia to be caused by protein Tau dysfunctions (Maccioni et al., 2010; Iqbal et al., 2014), deviant mitochondrial and oxidative stress functions (Swerdlow and Khan, 2004; Swerdlow et al., 2010), abnormal metabolic or neuro-inflammatory processes (Iqbal and Grundke-Iqbal, 2005; Morales et al., 2010). For all that, much remains to be accomplished to validate the true identity of the causative agent(s) of the pathology and unravel the associated downstream sequence of events.

VII. Animal models of Alzheimer's Disease

Because of the numerous molecular, cellular, histopathological and cognitive characteristics of Alzheimer's disease, modeling the pathology in animals has always been an immense challenge. Yet, a huge amount of work has been achieved over the last quarter century, and if current animal model do not recapitulate all AD features, we are definitely getting closer and closer to the notion of translational models (LaFerla and Green, 2012; Sabbagh et al., 2013).

From the late seventies, first animal models have been centered on the cholinergic hypothesis of the pathology (Contestabile, 2011). Indeed, before the beginning of the amyloid era, it was observed that administrating cholinergic antagonists caused memory impairments in healthy subjects (Drachman and Leavitt, 1974). Evidence stemming from post-mortem brain analysis demonstrated that certain markers of the cholinergic activity were crucially decreased in AD patients and correlated with the severity of the disease (Perry et al., 1981; Bartus et al., 1982). Similarly, systemic injections of anticholinergic agents such as scopolamine elicited significant deficits in various learning and memory tasks in animals. This pharmacological model also proved its predictive reliability from bench to bedside: partial/complete reversions of cognitive disruptions were found after acetylcholinesterase inhibitor treatments in rodents (Braida et al., 1996). Following these initial results, compounds like donepezil, galantamine and rivastigmine have demonstrated beneficial effects in clinical trials and are now considered as essential symptomatic treatments (Scarpini et al., 2003; Kaduszkiewicz et al., 2005). The preclinical potential efficacy of new therapeutic treatments is still often assessed via this method of investigation (Deiana et al., 2009). However, peripheral neurotransmitter modulation possesses non-specific effects.

Models of cholinergic lesions of the basal forebrain have been introduced with the purpose to counter this absence of specificity. When injected in nucleus basalis magnocellularis (NBM),

diagonal band of Broca (DBB) and/or medial septum (MS), glutamatergic excitotoxins produced behavioral deficits in animals (Mandel et al., 1989; Muir et al., 1993) but were not selective as other neurons than cholinergic ones were destroyed (McGaughy et al., 2000). Unlike classical neurotoxic agents, central administration of cholinergic immunotoxins (immunoglobulin 192IgG-saporin in rats, mu p75-saporin in mice) yielded to a higher selectivity (Wiley et al., 1991; Schliebs et al., 1996; Berger-Sweeney et al., 2001; Moreau et al., 2008): NBM/DBB/MS lesions massively reduced cholinergic outputs from this region towards the hippocampus and neocortex, which resulted in more or less important cognitive impairments, notably in working and spatial memory tasks (Leanza et al., 1998; Aztiria et al., 2009) but see also (Baxter et al., 1996; Waite et al., 1999). These models were nevertheless limited by low face validity in the absence of histopathological features of AD (McKinney, Jr. and Bunney, Jr., 1969).

Although age-related cognitive decline and Aß plaques deposition have been characterized in a few non transgenic animal models (Price et al., 1991; Cummings et al., 1996; Ito, 2013), the real revolution has started in the early nineties with the advent of transgenesis techniques (Gordon and Ruddle, 1981; Lannfelt et al., 1993). Notwithstanding initial failures, almost 100 chimeric mouse models have been generated to date (Alzheimer Forum website), most of which correspond to transgenic animals carrying one or several gene mutation(s) similar to those found in patients with EOFAD.

It is noteworthy that none of these current transgenic models develops all neuropathological hallmarks of AD (**Table 3**). More precisely, NFTs and cellular loss are often absent, notably in mice carrying only one or two human APP or PS1/2 mutations. Triple transgenic animals show no sign of neuronal death but bear NFTs at 12 months of age, likely due to a specific mutation directly affecting Tau (Oddo et al., 2003a). Conversely, quintuple mutated mice display no NFTs but harbor a significant cellular loss in the cortical layer V and subiculum area (Oakley et al., 2006). Nevertheless, in these models, exogenous promoters all drive a high transgene expression. Over time, massive production of Aß soluble peptides invariably leads to the appearance of amyloid plaques generally confined to hippocampal and cortical regions. Mice with several mutations usually present amyloid plaques earlier than mice with single-mutated gene insertion. Furthermore, the rise of pro-inflammatory and oxidative activities (Howlett and Richardson, 2009) coupled to the progressive decrease of pre- and postsynaptic markers (Pozueta et al., 2013) contributes to strengthen the homology with AD patients and the validity of the amyloid cascade hypothesis. Thus, gliosis, astrocytosis and dystrophic neurites are systematically found in brains of transgenic mice which have developed amyloid plaques.

Mouse model	Transgene	Promoter	Neuropathological hallmarks
PDAPP	hAPP V717F (Indiana)	PDGF promoter	Plaques: 9-10m (Johnson-Wood et al., 1997) No NFTs, but P-Tau (Masliah et al., 2001) No cellular loss (Irizarry et al., 1997b) Gliosis, astrocytosis, dystrophic neurites Synaptic loss: synaptophysin marker ↘ at 6-7m (Dodart et al., 2000)
Tg2576	hAPP K670N/M671L (Swedish)	Hamster PrP promoter	Plaques: 9-12m (Lee and Han, 2013) No NFTs, no cellular loss (Irizarry et al., 1997a) Gliosis, astrocytosis, dystrophic neurites (Hsiao et al., 1996) Neuro-inflammatory and oxidative stress markers ↗ (Yao et al., 2004;Parachikova et al., 2008)
APP/PS1	hAPPswe PSEN1ΔE9	Hamster PrP promoter	Plaques: 6m (Savonenko et al., 2005) No NFTs, no cellular loss Gliosis, astrocytosis (Wang et al., 2009) Neuro-inflammatory and oxidative stress markers ↗ (Garcia-Alloza et al., 2010;Puli et al., 2012)
3xTg-AD	hAPPswe MAPT P301L PSEN1 M146V	Thy1 promoter	Plaques: 6m (Oddo et al., 2003a) Intraneuronal Aß: 3m ; NFTs: 12m, no cellular loss Gliosis and astrocytosis: 7m (Caruso et al., 2013) Neuro-inflammatory and oxidative stress markers ↗ (Resende et al., 2008;Choi et al., 2013)
5xFAD	hAPPswe hAPP I716V (Florida) hAPP V717I (London) PSEN1 M146L PSEN1 L286V	Thy1 promoter	Soluble Aβ: 1.5m; plaques: 2m (Oakley et al., 2006) No NFTs Cellular loss in cortical layer V and subiculum: 9-12m Gliosis, astrocytosis, dystrophic neurites Oxidative stress markers ↗ (Devi and Ohno, 2012) Synaptic loss: synaptophysin, PSD95 markers ↘ at 9m (Oakley et al., 2006)

Table 3. Neuropathological features displayed by some of the most common transgenic mouse models of AD. 6m refers to 6 months for the onset of a given hallmark.

From a behavioral point of view, cognitive disturbances present in transgenic mice closely resemble those observed in AD patients (**Table 4**). More particularly, numerous memory

impairments have been outlined *in vivo* in these animals. Their performances are not only deteriorated in tasks assessing short-term memory (spontaneous alternation behavior in the T-Maze; spatial working memory in the MWM; etc.), but also in various paradigms evaluating long-term forms of memory (spatial reference memory in the MWM; associative memory in the CFC; recognition memory in the NORT; etc.). Consistent with human data summarized in Table 2, transgenic AD mice also present marked deficits in episodic-like memory tasks (Savonenko et al., 2005; Good et al., 2007; Volianskis et al., 2010) whilst acquisition of tasks requiring procedural memory is preserved in the same animals (Middei et al., 2004; Reiserer et al., 2007) but see (Dodart et al., 1999). Additionally, executive functions, for instance, are also disrupted in these animals when measured in reversal learning or set-shifting paradigms (Zhuo et al., 2007; Zhuo et al., 2008). In conclusion, cognitive symptoms in AD mouse models parallel those seen in AD patients.

Mouse model	SAB	CFC	NORT	MWM	RAM	BM
PDAPP	n/d	11m (Gerlai et al., 2002)	6m (Dodart et al., 1999)	4-6m (Hartman et al., 2005)	3m (Dodart et al., 1999)	n/d
Tg2576	4-6m (Ohno et al., 2004)	4m (Jacobsen et al., 2006)	12-14m (Oules et al., 2012)	6m (Westerman et al., 2002)	7m (Arendash et al., 2004)	8m (Yassine et al., 2013)
APP/PS1	n/d	6-11m (Cramer et al., 2012)	n/d	7m (Toth et al., 2013)	4m (Park et al., 2006)	7m (Reiserer et al., 2007)
3xTg-AD	9m (Carroll et al., 2007)	6m (Billings et al., 2005)	9m (Clinton et al., 2007)	4m (Clinton et al., 2007)	n/d	12m (Banaceur et al., 2013)
5XFAD	4-5m (Oakley et al., 2006)	6m (Kimura and Ohno, 2009)	4m (Giannoni et al., 2013)	12m (Bouter et al., 2014)	n/d	n/d

Table 4. Various cognitive deficits early pop up in different transgenic mouse models of AD. SAB: Spontaneous Alternation (measured in the T or Y-Maze); CFC: Contextual Fear Conditioning; NORT:

Novel Object Recognition Task; MWM: Morris Water Maze; RAM: Radial-Arm Maze; BM: Barnes Maze. 11m refers to 11 months for the onset of a given deficit. n/d: not determined.

At a cellular level, synaptic plasticity is thought to provide a solid substrate for learning and memory processes (Bliss and Collingridge, 1993; Malenka and Bear, 2004). This is especially the case for studies involving long-term potentiation (LTP). Using extracellular electrodes, (Bliss and Lomo, 1973) first described this phenomenon in anaesthetized rabbits: high-frequency stimulations of the perforant pathway successfully elicited a huge volley of responses that could continue for hours in post-synaptic corresponding DG neurons. Comparable results were later depicted in hippocampal slices of rodents stimulated in Schaffer's collaterals and recorded downstream at excitatory synapses located in the CA1 region. In agreement with their neuropathological and cognitive profile, transgenic animals of AD also differ from their wild-type littermates in electrophysiological experiments (**Table 5**). Whereas short-term plasticity measured through Paired Pulse Facilitation (PPF) is globally preserved in these animals, basal synaptic transmission and long-term potentiation interestingly decrease as early as 4-6 months of age.

Mouse model (publication)	Basal transmission	Paired Pulse Facilitation (PPF)	Long-Term Potentiation (LTP)
PDAPP (Larson et al., 1999)	No change	↗ (4-5m)	↘ (4-5m)
Tg2576 (Jacobsen et al., 2006)	↘ (4m)	n/d	↘ (4-5m)
APP/PS1 (Toth et al., 2013)	↗ (7m)	= (7m)	↘ (7m)
3xTg-AD (Oddo et al., 2003b)	↘ (6m)	= (6m)	↘ (6m)
5XFAD (Kimura and Ohno, 2009)	↘ (6m)	= (6m)	↘ (6m)

Table 5. Alterations in hippocampal synaptic transmission and short- and long-term forms of plasticity in glutamatergic excitatory synapses of different transgenic mouse models of AD. n/d: not determined.

The observation that both cognitive symptoms and synaptic defects exist well before the appearance of amyloid plaques in transgenic mouse models of AD has seriously undermined the initial amyloid cascade hypothesis. Yet, recent *in vitro*, *ex vivo* and *in vivo* studies have proposed to account for these findings. Different oligomeric species have been reported for their neurotoxic effects (Benilova et al., 2012). For example, non-fibrillar Aß-Derived Diffusible Ligands (ADDLs) trigger a higher apoptotic cell death in cultured primary neurons, abrogate synaptic signaling in hippocampal rat slices and hamper long-term forms of memory after infusion in the murine hippocampus (Lambert et al., 1998; Lesne et al., 2006; Shankar et al., 2008). Consequently, an avenue of research now utilizes transgenic models of AD to elucidate which soluble Aß assemblies are exactly implicated in the onset of cognitive and synaptic dysfunctions as well as to identify the underlying molecular mechanisms. At present the first point seems complicated to deal with because of the fragile equilibrium of oligomeric forms. Nevertheless, first studies have begun investigating distinct biological activities of Aß derivatives (Krafft and Klein, 2010). It has been notably demonstrated that soluble oligomeric ADDLs play a synaptotoxic role by excessively binding to N-Methyl-D-Aspartate receptors (NMDA-R) within excitatory synapses (De Felice et al., 2007), a finding in accordance with their noxious effects on NMDA-dependent LTP (Chen et al., 2002).

VIII. Alzheimer's disease: pharmacological therapies

As the pathology reaches epidemic proportions among worldwide elderly populations, there is an urgent need for effective AD treatments. So far, 5 drugs have obtained marketing approval from competent authorities: donepezil (Aricept^{TM}), galantamine (Reminyl^{TM}), rivastigmine (Exelon^{TM}), tacrine (Cognex^{TM}) and memantine (Namenda^{TM}). As initially found in various preclinical AD models (Iversen, 1997; Danysz and Parsons, 2003), these symptomatic treatments play a role in cholinergic or glutamatergic neurotransmission and allow a transient alleviation of cognitive and behavioral disturbances in AD patients (Birks, 2006; Nordberg, 2006; Winblad et al., 2007). Donepezil, galantamine, tacrine and rivastigmine are known to increase the availability of acetylcholine stocks in synaptic cracks by slowing down their degradation (acetylcholinesterase inhibitory action). By comparison, memantine is an uncompetitive, voltage-dependent NMDA receptor antagonist thought to preclude Aß toxicity and increase the release of neurotrophic factors from glial and astrocytic cells (Wu and Chen, 2009).

Still, beneficial effects resulting from these pharmacological treatments are limited and time-bounded (Ringman and Cummings, 2006; Raina et al., 2008), which has recently prompted the exploration of alternative approaches to endeavor to cure the disease. To this effect, the paucity of knowledge about APP and Aß functions and the variety of downstream effectors acting in the framework of the amyloid cascade hypothesis highly justify the diversity of targets represented in the current drug development pipeline of Alzheimer's Disease (Alzheimer Forum website). Thus, researchers have started developing drugs destined to modulate on request the biological activity of targets as diverse as Aß peptide, Tau protein, free radicals, phosphodiesterases (PDEs), cholesterol, neurotrophic, apoptotic or inflammatory factors (Mangialasche et al., 2010; Hong-Qi et al., 2012).

With regards to the essential role apparently played by Aß and its derivative oligomeric forms within the amyloid cascade hypothesis, different anti-amyloid strategies have been tested. To prevent an abundant Aß production through the amyloidogenic pathway, ß- or γ-secretase inhibitors/modulators among which rosiglitazone and semagacestat have been designed. In the same vein, α-secretase activators such as etazolate have been conceived to foster the non-amyloidogenic processing of APP. Another approach has consisted in creating synthetic Aß (bapineuzumab) or antibodies directed against Aß (AN-1792) to promote its clearance after active/passive immunotherapy. Finally, compounds like tramiprosate were also generated to destabilize Aß oligomers and therefore avoid their neurotoxic effects. However, until now, none of them has eventually proved clinical efficacy (Citron, 2004; Citron, 2010). This is all the more unfortunate that most of these compounds were effective in lowering the Aß load and improving cognitive symptoms in transgenic animal models of AD (Gervais et al., 2007; Escribano et al., 2010).

To sum up, Alzheimer's dementia is a complex and multifactorial neurodegenerative disorder clinically characterized by the progressive emergence of behavioral and cognitive disturbances. The formation of amyloid plaques and neurofibrillary tangles associated to an important cellular loss constitute the main histopathological hallmarks of the disease. Two major hypotheses have strongly influenced the development of preclinical models and therapeutic treatments. A first cholinergic hypothesis has promoted the loss of this essential neurotransmitter in specific brain regions of AD patients and its consequences on learning and memory processes. Consequently, a first generation of animal models with localized cholinergic depletion has been implemented to mimic memory impairments, following what most of current

symptomatic drugs have been developed and approved on the drug market. However, these treatments only postpone the worsening of the patient's state. More recently, the role of Aß and its various oligomeric and insoluble forms have been suggested through a complex sequence of molecular events. The amyloid cascade hypothesis would explain both clinical and neuropathological features in AD patients. Advances in genetics have rendered possible the creation of transgenic animals expressing human mutated genes responsible for familial cases of the pathology. If this second generation of models does not recapitulate all neuropathological hallmarks, cognitive symptoms are present in a selective fashion comparable to AD patients. Nevertheless, up to now, all disease-modifying compounds that have been successfully addressed against Aß and its derivatives in preclinical models have invariably failed in clinical trials.

Chapter 3: Introduction to the touchscreen-based methodology - Thesis objectives

Throughout the two first chapters, we have gathered an important bulk of evidence suggesting: 1) the existence of multiple, parallel memory systems in the mammalian brain and 2) a selective alteration of certain of these memory systems both in patients suffering from AD and transgenic animal models aiming at replicating the clinical spectrum of the pathology. We have also glimpsed classical methods to assess learning and memory processes in Humans and animals. However, if we set aside purely research-oriented tasks utilized in Humans to reproduce aspects of spatial navigation commonly investigated in mazes in rodents, cognitive testing is generally difficult to compare between the clinic and laboratory (Nithianantharajah and Grant, 2013).

Recent clinical failures in CNS drug development have unanimously pointed out the lack of concordance between promising results obtained from animal models and the subsequent absence of therapeutic effects in clinical trials (LaFerla and Green, 2012; McGonigle, 2014). Several counter measures have however been proposed. Because of the long asymptomatic phase of AD, (Selkoe, 2012) has suggested to treat patients in “prevention trials”, *i.e.* before the onset of first symptoms. In parallel, complete AD animal models that possess the broad range of neuropathological and cognitive features have now surfaced and could be of some interest in a near future (Cohen et al., 2013).

Beyond these considerations specifically based on the nature of the disease, one conceptual opportunity to bring species together concerns the methodology to assess cognitive functions. For that purpose, the enthronement of the Cambridge Neuropsychological Test Automated Battery (CANTAB) for the clinical diagnosis and follow-up of distinct pathological conditions has definitely constituted the first turning point toward a translational achievement (Sahakian et al., 1988; Fray and Robbins, 1996). Historically, original psychological tests were instructed by a clinician and realized by its patient during an oral/written examination (Bennett-Levy, 1984; Grober et al., 1985). They were relatively effective but presented confounding factors as the rule was subjectively introduced by the clinician. The advent of computerized cognitive tools has nonetheless brilliantly settled this problem. Within the testing environment, instructions appear on the sensitive touchscreen before the evaluation starts. During each test, patients are then assessed according to a determined rule via the

presentation of visual, culture-free stimuli. The intuitive nature of such tasks has been confirmed in various proportions of the human population including children, adult healthy volunteers or elderly people (Robbins et al., 1994; Robbins et al., 1998; Luciana, 2003). Another advantage of the method is the available number of tasks: no less than 25 tests measuring different cognitive domains among which memory, attention, executive function, decision making or social cognition have been developed (Cambridge Cognition website). Furthermore, most of these computerized neuropsychological tests have been showed to be at least as efficient as classical tasks to discriminate specifically between normal and neuropsychiatric conditions such as Alzheimer's Disease or depression (Egerhazi et al., 2007; Egerhazi et al., 2013).

In addition to these invaluable assets, the touchscreen-based methodology also has the virtue of being back-translatable. Studies have demonstrated the possibility to measure similar indexes of cognition in Non-Human Primates (NHPs) after minor adaptations (Weed et al., 1999; Nagahara et al., 2010). Moreover, in Humans and NHPs, memory impairments following a single scopolamine challenge are also quantifiable in such paradigms (Robbins et al., 1997; Taffe et al., 1999; Spinelli et al., 2006).

Broader efforts have been necessary to adapt the methodology from Humans to rodents. Acquisition of such paradigms usually takes a longer time, which has imposed to food-deprive animals and introduce a rewarding component in order to keep them motivated throughout the experiment (Bussey et al., 1997a). Learning of a defined rule also critically depends upon the accurate elaboration and maintenance of a certain number of parameters (Bussey et al., 2008; Horner et al., 2013; Mar et al., 2013; Oomen et al., 2013). Nevertheless, to date, around ten appetitive tasks targeting distinct cognitive functions have been designed. As in Humans, they are based on the identification and discrimination of visual stimuli followed by localized responses given to the touchscreen (nose-pokes in the case of rodents).

Table 6. Overview of the most common touchscreen paradigms currently available in rodents (see next page). 5-CSRTT: 5-Choice Serial Reaction Time Task; 5-CCPT: 5-Choice Continuous Performance Test; LD: Location Discrimination task; PAL: Paired Associates Learning task; PVD: Pairwise Visual Discrimination Task; TUNL: Trial Unique Non-matching to Location task; VMCL: Visuo-Motor Conditional Learning task. n/d: not determined. A star is placed in front of paradigms selected for our subsequent studies.

Task	Cognitive process	Brain regions involved	Neural systems implicated	Examples of associated publications
5-CSRTT **5-CCPT**	Attention	Prefrontal Cortex Basal forebrain	Cholinergic Serotoninergic Noradrenergic Dopaminergic	(Bartko et al., 2011a) (Romberg et al., 2011) (Barnes et al., 2012) (McTighe et al., 2013)
Autoshaping	Pavlovian conditioning	Ventral Striatum Amygdala Anterior Cingulate Cortex	Dopaminergic Glutamatergic	(Bussey et al., 1997a) (Parkinson et al., 2000) (Cardinal et al., 2002) (Dalley et al., 2005)
Extinction	Executive function	n/d	Glutamatergic	(Barkus et al., 2012) (Romberg et al., 2013)
LD	Pattern Separation	Hippocampus (dentate gyrus)	Glutamatergic	(Clelland et al., 2009) (McTighe et al., 2009) (Coba et al., 2012)
PAL*	Relational Memory	Hippocampus	Cholinergic Glutamatergic	(Talpos et al., 2009) (Bartko et al., 2011b) (Talpos et al., 2014)
PVD *	Long-term visual Memory (acquisition) Executive function (reversal learning)	Orbitofrontal Cortex Prefrontal Cortex Perirhinal Cortex Striatum Amygdala Mediodorsal nucleus of the thalamus	Dopaminergic Cholinergic Glutamatergic Serotoninergic	(Bussey et al., 1997b) (Chudasama et al., 2001) (Brigman et al., 2008) (Winters et al., 2010a) (Graybeal et al., 2011)
TUNL	Working Memory	Hippocampus Prefrontal Cortex	n/d	(Talpos et al., 2010) (McAllister et al., 2013)
VMCL*	Procedural Memory	Dorsal Striatum Posterior Cingulate Cortex	Dopaminergic	(Bussey et al., 1997b) (Chudasama et al., 2001)

Given the relative novelty of these translational paradigms, only a few teams of researchers have started exploiting the potential of the touchscreen technology. However, a sufficient bunch of lesion, genetic and pharmacological data (some of which are summarized in **Table 6**) has been collected to notice its usefulness and relevance. This is especially the case for schizophrenia where both preclinical and clinical paradigms have been recently validated in the framework of MATRICS (Measurement and Treatment Research to Improve Cognition in Schizophrenia) and CNTRICS (Cognitive Neuroscience approaches to the Treatment of Impaired Cognition in Schizophrenia) initiatives (Barnett et al., 2010; Bussey et al., 2012; Young et al., 2013). For instance, in animals, the 5-Choice Serial Reaction Time Task (5-CSRTT) is thought to reliably reflect a measure of sustained attention which is normally disrupted in schizophrenic patients evaluated in the Rapid Visual information Processing (RVP), the homolog version of this paradigm used in clinic (Kalkstein et al., 2010).

Some translational assays are also promising in the field of Alzheimer's Disease. Thus, it has been latterly suggested that human and rodent variants of the Paired Associates Learning (PAL) task could recruit similar brain regions and neural systems during the learning of "object-place" associations, *i.e.* linking different visual stimuli and their inherent spatial locations (Owen et al., 1995; Talpos et al., 2009; Bartko et al., 2011b; de Rover et al., 2011). Although an impairment in this task can be observed in other human pathological conditions like schizophrenia or drug abuse (Barnett et al., 2005; Ersche et al., 2006), it appears really early in AD patients and could even effectively predict the conversion of MCI into AD (Swainson et al., 2001; O'Connell et al., 2004). Besides, systemic administration / intrahippocampal infusion of classical cognitive enhancers aiming at glutamatergic or cholinergic systems increase the performance of rodents previously trained in the PAL assay (Talpos et al., 2009; Bartko et al., 2011b).

In spite of these encouraging results, very few studies appealing to the use of transgenic models of AD in touchscreen paradigms have been published so far (Romberg et al., 2011; Romberg et al., 2013). In this context, one of the initial purposes of this thesis was to assess for the first time the translational potential of touchscreen paradigms in one of these genetic models, with the perspective of later appreciating the effect of reference or putative cognitive enhancers.

To this end, we have selected three principal paradigms. Due to its aforementioned features, the PAL task has been immediately considered as a "must have" paradigm and added to our list of touchscreen tasks. In parallel, the PVD and VMCL tasks have been chosen in order to show the specificity of cognitive deficits: while it was expected that visual and procedural

memories would be spared in transgenic animal models of AD (Middei et al., 2004; Reiserer et al., 2007), we predicted that a deficit in executive function (through reversal learning) would conversely pop up in these mice (Zhuo et al., 2008).

In a first time, we have proceeded to the optimization or refinement of testing conditions for these tasks in young, male C57BL/6JRj mice. To satisfy the needs of another research project, the PVD task (Bussey et al., 2001; Morton et al., 2006) has been first adapted to allow the discrimination between two stimuli sharing comparable luminescent properties. Different parameters have then been introduced or modified so as to deepen our knowledge on the PAL task and permit the importation of the VMCL task in mice (Robbins et al., 1990; Reading et al., 1991). Finally, we also evaluated the possibility to combine some of these paradigms in a battery of tests, as previously emphasized (Bussey et al., 2012; Nithianantharajah and Grant, 2013). Corresponding results are fully described in the chapter 5 of this thesis and have given rise to a methodological publication (publication 1).

Once we have verified that normal mice were capable of learning each of these touchscreen tasks, we have initiated a serie of touchscreen experiments in wild-type and transgenic male animals belonging to the Tg2576 line, one of the most typical animal models of AD (Lee and Han, 2013; Webster et al., 2014). To measure the impact of the amyloid load (Aß monomers, oligomers and plaques when applicable) on cognitive performances, animals were tested at different ages (5-9.5 *vs* 12-16.5 months) in cross-sectional studies involving the assessment in the PAL task or in the VMCL and PVD tasks consecutively. Chapter 6 sums up the different data generated for this purpose.

As results stemming from the cognitive characterization of Tg2576 mice seriously challenged the legitimacy of our touchscreen paradigms, we have decided to explore in a third part the neurobiology of two of these tasks, the VMCL and PAL paradigms. In an initial experiment, young, male C57BL/6JRj mice damaged either in the whole (dorsal and ventral) hippocampus or in the dorsal (DMS and DLS) striatum after injection of an excitotoxic agent, the NMDA (Schwarcz et al., 1984). Respective effects of these lesions were then investigated on the acquisition of a battery of cognitive tasks including the PAL, VMCL and T-Maze alternation tasks (Gerlai, 1998; Spowart-Manning and van der Staay, 2004). In a second study, an independent batch of animals was first trained in the PAL task, then hippocampectomized before assessing the retrieval of the learned information. Reported in chapter 7 under the form of a lesion publication (publication 2), these studies provide parts of the solution to the issues stressed by transgenic experiments.

Materials & Methods

Chapter 4: Materials & Methods

I. Ethical statement

As all studies were led in the CNS Diseases Research Department of Boehringer Ingelheim Pharma GmbH & Co KG (Biberach an der Riss, Germany), procedures related to animal care and treatment (Tierschutzgesetz in der Fassung der Bekanntmachung vom 18/05/2006, BGBI IS.1206) were achieved with the specific approval of the appropriate governmental agency (Regierungspräsidium Tübingen, Germany; Ausnahmegenehmigung nach §9 TierSchG vom 04/05/2012 ; authorization number 35/9185.83 delivered to David Delotterie) and performed in an AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care International)-accredited facility in accordance with European Union guidelines (European Community Council Directive 2010/63/UE). All efforts were made to minimize animal suffering.

II. Animals

Methodological and lesion experiments were realized in young adult male C57BL/6JRj mice (supplier: Janvier Labs, France) aged 2-4 months at the beginning of the food-deprivation or surgery. The choice of this inbred strain was motivated by two main reasons. In comparison with other strains, these mice have been showed to possess good visual, learning and memory abilities in classical as well as touchscreen cognitive paradigms (Holmes et al., 2002; Graybeal et al., 2014). Moreover, they also share a part of their genetic pool with most of current genetic animal models of AD (Lee and Han, 2013).

Other experiments were conducted in adult male mice of different ages (5-17 months of age) belonging to the Tg2576 transgenic line (Hsiao et al., 1996). These mice were bred on a mixt C57BL/6 x SJL/J genetic background (supplier: Charles River, Germany). Before their arrival, mice had been genotyped for the APP Swedish mutation and wild-type (WT) and transgenic (TG) animals were consequently already identified at the beginning of experiments.

In any case, mice were stabulated in an animal facility where environmental conditions such as the temperature (T°C ~ 22 ± 2°C) and hygrometry (around 50 %) were carefully recorded

on a regular basis. They were kept on a 12h dark/light cycle, with lights turned on between 6:00 and 18:00. Animals were housed individually in plastic cages (dimensions: length = 26 cm; width = 21 cm; height = 14 cm) to better follow-up their daily food-intake and adjust each day the amount of food they needed during subsequent touchscreen studies. Cognitive deficits following a long-lasting social isolation have sometimes been indicated in the literature (Voikar et al., 2005; Koike et al., 2009). To preclude this phenomenon, some elements contributing to the environmental enrichment of the living space have therefore been added, among which a transparent red plastic nest box, shaving bedding and paper strips (Young et al., 1999; Jankowsky et al., 2005). Posterior measures of cognition in touchscreen have revealed that these compensation measures seemed adapted as animals were capable of learning effectively different rules.

Mice were given water *ad libitum* throughout the whole experiments. Nonetheless, just before the assessment in touchscreen devices, they were weighed 3-5 times over a one-week period and then food-deprived to reduce their free-feeding body weight to 85-90 % of the initial mean value. Once this weight range reached, the introduction to the first stages of a task could begin. During each touchscreen task, animals were rewarded with small amounts of half-diluted condensed milk (Milch Mädchen™, Germany) in case of correct responses. After completion of the daily session, they were directly weighed and fed in their home cage with pellets accordingly. The mild food restriction lasted until the end of the acquisition of the task. The same principle held for lesioned mice that had preliminarily undergone a stereotaxic surgery except that these animals were granted 4-5 weeks of recovery. That period allowed to the neurotoxic agent to cause sufficient excitotoxic damages to the lesioned area and gave time to an animal to gain back the weight it had lost following the intervention.

III. Surgical interventions

For lesion experiments, mice were first weighed and intraperitoneally anaesthetized with a cocktail of Xylazine at 10 mg/kg (Rompun™ 2 %; Bayer, Germany) and Ketamine at 100 mg/kg (Ketavet™ 100 mg/ml; Pfizer, Germany) dissolved in a physiological sodium chloride (NaCl) 0.9 % solution, as previously described (Van der Jeugd et al., 2009). They were placed in a stereotaxic frame (David Kopf Instruments, USA). In order to protect their eyes from a heat lamp, a moisturizing cream containing 5 % of dexpanthenol (Bepanthen™; Bayer, Germany) was abundantly applied.

Site name	AP (mm)	ML (mm)	DV (mm)	Volume injected (nL)
HPC 1 and 5	- 2.0	± 1.2	- 1.8	100
HPC 2 and 6	- 2.5	± 2.2	- 1.9	100
HPC 3 and 7	- 3.0	± 3.2	- 3.0	125
HPC 4 and 8	- 3.0	± 3.2	- 4.0	125
DS 1 and 3	+ 0.3	± 1.7	- 3.1	300
DS 2 and 4	+ 0.3	± 2.4	- 3.1	300

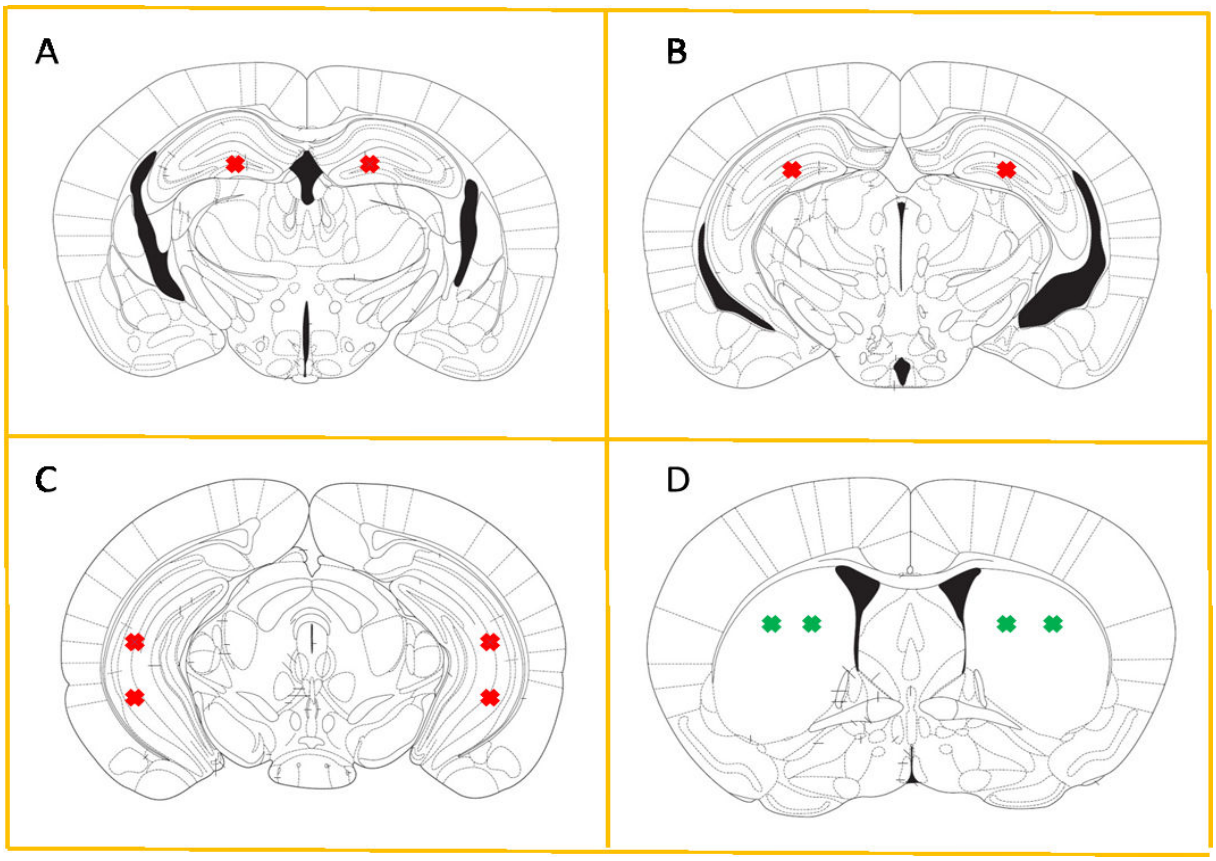


Figure 15. Theoretical stereotaxic coordinates defined from the mouse brain atlas and corresponding NMDA volumes injected to induce bilateral lesions of the dorsal and ventral parts of the hippocampus (panels A to C; in red) or the dorsal part of the striatum (panel D; in green). A total of 8 sites were targeted in the first case, against 4 sites in the latter case. AP: Antero-Posterior axis; ML: Medio-Lateral axis; DV: Dorso-Ventral axis.

The scalp was precisely incised to expose the skull. After determination of the Bregma and Lambda anatomical locations (Paxinos and Franklin, 2001), the horizontality of the skull ("flat skull") was assessed and corrected if necessary. Adapted from previous publications (Ohno et al., 2005; Yin, 2010), stereotaxic coordinates targeting the whole hippocampus or the dorsal striatum were then calculated (**Figure 15**). Small holes were then gently drilled to avoid destroying underlying cortical parts of the brain. Completion of this step was followed by injections of N-Methyl-D-Aspartate (NMDA dissolved at 90 mM in a PBS solution; Sigma-Aldrich, Germany) through a 2 µL Hamilton syringe (Hamilton, Switzerland) adapted with a 33-gauge stainless steel needle (beveled Nanofil needle; World Precision Instruments, USA). Lesions were induced chemically to spare fibers of passage (Guldin and Markowitsch, 1982). Given the low volumes of NMDA considered, a micro-pump (Ultra Micro Pump; World Precision Instruments, USA) was used to precisely deliver the excitotoxic agent in each site. NMDA was infused at the rate of 50 nL/min in the hippocampus or 75 nL/min in the striatum. In both cases, each injection was followed by a 4-5 min period during which the cannula was left in place. This permitted an appropriate diffusion of the excitotoxic agent. Sham-operated mice underwent the same procedure except that nothing was injected.

At the end of the surgery, the scalp was sutured. When reflexes appeared, animals received an intraperitoneal injection of Diazepam at 5 mg/kg (Diazepam 10 mg/2 mL dissolved in NaCl 0.9 %; Ratiopharm, Germany) to avoid the genesis and spreading of potential seizures (Deacon et al., 2002). Three hours later, mice were also subcutaneously administrated 1.5 mL of saline solution. They were also carefully weighed and observed over the course of the 3-4 following weeks before behavioral testing.

IV. Behavioral procedures

1. Touchscreen tasks

a) Apparatus

Animals were tested in operant chambers housed within sound and light attenuating boxes (**Figure 16**). Every trapezoidal-shaped chamber (respective dimensions: big basis= 25 cm; small basis= 6 cm; height= 18 cm) was individually equipped with a magazine, a house light, a tone generator, a liquid reward dispenser and a touchscreen (Campden Instruments, UK). The magazine was located at the small extremity of the trapezoidal chamber. By contrast, the touchscreen represented the opposite base of the trapezoidal chamber and was

permanently covered by a black Plexiglas mask with 2 or 3 holes. Square windows (side dimensions: length~ 7 cm; height= 7 cm) were separated by 0.4 cm and located at a height of 3.6 cm from the floor of the chamber. Through these windows, different visual stimuli could be shown on the screen geared to the stage and nature of the task (max. 1 stimulus per window). Moreover, infrared light beams were positioned at the rear (close to the magazine) and the front (close to the touchscreen) of each box and allowed quantifying the horizontal locomotor activity of each animal. According to the automated evaluation of animal actions by photocellular detection, operant chamber inputs and outputs were controlled via two softwares: the first was designed to control devices for behavioral research (Whisker Server; (Cardinal and Aitken, 2010) whereas the other was a graphical task design software (ABET II Touch software; Campden Instruments, UK).

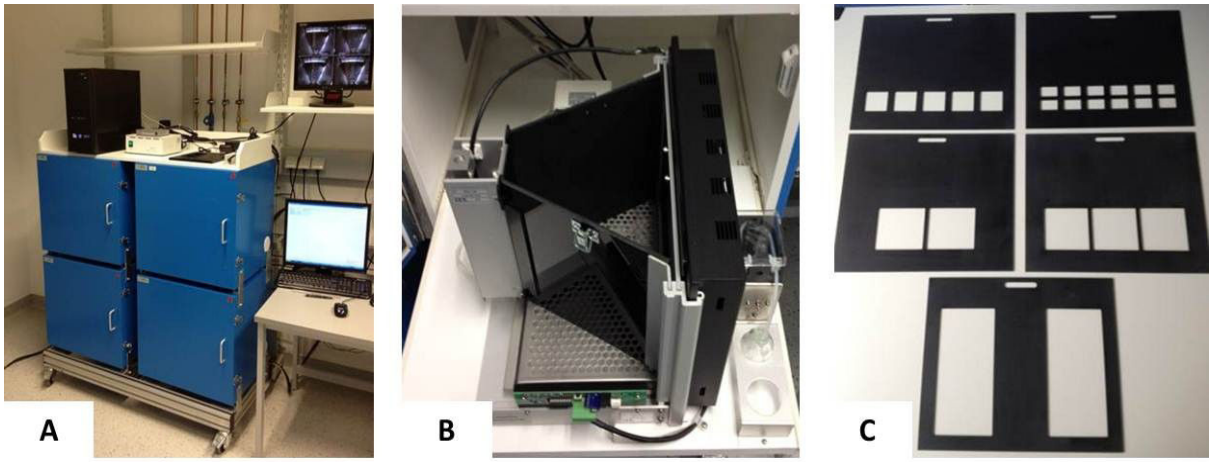


Figure 16. Illustrations of the device: a computer can handle until 4 chambers simultaneously (panel A); each testing environment is composed of a magazine, a trapezoidal-shaped chamber, a touchscreen and a pump delivering the reward (panel B, from left to right); the nature of the mask placed right next to the touchscreen depends on the task animals have to learn (panel C).

b) General considerations

In the different touchscreen paradigms, animals had to learn distinct rules involving the presentation of visual stimuli on the screen and responded during each trial by nose-poking a certain stimulus or location in order to get a small amount of the liquid reward into the magazine. However, such complex instrumental responses were not inborn in these mice. Therefore, their behavior was progressively shaped through a similar procedure before starting the main training phase, whatever the task subsequently considered (Horner et al.,

2013; Mar et al., 2013). After weight stabilization of newly food-restricted animals, they were first accustomed to the reward in the home cage (500 µL for 3 consecutive days), then in touchscreen boxes (250 µL into the magazine during a 20-min session of free exploration). Once it had been validated that mice quickly consumed the liquid reward in both environments, they were trained through a sequence of specific stages of increasing difficulty. At the end of that pokey training, they were eventually trained in the main task of interest (PAL, VMCL or PVD task). Naturally, the number of windows per mask (3 for PAL and VMCL tasks, 2 for the PVD task) and nature of stimuli presented on the screen depended on the chosen task.

A total of 9 different protocols (2 for the PAL task, 4 for the VMCL task, 3 for the PVD task) are described below. In each case, the initial protocol is extensively depicted, following what other protocols are briefly evoked on the basis of their principal differences. For each task, a table summarizes the main features of these protocols and indicates which one has been later selected for studies carried out with transgenic and lesioned animals (**Tables 7-9**).

c) PAL tasks and pertaining pokey training – Object in place memory

- Protocol 1 – dPAL task (conditions 1)

Prior to training in the main task, a four-step procedure took place in touchscreen devices: “initial touch” (IT), “must touch” (MT), “must initiate” (MI) and “punish incorrect” (PI) stages. In all these stages, animals were given a total of 36 trials or 60 min/session. Training stimuli consisted of 40 possible various shapes that were pseudo randomly chosen.

The IT stage corresponded to a Pavlovian training, during which a stimulus appeared in one of the three windows for 30 s. In the absence of nose-poke, the end of this period coincided with the offset of the training stimulus and the delivery of the reward (8 µL) accompanied by the illumination of the magazine light and a tone. A nose-poke towards the displayed stimulus immediately led to the same outcomes, except that the animal was rewarded with a more important amount of reward (24 µL) in that case. Collection of the condensed milk coincided with the beginning of the next trial and the occurrence of a new stimulus.

In the MT stage, each trial started in the same way, but the stimulus remained visible on the screen until the mouse had nose-poked it. A successful nose-poke was followed by the illumination of the food tray, a tone and the delivery of the liquid reward (8 µL). An intertrial

interval (ITI; 20 s) was introduced between the collection of the reward and the start of the next trial.

The MI stage was comparable to the previous stage, except that animals had to initiate new trials by nose-poking into the magazine before a training stimulus could be displayed on the screen.

Finally, animals were introduced in the PI stage. As before, a nose-poke towards the training stimulus was considered as a correct response and was followed by the usual outcomes described before. However, unlike other aforementioned stages, nose-poking one of the two blank windows was recognized as an incorrect response. In that case, the training stimulus disappeared, the house light was turned on for a time-out period of 10 s and no reward was given. After 10 s corresponding to the correction ITI, the mouse had to complete a correction trial procedure. For that purpose, the last used training stimulus and its position were kept the same and were re-presented to the animal until it responded correctly. Importantly, correction trials were not counted in the total number of completed trials. Mice were moved to the next phase once they had achieved 36 trials in less than 60 min. An additional criterion was used for the last stage, which consisted in an accuracy superior to 75% (minimum 27 correct responses) over two consecutive sessions.

After pokey training, each mouse was required to learn specific paired-associations of stimuli and locations in the dPAL task (Talpos et al., 2009; Bartko et al., 2011b). Three discriminative stimuli (flower, plane, and spider) were used for a total of 6 possible trial types. The flower was rewarded when presented in the left location, the plane in the central location, and the spider in the right location. Each trial was initiated by nose-poking into the magazine. The tray light then switched off and a pair of different stimuli appeared on the screen in 2 of the 3 possible locations: left, central, or right. Among the 2 stimuli shown on the screen, one stimulus was in a correct location (S+) and the other was in an incorrect location (S−). When a mouse nose-poked the correct stimulus (case 1: correct response), both stimuli disappeared and the mouse was rewarded for a correct response as previously described. Entry to collect the reward turned off the tray light and started a 20 s ITI. Afterwards, the tray light was again illuminated and the mouse could nose-poke into the magazine to trigger the next trial by triggering the appearance of a new pair of stimuli on the screen. By contrast, if the mouse nose-poked the incorrect stimulus (case 2: incorrect response), the stimuli disappeared, the house light was turned on for a time-out period of 10 s and no reward was given. After 10 s corresponding to the correction ITI, the mouse then

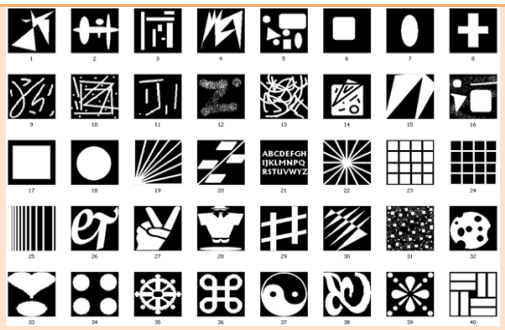
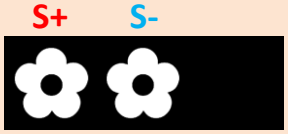
Conditions	1	2
Protocol N°	**1***	**2**
Pokey Training Stages	Initial Touch Must Touch Must Initiate Punish Incorrect	Initial Touch Must Touch Must Initiate Punish Incorrect
Pokey Training Images (randomly assigned)	 1 stimulus/trial among a list of 40 different ones	
Main Task	dPAL (different stimuli)	sPAL (similar stimuli)
Example of Trial Type		
Characteristics		
Session length	60 min	60 min
Max Number of Trials	36 trials	36 trials
Reward/trial	8 µL	8 µL
ITI	20 s	20 s
Correction (ITI)	10 s	10 s
Time-Out	10 s	10 s
Total Number of Sessions	50 sessions	50 sessions
Subsequent Changes	40 sessions of acquisition during lesion studies	/

Table 7. Overview of PAL protocols. The star indicates the selected protocol for transgenic and lesion studies. S+: rewarded stimulus; S-: non-rewarded stimulus; ITI: Inter –Trial Interval.

had to complete a correction trial procedure. A correction trial consisted of the re-presentation of the last pair of stimuli in the same spatial configuration and was repeated until a correct response was given to the screen. As for the last stage of the pokey training, correction trials were not counted in the total number of trials completed during the main training. Mice were evaluated for a total of 50 daily sessions, with a maximum of 36 trials or 60 min/session.

- Protocol 2 – sPAL task (conditions 2)

Inspired from a previous study led in rats (Talpos et al., 2009), this protocol was identical in all respects to protocol 1, except that similar stimuli were simultaneously displayed on the screen whenever a new trial was initiated in the main task.

d) Visuo-Motor Conditional Learning task – Procedural memory

- Protocol 3 – VMCL task (conditions 1)

Animals were trained in early pokey training stages (IT, MT and MI stages) almost as for the protocol 1. Four differences were however noticeable. First, training stimuli consisted of white squares this time. Second, locations where stimuli popped up over the different stages differed. They also appeared in one of the three possible locations during the IT stage but only in one of the two lateral windows during MT and MI stages. Third, completion of each pokey training stage was achieved when mice performed 30 trials in less than 60 min. Finally, mice were directly assessed in the VMCL task following completion of the MI stage.

The rule that needed to be learned in the VMCL task could be generally expressed as follows: “If stimulus A appears, then go left; if stimulus B appears, then go right” (Robbins et al., 1990; Reading et al., 1991). Basically, mice had to learn first to nose-poke the central window where a discriminative stimulus was displayed, then one of the 2 lateral locations depending on the nature of that central stimulus. Initiation of a new trial was followed by the appearance of a discriminative stimulus in the central window, which remained until the animal nose-poked it. This discriminative stimulus was chosen pseudo randomly among 2 possible stimuli that were different in shapes and colors (white icicle *vs* grey equal). After the first nose-poke, the central stimulus remained visible and 2 identical white squares appeared laterally on the left and on the right of the screen. The mouse then had to touch one of these 2 stimuli to get the reward according to the predefined rule. If the mouse nose-poked the

correct stimulus during the choice phase (case 1: correct trial), reward delivery was accompanied by illumination of the tray light and a tone. Collection of the condensed milk started the ITI. After the ITI period (20 s), the mouse could initiate a new trial. If the mouse nose-poked the wrong stimulus during the choice phase (case 2: incorrect trial), all stimuli disappeared, no reward was given to the animal and the house light was switched on for a 10 s (time out) punishment period. The house light was then turned off again, and a correction ITI period (10 s) elapsed. Following this period, a correction trial procedure could occur, during which the same discriminative stimulus was presented first and the same lateral nose-poke was expected. Correction trials continued until the animal responded correctly to the screen, but were not counted towards the total number of trials completed. Mice were recorded for a total of 30 daily sessions, with a maximum of 30 trials or 60 min/session. Furthermore, all groups were counterbalanced: half of the animals had to respond to the left when the grey equal was displayed and to the right when the white icicle was shown, whereas the other half had to learn the opposite rule.

- Protocol 4 – VMCL task (conditions 2)

Protocol 4 strongly resembled protocol 3 but a “pre-training” (PT) stage was recorded just after the MI. It was aimed to learn to the mouse to double nose-poke the touchscreen centrally, then laterally to get the reward. The onset of a new trial started with a nose-poke into the magazine. A first white square then appeared in the central window, and remained until the animal had nose-poked it. This first action had two consequences: the central stimulus disappeared and a second white square appeared pseudo randomly in the left or right window of the screen. When the mouse nose-poked the second stimulus, it disappeared, and the reward delivery (8 µL) was accompanied by illumination of the tray light and a tone. Collection of the condensed milk triggered an intertrial interval. After the ITI period (20 s), a new trial could start. Mice were expected to reach the criterion of 30 trials completed in less than 60 min for 2 consecutive days before they could start the main VMCL task.

- Protocol 5 – VMCL task (conditions 3)

Protocol 5 reproduced conditions of testing proposed in protocol 4 but a notion of limited holding time (LHT) was introduced both during PT (10 s) and reduced during the main task (5

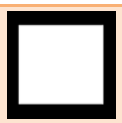
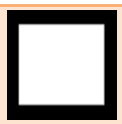
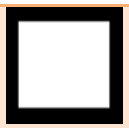
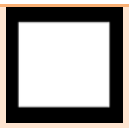
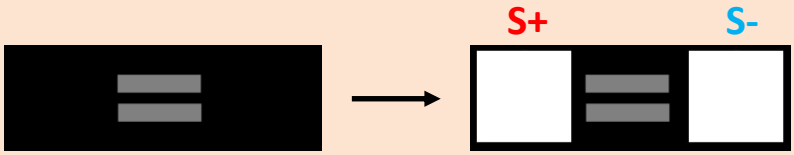
Conditions	1	2	3	4
Protocol N°	3	4*	5	6
Pokey Training Stages	Initial Touch Must Touch Must Initiate	Initial Touch Must Touch Must Initiate Pre-Training	Initial Touch Must Touch Must Initiate Pre-Training	Initial Touch Must Touch Must Initiate Pre-Training
Pokey Training Images (White Squares)				
Main Task	VMCL task			
Example of Trial Type				
Characteristics				
Session length	60 min	60 min	60 min	60 min
Max N° of Trials	30 trials	30 trials	30 trials	30 trials
Reward/trial	8 µL	8 µL	8 µL	8 µL
ITI	20 s	20 s	20 s	20 s
Correction (ITI)	10 s	10 s	10 s	10 s
Time-Out	10 s	10 s	10 s	10 s
Omissions	No	No	Yes	Yes
LHT (PT)	/	/	10 s	10 s
LHT (VMCL)	/	/	5 s	3 s
Total N° of Sessions	30 sessions	30 sessions	30 sessions	30 sessions

Table 8. Overview of VMCL protocols. The star indicates the selected protocol for transgenic and lesion studies. S+: rewarded stimulus; S-: non-rewarded stimulus; ITI: Inter-Trial Interval; LHT: Limited Holding Time; PT: Pre-Training.

s). In case the mouse did not nose-poke the second stimulus within the 10 s during PT (case 2: omission), the stimulus disappeared and no reward was given to the animal. Correction ITI period (10 s) followed a time out (10 s) during which the house light was illuminated. A

correction trial procedure then started with the re-presentation of the first stimulus, followed by that of the second stimulus in the last proposed spatial configuration. Omissions were counted in the total number of trials. Mice were trained in the main task if they reached the criterion of 30 trials completed in less than 60 min over 2 consecutive days (with less than 5 omissions per session). In the VMCL task, if the mouse didn't manage to respond to the screen within the allocated time (case 3: omission), the choice stimuli disappeared and no reward was given. A correction ITI period (10 s) followed a time out (10 s) during which the house light was illuminated. As for an incorrect trial, a correction trial procedure started. Importantly, and contrary to correction trials, omissions were counted in the total number of trials completed during the VMCL acquisition phase.

- Protocol 6 – VMCL task (conditions 4)

Protocol 6 mimicked protocol 5, except that the LHT associated to the main task was not equal to 5 but 3 s.

e) PVD task and pertaining pokey trainings – Visual discrimination / Executive functions

- Protocol 7 – PVD task (conditions 1)

In this protocol, animals were first trained in pokey training stages (IT, MT, MI and PI stages) as for protocol 1 apart from the criterion and duration of the ITI. Indeed, completion of each stage was achieved when mice performed 30 trials in less than 60 min while the duration of the ITI was reduced to 10 s.

During the visual discrimination acquisition phase (Morton et al., 2006; Bussey et al., 2008), mice were then required to learn to discriminate between a stimulus associated with reward (S+) and one associated with an absence of reward (S-). A trial began with the illumination of the reward receptacle with the house light turned on. Once the mice had nose-poked into the magazine (initiation), two stimuli were displayed in the two locations upon the screen. A response at the correct stimulus (case 1) triggered the removal of the stimuli from the screen, the reward tone, the delivery of the reward, and the illumination of the reward light. Once the pellet was collected, a short ITI would occur (10 s) and the reward light was deactivated. When the ITI had passed, the reward magazine light became again illuminated and signalled the beginning of the next trial. If the subject rather selected the incorrect stimulus (case 2), then a time-out period (10 s) occurred during which the house light was turned off.

Afterwards, the house light was turned on again and the correction ITI (10 s) started. At the end of the correction ITI, the next trial initiation started. This correction trial consisted of the re-presentation of the last pair of stimuli in the same spatial configuration and was repeated until a correct response was given to the screen. Correct and incorrect responses but not correction trials were counted towards the total number of trials. Each mice was maintained on the same S+/S- pairing across sessions, but counterbalanced between stimuli was also respected (half of the animal were rewarded on the Lines stimulus and half on the Ring stimulus). The locations of the S+ and S- varied randomly between trials. Mice received daily sessions of 30 trials or 45 min maximum.

Directly after mice had individually reached criteria (**≥** 23/30 correct responses for 3 consecutive days) in the acquisition phase, they started to be assessed in the reversal learning phase. Progress of that latter phase was nearly similar to the acquisition phase but the designation of correct and incorrect stimuli was reversed. Consequently, a stimulus previously associated with the liquid reward was no longer rewarded while an initial unrewarded stimulus became associated with the outcome. These changes imposed a behavioral flexibility. Mice were trained in the reversal learning until they reached comparable criteria to those of the acquisition phase.

- Protocol 8 – PVD task (conditions 2)

In this protocol, pokey training stages strongly differed from those described in protocol 7. In contrast, acquisition and reversal learning phases were kept exactly the same. Alternative pokey training consisted of 3 original stages: magazine training**,** rewarded response to the screen and rewarded selective response to the screen.

First, the magazine training started: mice were placed into the testing chamber with the magazine light turned on. A standard amount of reward (8 µL) was present in the reward magazine. Collecting the reward caused the reward light to go off. After an ITI of 10 s, the magazine light came on and a new reward was delivered, associated with a tone. The box stayed in this state until the next reward was collected. Touchscreens remained black during the whole stage. This continued for 60 trials and or 60 min.

The next habituation stage then started: the rewarded response to the screen (RRTTS). A trial began with the reward magazine illuminated. Once the mouse had nose-poked to the magazine, the light was extinguished and 2 white squares appeared in each of the two locations on the touchscreen.

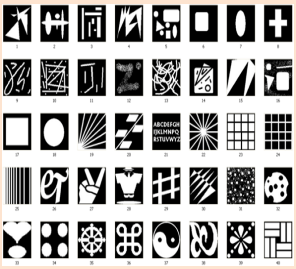
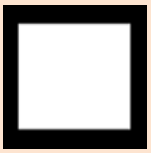
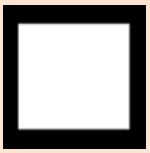
Conditions	1	2	3
Protocol N°	7	8	9*
Pokey Training Stages	Initial Touch Must Touch Must Initiate Punish Incorrect	Magazine Training RRTTS RSRTTS	Magazine Training RRTTS RSRTTS
Pokey Training Images	 1 stimulus/trial among a list of 40 different (randomly assigned)	 White square	 White square
Main Task		PVD task	
Example of Trial Type (Acquisition or Reversal learning)	 Size of each stimulus: 6.5 cm^2	 Size of each stimulus: 6.5 cm^2	 Size of each stimulus: 5.5 cm^2
Characteristics			
Session length	45 min	45 min	45 min
Max N° of Trials	30 trials	30 trials	30 trials
Reward/trial	8 µL	8 µL	8 µL
ITI	10 s	10 s	10 s
Correction (ITI)	10 s	10 s	10 s
Time-Out	10 s	10 s	10 s
Criteria	≥ 23/30 correct responses 3 consecutive days	≥ 23/30 correct responses 3 consecutive days	≥ 23/30 correct responses 3 consecutive days

Table 9. Overview of PVD protocols. The star indicates the selected protocol for transgenic and lesion studies. RRTTS: Rewarded Response To The Screen; RSRTTS: Rewarded Selective Response To The Screen; ITI: Inter-Trial Interval.

The mouse had to touch one of the 2 stimuli to elicit the reward response. Reward delivery (8 µL) was accompanied by illumination of the tray light and a tone. Entry to collect the reward turned off the tray light and started the ITI (10 s). Once the ITI had passed, the reward magazine was again illuminated, signaling the possibility to initiate a new trial. This continued for 60 trials or 60 trials. Criteria were to achieve the maximal number of trials in the allotted time for 2 consecutive sessions. Then began the final phase of habituation: the rewarded selective response to the screen (RSRTTS). It was included to avoid the mice developing a position bias. This stage of training was as above, except that only one location on the monitor became illuminated. Furthermore, only responses at the illuminated location triggered a reward (8 µL). The illuminated location pseudo randomly alternated between one of the two positions across trials. Responses at all non-illuminated locations were non-rewarded. This stage was achieved after 60 trials or 60 min. As for the RRTTS stage, criteria were to perform the maximal number of trials in the allotted time for 3 consecutive sessions.

- Protocol 9 – PVD task (conditions 3)

Protocol 9 was directly imported from protocol 8 with one major difference: the size of the two visual stimuli to discriminate during acquisition and reversal learning phases. Whereas Lines and Ring measured 6.5 cm^2 in protocol 8, they were a bit smaller (5.5 cm^2) in protocol 9, which allowed defining appropriate testing conditions.

f) Testing in a battery of touchscreen tasks

Mice were on several occasions sequentially evaluated over time in different touchscreen tasks. Yet, the construction of a battery of tasks required a few adjustments. Because the food-restriction was necessary during 6-12 weeks per task and prevented animals from growing normally, a minimal delay of 3 weeks with food *ad libitum* was respected between two behavioral assessments. Furthermore, in view of their preliminary experience, certain early stages of pokey training of the second task to acquire were optional. For this reason, mice were straight trained in the MI (PAL and VMCL tasks) or RSRTTS (PVD task) stages when they returned in touchscreen chambers.

2. T-Maze forced continuous alternation task – Spatial working memory

While touchscreen tasks measured distinct forms of cognition, in particular long-term memories or executive function, working memory was tested in a T Maze. Although different protocols have been developed since this maze has started to be used (Deacon and Rawlins, 2006), we here described a continuous alternation procedure adapted from Gerlai (1998) and Spowart-Manning and van der Staay (2004).

The apparatus consisted of an enclosed T-maze made of grey chlorure polyvinyl chloride, which was elevated one meter above the floor in a dimmed testing room (**Figure 17**). Extra maze cues were present on each arm side and directly illuminated by a low ceiling lighting (6-10 Lux). The start arm measured 54 X 8.5 X 20 cm, against 30 X 8.5 X 20 cm for the two horizontal arms facing each other (goal arms). Two removable guillotine doors allowed to manually controlling the access to these goal arms during the experiment. A third guillotine door, located in the bottom part of the start arm, permitted to restrict the tested mouse to a specific area (14 X 8.5 X 20 cm).

Figure 17. Photography illustrating the T Maze apparatus.

The evaluation of a mouse began with the opening of that third guillotine door. During the initial phase, a mouse was encouraged to explore the start arm and one of the two goal arms while the other was blocked. The choice of the first blocked arm (left or right) was

counterbalanced within each tested group. An animal was therefore forced to enter one of the two goal arms (full body including the tail), and eventually came back to the start arm. When it reached the extremity of the start arm, the guillotine door blocking one of the goal arms was raised and the choice phase started: the mouse was given the possibility to select which goal arm it wanted to visit. After the animal had completely got into one of the two goal arms, access to the other goal arm was immediately blocked by lowering the corresponding door. Again, the mouse had to go back to the terminal part of the start arm to trigger the lift of the guillotine door and allow the onset of a new free-choice trial. This protocol was repeated until animals had explored the T-Maze for a total of 14 free-choice trials or after 14 minutes. After each evaluation, partitions of the T-Maze were carefully cleaned with ethanol 70% and a paper towel to eliminate the smell and traces of each animal.

V. Euthanasia and tissue sampling

Following the completion of behavioral testing, mice from transgenic and lesion studies were deeply anaesthetized after intraperitoneal administration of the Xylazine and Ketamine cocktail and sacrificed after cervical dislocation. With respect to transgenic studies, the extremity of the tail (0.5 - 0.8 mm) of each wild-type or transgenic mouse was collected and directly frozen at -20°C before further genotyping. In comparison, brains from sham and lesioned animals were tidily removed, and postfixed in 4% paraformaldehyde (PFA; Boster Immunoleader, USA) for 2 days at 4°C before further immunohistochemical stainings.

VI. Histology and immunohistochemistry

Brain tissues from sham and lesioned mice were first dehydrated after successive baths of alcohol at 35°C (ethanol of increasing purity then xylol; total duration: 10 h). Afterwards, they were infiltrated with paraffin at 60 °C during 4 h (Tissue-Tek VIP® 6; Sakura Finetek Inc., USA). The day after, they were placed into molds and externally embedded with liquid paraffin wax in another device (Leica EG1150H; Leica GmbH, Germany). Once blocks were hardened, brains were ready to be severed. Thin (4-5 µm) coronal sections were cut on an automated rotary microtome (Microm HM355S; Thermo Scientific, Germany) and mounted on special adhesion slides (SuperFrost Ultra Plus; Thermo Scientific, Germany). Slices were dried during 2 days at 45 °C, following what they were processed for immunohistochemistry.

Steps	Number of baths	Total time needed (min)	Maximal Temperature (°C)
1) BOND Dewax Solution	3	1.5	72 °C
2) Alcohol (100 % Ethanol)	3	1.5	/
3) BOND Wash Solution	3	6	/
4) BOND ER Solution (Citrate)	4	42	95 °C
5) BOND Wash Solution	4	4.5	35 °C
6) Peroxide Block	1	5	/
7) BOND Wash Solution	3	1.5	/
8) Antibody anti-NeuN (primary)	1	30	/
9) BOND Wash Solution	3	1.5	/
10) Polymer	1	30	/
11) BOND Wash Solution	2	4	/
12) Deionizied Water	1	0.5	/
13) Mixed DAB Refine	2	10.5	/
14) Deionizied Water	3	1.5	/
15) Hematoxylin	1	3	/
16) Deionizied Water	1	0.5	/
17) BOND Wash Solution	1	0.5	/
18) Deionizied Water	1	0.5	/

Table 10. Detail of the successive steps necessary to achieve the NeuN immunostaining. Steps 1-3 correspond to the deparaffinization (in green), steps 4 and 5 to the pre-treatment (in red) and steps 6-18 to the proper immunostaining process (in blue). ER: Epitope Retrieval Solution; DAB: Antibody Diluent.

To estimate the importance of neuronal loss, we used a rabbit polyclonal antibody (Anti-NeuN, [1:500] dilution; Merck Millipore, USA) that recognized the neuron-specific protein NeuN (Wolf et al., 1996; Thelin et al., 2011). The whole process included three phases: deparaffinization, pre-treatment and immunostaining for a total duration of 4 h (BOND^{TM} Max immunostainer; Leica GmbH, Germany).

Deparaffinization stage aimed to remove the excess of paraffin surrounding the tissue. Pre-treatment stage unmasked antigen epitope by breaking cross-linkings previously established in the course of formalin fixation (Robinson and Vandre, 2001). Eventually, the immunostaining stage could occur, during which different reagents were successively applied (**Table 10**). At the end of the staining procedure, two drops of an aqueous mounting agent (Aquatex^{TM}; Merck Millipore, France) and a glas coverslip were positioned on each slide. Resulting slices dried overnight.

Lesions were verified by light microscope examination of areas of interest. Cell destruction was noticed in the absence of neuronal staining. The extent of hippocampal or dorso-striatal lesions was manually mapped onto standardized sections extracted in the mouse brain atlas (Paxinos and Franklin, 2001). Corresponding reconstruction of the smallest and biggest lesion extents and a few photographic illustrations are available in chapter 7.

VII. Polymerase Chain Reaction (PCR) genotyping

As aforementioned, mice from the Tg2576 line were bred on a mixt C57BL/6 x SJL/J background. Due to the choice of this second strain, they could possibly develop problems of visual acuity/blindness severely impacting behavioral and cognitive analyses (Brown and Wong, 2007). To exclude such animals from our cohorts, we consistently genotyped for the retinal degeneration (*rd*) mutation all wild-type and transgenic animals trained in our touchscreen tasks.

Tail biopsies were first individually digested overnight at 56 °C in a solution containing 20 µL of proteinase K and 180 µL of tissue lysis buffer (Qiagen, Germany). The day after, the content of each tube was centrifuged at 1000 rpm for 30 s to split the supernatant from remaining sediments (mainly, coat and bone). Genomic DNA present in the supernatant was then extracted and purified through a sequence of automated events using spin-column kits processed in a QIAcube system (Qiagen, Germany). Eventually, concentration of each eluted DNA (200 µL) was measured by ultraviolet spectrophotometry (NanoDrop; Thermo

Scientific, Germany) and generally comprised between 30 and 60 ng/µL. All DNA samples were directly stored at 4°C.

After extraction and purification steps, specific fragments of DNA were amplified *in vitro* according to a PCR procedure (Mullis et al., 1986;Bartlett and Stirling, 2003) for each mouse. Three primer sequences were used for that purpose:

RD3: 5'-TGACAATTACTCCTTTTCCCTCAGTCTG-3' (28-mer);

RD4: 5'-GTAAACAGCAAGAGGCTTTATTGGGAAC-3' (28-mer);

RD6: 5'-TACCCACCCTTCCTAATTTTTCTCAGCC-3' (28-mer).

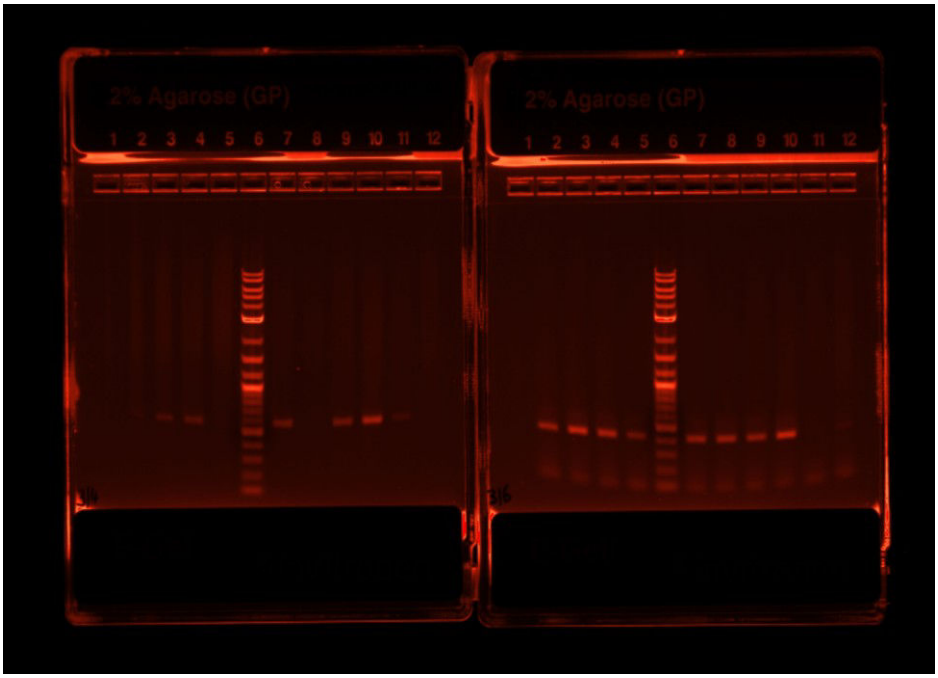


Figure 18. Analysis of PCR products by gel electrophoresis to distinguish blind from sighted mice of the Tg2576 line. After amplification in presence of RD3 and RD4 oligonucleotides (left panel), a 550 pb-band corresponding to the mutant allel (Pdeb^{rd1}) is observed while a 400 pb-band (right panel) is rather characteristic of the presence of RD3 and RD6 oligonucleotides (wild-type allel). Homozygots blind animals (lane 11) only present the heaviest band. Wild-type sighted animals (lanes 2, 5, 8 and 12) only possess the lightest band. As the *rd* mutation is recessive, heterozygots (lanes 3, 4, 7, 9 and 10), which present both bands, are sighted and can be kept into effectives. Lane 6: 1-kb DNA ladder.

These primers were paired (RD3/RD4 or RD3/RD6) within each PCR experiment. Indeed, the association of RD3 and RD4 primers allowed detecting the mutant allel (Pdeb^{rd1}) while the association of RD3 and RD6 primers permitted to promote the presence of the wild-type

allel (Gimenez and Montoliu, 2001). Each PCR experiment necessitated 1 µL of genomic DNA and 24 µL of a master mix (primers from Sigma-Aldrich, Germany; other reagents from Invitrogen, Germany) prepared with 1.25 µL of RD3 (20 µM), 1.25 µL of RD4 or RD6 (20 µM), 2.5 µL of dNTPs (2 mM), 2 µL of MgCl2 (25 mM), 0.5 µL of Taq Polymerase (5000 U/mL), 2.5 µL of Taq Buffer and 14 µL of RNase-free water. Each tube containing 25 µL was then incubated in a thermocycler set to reproduce a sequence of 3 stages for a total of 35 cycles: DNA denaturation (0.5 min at 94 °C), primer hybridization (1 min at 62°C) and DNA elongation (2 min at 72°C). During each cycle, these distinct steps respectively aimed to separate DNA strands, initiate and continue the synthesis of the complementary strand thanks to the different effectors available. In order to achieve the enzymatic reaction, samples were at length maintained during 10 more min at 72°C.

Following this phase, 20 µL of each tube were loaded on an agarose gel containing ethidium bromide (E-Gel™ Agarose 2 %; Invitrogen, Germany). After 30 min of electrophoretic migration, PCR products were visualized under UV light (ChemiDoc™ MP System; Bio-Rad, Germany) and interpreted to conclude about the genotype of each animal regarding the *rd* mutation (**Figure 18**). All experiments were duplicated to guarantee the accuracy of our results.

VIII. Data analysis

Many parameters can be measured in touchscreen chambers both during pokey training stages and main tasks. However, we noticed that animals assessed during the different studies all quickly integrated basic instrumental conditioning in early stages and showed no major difference (data not showed), whatever the condition tested. Therefore, we deliberately focused on the analysis of the PAL, VMCL or PVD tasks. In these paradigms, 6 parameters were initially recorded: the response accuracy (defined as the total number of correct responses divided by the total number of completed trials excluding correction trials, and expressed in %), the number of correction trials, the total number of completed trials and three different latencies (correct touch, incorrect touch and magazine latencies, respectively corresponding to the time necessary to nose-poke the correct/incorrect part of the screen or to get the reward into the magazine after a correct response). From experiment C presented in chapter 6, the number of correction trials, which brought relatively little information, was replaced by the specific locomotor activity (defined as the total number of back and front beams broken during a session divided by the time spent to achieve the total number of

trials, and expressed in beams/min). The first class of parameters (response accuracy, number of correction trials, total number of completed trials, specific locomotor activity) was directly analyzed (PVD task; note however that acquisition and reversal learning phases were treated independently) or plotted in blocks of 3 (dPAL or sPAL tasks) or 5 (VMCL task) sessions prior analysis. For all touchscreen tasks, the different latencies were averaged over the total number of sessions before proceeding to statistical comparisons. In the T-Maze forced continuous alternation task, two parameters were measured: the percentage of alternation (resulting from the number of correct choices divided by the total number of choices and multiplied by 100), and the total time animals needed to achieve the maximal number of trials. In any case, data were presented as means ± SEM. Statistical analyses were conducted with GraphPad Prism version 6.0 (GraphPad Software, San Diego, USA) using 1 or 2-way ANOVAs (with repeated measures on time factor when necessary) or unpaired *t*-tests. Data were considered as statistically significant when $p<0.05$. In that case, Bonferroni or Tukey *post-hoc* analyses allowed more detailed pairwise group comparisons.

Results

Chapter 5: Optimization of testing conditions, construction of a battery of touchscreen tasks

We have already broached in chapter 3 the different touchscreen tasks and their related cognitive functions of interest. Among the selected paradigms in this thesis, PVD and VMCL tasks had been early optimized using the touchscreen technology in rats and/or mice (Bussey et al., 1997b; Bussey et al., 2001; Morton et al., 2006). On one hand, these pioneering works had allowed further testing of animals within lesion studies (Chudasama et al., 2001; Chudasama and Robbins, 2003). On the other hand, they had also motivated the assessment of transgenic or pharmacological animal models of schizophrenia in the PVD task (Brigman et al., 2008; Brigman et al., 2009; Barkus et al., 2012). Concerning the PAL task, appropriate parameters had just been set up in rodents (Talpos et al., 2009; Bartko et al., 2011b) when I started my thesis.

Although these aspects originally went against the necessity of additional optimization steps, other arguments have incited us to take this direction. First, unlike previous studies, we wanted animals to discriminate between two stimuli of equivalent luminescence during the acquisition and reversal learning phases of the PVD task. Second, the VMCL task had never been adapted in mice (Horner et al., 2013) and was a prerequisite before further assessments with transgenic animals. Third, in the PAL task, the possibility was offered to differentiate the learning capacities of animals exposed to similar (sPAL) or different (dPAL) stimuli presented simultaneously on the screen. Eventually, our series of experiments also aimed to check the hypothesis according to which rodents could be successfully tested in a battery of touchscreen assays (Bussey et al., 2012; Nithianantharajah and Grant, 2013).

To achieve these diverse purposes, we have realized a total of 6 behavioral studies within touchscreen chambers. In the 2 first experiments (A and B), distinct groups of young C57BL/6JRj mice were tested according to protocols 1-6 (described in chapter 4) in the PAL or VMCL tasks. In a third experiment (C), animals previously tested in PAL variants were assessed in the VMCL task (protocol 4) while mice first tested in VMCL variants were evaluated either in the dPAL (protocol 1) or sPAL (protocol 2) tasks. All data generated by experiments A to C were gathered in publication 1. Over the last 3 experiments (D, E and F), different batches of young C57BL/6JRj mice were trained in the PVD task according to protocols 7-9. Corresponding results are presented in the pages below.

❖ Experiments A-C (publication 1)

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Optimization of Touchscreen-Based Behavioral Paradigms in Mice: Implications for Building a Battery of Tasks Taxing Learning and Memory Functions

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Abstract

Although many clinical pathological states are now detectable using imaging and biochemical analyses, neuropsychological tests are often considered as valuable complementary approaches to confirm diagnosis, especially for disorders like Alzheimer's or Parkinson's disease, and schizophrenia. The touchscreen-based automated test battery, which was introduced two decades ago in humans to assess cognitive functions, has recently been successfully back-translated in monkeys and rodents. We focused on optimizing the protocol of three distinct behavioral paradigms in mice: two variants of the Paired Associates Learning (PAL) and the Visuo-Motor Conditional Learning (VMCL) tasks. Acquisition of these tasks was assessed in naive *versus* pre-trained mice. In naive mice, we managed to define testing conditions allowing significant improvements of learning performances over time in the three aforementioned tasks. In pre-trained mice, we observed differential acquisition rates after specific task combinations. Particularly, we identified that animals previously trained in the VMCL paradigm subsequently poorly learned the sPAL rule. Together with previous findings, these data confirm the feasibility of using such behavioral assays to evaluate the power of different models of cognitive dysfunction in mice. They also highlight the risk of interactions between tasks when rodents are run through a battery of different cognitive touchscreen paradigms.

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Introduction

Neuropsychological tests historically represent valuable tools to diagnose and follow up neurodegenerative and psychiatric disorders. In many instances, they allow a precise discrimination between close pathological states affecting cognition [1]. Numerous cognitive domains can thereby be tested, among which executive functions, attention, different aspects of short- and long-term memories, etc. One of the best examples illustrating the importance of such tools is probably the mini mental state examination (MMSE). This composite cognitive test was introduced in 1975 in the field of clinical research to detect possible cognitive impairments/probable dementia in aged patients [2–4]. Almost forty years later, despite the increasing use of biomarkers for detection of Alzheimer's disease [5,6], it is worth noticing that this readout is still recommended for the evaluation of demented patients [7] or the recruitment of patients with mild to moderate dementia for clinical trials [8–11].

If various tests have been progressively implemented over the last decades, the Cambridge neuropsychological test automated battery (CANTAB) certainly deserves a particular attention due to its translational dimension [12]. Initially established in humans, neuropsychological tests included in this computerized battery are based on a universal principle: subjects have to respond to variously-shaped stimuli displayed on a sensitive touchscreen according to a defined rule. These tasks present the great advantage to be directly translatable from humans to non-human primates after no or sometimes only minor adaptations [13]. Interestingly, in macaques infected with the simian immunodeficiency virus (SIV) neuropsychological deficits appear similar to those described in human AIDS patients [14], and the same keeps true in aged rhesus monkeys compared to healthy aged humans [15]. Moreover, the assessment of cognitive abilities through this methodology is sensitive to drug manipulations in both monkeys and humans [16–19].

Recent reports have emphasized the need for more translational preclinical assays in animal models to better predict the efficacy of putative therapeutic agents in clinical studies [20–23]. Capitalizing on the additional value of new emerging models based on advances in transgenesis techniques [24,25], Bussey and collaborators gradually introduced the touchscreen-automated testing method in rats and mice [26–28]. As in humans, rodents are expected to respond to visual stimuli displayed on a touchscreen according to a specific rule. Nevertheless, each associative learning

of a given cognitive task requires extensive training. Correct nose pokes are thus rewarded with an appetitive reinforcer in food-deprived animals, which contributes to strengthen motivation and to decrease the stress component. Various behavioral touchscreen-based tasks pertaining to different cognitive functions and presenting the added benefit of automated measures in a controlled environment have thus been adapted in rodents [29–31]. Furthermore, several articles argue in favor of their use to screen or validate the predictivity of new animal models, especially with regard to schizophrenia and Alzheimer's disease [32–37].

The goals of the present work were to optimize in mice two cognitive touchscreen-based tasks, the paired-associates learning (PAL) and the visuo-motor conditional learning (VMCL) tasks and to validate whether they could be combined to evaluate the successive performances of animals tested in a battery of assays [38–40]. These paradigms have been studied in rats and are thought to depend on distinct brain structures, namely the hippocampus and the dorsal striatum, respectively [41–44]. Therefore, they could be, for instance, of high interest for the sequential cognitive evaluation of animal models of Alzheimer's disease, which are generally impaired in hippocampal-dependent tasks [45,46] but display preserved abilities in striatal-dependent procedural forms of learning [47]. So far, however, very few data are available in mice [48,49]. We first explored (experiment A) the acquisition of two versions of the PAL task using similar (sPAL) or different (dPAL) stimuli to examine in that spatial paradigm the role of the nature of presented objects [44]. In order to optimize the VMCL task (experiment B), we then investigated the impact of various training conditions that had been previously identified as critical factors for subsequent acquisition (with or without "pretraining"; different limited holding times to respond to the screen; data not shown, obtained in pilot studies). Finally, we determined (experiment C) whether the acquisition of a first rule affected the way mice learned a second rule in another task.

Results

Experiment a: dPAL *vs* sPAL Tasks in Naive Animals

Three mice (one from the dPAL group, two from the sPAL group) out of the 16 naive animals were excluded from data analysis because they displayed no evidence for learning after 50 testing sessions (accuracy<60%).

To determine if performance changed over time, we plotted accuracy data in 10 blocks of 5 sessions for the 2 variants of the PAL task (see figure 1). There was a significant effect of Time ($F(9,99) = 35.26$; $p<0.0001$) and Task ($F(1,11) = 17.18$; $p<0.01$) when looking at the accuracy parameter. A significant Time × Task interaction was also found ($F(9,99) = 2.19$; $p<0.05$). *Post-hoc* analysis revealed that the difference between the 2 variants of the PAL task appeared from 25 sessions onwards (block 5; $t(11) = 2.893$; $p<0.05$) and even became more important after 40 testing sessions (block 8; $t(11) = 3.988$; $p<0.01$), suggesting an easier acquisition of the sPAL task.

In agreement with this observation, planned comparisons against the group trained in the sPAL task showed a significant higher number of correction trials in animals recorded in the dPAL task (see table 1; $t(11) = 2.743$; $p<0.05$). Interestingly, there were no significant differences between dPAL and sPAL groups regarding correct touch ($t(11) = 0.411$; $p>0.05$), incorrect touch ($t(11) = 0.417$; $p>0.05$) and magazine ($t(11) = 0.508$; $p>0.05$) latencies.

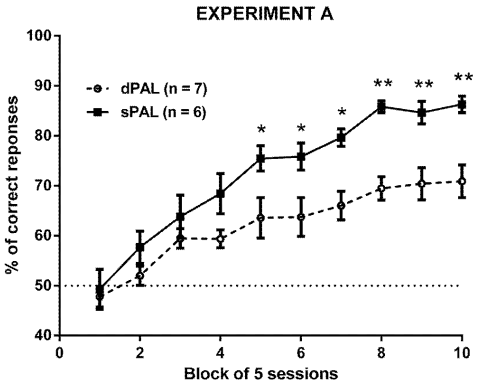

Figure 1. Acquisition curves in naive mice trained either in the sPAL (similar objects) or the dPAL (different objects) tasks. * $p<0.05$ and ** $p<0.01$ vs the dPAL group.
doi:10.1371/journal.pone.0100817.g001

Experiment B: VMCL Task in Naive Animals

Four different groups of 8 mice each were assessed in experiment B. Only two mice were excluded due to weak learning performance after 30 testing sessions (accuracy<75%). Consequently, groups 2 and 4 included only 7 mice.

Accuracy data were plotted in 10 blocks of 3 sessions for all groups trained in the VMCL task (see figure 2). Analysis of accuracy showed a significant effect of Time ($F(9,234) = 70.60$; $p<0.0001$) and Training condition ($F(3,26) = 3.00$; $p<0.05$), but no interaction between the two factors in the VMCL task ($F(27,234) = 1.37$; ns). Additional *post-hoc* analyses indicated a difference between groups 1 and 4 and groups 3 and 4 only during the earliest learning sessions (block 2: $t(13) = 3.480$ and $t(13) = 4.106$; $p<0.05$ and $p<0.01$, respectively).

Such subtle learning changes in group 4 did not affect most of the global measures reported in table 1: no significant differences were observed when comparing groups 1 to 4 on the number of correction trials ($F(3,29) = 1.769$; $p>0.05$), correct touch ($F(3,29) = 2.282$; $p>0.05$) and magazine latencies ($F(3,29) = 1.885$; $p>0.05$). Nevertheless, we found a significant effect of Training condition on incorrect touch latency ($F(3,29) = 6.293$; $p<0.01$): mice trained in conditions 1 presented a significantly higher incorrect touch latency than those trained in conditions 4 ($t(13) = 4.274$, $p<0.01$).

Experiment C: dPAL, sPAL and VMCL in Animals Previously Assessed in Touchscreen Tasks

Animals from experiment A (dPAL or sPAL) were assessed in the VMCL task, whereas animals from experiment B were assessed in 1 of the 2 variants of the PAL task. All groups were initially composed of 8 mice, but the same aforementioned criteria of accuracy were used to determine the final size of each group in experiment C: n = 6 mice in the dPAL task after the VMCL task; n = 7 mice in the sPAL task after the VMCL task; n = 7 mice trained in the VMCL task after the dPAL task; n = 7 mice trained in the VMCL task after the sPAL task. Accuracy data were plotted as described in experiments A and B (see figure 3); global measures were comparable to those shown in table 1.

Table 1. Additional parameters measured in touchscreen boxes during experiments A, B and C.

Experiment	Task	Group	N	N° of CT	CTL (s)	ITL (s)	ML (s)
A	1	dPAL	7	26.91±1.92*	2.05±0.18	2.07±0.15	1.26±0.05
A	1	sPAL	6	20.03±1.51	1.96±0.10	2.14±0.09	1.22±0.05
B	1	VMCL - Conditions 1	8	11.18±0.72	0.79±0.07	0.35±0.04##	1.10±0.03
B	1	VMCL - Conditions 2	7	9.52±1.00	0.77±0.07	0.25±0.04	1.11±0.04
B	1	VMCL - Conditions 3	8	12.21±0.99	0.75±0.07	0.29±0.03	1.04±0.04
B	1	VMCL - Conditions 4	7	9.28±1.46	0.58±0.04	0.16±0.02	1.16±0.03
C	2	dPAL (after VMCL)	6	27.92±1.97	2.17±0.28	2.26±0.25	1.32±0.05
C	2	sPAL (after VMCL)	7	32.43±2.49**	2.95±0.54	2.83±0.44	1.50±0.11
C	2	VMCL (after dPAL)	7	11.10±1.11	1.07±0.26	0.46±0.13	1.16±0.07
C	2	VMCL (after sPAL)	7	10.85±0.73	0.78±0.10	0.30±0.04	1.16±0.07

CT: Correction Trials; CTL: Correct Touch Latency; ITL: Incorrect Touch Latency; ML: Magazine Latency.
All parameters are expressed as mean values ± SEM.
*$p<0.05$,
**$p<0.01$ *vs* sPAL group (task 1);
##$p<0.01$ *vs* VMCL conditions 4 group.
doi:10.1371/journal.pone.0100817.t001

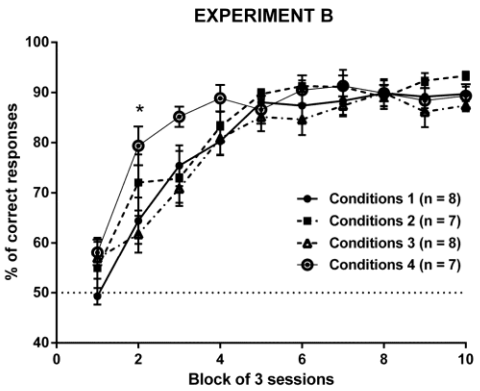

Figure 2. Acquisition curves in naive mice trained under various conditions in the VMCL task.
*$p<0.05$ group 4 vs group 1 and $p<0.01$ group 4 vs group 3.
doi:10.1371/journal.pone.0100817.g002

There was a main effect of Time ($F(9,99) = 19.02$; $p<0.0001$) on accuracy in the dPAL task (figure 3, left panel), but no effect of Task experience ($F(1,11) = 0.01$; $p>0.05$) and no interaction between the two factors ($F(9,99) = 1.28$; $p>0.05$). Moreover, no significant difference was found among the different global measures (table 1) within tested groups: number of correction trials ($t(11) = 0.367$; $p>0.05$), correct touch ($t(11) = 0.372$; $p>0.05$), incorrect touch ($t(11) = 0.679$; $p>0.05$), and magazine ($t(11) = 0.902$; $p>0.05$) latencies.

Likewise, analysis of the accuracy in the VMCL task (figure 3, central panel) revealed a significant effect of Time ($F(9,162) = 52.97$; $p<0.0001$), but no effect of Task experience ($F(2,18) = 3.16$; $p>0.05$) and no interaction between the two factors ($F(18,162) = 0.80$; $p>0.05$). Furthermore, none of the additional measures summarized in table 1 was significantly different within tested groups: number of correction trials ($F(2,18) = 0.783$; $p>0.05$), correct touch ($F(2,18) = 1.089$; $p>0.05$), incorrect touch ($F(2,18) = 1.860$; $p>0.05$), and magazine ($F(2,18) = 0.195$; $p>0.05$) latencies. Altogether, these results suggest that an experience in a VMCL touchscreen task does not influence subsequent acquisition of a dPAL touchscreen task, and both versions of the PAL task do not influence the subsequent acquisition of the VMCL task.

However, unlike the 2 other tasks, analysis of the accuracy in the sPAL task (figure 3, right panel) indicated significant effects of Time ($F(9,99) = 20.68$; $p<0.0001$), Task experience ($F(1,11) = 71.13$; $p<0.0001$; from block 4, $p<0.05$) and an interaction between these factors ($F(9,99) = 5.19$; $p<0.0001$). Mice previously trained in the VMCL task were slower to acquire the sPAL task than naive mice. Importantly, this significant effect was accompanied by a significant increase of the number of correction trials (table 1; $t(11) = 4.079$; $p<0.01$), as well as a non-significant increase of both correct touch ($t(11) = 1.677$; $p>0.05$), incorrect touch ($t(11) = 1.439$; $p>0.05$), and magazine ($t(11) = 2.135$; $p>0.05$) latencies.

Preference for a Specific Stimulus or Location in Touchscreen Tasks

To explore the possibility of a preference for a specific configuration of stimuli among the different trial types, we

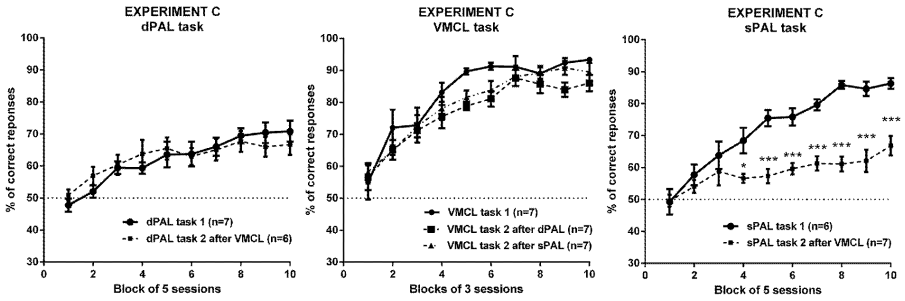

Figure 3. Effect of a previous training experience in touchscreen boxes on the acquisition of a new task. Comparison of the acquisition curves of naive vs trained mice in the dPAL (left panel), the VMCL (central panel) or the sPAL task (right panel). *p<0.05 and ***p<0.001 vs the sPAL (task 1).
doi:10.1371/journal.pone.0100817.g003

analyzed for both variants of the PAL task the repartition of the total number of correct responses recorded in experiments A and C, independently of previous touchscreen experience. A similar calculation was made with regard to the VMCL task to determine whether mice presented a preference for a certain location (left *vs* right) from experiments B and C. Corresponding results are illustrated in figure 4.

In the dPAL task, we initially found a significant effect of the Trial type ($F(5,66) = 3.050$; $p<0.05$). A complementary *post-hoc* analysis showed that animals responded significantly more when trial types 3 or 4 were presented on the screen as compared to trial type 6 (respectively, $t(22) = 3.189$; $p<0.05$ et $t(22) = 3.416$; $p<0.05$). However, a close inspection of performance showed that this effect was mainly due to 2 mice which specifically occulted that trial type. After their exclusion (figure 4, left panel), significance vanished ($F(5,54) = 1.760$; $p>0.05$). In parallel, a similar analysis led for the sPAL task with raw effectives did not reveal any effect of the Trial type ($F(5,66) = 0.830$; $p>0.05$). These results suggest that mice acquiring one of the two variants of the PAL task do not learn the rule by partially using some of the displayed stimuli, but rather consider all combinations of visual stimuli to progressively define the nature of the rule.

In the VMCL task, there was no significant effect of the Trial type ($t(86) = 1.344$; $p>0.05$). This result is in agreement with the balanced expression of left *vs* right correct responses observed in these mice (figure 4, right panel) and confirms the absence of side preference in this task.

Discussion

A first goal of this study was to validate testing conditions demonstrating the acquisition of three distinct cognitive, touchscreen-based tasks. Two variants of the paired-associates learning (PAL) task and one of the visuo-motor conditional learning (VMCL) task were used in mice. If instrumental touchscreen tasks present many advantages, as multiple cognitive domains can be assessed [50], their development remains quite challenging due to the numerous parameters that must be considered and adjusted.

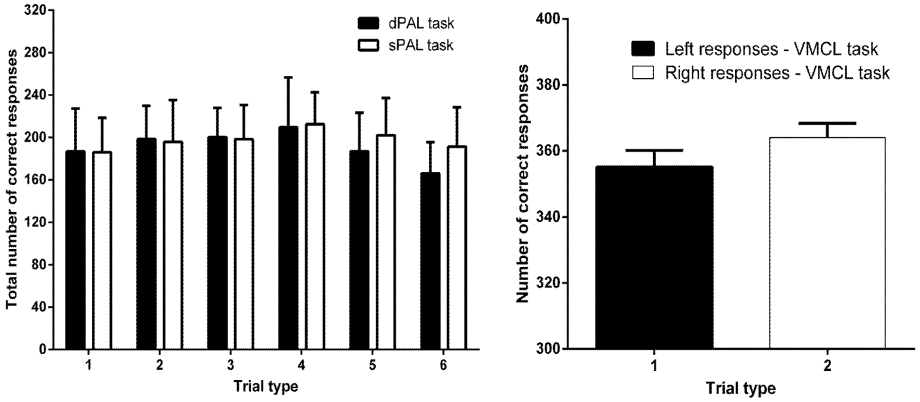

Figure 4. Global repartition of the correct responses of all animals assessed in the PAL tasks (left panel) or in the VMCL task (right panel). In both PAL paradigms, there are six possible object-place combinations. In the VMCL task, mice can only respond to the left or the right part of the screen after the first central nose-poke.
doi:10.1371/journal.pone.0100817.g004

Critical factors such as the duration of the inter-trial interval (ITI), the number of trials per session, the nature and size of the stimuli can deeply influence performance [27,28]. However, tasks presented in this paper have been adapted on the basis of previous works in rats [26,44] and mice [48], facilitating their optimization in mice. Mice were able to perform all tasks with a significant improvement over time. Nose-pokes given to the correct or incorrect stimuli occurred quickly (always <3 s), indicating good reaction times and short decision-making. Moreover, low magazine latencies (around 1 s) demonstrated intact motivation towards a liquid reward (diluted condensed milk solution). Finally, it could be argued that if naive/previously trained mice were apparently able to learn the 3 different tasks, they might have developed preferences for stimuli or locations over time. However, there was no preference for stimuli or locations in our different experiments.

In experiment A, naive animals were trained in one if the two PAL variants (sPAL or dPAL tasks). Over extensive training, mice were able to identify the different stimuli (Flower, Plane, and Spider), to distinguish between locations (Left, Central, and Right) and to remember in which specific location each stimulus was systematically rewarded. The cognitive demand was expected to be lower in the sPAL task than in the dPAL task, as similar stimuli were presented within a same trial in that case. Indeed, the former task was acquired faster than the latter in rats [44], although the sPAL and dPAL learning curves merged at 85% of correct responses after 45 sessions. In our experiments, we observed similar initial patterns of learning, with the sPAL task being acquired quicker than the dPAL task, but unlike rats, dPAL and sPAL learning curves still diverged after 50 sessions of training (70% *vs* 85% of correct responses, respectively). The final performance of our mice trained in the dPAL task was also in accordance with a previous study in which mice tested in the same paradigm reached 80% correct responses after 95 testing sessions [48].

With regards to the VMCL task, for the first time we successfully transposed the task from rats [26] to mice. In a first experiment (data not shown), we had measured the ability of young naive male C57BL/6 mice to learn the VMCL rule using a rat paradigm with a few differences on the nature of the reward (pellets), the type of boxes (Med Associates boxes) and the characteristics of the ITI (a variable ITI of 40 ± 30 s, which means a random value between 10 and 70 s). In these conditions, mice trained in similar pokey training stages reached 70% correct after 30 sessions in the VMCL task, which was in conflict with the quick acquisition of the task in rats [51]. We suspected the value of the ITI to be the determining factor and therefore decided to reduce its duration to 20 s as a fixed interval for both "pretraining" and VMCL tasks in experiment B. Other parameters were also adjusted as all naive mice were trained in touchscreen boxes using condensed milk as the reward. Groups 1 and 2 were recorded to evaluate the impact of a "pretraining" phase. Because the quick disappearance of the stimuli could also incite mice to approach the touchscreen and nose-poke more efficiently the stimuli, we also measured whether mice trained with a limited holding time during both "pretraining" and VMCL task (5 s for group 3; 3 s for group 4) would learn easier the VMCL task. Surprisingly, all groups quickly learned the VMCL task under these conditions, achieving 90% of correct responses after 15–18 sessions of 30 trials. This acquisition rate was almost comparable to the performance of rats in the VMCL task (90% of correct responses after 6 sessions of 100 trials), although rats had been trained with more difficult conditions, especially the disappearance of the discriminative central stimulus which increases the mnesic component of the task during each choice phase [51].

The use of a battery of cognitive touchscreen tasks using similar stimuli, responses and outcomes has been recently highlighted and emphasized [50,52]. Therefore, we decided to investigate to which extent a first assessment in a touchscreen task would influence the acquisition of a second task differing by the nature of its rule. We noticed no difference between acquisition of the VMCL or the dPAL tasks between naive or trained mice, but observed an interesting gap in the sPAL task: whilst animals first trained in the sPAL task normally acquired the VMCL task, those first trained in the VMCL task displayed a learning deficit in the sPAL task, reaching only 65% of correct responses after a total of 50 sessions. These results suggest that under certain circumstances, one form of learning could interfere with the subsequent acquisition of a second, harder task. They also underline the putative involvement of common neural substrates in the sPAL and VMCL tasks. Observing that intra-hippocampal infusions of drugs had no effect on post-acquisition performance of the sPAL task in rats [44], Talpos hypothesized that similarly to the VMCL task, the sPAL task could be solved via a conditional rule of the type "If stimulus A appears, then choose location 1; if stimulus B appears, then choose location 2; if stimulus C appears, then choose location 3". We globally agree with that view. Final performance of naive mice trained in the sPAL task converged towards those of naive mice trained in the VMCL task (85–90% of correct responses), albeit the acquisition process required a higher number of sessions in the first case, probably due to task difficulty. Additionally, if we admit that animals base their responses on conditional rules to achieve both VMCL and sPAL tasks, it makes sense noticing that mice first trained in the most difficult task (sPAL task) can efficiently learn a simpler rule (VMCL task), whereas mice first trained in the easiest task (VMCL task) struggle to learn a harder rule (sPAL task).

In parallel, our results also confirm the possibility to measure dPAL and VMCL performances in mice and to combine these tasks to evaluate cognitive impairment. In particular, it should be of great interest to investigate the effects of hippocampal (HPC) *vs* dorso-striatal (DS) lesions on the acquisition of both tasks. Indeed, compelling evidence indicate that the dPAL task primarily depends upon the hippocampal integrity. First, the human version of the task [12,53] allows measuring direct episodic memory performance and detecting early impairments in patients with mild cognitive impairment (MCI), Alzheimer's disease [54–56] or schizophrenia [57,58]. Moreover, using fMRI, it has recently been shown that the low performance of MCI patients in the dPAL task specifically coincided with a lower hippocampal activation when the task demand was increased [59]. Second, although the rodent version of the dPAL task assesses object-in-place memory rather than episodic memory – with object-place (what-where) associations being gradually encoded during training –, the hippocampus plays an important role during retention of this type of information. Indeed, post-acquisition, intra-hippocampal infusion of MK-801, lidocaine or CNQX produces a significant decrease of dPAL performance in rats [44]. Third, data from other paired-associates learning tasks support a hippocampal implication during the acquisition of the task in rats, especially when one of the two dimensions to associate is a spatial feature [60–62].

By contrast, the VMCL task has historically been introduced as a stimulus-response learning task in rats thirty years ago [63]. In this construct, animals had initially to learn a conditional rule of the type "If lights are flashing FAST, press the right lever; if lights are flashing SLOW, press the left one". Later this procedure was replaced by another conditional rule of the type "If stimulus A

appears, then go left; if stimulus B appears, then go right" along with the emergence of the touchscreen method [26]. As expected in such a habitual task, control rats quickly learned the rule and reached a plateau performance (about 90% correct responses) after only a few sessions. Different studies based on excitotoxic lesions have shown the involvement of a corticostriatal network relying on intact dorso-lateral striatum [41,42] and cingulate cortex [26] in this task. Accordingly, animals' acquisition and subsequent performance were spared after lesions of the hippocampus [43], the prelimbic cortex, thalamic nuclei [64], perirhinal and postrhinal cortices or after a fornix transection [51]. Given that a similar rule resulted in fast acquisition curves in our mice, the task is most likely linked to the integrity of the same brain regions than in rats.

Conclusion

Optimizing training conditions in translational paradigms is an important step as mice represent an increasingly used species in preclinical research, notably since the emergence of genetic models. Here, we demonstrate that, like rats, normal mice can successfully learn three appetitive touchscreen rules defining associations between objects and locations: the dPAL task, the sPAL task and the VMCL task. Using the touchscreen method, reliable parameters make it possible to monitor the animals' performance in the absence of object/location preference and to check their motivational state throughout the experiment. We also show that although it may be appropriate to use the dPAL task and the VMCL task in a cognitive testing battery, as numerous papers underpin the involvement of distinct neural substrates in similar tasks, the cumulative assessment of mice in both the sPAL and VMCL task appears to be more risky. Future studies should now examine the effects of hippocampal *vs* dorso-striatal lesions in mice trained in the dPAL or the VMCL tasks.

Materials & Methods

Ethics Statement

All protocols included in this study and procedures related to Animal Care and Treatment were conducted with the specific approval of the appropriate governmental agency (Regierungspräsidium Tübingen, Germany) and performed in an AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care International)-accredited facility in accordance with European Union guidelines (European Community Council Directive 2010/63/UE). All efforts were made to minimize animal suffering.

Animals

48 male C57BL/6JRj mice were obtained from Janvier (France). They were 8–10 weeks old (23–27 g) at the start of food deprivation. Upon their arrival, mice were placed in a temperature- and humidity-controlled environment under a 12 h light/dark cycle (lights on 06:00 h). They were individually housed in plastic cages (dimensions: length = 26 cm; width = 21 cm; height = 14 cm) to allow a more accurate follow-up of their daily food-intake. Each cage contained wood shaving bedding, and a red transparent plastic nest box and paper strips to provide some environmental enrichment. Animals were first given a week of habituation to the environmental conditions of our animal facility. Meanwhile, mice were weighed three times to determine their respective basal free-feeding body weight. The body weight was then slowly reduced and maintained at 85–90% of its free-feeding value throughout behavioral testing. Behavioral assessments were conducted during the light phase of the light/dark cycle. Mice were trained 5–6 days/week and rewarded in touchscreen devices with a liquid reward (condensed milk, Milch Mädchen, Nestlé, Germany; half diluted in water). They were directly weighed and fed upon return to the home cage after each daily session. Water was available *ad libitum*.

Apparatus

The touchscreen-based apparatus consisted in an operant chamber housed within a sound and light attenuating box. Every trapezoidal-shaped chamber (respective dimensions: big basis = 25 cm; small basis = 6 cm; height = 18 cm) was individually equipped with a house light and a tone generator, and had been especially designed to focus the attention of the animal towards the touchscreen placed at one end of the chamber (model #80614, Bussey Mouse Touchscreen Chamber, Campden Instruments, U.K.). The liquid reward dispenser delivering condensed milk into a magazine was located at the opposite end of the chamber. The touchscreen was permanently covered by a black Plexiglas 3-holes mask. Three square windows (side dimensions: length = 7 cm; height = 7 cm) were separated by 0.4 cm and located at a height of 3.6 cm from the floor of the chamber. Through these windows, different visual stimuli could be shown on the screen (max. 1 stimulus per window). Stimulus presentation and reward delivery timing were both controlled by a graphical task design software (ABET II Touch software, model #89505, Campden Instruments, U.K.) according to the automated detection of animal nose-pokes specifically oriented towards the screen and the magazine.

Behavioral Procedures

Experiment A: dPAL vs sPAL tasks in naive animals. 16 male C57BL/6JRj mice were randomly assigned to 2 groups (n = 8 animals) and tested in one of the two versions of an "object-in-place" memory task involving the presentation of different (dPAL) *vs* similar (sPAL) stimuli during the main training phase (see figure 5).

Mice were food-deprived, then acclimated to the liquid reward in their home cage with 500 µL of condensed milk placed in a cup for 3 consecutive days. Afterwards, they were introduced in boxes with 250 µL of condensed milk into the magazine for a 20-min session of habituation. All mice had consumed the reward at the end of the session.

A pokey training procedure then started to train each animal to progressively detect and respond specifically to the window where a training stimulus appeared. In total, four different stages were included, namely "initial touch", "must touch", "must initiate" and "punish incorrect" stages. In all pokey training stages, only one training stimulus was displayed on the screen per trial, in one of the 3 possible windows. Training stimuli consisted of 40 possible various shapes that were pseudo randomly chosen.

In the "initial touch" paradigm, each trial started with the presentation of a training stimulus for a fixed duration (30 s) in one of the three possible locations of the screen. The end of this period coincided with the offset of the training stimulus and the delivery of the reward (8 µL) accompanied by the illumination of the magazine light and a tone. There was no inter-trial interval (ITI) at this stage: once the mouse had nose-poked into the food tray, a new trial started with the onset of a new training stimulus. Importantly, if the animal touched the training stimulus during its presentation, it received three times as much as the normal amount of reward (24 µL). Mice reached criterion when they were able to complete 36 trials in less than 60 min.

In the "must touch" paradigm, each trial started also with a training stimulus displayed in one of the three windows, but it

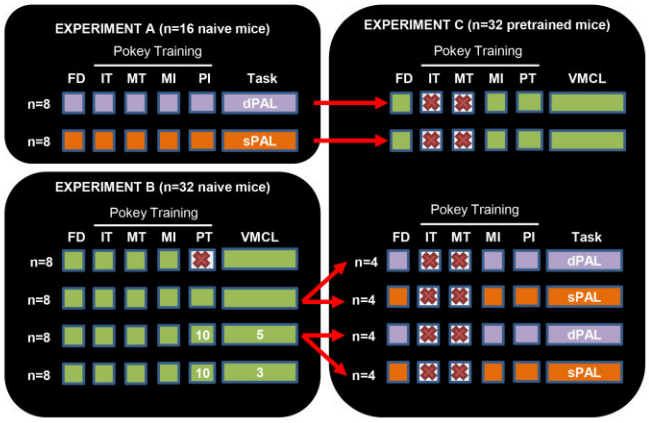

Figure 5. Global design of touchscreen experiments. In experiments A and B, naive mice were trained in the dPAL, sPAL or VMCL tasks according to specific learning conditions (groups 1–4, from top to bottom). In experiment C, most of the mice previously trained in a first touchscreen paradigm were assessed in a different task; because of their experience, early stages of pokey training were purposely skipped. For animals trained in stages with defined limited holding times (LHT), corresponding values are given in white. FD: Food Deprivation; IT: "Initial Touch"; MT: "Must Touch"; MI: "Must Initiate"; PI: "Punish Incorrect"; PT: "Pre-Training".
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remained visible until the mouse had nose-poked it. As previously, a successful nose-poke was followed by the illumination of the food tray, a tone and the delivery of the liquid reward (8 µL). An ITI (20 s) was introduced before the beginning of each new trial. When a mouse completed 36 trials in less than 60 min, the third stage of pokey training was started: the "must initiate" paradigm, during which the principle remained the same, except that animals had to nose-poke in the magazine before a training stimulus could be displayed on the screen. Same criteria as initial touch and must touch stages allowed to determine the start of the next stage.

In the "punish incorrect" paradigm, as before, a nose-poke of the training stimulus (correct response) was followed by the illumination of the food tray, a tone, and the delivery of the liquid reward (8 µL) with a 20 s ITI before a new trial could start. However, after a nose-poke of one of the two other blank windows (incorrect response), the training stimulus disappeared, the house light was turned on for a time-out period of 10 s and no reward was given. After 10 more seconds corresponding to the correction ITI, the mouse then had to complete a correction trial procedure. For that purpose, the last used training stimulus and its position were kept the same and were re-presented to the animal until it responded correctly. Importantly, correction trials were not counted in the total number of completed trials. Mice were directly brought to the next phase (dPAL or sPAL) when they achieved 36 trials in less than 60 min with an accuracy superior to 75% (minimum 27 correct responses) over two consecutive sessions.

In both variants of the PAL task, each mouse was required to learn specific paired-associations of stimuli and locations. Therefore, three discriminative stimuli (flower, plane, and spider) were used for a total of 6 possible trial types. Contrary to a previous paper [44], if the flower was also rewarded when presented in the left location, the plane was this time rewarded when presented in the central location, whereas the spider was rewarded when presented in the right location. Mice were recorded for a total of 50 sessions, with 36 trials per session. Each trial was initiated by nose-poking into the magazine. The tray light then switched off and a pair of stimuli appeared on the screen in 2 of the 3 possible locations: left, central, or right. These stimuli were different (dPAL) or similar (sPAL) ones; the latter condition was expected to be easier as animals did not have to discriminate between stimuli and locations within a same trial, but to discriminate between locations only (see figure 6). Among the 2 stimuli shown on the screen, one stimulus was the correct one (S+) and the other was the incorrect one (S−). When a mouse nose-poked the correct stimulus (case 1: correct response), both stimuli disappeared and the mouse was rewarded for a correct response as previously described. Entry to collect the reward turned off the tray light and started a 20 s ITI. Afterwards, the tray light was again illuminated and the mouse could nose-poke into the magazine to trigger the next trial by initiating the apparition of a new pair of stimuli on the screen. By contrast, if the mouse nose-poked the incorrect stimulus (case 2: incorrect response), the stimuli disappeared, the house light was turned on for a time-out period of 10 s and no reward was given. After 10 more seconds corresponding to the correction ITI, the mouse then had to complete a correction trial procedure. A correction trial consisted of the re-presentation of the last pair of stimuli in the same spatial configuration and was repeated until a correct response was given to the screen. As for the "punish incorrect" stage, correction trials were not counted in the total number of trials completed during the main training.

Experiment B: VMCL task in naive animals. 32 male C57BL/6JRj mice were randomly divided into 4 groups (n = 8 animals) and trained under similar pokey training conditions before following specific "pretraining" and training programs. More precisely, in those two latter stages, animals had to nose-poke the stimuli in a limited holding time (LHT). Group 1 (no "pretraining", VMCL with no LHT), group 2 (both "pretraining" and VMCL with no LHT), group 3 ("pretraining" LHT 10 s, VMCL LHT 5 s) and group 4 ("pretraining" LHT 10 s, VMCL LHT 3 s) thus aimed at defining the ideal conditions of learning (see figure 5).

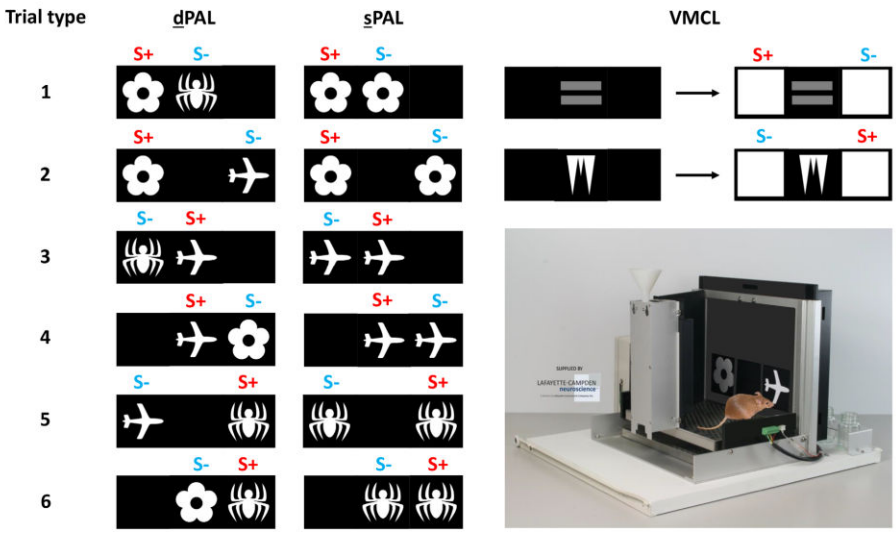

Figure 6. The different trial types in the two versions of the Paired-Associates Learning (dPAL and sPAL) tasks and in the Visuo-Motor Conditional Learning (VMCL) task. In both PAL paradigms, stimuli are rewarded when located in a specific location: left for the Flower, central for the Plane, right for the Spider. However, in a first variant of the task (dPAL), two different stimuli are presented at the same time, whereas two similar stimuli are presented in the second variant of the task (sPAL). In the VMCL paradigm, only two different trial types coexist for a given rule; note that the current rule can be inverted ("If Equal appears, go Right; if Icicle appears, go Left"), which is why all groups were counterbalanced. S+: rewarded stimulus (correct response); S-: non-rewarded stimulus (incorrect response). Picture from Campden Instruments Ltd.; reprinted with permission.
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As described above, food-deprivation, acclimation to the liquid reward and habituation to the environment also preceded the beginning of the testing procedure. All animals were trained in early pokey training stages ("initial touch", "must touch" and "must initiate" stages) as for the PAL task with 3 differences: the nature of training stimuli, the locations where stimuli appeared over the different stages and finally the criterion. We used white squares as training stimuli. They appeared in one of the three possible locations during the initial touch stage, but only in one of the two lateral windows during "must touch" and "must initiate" stages. Finally, completion of each pokey training stage was achieved when mice performed 30 trials in less than 60 min.

Subsequent to this pokey training, groups 2 to 4 were given an additional "pretraining" stage to learn to nose-poke the touchscreen centrally, then laterally to get the reward. Before every new trial started, the mouse had to nose-poke into the magazine and exit the reward tray. A first white square then appeared in the central window, and remained until the animal nose-poked it. After the animal had touched the first stimulus, the stimulus disappeared and a second white square appeared in the left or right window of the screen. The position of this second stimulus was chosen pseudo randomly. The mouse then had to touch this second stimulus in a limited holding time (10 s for both groups 3 and 4) or not (group 2) to get the reward. If the mouse nose-poked the second stimulus before the fixed time limit was reached (case 1: correct trial), reward delivery was accompanied by illumination of the tray light and a tone as the stimulus disappeared. Entry to collect the condensed milk turned off the tray light and started the ITI. After the ITI period (20 s), the tray light was again illuminated, and a new trial could start. On the contrary, if the mouse did not nose-poke the second stimulus within the 10 s (case 2: omission, only for groups 3 and 4), the stimulus disappeared and no reward was given to the animal. Correction ITI period (10 s) followed a time out (10 s) during which the house light was illuminated. A correction trial procedure then started with the re-presentation of the first stimulus, followed by that of the second stimulus in the last proposed spatial configuration. Omissions were counted in the total number of trials. All groups were finally trained in the main task after groups 2, 3 and 4 reached the criterion of 30 trials completed in less than 60 min over 2 consecutive days (with less than 5 omissions per session for groups 3 and 4).

In the VMCL task, mice had to learn first to nose-poke the central window where a discriminative stimulus was displayed, then one of the 2 lateral locations depending on the nature of that central stimulus (see figure 6). They were recorded for a total of 30 sessions, with 30 trials per session. Each mouse first had to nose-poke into the magazine and exit the reward tray to initiate a trial. A first discriminative stimulus was then displayed in the central window, and remained until the animal nose-poked it. This discriminative stimulus was chosen pseudo randomly among 2 possible stimuli that were different in shapes and colors (white icicle *vs* grey equal). After the first central nose-poke, the initial stimulus remained visible and 2 white squares appeared laterally on the left and on the right of the screen. The mouse then had to touch one of these 2 stimuli to get the reward according to the predefined rule "If stimulus A appears, then go left; if stimulus B appears, then go right", without (groups 1 and 2) or with a limited

holding time (groups 3 and 4, respectively LTH = 5 and 3 s). Within each group trained in the VMCL task, the nature of visuo-motor associations was counterbalanced: half of the animals had to respond to the left panel when the grey equal was displayed and to the right panel when the white icicle was shown, whereas the other half had to respond to the right panel when the grey equal was displayed and to the left panel when the white icicle was shown (opposite rule).

If the mouse nose-poked the correct stimulus during the choice phase (case 1: correct trial), reward delivery was accompanied by illumination of the tray light and a tone. Entry to collect the condensed milk turned off the tray light and started the ITI. After the ITI period (20 s), the tray light was again illuminated and the mouse could initiate a new trial. If the mouse nose-poked the wrong stimulus during the choice phase (case 2: incorrect trial), all the stimuli disappeared, no reward was given to the animal and the house light was switched on for a 10 s time out period. After that, the house light was turned off again, and a correction ITI period (10 s) occurred before the tray light was switched on, after which a correction trial procedure occurred during which the same discriminative stimulus was presented first and the same lateral nose-poke was expected. Correction trials continued until the animal responded correctly to the screen. Finally, if the mouse didn't manage to respond to the screen within the allocated time (case 3: omission in groups 3 and 4 only), the choice stimuli disappeared and no reward was given. A correction ITI period (10 s) followed a time out (10 s) during which the house light was illuminated. As for an incorrect trial, a correction trial procedure started. Importantly, and contrary to correction trials, omissions were counted in the total number of trials completed during the VMCL acquisition phase.

Experiment C: dPAL, sPAL and VMCL in animals previously assessed in touchscreen tasks. The end of experiments A and B coincided with the end of food restriction for all mice. Mice were then left in their cage with food and water *ad libitum* for 3 to 4 weeks. Afterwards, 32 out of the 48 male C57BL/6JRj mice that had been assessed in a first cognitive task were selected to acquire a new rule in the same touchscreen-equipped boxes (see figure 5): n = 8 mice tested in dPAL as task 1; n = 8 mice tested in sPAL as task 1; n = 8 mice tested in VMCL, conditions 2 as task 1; finally, n = 8 mice tested in VMCL, conditions 3 as task 1. We decided to use animals previously trained in conditions 2 and 3 in the VMCL task because those mice displayed really similar learning abilities. To our opinion, and contrary to conditions 1 (no "pretraining") and 4 (different pattern of learning observed), those conditions allowed further comparisons in another touchscreen task.

Selected mice were again food-deprived and then maintained at 85% of their free-feeding weight. After two sessions of re-acclimation to the liquid reward in the home cage, the mice were returned to the operant chambers, starting directly at the "must initiate" stage due to their previous experience in touchscreens. All mice initially trained in variants of the PAL task were then trained in the VMCL task (conditions 2: both "pretraining" and VMCL with no LHT; counterbalanced stimuli as described in experiment B). By comparison, mice first trained in conditions 2 and 3 in the VMCL task started to acquire either the dPAL or the sPAL task (counterbalanced groups).

Data Analysis

Five main parameters were explored: the accuracy (percentage of correct responses), the number of correction trials, correct/incorrect touch (time to nose-poke the correct/incorrect stimulus presented on the screen during the first presentation of stimuli on the screen) and magazine latencies (time to nose-poke into the magazine after giving the correct response on the screen). All collected data were expressed as means ± SEM. Accuracy data were plotted in blocks of 3 or 5 sessions depending on the nature of the task and therefore analyzed using a 2-way ANOVA with repeated measures on time, with a Bonferroni post-hoc analysis. By comparison, all other parameters, as global measures, were submitted to a 1-way ANOVA (VMCL task) with a Bonferroni post-hoc analysis or an unpaired t-test (sPAL or dPAL tasks) to detect any effect of the type of learning or conditions of testing.

Finally, to determine if there was a bias due to the use of discriminative locations in the touchscreen boxes, global measures corresponding to the repartition of correct responses among the 2 (VMCL task) or 6 (sPAL and dPAL tasks) possible trial types were generated from experiments A to C. These measures were analyzed with a 1-way ANOVA (sPAL or dPAL tasks) or with an unpaired t-test (VMCL task). All statistical analyses were performed with GraphPad Prism version 5.0 (GraphPad Software, San Diego, USA) and conducted with a significance level of $p < 0.05$.

Author Contributions

Conceived and designed the experiments: DD AM. Performed the experiments: DD. Analyzed the data: DD. Wrote the paper: DD. Critical revision of the manuscript: DD CM JCC CDC AM. Final approval of the version to be published: DD CM JCC CDC AM.

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❖ Experiment D

In this experiment, we aimed to determine if young C57BL/6JRj animals would be able of assimilating the PVD rule following the presentation of a pair of novel visual stimuli. Effectives were as follows: n=8 mice for which the reinforced conditioned stimulus (CS+) was the Lines during the acquisition phase and the Ring during the reversal learning phase (Lines, then Ring); n=8 mice for which the CS+ was the Ring during the acquisition phase and the Lines during the reversal learning phase (Ring, then Lines). These animals were trained in the PVD task according to the protocol 7 previously described in the Materials & Methods section.

Accuracy – **Figure 19 (panel A)**

During acquisition, repeated measures two-way ANOVA revealed a significant effect of the nature of the rewarded stimulus ($F(1,14)=22.66$; $p<0.001$) and time ($F(11,154)=40.63$; $p<0.0001$) on accuracy parameter. An interaction between these two factors was also found ($F(11,154)=7.94$; $p<0.0001$). *Post-hoc* Bonferroni analyses showed a significant difference between both groups from session 1 ($t(168)=7.14$; $p<0.0001$) to session 4 ($t(168)=3.87$; $p<0.01$).

Similarly, repeated measures two-way ANOVA revealed a significant effect of the nature of the rewarded stimulus ($F(1,14)=49.85$; $p<0.0001$) and time ($F(15,210)=68.41$; $p<0.0001$) on accuracy parameter during the reversal learning phase. An interaction between these two factors was also found ($F(15,210)=4.27$; $p<0.0001$). *Post-hoc* Bonferroni analyses showed a significant difference between both groups from session 16 ($t(224)=3.21$; $p<0.05$) to session 21 ($t(224)=3.29$; $p<0.05$).

Number of correction trials – **Figure 19 (panel B)**

During acquisition, repeated measures two-way ANOVA revealed a significant effect of the nature of the rewarded stimulus ($F(1,14)=9.53$; $p<0.01$) and time ($F(11,154)=16.80$; $p<0.0001$) on the number of correction trials. An interaction between these two factors was also found ($F(11,154)=3.72$; $p=0.0001$). *Post-hoc* Bonferroni analyses showed a significant difference between both groups from session 1 ($t(168)=4.74$; $p<0.0001$) to session 3 ($t(168)=4.05$; $p<0.0001$).

Similarly, repeated measures two-way ANOVA revealed a significant effect of the nature of the rewarded stimulus ($F(1,14)=36.00$; $p<0.0001$) and time ($F(15,210)=106.1$; $p<0.0001$) on

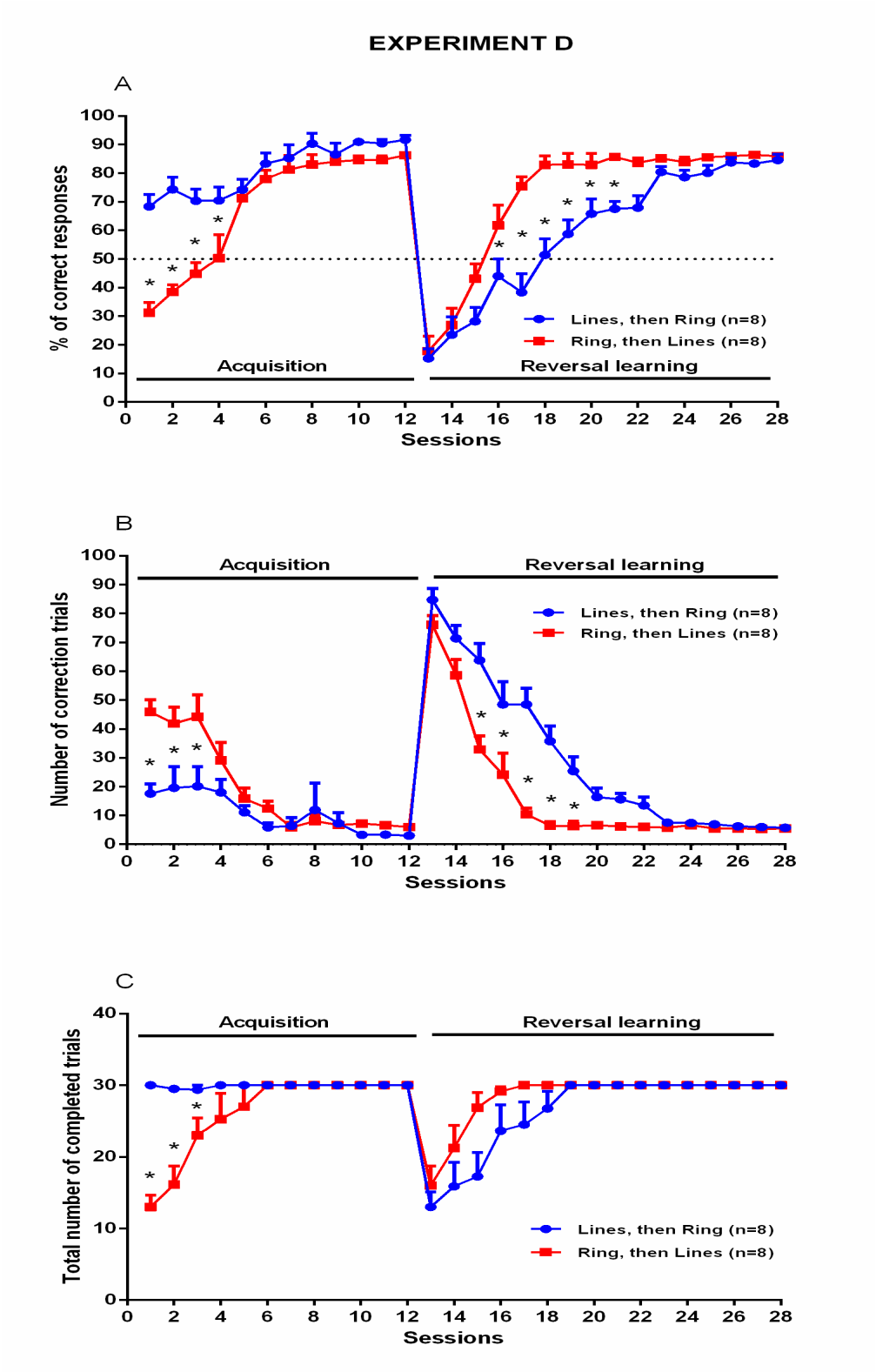


Figure 19. Percentage of correct responses (panel A), number of correction trials (panel B) and total number of completed trials (panel C) measured in mice trained in the PVD task (experiment D, protocol 7). * $p<0.05$ between groups.

the number of correction trials during the reversal learning phase. An interaction between these two factors was also found ($F(15,210)=7.56$; $p<0.0001$). *Post-hoc* Bonferroni analyses showed a significant difference between both groups from session 15 ($t(224)=6.40$; $p<0.0001$) to session19 ($t(224)=3.95$; $p<0.01$).

Total number of completed trials – **Figure 19 (panel C)**

During acquisition, repeated measures two-way ANOVA revealed a significant effect of the nature of the rewarded stimulus ($F(1,14)=31.11$; $p<0.0001$) and time ($F(11,154)=12.24$; $p<0.0001$) on the total number of completed trials. An interaction between these two factors was also found ($F(11,154)=11.38$; $p<0.0001$). *Post-hoc* Bonferroni analyses showed a significant difference between both groups from session 1 ($t(168)=9.52$; $p<0.0001$) to session 3 ($t(168)=3.57$; $p<0.01$).

Repeated measures two-way ANOVA revealed a significant effect of time ($F(15,210)=22.18$; $p<0.0001$) on the total number of completed trials during the reversal learning phase. However, there was no effect of the nature of the rewarded stimulus on this parameter ($F(1,14)=4.07$; $p=0.06$, n.s.). An interaction between these two factors was found ($F(15,210)=2.21$; $p<0.01$).

As demonstrated by the different parameters, training conditions defined within protocol 7 allowed measuring significant learning during both acquisition and reversal learning phases of the PVD task in mice. Nevertheless, the speed of learning depended on the nature of the reinforced stimulus, with a marked preference for the stimulus Lines.

❖ Experiment E

Following the failure of previous training conditions to induce a balanced learning within the different subgroups evaluated, animals were this time trained in the PVD task according to the protocol 8 previously described in the Materials & Methods section. In this experiment, effectives were as follows: n=7 mice (Lines, then Ring); n=8 mice (Ring, then Lines).

Accuracy – **Figure 20 (panel A)**

During acquisition, repeated measures two-way ANOVA revealed a significant effect of the nature of the rewarded stimulus ($F(1,13)=13.13$; $p<0.01$) and time ($F(10,130)=15.64$; $p<0.0001$) on accuracy parameter. There was no interaction between these two factors ($F(10,130)=1.04$; $p>0.05$). *Post-hoc* Bonferroni analyses showed a significant difference between both groups for session 2 ($t(143)=3.00$; $p<0.05$), session 5 ($t(143)=3.90$; $p<0.01$) and session 8 ($t(143)=3.32$; $p<0.05$).

Repeated measures two-way ANOVA revealed a significant effect of the nature of the rewarded stimulus ($F(1,13)=9.41$; $p<0.01$) and time ($F(11,143)=46.10$; $p<0.0001$) on accuracy parameter during the reversal learning phase. An interaction between these two factors was also found ($F(11,143)=4.53$; $p<0.0001$). *Post-hoc* Bonferroni analyses showed a significant difference between both groups from session 13 ($t(156)=4.15$; $p=0.0001$) to session 16 ($t(156)=3.47$; $p<0.01$).

Number of correction trials – **Figure 20 (panel B)**

During acquisition, repeated measures two-way ANOVA revealed a significant effect of the nature of the rewarded stimulus ($F(1,13)=11.36$; $p<0.01$) and time ($F(10,130)=11.27$; $p<0.0001$) on the number of correction trials. There was no interaction between these two factors ($F(10,130)=0.79$; $p>0.05$). *Post-hoc* Bonferroni analyses showed a significant difference between both groups during session 1 ($t(143)=2.92$; $p<0.05$) and session 2 ($t(143)=3.38$; $p<0.05$).

Repeated measures two-way ANOVA revealed a significant effect of time ($F(11,143)=84.43$; $p<0.0001$) on the number of correction trials during the reversal learning phase. However, there was no effect of the nature of the rewarded stimulus on this parameter ($F(1,13)=3.99$; $p=0.07$, n.s.). An interaction between the two factors was found ($F(11,143)=3.80$; $p<0.0001$).

Total number of completed trials – **Figure 20 (panel C)**

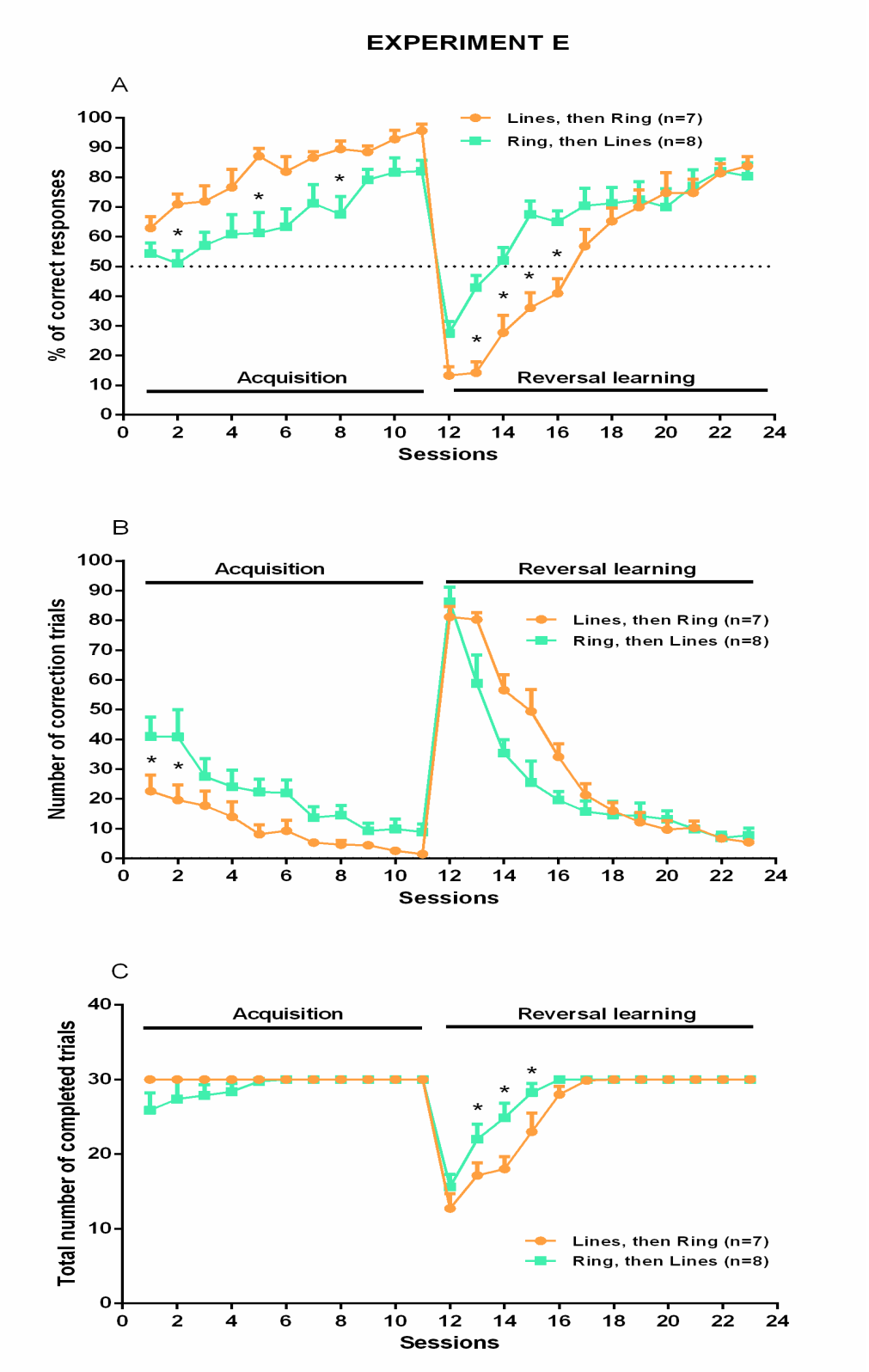


Figure 20. Percentage of correct responses (panel A), number of correction trials (panel B) and total number of completed trials (panel C) measured in mice trained in the PVD task (experiment E, protocol 8). * $p<0.05$ between groups.

During acquisition, repeated measures two-way ANOVA revealed no significant effect of the nature of the rewarded stimulus ($F(1,13)=2.37$; $p>0.05$) nor time ($F(10,130)=1.54$; $p>0.05$) on the total number of completed trials. Furthermore, there was no interaction between these two factors ($F(10,130)=0.13$; $p>0.05$).

By contrast, repeated measures two-way ANOVA revealed a significant effect of the nature of the rewarded stimulus ($F(1,13)=7.92$; $p<0.05$) and time ($F(11,143)=53.76$; $p<0.0001$) on the total number of completed trials during the reversal learning phase. An interaction between these two factors was also found ($F(11,143)=2.98$; $p<0.01$). *Post-hoc* Bonferroni analyses showed a significant difference between both groups from session 13 ($t(156)=3.14$; $p<0.05$) to session 15 ($t(156)=3.39$; $p<0.05$).

As for experiment D, training conditions defined within experiment E allowed measuring significant learning during both acquisition and reversal learning phases of the PVD task in mice. Because a stimulus bias was again observable, we introduced a new training procedure in experiment F.

❖ Experiment F

Experiments D and E had failed to show unbiased learnings in groups trained with different rewarded stimuli. In this experiment, we therefore sought to evaluate the impact of the size (5.5 cm^2 instead of 6.5 cm^2) of these discriminative stimuli on the acquisition of the PVD task. Effectives were as follows: n=8 mice (Lines, then Ring); n=8 mice (Ring, then Lines). These animals were trained according to the protocol 9 previously described in the Materials & Methods section.

Accuracy – **Figure 21 (panel A)**

During acquisition, repeated measures two-way ANOVA revealed a significant effect of the nature of the rewarded stimulus ($F(1,14)=6.98$; $p<0.05$) and time ($F(9,126)=26.38$; $p<0.0001$) on accuracy parameter. There was no interaction between these two factors ($F(9,126)=1.21$; $p>0.05$). Moreover, *post-hoc* Bonferroni analyses failed to find a significant difference between groups, whatever the session considered.

Repeated measures two-way ANOVA revealed a significant effect of time ($F(11,154)=68.84$; $p<0.0001$) on accuracy parameter during the reversal learning phase. However, there was no effect of the nature of the rewarded stimulus on this parameter ($F(1,14)=0.49$; $p>0.05$). No interaction between these two factors was found ($F(11,154)=0.67$; $p>0.05$).

Number of correction trials – **Figure 21 (panel B)**

During acquisition, repeated measures two-way ANOVA revealed a significant effect of the nature of the rewarded stimulus ($F(1,14)=13.90$; $p<0.01$) and time ($F(9,126)=25.30$; $p<0.0001$) on the number of correction trials. There was no interaction between these two factors ($F(9,126)=1.94$; $p=0.052$, n.s.). *Post-hoc* Bonferroni analyses showed a significant difference between both groups during session 1 ($t(140)=2.93$; $p<0.05$) and session 2 ($t(140)=4.44$; $p=0.0001$).

Repeated measures two-way ANOVA revealed a significant effect of time ($F(11,154)=109.1$; $p<0.0001$) on the number of correction trials during the reversal learning phase. However, there was no effect of the nature of the rewarded stimulus on this parameter ($F(1,14)=1.05$; $p>0.05$). There was no interaction between these two factors ($F(11,154)=1.44$; $p>0.05$).

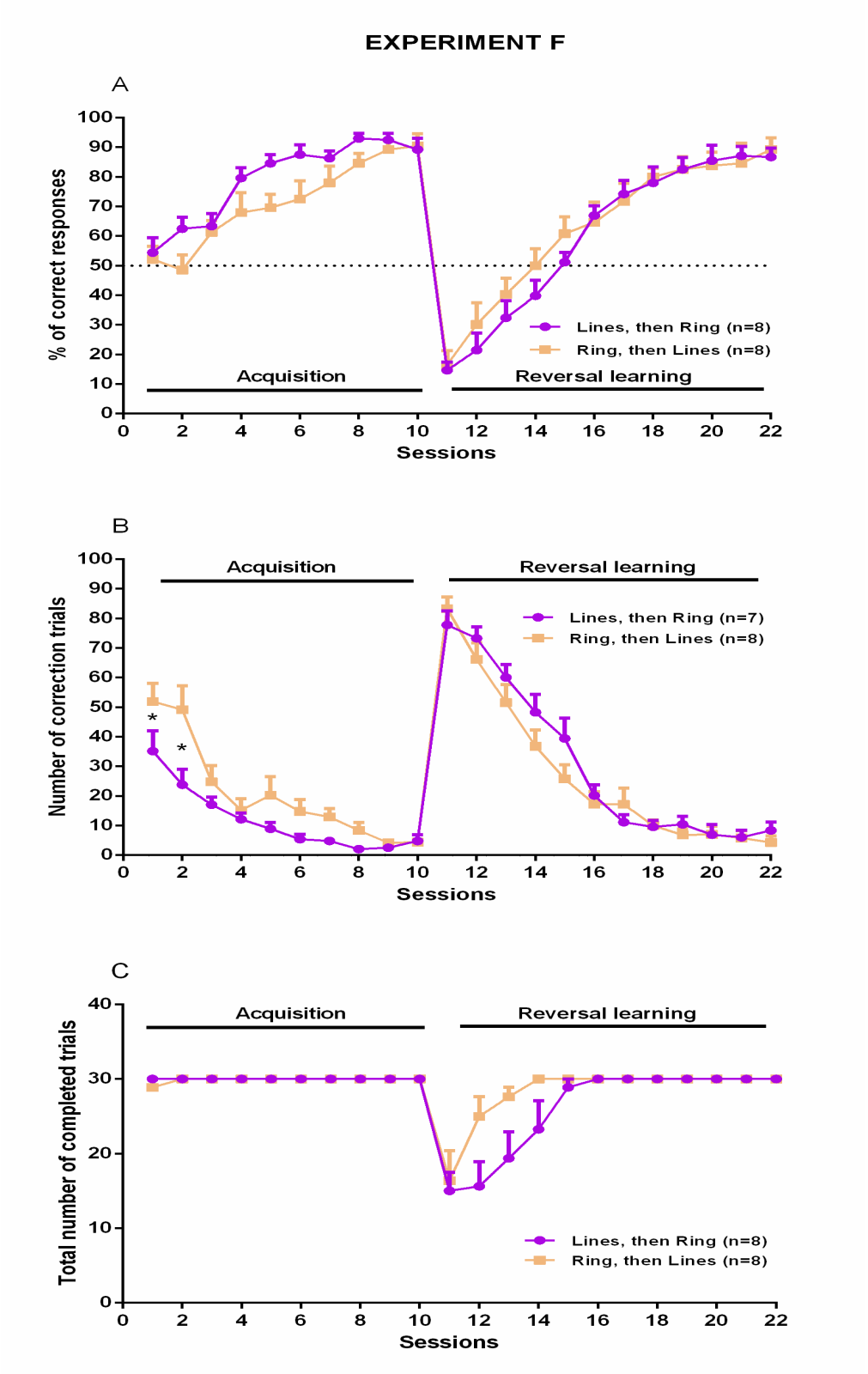


Figure 21. Percentage of correct responses (panel A), number of correction trials (panel B) and total number of completed trials (panel C) measured in mice trained in the PVD task (experiment F, protocol 9). * $p<0.05$ between groups.

Total number of completed trials – **Figure 21 (panel C)**

During acquisition, repeated measures two-way ANOVA revealed no effect of the nature of the rewarded stimulus ($F(1,14)=1.00$; $p>0.05$) nor time ($F(9,126)=1.00$; $p>0.05$) on the total number of completed trials. No interaction between factors was found ($F(9,126)=1.00$; $p>0.05$).

Repeated measures two-way ANOVA revealed a significant effect of time ($F(11,154)=19.00$; $p<0.0001$) on the total number of completed trials during the reversal learning phase. However, there was no effect of the nature of the rewarded stimulus on this parameter ($F(1,14)=3.50$; $p=0.08$, n.s.). An interaction was found between these two factors ($F(11,154)=2.70$; $p<0.01$).

Phase	Acquisition		Reversal Learning	
CS+ stimulus	Lines	Ring	Lines	Ring
CTL	2.21 ± 0.12	2.31 ± 0.20	2.80 ± 0.33	2.80 ± 0.13
ITL	2.07 ± 0.10	2.24 ± 0.24	2.77 ± 0.37	2.50 ± 0.13
ML	1.24 ± 0.04	1.42 ± 0.05 *	1.35 ± 0.04	1.56 ± 0.14

Table 11. Mean latencies (in s) recorded in mice trained in the PVD task (experiment F, protocol 9). ***** $p<0.05$ *vs* group Lines during the same stage of training. CS+ stimulus: reinforced stimulus; CTL: Correct Touch Latency; ITL: Incorrect Touch Latency; ML: Magazine Latency.

Correct touch, incorrect touch and magazine latencies – **Table 11**

According to unpaired *t*-tests, there was no main effect of the nature of the rewarded stimulus on correct touch latency during acquisition ($t(14)=0.41$; $p>0.05$) or reversal learning ($t(14)=0.01$; $p>0.05$) phases. On a comparable basis, no significant effect of the nature of the reinforced stimulus was found for incorrect touch latency during acquisition ($t(14)=0.65$; $p>0.05$) or reversal learning ($t(14)=0.68$; $p>0.05$) phases. Analysis of the magazine latency

revealed a significant effect of the nature of the rewarded stimulus during acquisition ($t(14)=3.02$; $p<0.01$) but not reversal learning ($t(14)=1.48$; $p>0.05$).

In this experiment, minimal variations were observed through the different parameters measured, whatever the nature of the reinforced stimulus. We therefore selected protocol 9 for further studies.

❖ Intermediary discussion

An overview of main results described in this chapter is presented below (**Table 12**). After a global summary, we will tackle each task individually to compare our data with the existing literature.

Global outcomes

From a general standpoint, we managed to define or adapt testing conditions that all resulted in significant learnings over time in 3 cognitive tasks of interest: the PAL, VMCL and PVD tasks. Furthermore, our different methodological approaches conclusively proved the usefulness of the diluted condensed milk as an appropriate alternative to the food pellets. Indeed, if most of paradigms dealing with instrumental conditioning are historically based on food reward, mice trained in touchscreen chambers showed outstanding reaction times (correct and incorrect touch latencies < 3 s) and demonstrated an intact motivation (magazine latencies < 2 s; total number of completed trials per session), whatever the task considered. Eventually, results from experiment A, B and F (dPAL, VMCL and PVD tasks, respectively) confirmed for the first time the absence of preference for specific visual stimuli in each touchscreen assay selected for following studies.

Experiment A: object-place associations in dPAL or sPAL tasks

The first attempt to import the PAL task from Humans to rodents through a similar touchscreen technology had been proposed by (Talpos et al., 2009). In this publication, he introduced in rats two variants of the task that only differed by the nature of the two objects simultaneously displayed on the screen. On one hand, different stimuli (dPAL task) appeared in 2 windows when a new trial started. On the other hand, the initiation of a new trial coincided with the appearance of 2 similar stimuli (sPAL task). The former task was reported to be more demanding than the latter in rats. In experiment A, we validated a comparable effect in our mice. In point of fact, animals trained in the dPAL task (protocol 1) acquired the inherent rule slower than their littermates trained in the sPAL task (protocol 2), ultimately reaching 70 % (against 85 %) of correct responses after a total of 50 sessions. Concerning the first variant of the task, our results were also in accordance with more recent data obtained in mice (Clelland et al., 2009; Bartko et al., 2011b; Nithianantharajah et al., 2013). As protocol 1 was the nearest from the human counterpart of the PAL task, we applied it for subsequent studies of visuo-spatial function performed in transgenic and lesioned animals.

Experiment	Task	Protocol N°	% correct (final)	Total N° of sessions	Latencies	Stimulus preference	Conditions validated?
A	dPAL	1	~ 70 %	50	< 3 s	No	Yes
	sPAL	2	~ 85 %			No	/
B	VMCL	3-6	~ 90 %	30	< 3 s	No	Yes
C	dPAL	1	~ 70 %	50	< 3 s	No	/
	sPAL	2	~ 65 %	50			/
	VMCL	4	~ 85 %	30			/
D	PVD	7	~ 80-85 %	12 (A) 15 (RL)	n/d	Yes	No
E	PVD	8	~ 80-90 %	11 (A) 12 (RL)	n/d	Yes	No
F	PVD	9	~ 80-85 %	10 (A) 12 (RL)	< 3 s	No	Yes

Table 12. Summary of the principal results obtained in the different touchscreen tasks during optimization studies performed in young (3-4 months of age when placed under food-restricted regimen) male C57BL/6JRj mice. n/d: not determined; (A): acquisition of the PVD task; (RL): reversal learning of the PVD task.

Experiment B: enthronement of the VMCL task in mice

First efforts to develop the VMCL task in rodents dated back 25 years. In an ancestoral version of the task involving the presentation of slow *vs* fast flashing lights followed by specific lever responses (Robbins et al., 1990; Reading et al., 1991), rats quickly learned stimulus-response associations. This paradigm was later successfully relieved in a touchscreen environment (Bussey et al., 1997b; Chudasama et al., 2001) where animals had to associate visual stimuli with lateral nose-pokes according to a conditional rule ("If stimulus A appears, then nose-poke the left window; if stimulus B appears, then nose-poke the right window"). Probably due to unsuited training conditions (see the discussion section of publication 1 for details) preliminary studies in mice only revealed 70 % of accuracy after 900 trials, which contrasted with the performance of rats reaching 90 % after only 600 trials. We therefore modified the testing conditions in experiment B in order to facilitate the acquisition of this task. Unlike rats, the central stimulus did not disappear during each choice phase in mice, alleviating the mnesic component of the task. Besides, other valuable elements such as the shortening of the ITI duration, implementation of a pre-training phase or integration of limited holding times contributed to significantly improve the task acquisition in mice assessed with protocols 3 to 6 (90 % of accuracy after 15-20 sessions of 30 trials). Protocol 4, which combined a pre-training stage but no limited holding time during the main task, was therefore chosen for subsequent evaluations of transgenic and lesioned mice.

Experiment C: building a battery of touchscreen tasks

Over the last decades, batteries of behavioral tasks have progressively become widespread in rodents, primarily influenced by the establishment of gold standards in animal experimentation (3Rs: Replacement, Reduction and Refinement; Russell and Burch, 1959) or more practical reasons (*e.g.* elevated costs associated with the breeding of transgenic animals). A consensual idea prevailed, according to which the organization of the global procedure had to take into account the degree of stress induced by each behavioral test. Yet, it is noteworthy that the parallel effect of training history on subsequent performance had rarely been examined (McIlwain et al., 2001; Voikar et al., 2004). Among the strongest advantages of the touchscreen-based methodology figured the homogeneity of presented stimuli, expected responses and associated rewards in all developed paradigms (Bussey et al., 2012; Romberg et al., 2013; Nithianantharajah and Grant, 2013). These comparable aspects theoretically favored the possibility to successively evaluate the same animals in

touchscreen tasks targeting distinct learning and memory functions. Thus, experiment C aimed to verify the feasibility of combining tasks previously optimized in the framework of experiments A and B. To this end, mice pre-trained in the VMCL task later acquired the dPAL task as quickly as naive animals (70 % of correct responses after 50 sessions). Moreover, mice pre-trained in the dPAL or sPAL variants learned the VMCL rule (85-90 % of correct responses after 20 sessions) in a comparable manner to naive animals. More surprising was the finding, however, that animals pre-trained in the VMCL task later struggled to learn the sPAL task (65 % of correct responses after 50 sessions) compared with naive animals. These results had several implications. First, a deficit of acquisition was only observed when certain cognitive assays were performed in a specific order, which indicated the necessity to assess animals with paradigms of decreasing difficulty (sPAL task followed by VMCL task) to minimize the risk of task interactions. Second, the appearance of memory interference after the successive learning of 2 cognitive assays suggested that common neural substrates were conceivably involved in these respective paradigms. In this context, post-training intra-hippocampal pharmacological manipulations had showed no effect on sPAL performance in rats (Talpos et al., 2009). Our main hypothesis was that similarly to the VMCL task, and notwithstanding its object-place properties, the sPAL task depended on the integrity of the striatum in mice as it could be solved via a conditional rule of the type ‘‘If stimulus A appears, then choose location 1; if stimulus B appears, then choose location 2; if stimulus C appears, then choose location 3”. In parallel, our results confirmed the possibility to measure acquisition performances in dPAL and VMCL tasks in mice and to combine them to evaluate cognitive impairment in a battery of tests taxing distinct forms of learning and memory.

Experiments D-F: importance of training conditions in the PVD task

Because the PVD assay was one of the first touchscreen tasks and had been extensively adapted in mice (Bussey et al., 2001; Morton et al., 2006; Horner et al., 2013), our initial training conditions (protocol 7) were close to those usually defined to measure visual memory (acquisition phase) and behavioral flexibility (reversal learning phase) within that species. Adaptations concerned the maximal number of trials per session, the duration of the ITI and the nature and size of newly introduced equiluminescent stimuli (Ring and Lines). Whilst such changes are generally susceptible to affect the way animals learn a given rule (Bussey et al., 2008), the variations imposed by our own protocol did not prevent animals from learning the task during experiment D (80-85 % of accuracy after 12 sessions of acquisition or 15 sessions of reversal learning). Even so, mice exposed a clear preference

for the Lines stimulus in both learning phases. In order to get rid of that stimulus bias, we tried to modify the protocol. In spite of significant learnings (80-90 % of accuracy after 11 sessions of acquisition or 12 sessions of reversal learning), experiment E (protocol 8) ended in failure for the same reason after the substitution of classical pokey training stages by magazine training, RRTTS and RSRTTS stages. Eventually, in experiment F, a new batch of animals was tested as in protocol 8 except that the size of the Ring and Lines stimuli was reduced (5.5 cm^2 instead of 6.5 cm^2). These training conditions (protocol 9) gave rise to satisfactory improvements of learning performance (80-85 % of accuracy after 10 sessions of acquisition or 12 sessions of reversal learning) in the absence of stimulus preference and were therefore used during the evaluation of transgenic mice.

Chapter 6: Behavioral characterization of a murine transgenic model of Alzheimer's Disease

After the validation of appropriate training conditions for the 3 touchscreen tasks of interest (dPAL, VMCL and PVD tasks), we have focused our attention on one of the most common transgenic models of Alzheimer's Disease, the Tg2576 mouse (Hsiao et al., 1996). As extensively reviewed in chapter 2, transgenic animals of this murine line were known to carry out a "Swedish" double mutation (K670N / M671L) accounting for certain EOFAD cases in Humans and resulting in an aberrant cleavage of APP and exaggerated production of Aß throughout life. This single molecular abnormality had important neuropathological, behavioral and electrophysiological consequences. First, Tg2576 mice started accumulating cerebral Aß under soluble monomeric and oligomeric forms from 6 months of age (Kawarabayashi et al., 2001). Amyloid plaques, mainly localized in the hippocampus and cortex of mice, appeared between 9 and 12 months of age (Lee and Han, 2013), other hallmarks including gliosis, astrocytosis and dystrophic neurites. Second, these mice displayed age-dependent cognitive deficits, notably in tasks appealing to spatial or relational forms of memory (Westerman et al., 2002; Arendash et al., 2004; Good and Hale, 2007; Yassine et al., 2013) as well as executive functions (Zhuo et al., 2007; Zhuo et al., 2008). Third, synaptic defects (basal transmission and LTP) were also quantifiable as early as 4-5 months of age in the hippocampus (Jacobsen et al., 2006).

Given the specific nature of cognitive disturbances noticed in this transgenic line, evaluation of WT and TG animals in our innovative touchscreen tasks was obviously of great interest. However, taking heed to some essential aspects, among which their *rd* status, turned out necessary to correctly interprete our results. Indeed, a previous study realized within the laboratory (Yassine et al., 2013) had revealed the selective effects of this factor on acquisition of certain learning tasks (such as the Morris Water Maze) where the use of visual cues could not be compensated by other modalities. We therefore controlled by PCR the *rd* status of each Tg2576 mouse after behavioral testing and excluded blind mice. Additionally, some animals of both genotypes that exhibited extreme levels of stereotypical activity in touchscreen chambers were also discarded.

Relying on an approach integrating behavioral and molecular methods, we have realized a total of 8 studies implicating this mouse model. In pilot experiments (A and B), distinct groups

of young and aged WT mice were first tested in the dPAL or VMCL tasks. This allowed confirming the possibility to measure different aspects of cognition in WT mice of various ages bred on a C57BL/6 x SJL/J background. Following these encouraging results, we similarly trained different batches of young (experiments C and E) and aged (experiments D and F) WT and TG animals in dPAL or VMCL paradigms. Furthermore, mice that had successfully learned the VMCL task within experiments E and F were thereafter assessed in the PVD task (experiments G and H), notably to investigate their behavioral flexibility during the reversal learning phase. Corresponding data are presented in the pages below.

❖ Experiment A

In this first pilot experiment, we aimed to determine whether WT mice of the Tg2576 line were capable of learning the dPAL task. Initial effectives were as follows: n=12 and n=4 mice, respectively aged of 5-6 and 13-14 months when the food restriction started. 3 mice genotyped for the *rd* mutation were excluded, final effectives were therefore composed of n=10 young and n=3 aged mice.

Accuracy – **Figure 22 (panel A)**

Repeated measures two-way ANOVA on accuracy parameter revealed a main effect of time ($F(9,99)=8.76$; $p<0.0001$), but no group effect ($F(1,11)=0.46$; $p>0.05$) nor interaction between time and group factors ($F(9,99)=0.41$; $p>0.05$) during acquisition of the task.

Total number of completed trials – **Figure 22 (panel B)**

Repeated measures two-way ANOVA on the total number of completed trials revealed a main effect of time ($F(9,99)=4.48$; $p<0.0001$), but no group effect ($F(1,11)=1.57$; $p>0.05$) nor interaction between time and group factors ($F(9,99)=1.21$; $p>0.05$) during acquisition of the task.

Number of correction trials – **Figure 22 (panel C)**

As for the two previous parameters, repeated measures two-way ANOVA on the number of correction trials revealed a main effect of time ($F(9,99)=19.50$; $p<0.0001$), but no group effect ($F(1,11)=2.68$; $p>0.05$) nor interaction between time and group factors ($F(9,99)=0.74$; $p>0.05$) during acquisition of the task.

Rd status and accuracy – **Figure 22 (panel D)**

All animals (n=3) carrying the *rd* mutation subsequently identified by PCR presented over time fluctuating performances around the chance threshold (~50 % of correct responses). This contrasted with the progressive learning measured in sighted animals.

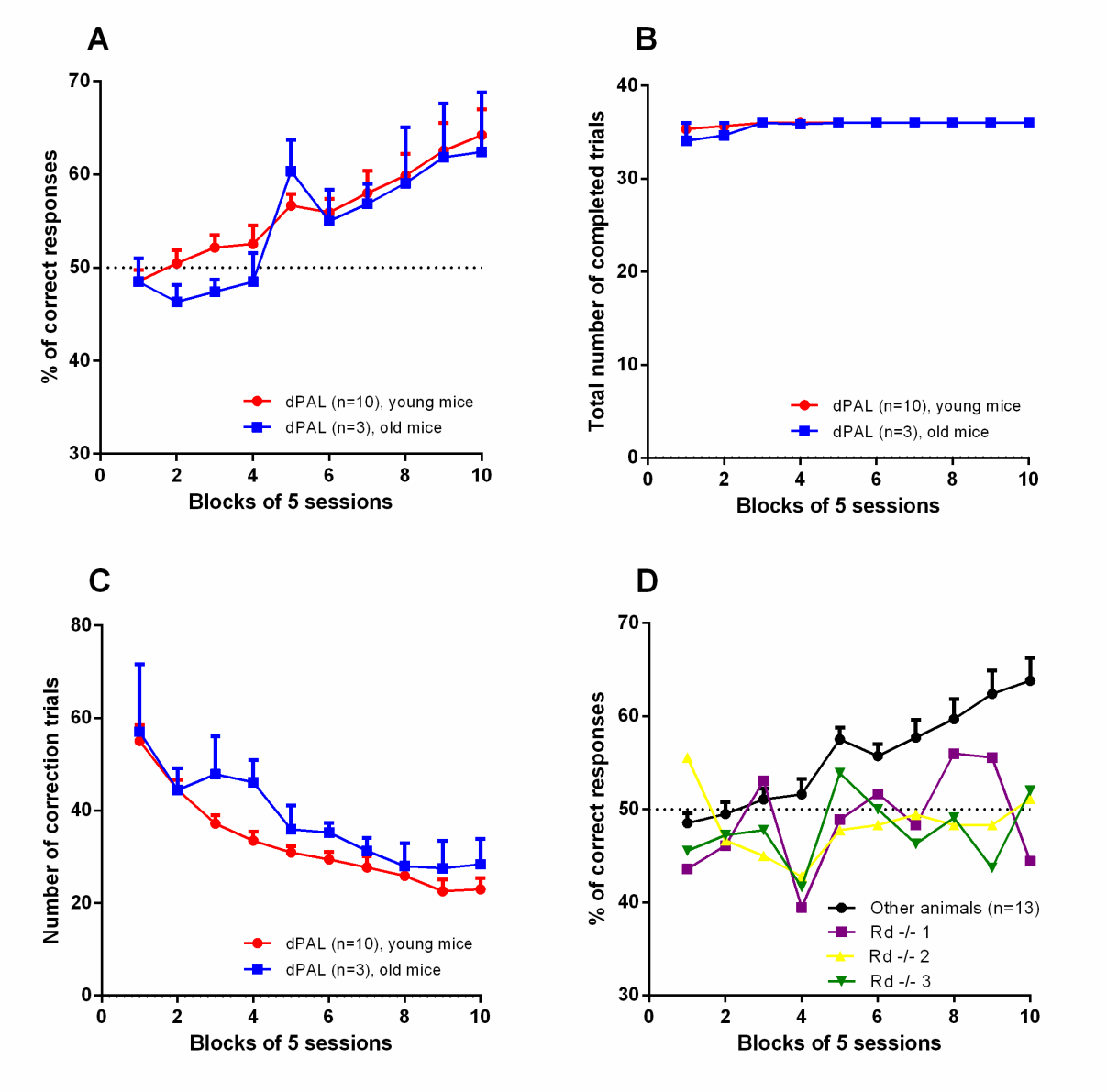


Figure 22. Percentage of correct responses (panel A), total number of completed trials (panel B) and number of correction trials (panel C) measured in WT mice of the Tg2576 line in the dPAL task (experiment A). For the sake of comparison, individual performances of blind animals (*rd* -/-) are displayed in panel D.

Young and aged WT mice of the Tg2576 line were able to learn the dPAL task in touchscreen chambers. Moreover, animals of both ages were motivated to perform the task. However, the *rd* mutation deeply affected the learning of the rule. Thus, blind mice could not discriminate visual stimuli and presented no learning improvement over time.

❖ Experiment B

In this second pilot experiment, we examined whether WT mice of the Tg2576 line were capable of learning the VMCL task. Initial effectives were as follows: n=11 and n=4 mice, respectively aged of 5-6 and 13-14 months when the food restriction started. Again, 3 mice genotyped for the *rd* mutation were excluded, final effectives were therefore composed of n=8 young and n=4 aged mice.

Accuracy – **Figure 23 (panel A)**

Repeated measures two-way ANOVA on accuracy parameter revealed a main effect of time ($F(9,90)=22.55$; $p<0.0001$) and group ($F(1,10)=6.30$; $p<0.05$), accompanied by an interaction between time and group factors ($F(9,90)=2.89$; $p<0.01$) during acquisition of the task. *Post-hoc* Bonferroni analysis showed a significant difference between groups during blocks 4 ($t(100)=2.97$; $p<0.05$) and 7 ($t(100)=3.75$; $p<0.01$).

Total number of completed trials – **Figure 23 (panel B)**

Repeated measures two-way ANOVA on the total number of completed trials also revealed a main effect of time ($F(9,90)=7.71$; $p<0.0001$) and group ($F(1,10)=5.11$; $p<0.05$), accompanied by an interaction between time and group factors ($F(9,90)=3.45$; $p<0.01$) during acquisition of the task. *Post-hoc* Bonferroni analysis showed a significant difference between groups during blocks 3 ($t(100)=3.61$; $p<0.01$) and 7 ($t(100)=3.69$; $p<0.01$).

Number of correction trials – **Figure 23 (panel C)**

As for the two previous parameters, repeated measures two-way ANOVA on the number of correction trials also revealed a main effect of time ($F(9,90)=15.02$; $p<0.0001$). There was no group effect ($F(1,10)=2.92$; $p>0.05$), despite an interaction between time and group factors ($F(9,90)=2.89$; $p<0.01$) during acquisition of the task.

Rd status and accuracy – **Figure 23 (panel D)**

All animals (n=3) carrying the *rd* mutation subsequently identified by PCR presented over time fluctuating performances around the chance threshold (~50 % of correct responses). As in experiment A, this contrasted with the gradual learning measured in sighted animals.

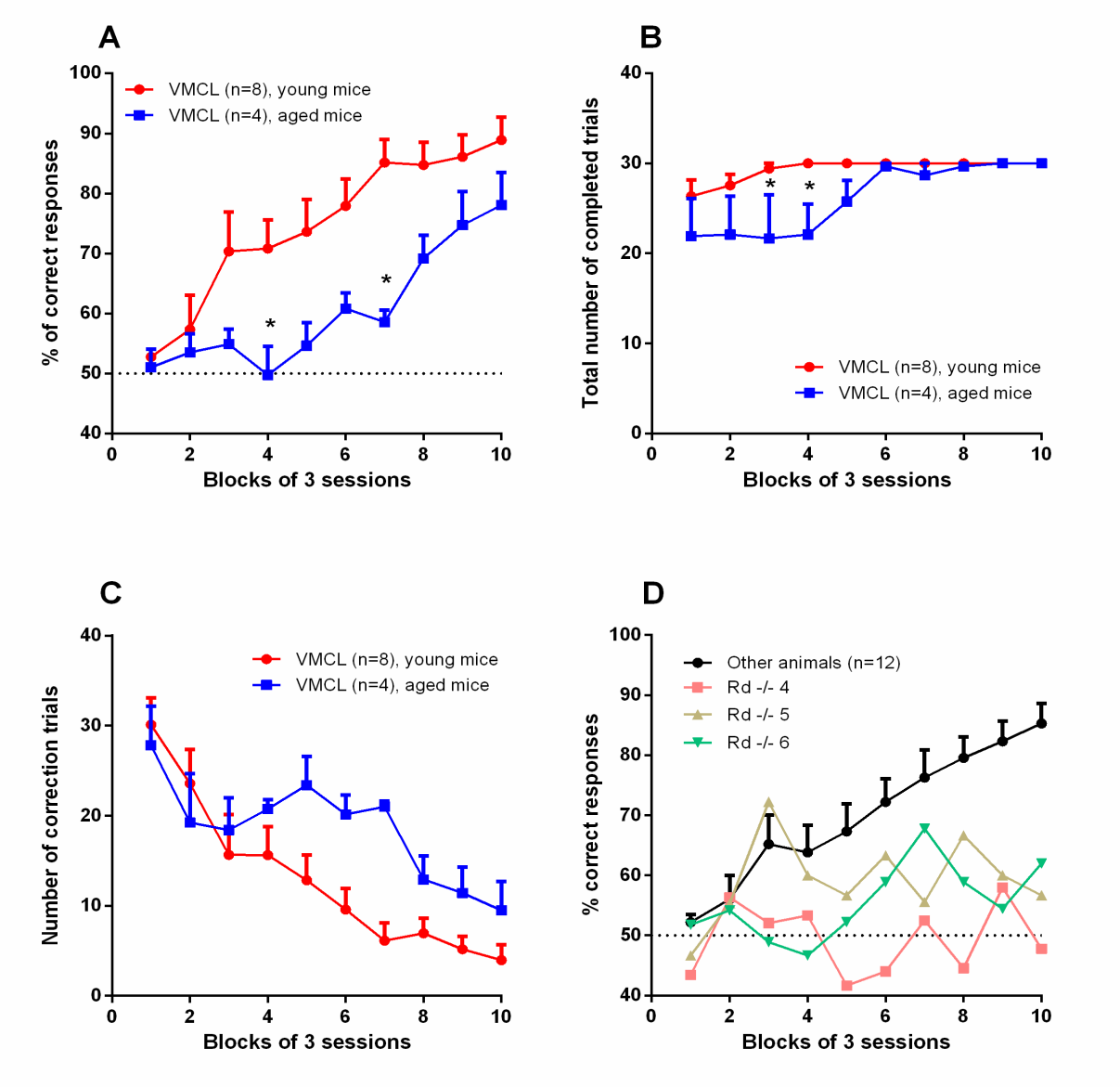


Figure 23. Percentage of correct responses (panel A), total number of completed trials (panel B) and number of correction trials (panel C) measured in WT mice of the Tg2576 line in the VMCL task (experiment B). For the sake of comparison, individual performances of blind animals (*rd* -/-) are displayed in panel D. * p<0.05 between groups.

Young and aged WT mice of the Tg2576 line were able to learn the VMCL task in touchscreen chambers. Moreover, animals of both ages were motivated to perform the task. However, the *rd* mutation deeply affected the learning of the rule. Thus, blind mice could not discriminate visual stimuli and presented no learning improvement over time. The task was also acquired differently by sighted animals, with young WT mice conceivably learning quicker than old ones.

❖ Preamble to experiments C-H

As specified above, preliminary experiments A and B demonstrated the possibility to assess young and aged WT animals of the tg2576 line in dPAL and VMCL tasks. Consequently, we initiated a serie of studies (experiments C-H) to investigate the parallel acquisition of these tasks just as the PVD assay in young and aged WT or TG animals of the same transgenic line. In addition to its *rd* status, we qualitatively took into consideration the general behavior of each mouse to eliminate, when necessary, animals that showed excessive stereotypies (primarily intensive wall-climbing and circling behaviors; see (Kelley, 2001) in touchscreen chambers). Indeed, a few WT and TG mice were disabled by the continuous presence of such repetitive movements and performed poorly in tasks of interest.

❖ Experiment C

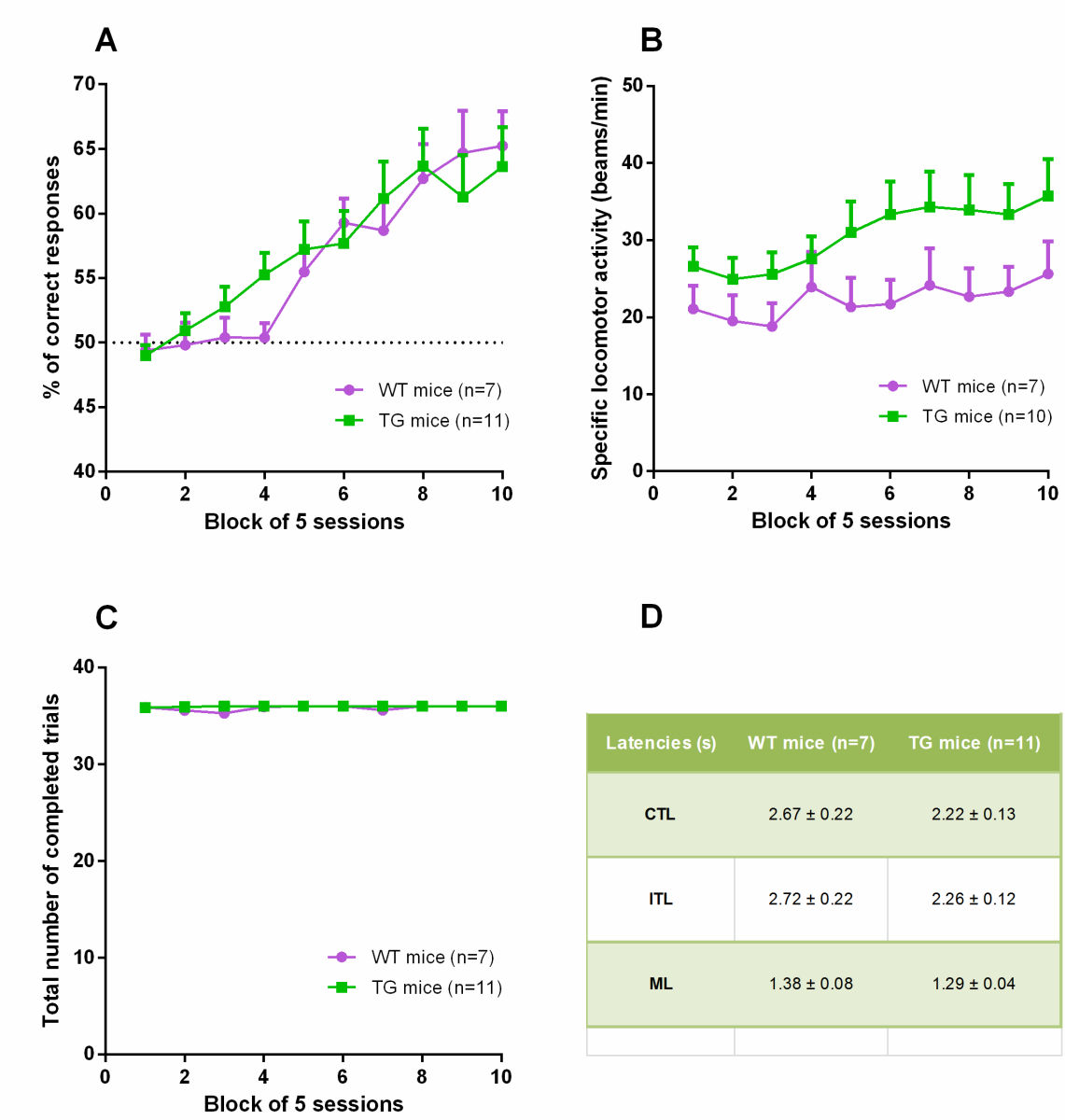
In this first experiment, we examined whether young WT and TG mice of the Tg2576 line exhibited similar or different cognitive capacities in the dPAL task. Initial effectives were as follows: n=10 WT and n=13 TG aged of 5 months when the food restriction started. A total of 5 mice (*rd* mutation: 3 WT and 2 TG) were excluded. Final effectives were therefore composed of n=7 WT and n=11 TG mice.

Accuracy – **Figure 24 (panel A)**

Repeated measures two-way ANOVA on accuracy parameter revealed a main effect of time ($F(9,144)=17.58$; $p<0.0001$), but no genotype effect ($F(1,16)=0.09$; $p>0.05$) nor interaction between time and genotype factors ($F(9,144)=0.80$; $p>0.05$) during acquisition of the task.

Specific locomotor activity – **Figure 24 (panel B)**

Repeated measures two-way ANOVA on the specific locomotor activity revealed a main effect of time ($F(9,135)=4.47$; $p<0.0001$), but no genotype effect ($F(1,15)=3.01$; $p>0.05$) nor interaction between time and genotype factors ($F(9,135)=1.03$; $p>0.05$) during acquisition of the task.



Latencies (s)	WT mice (n=7)	TG mice (n=11)
CTL	2.67 ± 0.22	2.22 ± 0.13
ITL	2.72 ± 0.22	2.26 ± 0.12
ML	1.38 ± 0.08	1.29 ± 0.04

Figure 24. Percentage of correct responses (panel A), specific locomotor activity (panel B), total number of completed trials (panel C) and mean latencies (panel D) measured in young (5-8 months old) WT and TG mice of the Tg2576 line in the dPAL task (experiment C). CTL: Correct Touch Latency; ITL: Incorrect Touch Latency; ML: Magazine Latency.

Total number of completed trials – **Figure 24 (panel C)**

Repeated measures two-way ANOVA on the total number of completed trials revealed a main effect of time ($F(9,144)=2.88$; $p<0.01$) but no genotype effect ($F(1,16)=2.81$; $p>0.05$). There was however an interaction between time and genotype factors ($F(9,144)=2.75$; $p<0.01$) during acquisition of the task.

Correct touch, incorrect touch and magazine latencies – **Figure 24 (panel D)**

According to unpaired *t*-tests, there was no main effect of the genotype on correct touch ($t(16)=1.87$; $p>0.05$), incorrect touch ($t(16)=1.97$; $p>0.05$) or magazine ($t(16)=1.11$; $p>0.05$) latencies during acquisition of the task.

These data clearly indicated that young (5-8 months) WT and TG mice learned the dPAL touchscreen task on a comparable basis. TG animals were as active as their WT littermates in touchscreen boxes. Animals of both genotypes were motivated to perform the task and showed similar reaction times to nose poke the screen or retrieve the liquid reward.

❖ Experiment D

In this second experiment, we sought to find out whether old WT and TG mice of the Tg2576 line exhibited similar or different cognitive capacities in the dPAL task. Initial effectives were as follows: n=15 WT and n=16 TG aged of 12 months when the food restriction started. A total of 7 mice (*rd* mutation: 2 WT and 2 TG; exacerbated stereotypies: 1 WT and 2 TG) were excluded. Final effectives were therefore composed of n=12 WT and n=12 TG mice.

Accuracy – **Figure 25 (panel A)**

Repeated measures two-way ANOVA on accuracy parameter revealed a main effect of time ($F(9,198)=28.80$; $p<0.0001$), but no genotype effect ($F(1,22)=0.06$; $p>0.05$) nor interaction between time and genotype factors ($F(9,198)=0.85$; $p>0.05$) during acquisition of the task.

Specific locomotor activity – **Figure 25 (panel B)**

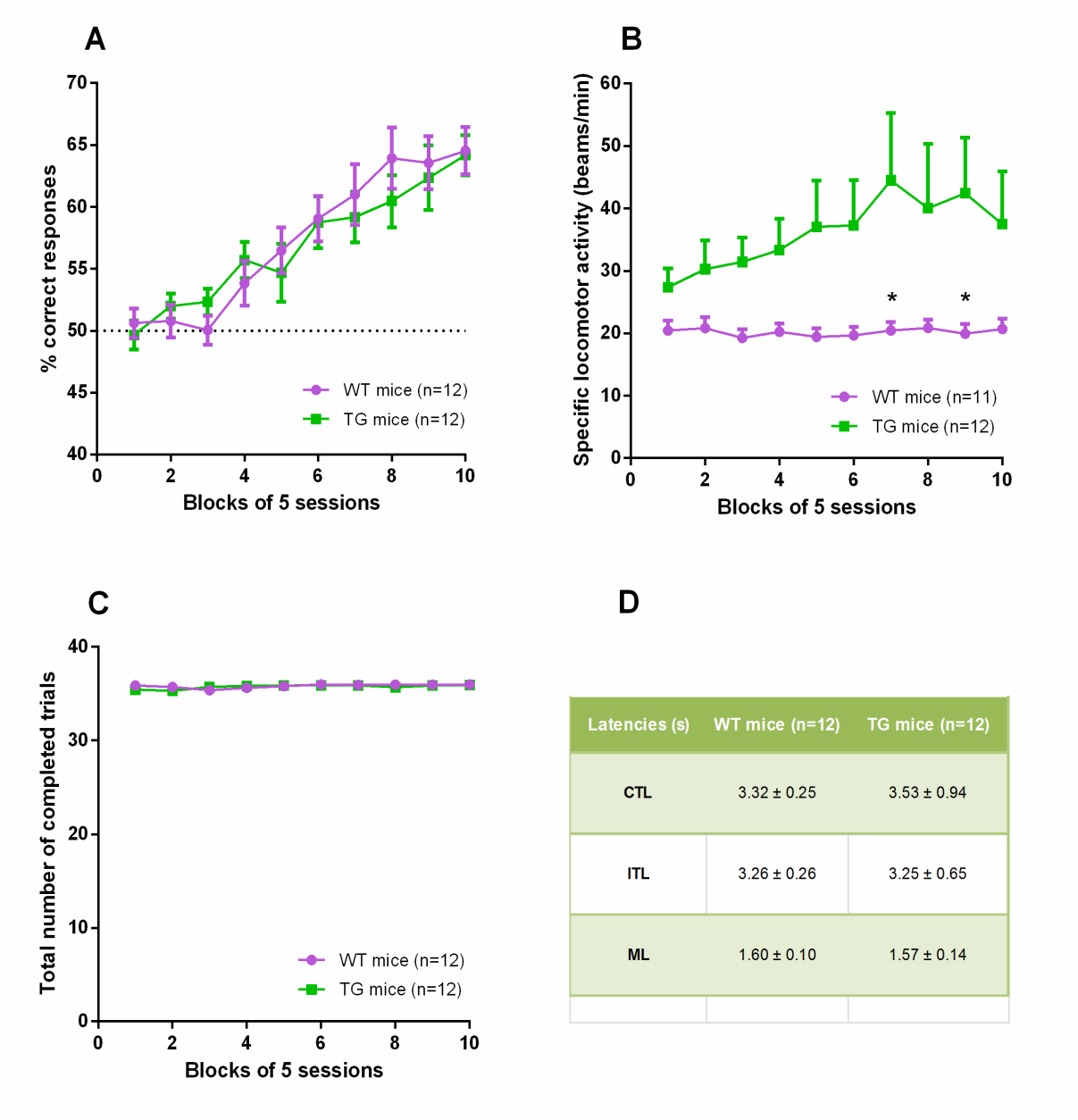
Repeated measures two-way ANOVA on the specific locomotor activity revealed a main effect of genotype ($F(1,21)=4.67$; $p<0.05$), but no time effect ($F(9,189)=1.86$; $p>0.05$) nor interaction between time and genotype factors ($F(9,189)=1.83$; $p>0.05$) during acquisition of the task. *Post-hoc* Bonferroni analysis showed a significant difference between WT and TG mice during blocks 7 ($t(210)=3.03$; $p<0.05$) and 9 ($t(210)=2.85$; $p<0.05$).

Total number of completed trials – **Figure 25 (panel C)**

Repeated measures two-way ANOVA on the total number of completed trials revealed no main effect of time ($F(9,198)=1.41$; $p>0.05$) or genotype ($F(1,22)=0.20$; $p>0.05$). Besides, there was no interaction between time and genotype factors ($F(9,198)=0.84$; $p>0.05$) during acquisition of the task.

Correct touch, incorrect touch and magazine latencies – **Figure 25 (panel D)**

According to unpaired *t*-tests, there was no main effect of the genotype on correct touch ($t(22)=0.22$; $p>0.05$), incorrect touch ($t(22)=0.01$; $p>0.05$) or magazine ($t(22)=0.16$; $p>0.05$) latencies during acquisition of the task.



Latencies (s)	WT mice (n=12)	TG mice (n=12)
CTL	3.32 ± 0.25	3.53 ± 0.94
ITL	3.26 ± 0.26	3.25 ± 0.65
ML	1.60 ± 0.10	1.57 ± 0.14

Figure 25. Percentage of correct responses (panel A), specific locomotor activity (panel B), total number of completed trials (panel C) and mean latencies (panel D) measured in aged (12-15 months old) WT and TG mice of the Tg2576 line in the dPAL task (experiment D). CTL: Correct Touch Latency; ITL: Incorrect Touch Latency; ML: Magazine Latency.

Altogether, these data suggested that aged (12-15 months) WT and TG mice learned the dPAL touchscreen task on a comparable basis. Additionally, TG mice were this time significantly more active than their WT littermates in touchscreen boxes. Animals of both genotypes were motivated to perform the task and showed similar reaction times to nose poke the screen or retrieve the liquid reward.

❖ Experiment E

In this third experiment, we examined whether young WT and TG mice of the Tg2576 line exhibited similar or different cognitive capacities in the VMCL task. Initial effectives were as follows: n=10 WT and n=10 TG aged of 5 months when the food restriction started. A total of 3 mice (*rd* mutation: 1 WT and 1 TG; exacerbated stereotypies: 1 TG) were excluded. Final effectives were therefore composed of n=9 WT and n=8 TG mice.

Accuracy – **Figure 26 (panel A)**

Repeated measures two-way ANOVA on accuracy parameter revealed a main effect of time ($F(9,135)=51.08$; $p<0.0001$), but no genotype effect ($F(1,15)=1.59$; $p>0.05$) nor interaction between time and genotype factors ($F(9,135)=1.83$; $p>0.05$) during acquisition of the task.

Specific locomotor activity – **Figure 26 (panel B)**

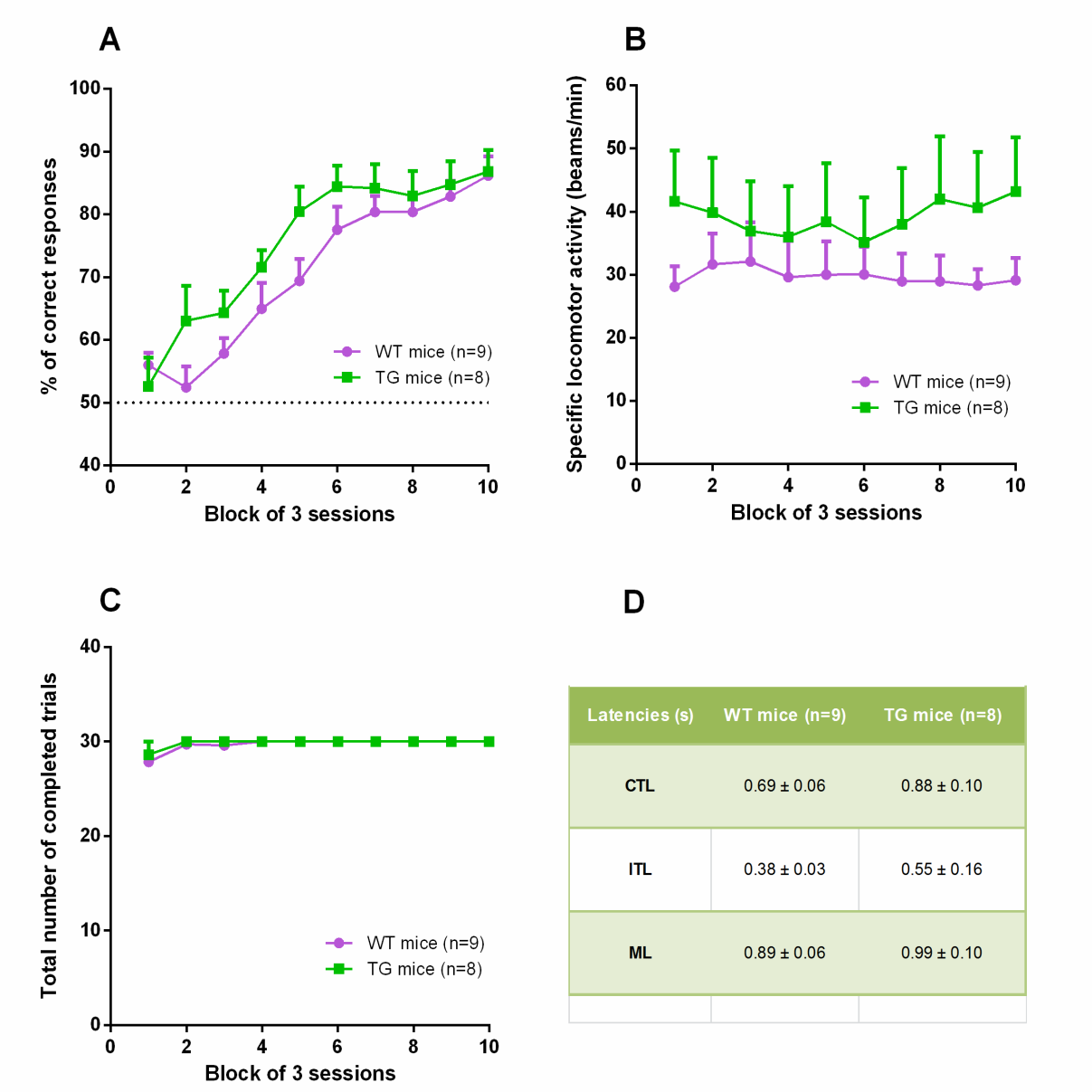
Repeated measures two-way ANOVA failed to revealed a main effect of time ($F(9,135)=0.91$; $p>0.05$) or genotype ($F(1,15)=1.09$; $p>0.05$) on specific locomotor activity during acquisition of the task. There was an interaction between time and genotype factors ($F(9,135)=1.97$; $p<0.05$).

Total number of completed trials – **Figure 26 (panel C)**

Repeated measures two-way ANOVA on the total number of completed trials revealed a main effect of time ($F(9,135)=4.62$; $p<0.0001$) but no effect of genotype ($F(1,15)=0.60$; $p>0.05$). Besides, there was no interaction between time and genotype factors ($F(9,135)=0.27$; $p>0.05$) during acquisition of the task.

Correct touch, incorrect touch and magazine latencies – **Figure 26 (panel D)**

According to unpaired *t*-tests, there was no main effect of the genotype on correct touch ($t(15)=1.69$; $p>0.05$), incorrect touch ($t(15)=1.11$; $p>0.05$) or magazine ($t(15)=0.92$; $p>0.05$) latencies during acquisition of the task.



Latencies (s)	WT mice (n=9)	TG mice (n=8)
CTL	0.69 ± 0.06	0.88 ± 0.10
ITL	0.38 ± 0.03	0.55 ± 0.16
ML	0.89 ± 0.06	0.99 ± 0.10

Figure 26. Percentage of correct responses (panel A), specific locomotor activity (panel B), total number of completed trials (panel C) and latencies (panel D) measured in young (5-7 months old) WT and TG mice of the Tg2576 line in the VMCL task (experiment E). CTL: Correct Touch Latency; ITL: Incorrect Touch Latency; ML: Magazine Latency.

These data clearly indicated that young (5-7 months) WT and TG mice learned the VMCL touchscreen task on a comparable basis. Despite high variability, TG animals were as active as their WT littermates in touchscreen boxes. Animals of both genotypes were motivated to perform the task and showed similar reaction times to nose poke the screen or retrieve the liquid reward.

❖ Experiment F

In this fourth experiment, we examined whether aged WT and TG mice of the Tg2576 line exhibited similar or different cognitive capacities in the VMCL task. Initial effectives were as follows: n=10 WT and n=15 TG aged of 12 months when the food restriction started. A total of 3 mice (*rd* mutation: 1 WT and 1 TG; exacerbated stereotypies: 1 WT) were excluded. Final effectives were therefore composed of n=8 WT and n=14 TG mice.

Accuracy – **Figure 27 (panel A)**

Repeated measures two-way ANOVA on accuracy parameter revealed a main effect of time ($F(9,180)=32.32$; $p<0.0001$), but no genotype effect ($F(1,20)=0.65$; $p>0.05$) nor interaction between time and genotype factors ($F(9,180)=0.89$; $p>0.05$) during acquisition of the task.

Specific locomotor activity – **Figure 27 (panel B)**

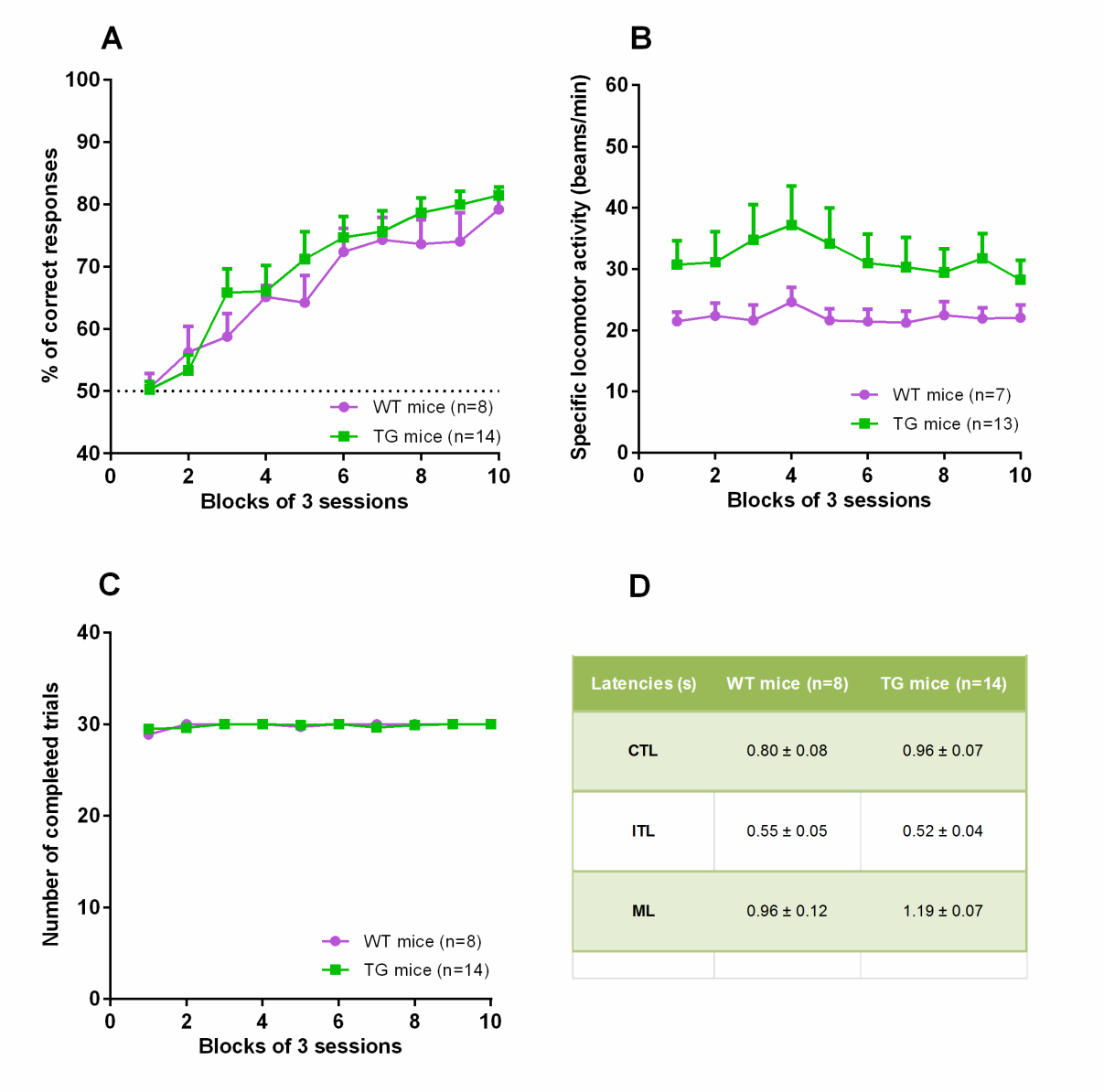
Repeated measures two-way ANOVA on specific locomotor activity failed to reveal an effect of time ($F(9,162)=1.62$; $p>0.05$), genotype ($F(1,18)=2.34$; $p>0.05$) or an interaction between these two factors ($F(9,162)=0.80$; $p>0.05$) during acquisition of the task.

Total number of completed trials – **Figure 27 (panel C)**

Repeated measures two-way ANOVA on the total number of completed trials revealed a main effect of time ($F(9,180)=2.50$; $p<0.05$), but no genotype effect ($F(1,20)=0.01$; $p>0.05$) nor interaction between time and genotype factors ($F(9,180)=0.73$; $p>0.05$) during acquisition of the task.

Correct touch, incorrect touch and magazine latencies – **Figure 27 (panel D)**

According to unpaired *t*-tests, there was no main effect of the genotype on correct touch ($t(20)=1.43$; $p>0.05$), incorrect touch ($t(20)=0.32$; $p>0.05$) or magazine ($t(20)=1.82$; $p>0.05$) latencies during acquisition of the task.



Latencies (s)	WT mice (n=8)	TG mice (n=14)
CTL	0.80 ± 0.08	0.96 ± 0.07
ITL	0.55 ± 0.05	0.52 ± 0.04
ML	0.96 ± 0.12	1.19 ± 0.07

Figure 27. Percentage of correct responses (panel A), specific locomotor activity (panel B), total number of completed trials (panel C) and latencies (panel D) measured in aged (12-14 months old) WT and TG mice of the Tg2576 line in the VMCL task (experiment F). CTL: Correct Touch Latency; ITL: Incorrect Touch Latency; ML: Magazine Latency.

Altogether, these data suggested that aged (12-14 months) WT and TG mice learned the VMCL touchscreen task on a comparable basis. TG animals were as active as their WT littermates in touchscreen boxes. Animals of both genotypes were motivated to perform the task and showed similar reaction times to nose poke the screen or retrieve the liquid reward.

❖ Experiment G

In this penultimate experiment, we examined whether young WT and TG mice of the Tg2576 line exhibited similar or different cognitive capacities in the PVD task. It is noteworthy to precise that animals used in this study had been previously tested in the VMCL task (experiment E). Initial effectives were thus as follows: n=9 WT and n=8 TG aged of 7 months when the second food restriction started. A total of 2 mice (failure to meet criteria at the end of the acquisition phase: 2 WT) were excluded. Final effectives were therefore composed of n=7 WT and n=8 TG mice.

Accuracy – **Figure 28 (panel A)**

During the acquisition, repeated measures two-way ANOVA revealed a significant effect of time ($F(21,273)=20.70$; $p<0.0001$) on accuracy parameter. However, there was no main effect of genotype ($F(1,13)=0.14$; $p>0.05$) nor interaction between the two factors ($F(21,273)=0.61$; $p>0.05$).

Similarly, repeated measures two-way ANOVA revealed a significant effect of time ($F(21,273)=49.49$; $p<0.0001$) on accuracy parameter during the reversal learning phase. However, there was no main effect of genotype ($F(1,13)=0.26$; $p>0.05$) nor interaction between the two factors ($F(21,273)=1.28$; $p>0.05$).

Specific locomotor activity – **Figure 28 (panel B)**

No main effect of time (acquisition: $F(21,273)=0.92$, $p>0.05$; reversal learning: $F(21,273)=0.78$, $p>0.05$) or genotype (acquisition: $F(1,13)=0.26$, $p>0.05$; reversal learning: $F(1,13)=0.33$, $p>0.05$) was found when analyzing the specific locomotor activity. Furthermore, there was no interaction between factors during acquisition ($F(21,273)=1.00$; $p>0.05$) or reversal learning ($F(21,273)=0.45$; $p>0.05$) phases.

Total number of completed trials – **Figure 28 (panel C)**

During the acquisition, repeated measures two-way ANOVA revealed no significant effect of time ($F(21,273)=0.91$; $p>0.05$) or genotype ($F(1,13)=0.84$; $p>0.05$) on the total number of completed trials. Besides, there was no interaction between the two factors ($F(21,273)=0.83$; $p>0.05$).

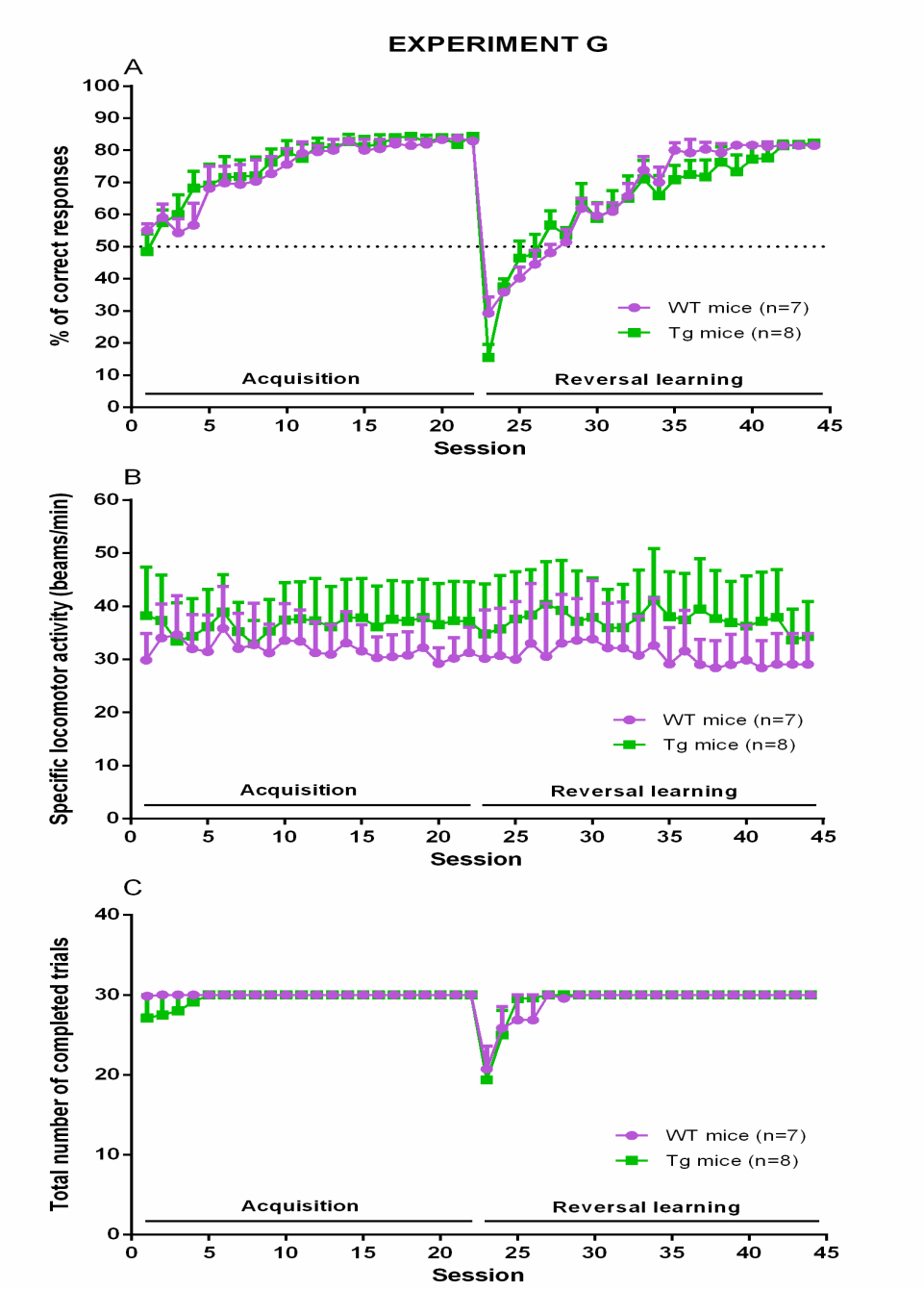


Figure 28. Percentage of correct responses (panel A), specific locomotor activity (panel B) and total number of completed trials (panel C) measured in young (7-9.5 months old) WT and TG mice of the Tg2576 line in the PVD task (experiment G).

In contrast, there was a significant effect of time ($F(21,273)=7.94$; $p<0.0001$) on the same parameter during the reversal learning phase. In parallel, no main effect of genotype

(F(1,13)=0.10; p>0.05) nor interaction between the two factors (F(21,273)=0.30; p>0.05) were observed.

Correct touch, incorrect touch and magazine latencies – **Table 13**

According to unpaired *t*-tests, there was no main effect of the genotype on correct touch (*t*(13)=1.75; p>0.05), incorrect touch (*t*(13)=1.89; p>0.05) or magazine (*t*(13)=1.18; p>0.05) latencies during acquisition of the task. In the same way, no significant effect of genotype was observed for correct touch (*t*(13)=1.46; p>0.05) or magazine (*t*(13)=1.26; p>0.05) latencies during the reversal learning phase. Nevertheless, an unpaired *t*-test revealed a main effect of genotype on incorrect touch latency (*t*(13)=2.43; p<0.05).

Phase	Acquisition		Reversal Learning	
Genotype	WT (n=7)	TG (n=8)	WT (n=7)	TG (n=8)
CTL	1.54 ± 0.10	2.10 ± 0.29	2.01 ± 0.19	2.53 ± 0.29
ITL	1.63 ± 0.10	2.02 ± 0.17	1.77 ± 0.15	2.33 ± 0.17 *
ML	1.18 ± 0.09	1.36 ± 0.12	1.26 ± 0.06	1.39 ± 0.08

Table 13. Mean latencies (in s) recorded in young (7-9.5 months old) WT and TG mice of the Tg2576 line trained in the PVD task (experiment G). CTL: Correct Touch Latency; ITL: Incorrect Touch Latency; ML: Magazine Latency. * p<0.05 *vs* WT mice.

These data clearly indicated that young (7-9.5 months) WT and TG mice learned the PVD touchscreen task on a comparable basis. TG animals were as active as their WT littermates in touchscreen boxes. Animals of both genotypes were motivated to perform the task and generally showed similar reaction times to nose poke the screen or retrieve the liquid reward.

❖ Experiment H

In this last experiment, we examined whether old WT and TG mice of the Tg2576 line exhibited similar or different cognitive capacities in the PVD task. It is noteworthy to precise that animals used in this study had been previously tested in the VMCL task (experiment F). Initial effectives were thus as follows: n=8 WT and n=14 TG aged of 14 months when the second food restriction started. A total of 3 mice (failure to meet criteria at the end of the acquisition phase: 1 WT and 6 TG) were excluded. Final effectives were therefore composed of n=7 WT and n=8 TG mice.

Accuracy – **Figure 29 (panel A)**

During the acquisition, repeated measures two-way ANOVA revealed a significant effect of time ($F(19,247)=27.35$; $p<0.0001$) on accuracy parameter. However, there was no main effect of genotype ($F(1,13)=0.70$; $p>0.05$) nor interaction between the two factors ($F(19,247)=1.57$; $p>0.05$).

Similarly, repeated measures two-way ANOVA revealed a significant effect of time ($F(29,377)=37.42$; $p<0.0001$) on accuracy parameter during the reversal learning phase. However, there was no main effect of genotype ($F(1,13)=3.15$; $p>0.05$) nor interaction between the two factors ($F(29,377)=0.75$; $p>0.05$).

Specific locomotor activity – **Figure 29 (panel B)**

During the acquisition, repeated measures two-way ANOVA revealed a significant effect of genotype ($F(1,12)=8.54$; $p<0.05$) on specific locomotor activity. However, there was no main effect of time ($F(19,228)=0.89$; $p>0.05$) nor interaction between the two factors ($F(19,228)=1.03$; $p>0.05$). *Post-hoc* Bonferroni analysis showed a significant difference between WT and TG groups during sessions 2, 3, 5 and 6 (respectively: $t(240)=3.50$, $t(240)=3.49$, $t(240)=3.45$, $t(240)=3.31$; in all cases, $p<0.05$).

Similarly, repeated measures two-way ANOVA revealed a significant effect of genotype ($F(1,12)=5.13$; $p<0.05$) on specific locomotor activity during the reversal learning phase. However, there was no main effect of time ($F(29,348)=0.75$; $p>0.05$) nor interaction between the two factors ($F(29,348)=1.26$; $p>0.05$). *Post-hoc* Bonferroni analysis did not demonstrate a significant difference between WT and TG groups, whatever the session considered.

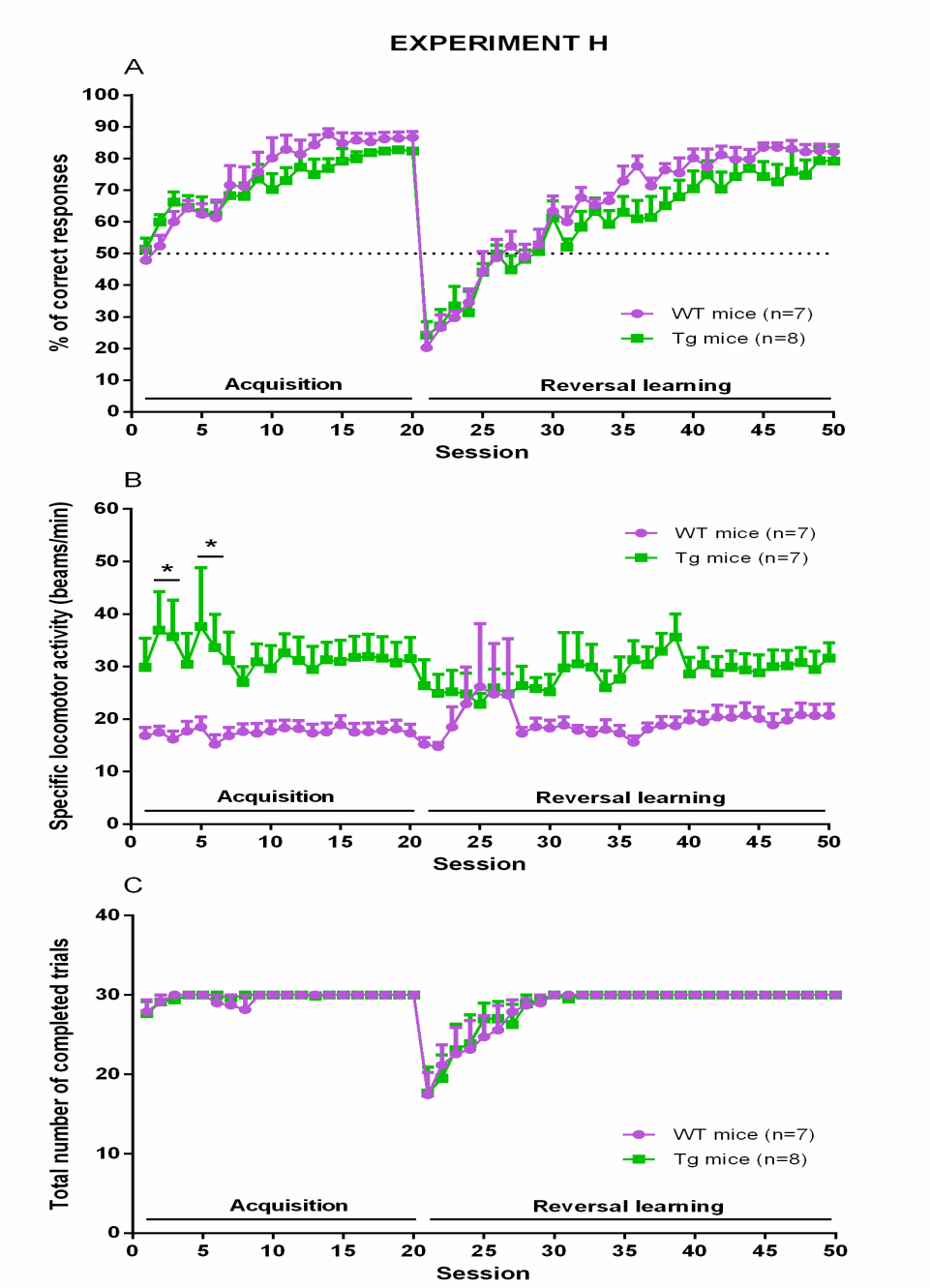


Figure 29. Percentage of correct responses (panel A), specific locomotor activity (panel B) and total number of completed trials (panel C) measured in old (14-16.5 months old) WT and TG mice of the Tg2576 line in the PVD task (experiment H). * $p<0.05$ *vs* WT animals (sessions 2, 3, 5 and 6).

Total number of completed trials – **Figure 29 (panel C)**

During the acquisition, repeated measures two-way ANOVA revealed a significant effect of time ($F(19,247)=2.17$; $p<0.05$) on total number of completed trials. However, there was no

main effect of genotype ($F(1,13)=0.38$; $p>0.05$) nor interaction between the two factors ($F(19,247)=0.48$; $p>0.05$).

In the same way, there was a significant effect of time ($F(29,377)=12.10$; $p<0.0001$) on the same parameter during the reversal learning phase, whereas no main effect of genotype ($F(1,13)=0.01$; $p>0.05$) nor interaction between the two factors ($F(29,377)=0.14$; $p>0.05$) were observed.

Correct touch, incorrect touch and magazine latencies – **Table 14**

According to unpaired *t*-tests, there was no main effect of the genotype on correct touch, incorrect touch or magazine latencies during acquisition (respectively: $t(13)=0.65$, $t(13)=0.57$ and $t(13)=0.10$; all $p>0.05$) and reversal learning (respectively: $t(13)=0.66$, $t(13)=0.64$ and $t(13)=0.82$; all $p>0.05$) phases of the task.

Phase	Acquisition		Reversal Learning	
Genotype	WT (n=7)	TG (n=8)	WT (n=7)	TG (n=8)
CTL	3.09 ± 0.36	2.80 ± 0.28	3.39 ± 0.32	3.09 ± 0.33
ITL	3.00 ± 0.32	2.74 ± 0.32	3.42 ± 0.27	3.31 ± 0.34
ML	1.72 ± 0.16	1.74 ± 0.05	1.51 ± 0.13	1.63 ± 0.08

Table 14. Mean latencies (in s) recorded in aged (14-16.5 months old) WT and TG mice of the Tg2576 line trained in the PVD task (experiment H). CTL: Correct Touch Latency; ITL: Incorrect Touch Latency; ML: Magazine Latency.

Altogether, these data suggested that aged (14-16.5 months) WT and TG mice learned the PVD touchscreen task on a comparable basis. TG animals were significantly more active than their WT littermates during first sessions of acquisition. Animals of both genotypes were otherwise motivated to perform the task and showed similar reaction times to nose poke the screen or retrieve the liquid reward.

❖ Intermediary discussion

An overview of main results cumulated during these different studies is presented below (**Table 15**). After a general summary, we will put forward some hypotheses to explain discrepancies concerning data obtained in our dPAL and PVD tasks.

Global outcomes: motivational, learning and motoric functions

Although WT and TG mice of the Tg2576 line were bred on a mixt C57BL/6 x SJL/J genetic background, their evaluation in touchscreen boxes was fully possible, as indicated by the constant completion of daily sessions and low response (< 4 s) and magazine (< 2 s) latencies. This aspect was worth to be noticed, as previous publications dealing with Pavlovian or instrumental conditioning in appetitive paradigms had led to contradictory conclusions regarding the motivational state of TG animals (Blackshear et al., 2011; Lelos et al., 2011). Incidentally, measures of locomotor function in touchscreen boxes revealed a higher, although rarely significant, activity in young and aged TG animals compared with their WT littermates, a finding rather in accordance with previous studies (Lalonde et al., 2003; Ognibene et al., 2005; Deacon et al., 2009) considering the important variability of data and subsequent elimination of certain animals. Besides, whatever the age considered, WT and TG animals generally learned in a similar manner the touchscreen task(s) to which they had been assigned.

Experiments A-B: learning in WT animals, blindness and stereotypies

Although the different latencies and locomotor activity were not measured during these pilot studies, other parameters were sufficient to carry first important lessons. Available parameters (accuracy, numbers of correction and completed trials) validated the possibility to evaluate the Tg2576 line at different ages in touchscreen tasks. Thus, WT mice significantly improved their performance over time in dPAL (60-65 % of correct responses after 50 sessions) and VMCL (75-85 % of correct responses after 30 sessions) tasks. This was better stressed after exclusion of animals expressing the *rd* mutation responsible for the degeneration of specific retinal cell populations, namely rods and cones (Brown and Wong, 2007; Errijgers et al., 2007; Farley et al., 2011). Indeed, as reported in previous publications (Garcia et al., 2004; Yassine et al., 2013), blind *rd -/-* mice were unable to correctly learn cognitive tasks primarily relying upon vision. Therefore, they did not discriminate the various

Experiment	Task	Genotype and age (start of testing)	% correct	N° of completed trials	Locomotor activity	Latencies
A	dPAL	WT (5-6m *vs* 13-14m)	=	=	n/d	n/d
B	VMCL	WT (5-6m *vs* 13-14m)	↘ (aged)	↘ (aged)	n/d	n/d
C	dPAL	WT *vs* TG (5m)	=	=	=	=
D	dPAL	WT *vs* TG (12m)	=	=	↗ (TG)	=
E	VMCL	WT *vs* TG (5m)	=	=	=	=
F	VMCL	WT *vs* TG (12m)	=	=	=	=
G	PVD	WT *vs* TG (7m)	=	=	=	↗ ITL (TG) Reversal learning phase only
H	PVD	WT *vs* TG (14m)	=	=	↗ (TG) Acquisition phase only	=

Table 15. Summary of the principal results obtained in the different touchscreen tasks during transgenic studies performed in young and aged animals of the Tg2576 line. n/d: not determined; =: no difference between WT and TG mice; ↗: significant increase; ↘: significant decrease; ITL: Incorrect Touch Latency.

stimuli appearing on the screen and failed to learn inherent rules to touchscreen assays (experiments A and B). A closer look at a few animals' behavior in touchscreen chambers urged us to also consider the massive occurrence of certain stereotypies – especially wall-climbing and circling behaviors – as a fundamental confounding factor (experiment B). Added to the low effectives, these repetitive and uncontrolled actions (Kelley, 2001) established in 2 of the 4 aged WT mice might have caused the significant delay in the acquisition of the VMCL task. To counter these unwanted effects, we qualitatively determined in subsequent studies (experiments C to H) whether WT and TG animals were blind or displayed exacerbated stereotypic behaviors, and discarded them when one of these conditions was encountered. This double selection process, recommended by some authors (Garcia et al., 2004; Hunsaker, 2012), was intended to decrease the risk to yield discrepant data in the framework of the behavioral evaluation of the Tg2576 model (Holcomb et al., 1999; King et al., 1999; Stewart et al., 2011; Yassine et al., 2013).

Experiments C-D: preserved learning in the dPAL task

Contrary to our predictions, the dPAL task, which implicated the formation of a memory based on the construction of object-place associations, was acquired in a comparable way by young or aged (5-8 months and 12-15 months old, respectively) WT and TG animals. In both cases, mice performed around 65 % of accuracy after a total of 50 sessions. These unexpected results heavily contrasted with the long-standing deficits established in these mice in diverse spatial tasks (Hsiao et al., 1996; Westerman et al., 2002; Arendash et al., 2004; Ognibene et al., 2005; Stewart et al., 2011; Yassine et al., 2013). Above all, it completely stood out from another behavioral procedure, the spatial object recognition task, which enabled to dissociate visuospatial memory performances of 14 to 16-months old TG animals from their WT littermates (Hale and Good, 2005; Good and Hale, 2007). Notwithstanding a certain age-related variability of appearance of memory deficits in TG mice of the Tg2576 line (Webster et al., 2014), age ranges chosen in the case of our touchscreen experiments could probably account for the absence of cognitive impairment in young (low amyloid load) but not aged (high amyloid load) TG mice. In other words, these results rather pointed out the behavioral task than the animal model of Alzheimer's Disease used in these studies.

With respect to that last idea, it was noteworthy that a certain number of critical changes had been made to allow the back-translation of the task from Humans to rodents. Thus, while the learning of the dPAL rule required about 50 training sessions of 36 rewarded trials, patients evaluated in the equivalent neuropsychological CANTAB-PAL task (**Figure 30**) executed a

unique session (potentially, 8 stages of increasing difficulty for a maximum of 80 trials) and did not receive any positive reinforcer after completion of each testing condition (Sahakian et al., 1988; Swainson et al., 2001; Blackwell et al., 2004). In light of these different elements, the absence of deficit glimpsed in old TG mice of the Tg2576 line clearly questioned the hippocampal-dependent nature of the task and highlighted the necessity to investigate the involvement of other potential neural substrates.

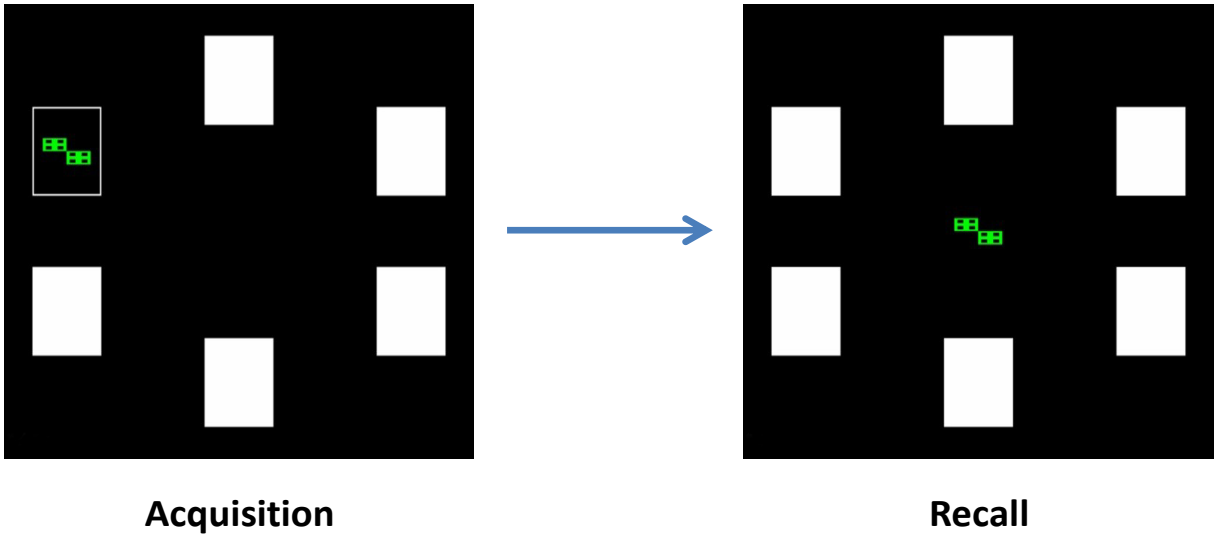


Figure 30. Illustration of the CANTAB Paired Associates Learning (PAL) paradigm for clinical use. This task is organized in 2 phases: acquisition and recall. In a first time, the patient is presented a certain number of patterns (here, 6 colored stimuli in total) that appear one by one in distinct windows for a short duration. Then, the recall phase starts, during which each pattern previously showed is individually displayed in the center of the touchscreen and the patient must remember and choose the corresponding window. As the task exists in different versions that comprise 1 to 8 pattern(s), the difficulty gradually increases whenever all given responses are correct (2 stages with 1 pattern, 2 stages with 2 patterns, 2 stages with 3 patterns, 1 stage with 6 patterns and 1 stage with 8 patterns). Subjects are usually granted a maximal of 10 trials (acquisition followed by recall) for each pattern condition. In this context, most of AD patients generally manage to complete 4-5 first stages but make significantly more errors than depressive or control subjects in the 6-pattern condition (Swainson et al., 2001).

Experiments E-F: no deficit in the VMCL task

There was no difference between WT and TG animals tested in the VMCL task, no matter their age. Young (5-7 months old) mice of both genotypes reached 85 % of accuracy after a total of 30 sessions whereas in the same time, corresponding aged (12-14 months old)

animals reached a plateau around 80 % of accuracy. Interestingly, these results coincided with previous studies demonstrating the maintenance or even enhancement of procedural learning in TG mice of the Tg2576 line (Middei et al., 2004; Lelos et al., 2011).

Experiments G-H: spared visual memory and cognitive flexibility in the PVD task

Executive functions encompass high-order cognitive processes that allow planning and controlling the behavior of an organism in an adaptative manner in order to respond to environmental changes. In rodents, cognitive flexibility is generally tested through set-shifting, reversal learning or response inhibition tasks (Webster et al., 2014). Among these different classes of behavioral assays, reversal learning paradigms have been historically emphasized (Brigman et al., 2010). The PVD assay was among the first touchscreen tasks developed in rodents and had been successfully utilized to characterize executive dysfunctions in animal models of schizophrenia (Brigman et al., 2008; Barkus et al., 2012). The absence of significant deficit in our young or old TG animals recorded in that task was however intriguing as previous works had demonstrated the existence of reversal learning deficits in transgenic animals of the Tg2576 line as early as 6 months of age (Zhuo et al., 2007; Zhuo et al., 2008; Papadopoulos et al., 2013). Instead, young (7-9.5 months old) and aged (14-16.5 months old) WT and TG mice similarly acquired the initial rule (80-85 % of accuracy after 20-22 sessions of acquisition) and its opposite version (80 % of accuracy after 23-28 sessions of reversal learning).

Given the low number of acquisition and reversal learning sessions that young C57BL/6JRj mice needed to reach similar criteria (chapter 5), slow learning of both phases in WT and TG mice of the Tg2576 line deserved to be further considered. Alone, the possible presence of undetected blind mice or animals with highly frequent stereotypies could not account for such a difference in both genotypes. However, noticeably, these animals had been beforehand tested in the VMCL task, which relied upon the integrity of cortico-striatal networks in rats (Reading et al., 1991; Bussey et al., 1997b). Following potential issues of task combinations evoked in chapter 5, it was easy to hypothesize the existence of memory interferences due to common neural substrates solicited during the posterior evaluation of Tg2576 mice in the PVD task. This assumption was thoroughly supported by recent studies describing the importance of a neural circuitry implicating dorso-striatal and prefrontal regions in the PVD task in mice (Graybeal et al., 2011; Brigman et al., 2013). Yet, the nature of cerebral regions whose integrity enabled the acquisition of the VMCL task still remained to be verified in mice to validate this theory.

Chapter 7: Investigation of the neural substrates involved in dPAL and VMCL tasks: effects of HPC vs. DS lesions

In view of the ambiguous results uncovered during transgenic studies addressed in chapter 6, the examination of the role of certain brain regions in touchscreen tasks turned out both necessary and valuable to better comprehend the particular nature of entailed learnings. As repeatedly mentioned, the dPAL task had been adapted from a neuropsychological task used in clinic to detect patients suffering from AD or to predict the conversion of subjects presenting a MCI into AD (Owen et al., 1995; Swainson et al., 2001). In as much as the human version of the task had been identified to be hippocampal-dependent (de Rover et al., 2011), it was worth investigating whether the permanent disruption of this subcortical structure would likewise influence the acquisition of the rodent dPAL touchscreen task. This was especially true since PAL paradigms that relied on a different approach than the touchscreen-based methodology had been markedly demonstrated to be hippocampal-dependent according to lesion studies realized in rats (Gilbert and Kesner, 2002; Langston et al., 2010). In comparison, we had successfully imported the VMCL task in mice but wanted to confirm if, as rats evaluated in the counterpart of that paradigm (Reading et al., 1991), our animals learned the related rule thanks to the specific pattern of dorso-striatal activity.

In answering these questions, we have conceived an experimental design (experiment A) that permitted to quantify the cognitive effects of hippocampal or dorso-striatal lesions on the acquisition of the 2 aforementioned tasks as well as a more classical forced alternation task. The risk of interactions between dPAL and VMCL touchscreen tasks had been rejected during optimization studies (chapter 5). Pilot studies were therefore confined to the determination of relevant stereotaxic coordinates, concentration and volumes of excitotoxic agent (Schwarcz et al., 1984) to bilaterally elicit fiber-sparing lesions of the whole (dorsal and ventral) hippocampus or dorsal striatum. Following these adjustments and the realization of behavioral procedures within the experiment A, animals were killed. NeuN immunostainings (Wolf et al., 1996), that dissociated surviving from dead neurons, were then realized to ascertain the size and localization of lesions. This allowed excluding animals whose lesions were incorrectly placed or affected other brain structures. In a complementary study (experiment B), chemically-induced hippocampal lesions were this time generated post-training to selectively assess the putative involvement of this structure during the retrieval of

learned information in the dPAL task. Data corresponding to experiments A and B (gathered in publication 2 and completed by some additional results) are presented in the following pages.

❖ Publication 2 (before submission)

Title: Touchscreen tasks in mice to demonstrate differences between hippocampal and striatal functions.

Abbreviated title: DS or HPC lesions in touchscreen paradigms

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N° of pages/figures/tables/words (Abstract/Introduction/Discussion): 34/7/2/ (255/501/1503)

Conflicts of interest

DD has the following disclosure: Boehringer Ingelheim Pharma GmbH & Co KG, PhD contract. HR, CDC and AM have the following disclosure: Boehringer Ingelheim Pharma GmbH & Co KG, contract of employment. CM and JCC declare no competing interest, whatever their nature. This does not alter our adherence to policies of the Journal of Neuroscience.

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Abstract

In mammals, hippocampal and striatal regions are engaged in separable cognitive processes usually assessed through species-specific paradigms. To reconcile cognitive testing among species, translational advantages of the touchscreen-based automated method have been recently promoted. However, it remained undetermined whether similar neural substrates would be involved in such behavioral tasks both in humans and rodents. To address this question, the effects of hippocampal or dorso-striatal fiber-sparing lesions were first assessed in mice through a battery of tasks (experiment A) comprising the acquisition of two touchscreen paradigms, the Paired Associates Learning (dPAL) and Visuo-Motor Conditional Learning (VMCL) tasks, as well as a more classical T-maze alternation task. Additionally, we sought to determine whether post-acquisition hippocampal lesions would alter memory retrieval in the dPAL task (experiment B). Pre-training lesions of dorsal striatum caused major impairments in all paradigms. In contrast, pre-training hippocampal lesions disrupted the performance of animals trained in the T-maze assay, but spared the acquisition in all touchscreen tasks. Nonetheless, post-training hippocampal lesions severely impacted the recall of the previously learned dPAL task. Altogether, our data show that, after having demonstrated their potential in genetically modified mice, touchscreens also reveal perfectly adapted to taxing functional implications of brain structures in mice by means of lesion approaches. Unlike its human counterpart soliciting the hippocampus, the acquisition of the dPAL task requires the integrity of the dorsal striatum in mice. The hippocampus only later intervenes, when consolidated information needs to be retrieved. Touchscreen assays may therefore appear adapted to study striatal- or hippocampal-dependent forms of learnings in mice.

Keywords

Touchscreen tasks; Excitotoxic lesions; Hippocampus; Dorsal striatum; Spatial memory; Mice

1) Introduction

It is now well established that memory is not a unitary process. Instead, different forms of memory exist and are supported by distinct brain regions (White and McDonald, 2002). Lesion studies have demonstrated a functional dissociation between the hippocampus (HPC) and the dorsal striatum (DS). Indeed, each lesion produces specific learning deficits (Packard et al., 1989; Knowlton et al., 1996). Although dissociation studies have pointed to a contribution of other brain areas to specific memory processes (Squire, 2004), the relative functional roles of the HPC and DS have been given particular interest. Such interest is motivated by the fact that memory and/or motor processes involving these regions are strongly disturbed in patients with neurodegenerative or neuropsychiatric disorders (Holtzman et al., 2011; Schapira 2009). Work in rodents has widely promoted the role of the HPC in spatial learning and navigation (Paul et al., 2009), in contextual memories (Phillips and LeDoux, 1992; Maren et al., 1997), and in configural or relational forms of memory (Alvarado and Rudy, 1995; Alvarez et al., 2002). Furthermore, converging evidence has shed light on the nature of cognitive functions supported by the basal ganglia. Mainly based on conditioning paradigms, lesion studies have notably demonstrated the importance of three striatal subregions in learning processes: the nucleus accumbens is presented as a superintendent in Pavlovian conditioning learning (Parkinson et al., 1999), whereas the dorsomedial striatum (DMS) orchestrates flexible “response-outcome” (R-O) associations resulting in goal-directed actions, and the dorsolateral striatum (DLS) supports habit learning through the establishment of rigid “stimulus-response” (S-R) associations (Yin and Knowlton, 2006; Balleine et al., 2009). The advent of neuroimaging techniques globally confirmed similar functions for the HPC and DS in humans (Maguire et al., 2000; Balleine and O’Doherty, 2010).

Cognitive tasks in rodents still deeply differ from those realized in humans, which might explain the current difficulty to translate preclinical results into clinical stages (Keeler and Robbins, 2011; Homberg, 2013). To bridge the gap of cognitive testing across species, appetitive touchscreen tasks have been latterly developed in rodents (Bussey et al., 2012). In such paradigms, the situation is comparable to that of patients tested in computerized neuropsychological tasks (Robbins et al., 1994), as the animal must respond to a touchscreen after the presentation of visual stimuli obeying a defined rule. However, the neurobiology of such assays has not been systematically examined.

Herein, we focused on the Paired Associates Learning (dPAL) and the Visuo-Motor Conditional Learning (VMCL) tasks. Acquisition of the first task depends on the HPC in humans, as demonstrated by the poor performance of Alzheimer's disease patients (Swainson et al., 2001; de Rover et al., 2011). Moreover, it is sensitive to post-training pharmacological manipulations of HPC functions in rats (Talpos et al., 2009). By contrast, DLS but not DMS lesions disrupt the acquisition of the second task in rats (Reading et al., 1991). We therefore report on the effect of excitotoxic lesions circumscribed to the HPC or DS on the acquisition/retrieval of these tasks as compared to a more classical T-maze alternation task in mice.

2) Material & methods

2.1 Ethics Statement

All procedures described in this article and related to the Care, Treatment and Use of animals were performed with the specific approval of the appropriate governmental agency (Regierungspräsidium Tübingen, Germany) in an AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care International)-accredited facility. These procedures were also in compliance with European Union guidelines (European Community Council Directive 2010/63/UE). All efforts were made to minimize animal suffering and to respect the concept of the 3 Rs (reduce, refine, replace).

2.2 Animals

Three to four-month old male C57BL/6JRj mice (n=54 for experiment A, n=25 for experiment B; Janvier, France) were used in this study. Upon their arrival, they were housed individually (temperature- and humidity-controlled room; 12 h light/dark cycle (lights on 06:00h) in a cage (26 X 21 X 14 cm) with wood shaving bedding, and a red transparent plastic nest box and paper strips as environmental enrichment. Behavioral assessments were conducted during the light phase of the cycle. Food and water were available *ad libitum*, except during periods of evaluation in touchscreen devices. A mouse was first weighed 5 times over a one-week period to establish a baseline weight. Its free-feeding body weight was then slowly reduced to 85-90% of the initial value and maintained throughout the whole testing duration. In appetitive touchscreen tasks, animals were rewarded with half-diluted condensed milk (Milch Mädchen; Nestlé, Germany). After each daily session, they were immediately weighed and

fed upon return to the home cage. By contrast, water was always available *ad libitum*. Mice were trained 5-6 days/week in touchscreen devices.

2.3 Surgery

Mice were intraperitoneally anaesthetized with a cocktail of Xylazine at 10 mg/kg (Rompun® 2 %; Bayer, Germany) and Ketamine at 100 mg/kg (Ketavet® 100 mg/ml; Pfizer, Germany) as described before (Van der Jeugd et al., 2009). After being placed in a stereotaxic frame (David Kopf Instruments, USA), N-methyl D-aspartic acid (NMDA dissolved at 90 mM in a PBS solution; Sigma-Aldrich, Germany) was injected *in situ* through a 2 µL Hamilton syringe (Hamilton, Switzerland) adapted with a 33-gauge stainless steel needle (beveled Nanofil needle; World Precision Instruments, USA), either in the whole hippocampus (dorsal and ventral parts) or in the dorsal striatum. Appropriate coordinates had been defined according to Bregma and Lambda references on the basis of pilot studies. A total of 8 sites were used for HPC lesions, against 4 sites for the striatal ones (coordinates given in Table 1). A micro-pump (Ultra Micro Pump; World Precision Instruments, USA) was used to precisely deliver the excitotoxic agent in each site (flow: 50-75 nL/min). Sham-operated mice underwent the same procedure except that no NMDA was injected. When reflexes reappeared, animals received an intraperitoneal injection of Diazepam at 5 mg/kg (Diazepam 10 mg/2 mL dissolved in NaCl 0.9 %; Ratiopharm, Germany) to avoid the genesis and spreading of potential seizures (Deacon et al., 2002). Three hours later, mice were also subcutaneously administrated 1 mL of NaCl 0.9 %. Mice were carefully weighed and observed over the course of the following week. In total the following numbers of operated mice were used for the behavioral studies: 11 HPC sham controls, 13 HPC lesioned mice, 12 DS sham controls, 14 DS lesioned mice in experiment A; 12 HPC sham controls and 13 HPC lesioned mice in experiment B.

2.4 Behavioral procedures

In experiment A, animals were lesioned, then successively trained in a battery of three cognitive tasks: the Paired-Associates Learning (dPAL) and the Visuo-Motor Conditional Learning (VMCL) tasks both recorded in touchscreen devices, and a continuous alternation task measured in a T-Maze (Figure 1). In experiment B, a new batch of mice was first trained in the dPAL task, then lesioned and later re-tested in the same paradigm. To allow a

sufficient post-surgical recovery time and make sure those excitotoxic lesions would be effective, the behavioral training phase started or started again 4 weeks post-surgery.

2.4.1 Touchscreen tasks

Procedures presented below are largely inspired from our recent work (Delotterie et al., 2014) with a few minor changes. We had notably defined optimal testing conditions for both dPAL and VMCL paradigms and demonstrated that a same mouse could be sequentially assessed in the two tasks over time without any impact on its acquisition performance.

2.4.1.1 Apparatus

Animals were tested in operant chambers housed within sound and light attenuating boxes. Every trapezoidal-shaped chamber (respective dimensions: big basis=25 cm; small basis=6 cm; height=18 cm) was individually equipped with a magazine, a house light, a tone generator, a liquid reward dispenser and a touchscreen (Bussey Mouse Touchscreen Chamber; Campden Instruments, UK). The magazine was located at the small extremity of the trapezoidal chamber. By contrast, the touchscreen represented the opposite base of the trapezoidal chamber. It was permanently covered by a black Plexiglas mask with three square windows (side dimensions: length= 7 cm; height= 7 cm) separated by 0.4 cm and located at a height of 3.6 cm from the floor of the chamber. Through these windows, visual stimuli could be shown on the screen (max. 1 stimulus per window). Moreover, infrared light beams were positioned at the rear (close to the magazine) and front (close to the touchscreen) of each box and allowed quantifying the horizontal locomotor activity of each animal. According to the automated evaluation of animal actions by photocellular detection, operant chamber inputs and outputs were controlled by a graphical task design software (ABET II Touch software; Campden Instruments, UK).

2.4.1.2 Paired Associates Learning (dPAL) task – Object in place memory

Pokey training

In experiments A and B, food-deprived animals were first acclimated to the liquid reward (500 µL) in their home cage for 3 days, and then habituated to the chamber environment during a single 20-min session with 250 µL of condensed milk in the magazine. Prior to initial training

in the dPAL task, the behavior of mice was progressively shaped through a four-step procedure: “initial touch”, “must touch”, “must initiate” and “punish incorrect” stages. In all these stages, animals were given a total of 36 trials or 60 min/session. Training stimuli consisted of 40 possible various shapes that were pseudo randomly chosen. The “initial touch” stage corresponded to a Pavlovian training, during which a stimulus appeared in one of the three windows for 30 s. In the absence of nose-poke, the end of this period coincided with the offset of the training stimulus and the delivery of the reward (8 µL) accompanied by the illumination of the magazine light and a tone. A nose-poke towards the displayed stimulus immediately led to the same outcomes, except that the animal was rewarded with a more important amount of reward (24 µL). Collection of the condensed milk coincided with the beginning of the next trial and the occurrence of a new stimulus. In the second training stage, called “must touch”, each trial started in the same way, but the stimulus remained visible on the screen until the mouse had nose-poked it. A successful nose-poke was followed by the illumination of the food tray, a tone, and the delivery of the liquid reward (8 µL). An inter-trial interval (ITI; 20 s) was introduced between the collection of the reward and the start of the next trial. The “must initiate” stage was comparable to the previous stage, except that animals had to initiate new trials by nose-poking into the magazine. Finally, animals were introduced in the “punish incorrect” stage. As before, a nose-poke towards the training stimulus was considered as a correct response and was followed by the usual outcomes described before. However, unlike other aforementioned stages, nose-poking one of the two blank windows was recognized as an incorrect response. In that case, the training stimulus disappeared, the house light was turned on for a time-out period of 10 s and no reward was given. After 10 other seconds corresponding to the correction ITI, the mouse had to complete a correction trial procedure. For that purpose, the last training stimulus used and its position were kept the same and were re-presented to the animal until it responded correctly. Importantly, correction trials were not counted in the total number of completed trials. Mice were moved to the next phase once they had achieved 36 trials in less than 60 min. An additional criterion was used for the last stage, which consisted in an accuracy superior to 75% (minimum 27 correct responses) over two consecutive sessions.

Main dPAL task

After pokey training, each mouse was required to learn specific paired-associations of stimuli and locations in the dPAL task. Three discriminative stimuli (flower, plane, and spider) were used for a total of 6 possible trial types. The flower was rewarded when presented in the left

location, the plane in the central location, and the spider in the right location. Each trial was initiated by nose-poking into the magazine. The tray light then switched off and a pair of different stimuli appeared on the screen in 2 of the 3 possible locations: left, central, or right. Among the 2 stimuli shown on the screen, one stimulus was in a correct location (S+) and the other was in an incorrect location (S−). When a mouse nose-poked the correct stimulus (case 1: correct response), both stimuli disappeared and the mouse was rewarded for a correct response as previously described. Entry to collect the reward turned off the tray light and started a 20 s ITI. Afterwards, the tray light was again illuminated and the mouse could nose-poke into the magazine to initiate the next trial by triggering the appearance of a new pair of stimuli on the screen. By contrast, if the mouse nose-poked the incorrect stimulus (case 2: incorrect response), the stimuli disappeared, the house light was turned on for a time-out period of 10 s and no reward was given. After 10 more seconds corresponding to the correction ITI, the mouse then had to complete a correction trial procedure. A correction trial consisted of the re-presentation of the last pair of stimuli in the same spatial configuration and was repeated until a correct response was given to the screen. As for the last stage of pokey training, correction trials were not counted in the total number of trials completed during the main training. Mice were evaluated for a total of 40 daily sessions, with a maximum of 36 trials or 60 min/session. Following the end of the task (experiment A), they were given free access to the food and water to facilitate their weight gain outside testing periods. The same kept true in experiment B, except that post-training hippocampal lesioned and sham control animals were subsequently re-tested in the dPAL task for a total of 5 additional sessions.

2.4.1.3 Visuo-Motor Conditional Learning (VMCL) task – Habit learning

Pokey training

After a new period of progressive introduction to a restricted food diet (experiment A), animals were trained in a new short pokey training procedure. Mice were this time trained in only two different stages, “initial touch” and “must touch”, which were sufficient for mice previously trained in touchscreen devices (Delotterie et al., 2014). The “must initiate” stage was globally comparable to the procedure detailed above, but white squares were used as training stimuli. Completion of this pokey training stage was achieved when mice performed 30 trials in less than 60 min. Because of the specific nature of the VMCL task, the “pre-training” stage replaced the “punish incorrect” stage and aimed to teach the mouse to double

nose-poke the touchscreen centrally, then laterally to get the reward. The onset of a new trial started with a nose-poke into the magazine. A first white square then appeared in the central window, and remained until the animal had nose-poked it. This first action had two consequences: the central stimulus disappeared and a second white square appeared pseudo randomly in the left or right window of the screen. When the mouse nose-poked the second stimulus, it disappeared, and the reward delivery was accompanied by illumination of the tray light and a tone. Collection of the condensed milk triggered an inter-trial interval. After the ITI period (20 s), a new trial could start. Mice were expected to reach the criterion of 30 trials completed in less than 60 min for 2 consecutive days before they could start the main VMCL task.

Main VMCL task

The rule that must be learned in the VMCL task is generally expressed as follows: “If stimulus A appears, then go left; if stimulus B appears, then go right”. Basically, mice had to learn first to nose-poke the central window where a discriminative stimulus was displayed, then one of the 2 lateral locations depending on the nature of that central stimulus. Initiation of a new trial was followed by the appearance of a discriminative stimulus in the central window, which remained until the animal nose-poked it. This discriminative stimulus was chosen pseudo randomly among 2 possible stimuli that were different in shapes and colors (white icicle *vs* grey equal). After the first nose-poke, the central stimulus remained visible and 2 identical white squares appeared laterally on the left and on the right of the screen. The mouse then had to touch one of these 2 stimuli to get the reward according to the predefined rule. If the mouse nose-poked the correct stimulus during the choice phase (case 1: correct trial), reward delivery was accompanied by illumination of the tray light and a tone. Collection of the condensed milk started the ITI. After the ITI period (20 s), the mouse could initiate a new trial. If the mouse nose-poked the wrong stimulus during the choice phase (case 2: incorrect trial), all stimuli disappeared, no reward was given to the animal and the house light was switched on for a 10 s (time out) punishment period. The house light was then turned off again, and a correction ITI period (10 s) elapsed. Following this period, a correction trial procedure could occur, during which the same discriminative stimulus was presented first and the same lateral nose-poke was expected. Correction trials continued until the animal responded correctly to the screen, but were not counted towards the total number of trials completed. Mice were recorded for a total of 30 daily sessions, with a maximum of 30 trials or 60 min/session. Furthermore, all groups were counterbalanced: half

of the animals had to respond to the left when the grey equal was displayed and to the right when the white icicle was shown, whereas the other half had to learn the opposite rule.

2.4.2 T-maze continuous alternation task (TCAT) – Spatial working memory

The continuous alternation procedure used during experiment A was adapted from Gerlai (1998) and Spowart-Manning and Van der Staay (2004). Briefly, the apparatus consisted of an enclosed T-maze made of grey polyvinyl chloride, which was elevated one meter above the floor in a dimmed testing room. Extra maze cues were present on each arm side and directly illuminated by a low ceiling lighting (6-10 Lux). The start arm measured 54 X 8.5 X 20 cm, against 30 X 8.5 X 20 cm for the two horizontal arms facing each other (goal arms). Two removable guillotine doors permitted a manual control of the access to these goal arms during the experiment. A third guillotine door, located in the bottom part of the start arm, permitted to restrict the mouse to a specific area (14 X 8.5 X 20 cm) for a few seconds (around 5 s) before starting the behavioral testing.

Once this guillotine door of the start arm was opened, the evaluation began. During the initial phase, a mouse was encouraged to explore the start arm and one of the two goal arms while the other was blocked (left or right arm counterbalanced within each group). An animal was therefore forced to enter one of the two goal arms (full body including the tail), and eventually came back to the start arm. When it reached the extremity of the start arm, the guillotine door blocking one of the goal arms was raised and the choice phase started. After the animal had completely entered one of the two goal arms, access to the other goal arm was immediately blocked by lowering the corresponding door. Again, the mouse had to go back to the terminal part of the start arm to trigger the lift of the guillotine door and allow the onset of a new choice phase. This protocol was repeated until animals had explored the T-maze for a total of 14 trials or after 14 minutes. It was expected that animals with an intact working memory would remember which goal arm they had visited during the initial phase and that their natural trend to alternate (Deacon and Rawlins, 2006) would subsequently guide them to choose the other goal arm during the choice phase. After each evaluation, partitions of the T-maze were carefully cleaned with ethanol 70 % and a paper towel to eliminate the smell and traces of each animal.

2.5 Histological analysis

Following completion of behavioral testing, mice from experiments A and B were deeply anaesthetized after intraperitoneal administration of the Xylazine/Ketamine cocktail and killed by cervical dislocation. Brains were tidily removed, and postfixed in 4% paraformaldehyde (PFA; Boster Immunoleader, USA) for 2 days. Brain tissues were then dehydrated (Tissue-Tek VIP® 6; Sakura Finetek Inc., USA) and embedded in paraffin (Leica EG1150H; Leica GmbH, Germany). Thin (4-5 µm) coronal sections were cut on an automated rotary microtome (Microm HM355S; Thermo Scientific, Germany), mounted on special adhesion slides (SuperFrost Ultra Plus; Thermo Scientific, Germany) and processed for immunohistochemistry with the neuron-specific nuclear protein NeuN (Anti-NeuN, rabbit polyclonal antibody, [1:500] dilution; Merck Millipore, USA). Lesions were verified by light microscope examination of areas of interest. Cell destruction was noticed in the absence of neuronal staining. The extent of lesions was manually mapped onto standardized sections extracted from the mouse brain atlas (Paxinos and Franklin, 2001). Corresponding reconstructions of the smallest and biggest lesion extents are shown for each structure in Figures 2 and 3.

2.6 Data analysis

All data are presented as means ± SEM. Their representation and analysis used GraphPad Prism version 6.0 (GraphPad Software, San Diego, USA). Comparisons were considered as statistically significant when $p<0.05$. Five parameters were measured in touchscreen boxes: the response accuracy (% of correct responses), the specific locomotor activity (defined as the total number of back and front beams broken during a session divided by the time spent to achieve the total number of trials, and expressed in beams/min), and three different latencies (correct touch latency, incorrect touch latency, and magazine latency). These latencies correspond to the time necessary to nose-poke the correct part of the screen, the incorrect one, or to get the reward into the magazine after a correct response, respectively. While accuracy and specific locomotor activity parameters were plotted in blocks of 3 (dPAL task) or 5 (VMCL task) sessions and analyzed using a 2-way ANOVA (factors: nature of the lesion/group and time) with repeated measures on the latter factor, other measures were subject to a 1-way ANOVA (factor nature of the lesion, experiment A) or an unpaired t-test (factor group, experiment B). In both cases, comparisons were performed using Tukey (against the corresponding control) or Bonferroni post-hoc analyses. In the TCAT, two parameters were measured: the percentage of alternation resulting from the number of correct choices divided by the total number of choices and multiplied by 100, and the total

time to achieve the maximal number of trials. As for latencies in touchscreens, both measures were statistically analyzed using a 1-way ANOVA (factor nature of the lesion) completed by a Tukey post-hoc test.

3) Results

***Post-mortem* evaluation of brain lesions (experiments A and B)**

Injections of the excitotoxic agent produced a massive neuronal death in the areas of interest, reaching either the whole HPC formation (the dentate gyrus, the three subregions of the Ammon's horn and the subiculum; figure 2) or the DS (dorso-medial and dorso-lateral parts of the caudate-putamen nucleus; figure 3). In experiment A, 3 animals with incomplete HPC lesions were excluded from the corresponding group, whilst 5 animals were set aside of the DS lesioned group (unilateral/bilateral damage restricted to the DMS). Final sample size was 11 HPC sham controls; 10 HPC lesioned mice; 12 DS sham controls; 9 DS lesioned mice. In experiment B, 3 animals with incomplete HPC lesions and 2 sham controls with cortical damage were ruled out. Final effectives consisted in n=10 post-training HPC lesioned mice and n=10 post-training HPC sham controls.

Experiment A: pokey training and acquisition in the dPAL task

During the habituation to the reward, all groups clearly showed a high interest for the condensed milk (data not shown), demonstrating an unaltered motivational state. Additionally, they needed an equivalent number of pokey training sessions to reach pre-defined criterion (group HPC Sham: 5.27 ± 0.27 sessions, group HPC Les: 5 sessions, group DS Sham: 5 sessions, group DS Les: 5.56 ± 0.24 sessions). After mice had achieved the last pokey training stage, the assessment in the dPAL task started. Corresponding results are presented in figure 4 and table 2.

Over training sessions, performance improved substantially in 3 out of 4 groups (figure 4A), explaining the overall effect of Time on % of correct responses (RM 2-way ANOVA: $F(7,266)=59.32$; $p<0.0001$). Performance of animals either sham-operated or with lesions of the HPC did not differ from each other. Conversely, in mice subjected to DS lesions, there was almost no improvement, explaining the significant Group x Time interaction (RM 2-way ANOVA: $F(21,266)=4.39$; $p<0.0001$). From the fourth 5-session block onwards, their performance was significantly below that of the three other groups (Tukey's *post-hoc* tests: all $q>4$; all $p<0.05$), indicating an impossible acquisition of the task over the 8 5-session blocks following DS lesions.

In parallel, a similar pattern of activity was observed in all groups except animals bearing HPC lesions, as evidenced by the main Group effect (RM 2-way ANOVA: $F(3,36)=30.80$; $p<0.0001$) and Group x Time interaction (RM 2-way ANOVA: $F(21,252)=2.58$; $p=0.0003$) on specific locomotor activity parameter over time (figure 4B). Compared with sham control or DS lesioned groups, HPC lesioned mice were significantly more active throughout the learning phase (Tukey's *post-hoc* tests: all $q>7$; all $p<0.0001$).

Furthermore, the learning deficit previously exposed in DS lesioned animals was accompanied by a global decrease of reactivity in these animals (table 2), in accordance with the significant Group effect observed on correct, incorrect and magazine latencies (1-way ANOVA: $F(3,38)=13.98$, $p<0.0001$; $F(3,38)=14.29$, $p<0.0001$ and $F(3,38)=26.57$, $p<0.0001$, respectively). Indeed, significantly increased latencies were uniquely observable in animals with DS lesions as compared with all other groups (Tukey's *post-hoc* tests: all $q>5$; all $p<0.01$).

Experiment A: pokey training and acquisition in the VMCL task

To investigate the neural substrates involved in habit learning processes, we studied the effect of HPC or DS lesions on the acquisition of the VMCL task in touchscreen boxes. Following the acquisition of the dPAL task and 3 weeks of free access to food, mice were again placed under restricted food diet and trained in a shortened version of the pokey training. All groups needed a comparable number of pokey training sessions to reach pre-defined criterion (group HPC Sham: 3 sessions, group HPC Les: 3.20 ± 0.20 sessions, group DS Sham: 3 sessions, group DS Les: 3 sessions). Upon completion of the last pokey training stage, mice began to be evaluated in the VMCL task. Results of the different parameters measured are shown in figure 5 and table 2.

As for the dPAL task, 3 out of 4 groups readily learned the VMCL task over time (figure 5A), clarifying the overall effect of Time on accuracy (RM 2-way ANOVA: $F(9,342)=47.76$; $p<0.0001$). Learning curves of animals either sham-operated or with lesions of the HPC overlapped most of the time. Conversely, in mice subjected to DS lesions, performance stagnated around 60-65 % of correct responses after 10 3-session blocks, explaining the significant Group x Time interaction (RM 2-way ANOVA: $F(27,342)=3.79$; $p=<0.0001$). As early as block 3, and later from the fifth 3-session block onwards, performance of mice with DS lesions was significantly lower than those of other groups (Tukey's *post-hoc* tests: all $q>4$; all $p<0.05$), suggesting an impairment of the acquisition of the VMCL task in these animals.

Measures of locomotion disclosed equivalent levels of activity in all groups apart from HPC lesioned animals, reflecting the main Group effect (RM 2-way ANOVA: $F(3,35)=26.00$; $p<0.0001$) and Group x Time interaction (RM 2-way ANOVA: $F(27,315)=2.01$; $p=0.003$) on specific locomotor activity parameter over time (figure 5B). Thus, in comparison with sham control and DS lesioned groups, hippocampectomized mice were hyperactive during the whole acquisition of the VMCL task (Tukey's post-hoc tests: all $q>8$; all $p<0.0001$).

Similar correct, incorrect and magazine latencies were recorded in sham control and HPC lesioned groups. This was in contrast with higher corresponding measures in animals with DS lesions (table 2), explaining a main Group effect for all parameters considered (1-way ANOVA: $F(3,38)=4.11$, $p=0.013$; $F(3,38)=15.04$, $p<0.0001$; $F(3,38)=4.57$, $p=0.008$, respectively). Nevertheless, in most cases, planned comparisons against other groups failed to reveal a significant effect in DS lesioned mice (Tukey's *post-hoc* test: all $q<3$, all $p>0.05$ for correct latencies; all $q>5$, all $p<0.01$ for incorrect latencies; all $q<4$, all $p>0.05$ for magazine latencies). *In fine*, these results rather suggested similar reaction times in all groups tested in the VMCL task.

Experiment A: evaluation in the T-maze Continuous Alternation Task (TCAT)

After completion of the second touchscreen task, mice were given food *ad libitum* and let at rest in their home cages for 3 weeks. They were subsequently tested in a T-maze modified procedure involving an initial randomly assigned forced trial that preceded a total of 14 free-choice trials. Corresponding results are presented in figure 6.

In this paradigm, response accuracy (64.29 ± 3.85 % in HPC Sham; 38.57 ± 4.15 % in HPC Les; 62.50 ± 2.93 % in DS Sham; 46.03 ± 4.31 % in DS Les) differed according to the nature of the lesion (figure 6A), from whence a main Group effect found on the percentage of alternation parameter (1-way ANOVA: $F(3,38)=11.15$; $p<0.0001$). More particularly, whereas HPC and DS sham controls performed similarly (Tukey's *post-hoc* test: $q(38)=0.50$; $p=0.985$), a significant difference was showed between HPC lesioned and HPC sham groups on one hand (Tukey's *post-hoc* test: $q(38)=6.82$; $p=0.0001$), and between DS lesioned and DS sham groups on the other hand (Tukey's *post-hoc* test: $q(38)=4.33$; $p=0.020$). The total time spent in the T-maze (560.40 ± 57.62 s in HPC Sham; 371.60 ± 14.76 s in HPC Les; 473.90 ± 38.05 s in DS Sham; 456.80 ± 58.17 s in DS Les) was also influenced by the nature of the lesion (figure 6B), thus in agreement with a significant Group effect observed on this parameter (1-way ANOVA: $F(3,38)=2.91$; $p=0.047$). HPC and DS sham groups (Tukey's *post-hoc* test: $q(38)=1.99$; $p=0.500$) spent a comparable amount of time to perform the maximal number of trials in the T-maze, so did DS lesioned and sham groups (Tukey's *post-*

hoc test: q(38)=0.37; p=0.993). Conversely, HPC lesioned animals achieved the total number of trials significantly quicker than HPC sham controls (Tukey's *post-hoc* test: q(38)=4.16; p=0.027). Overall, these results demonstrated that both HPC and DS lesioned groups were impaired in the spatial working memory task. Additionally, the former group needed less time for an equivalent number of trials, which was in accordance with the hyperactivity pattern previously observed in touchscreen devices.

Experiment B: pokey training, acquisition and recall in the dPAL task

To determine the role of the hippocampus in recall processes occurring in the dPAL task, a second batch of animals was first trained in the paradigm. As for mice of experiment A, they were all motivated by the condensed milk, quickly reached criteria imposed by pokey training sessions (post-training HPC Sham and HPC Les groups: 5 sessions) and performed the total number of trials during each session. Corresponding results are presented in figure 7 and table 2.

Acquisition of the dPAL task was characterized by a major improvement of the performance in both groups (figure 7A), accounting for the main Time effect on accuracy (RM 2-way ANOVA: $F(7,126)=46.10$; $p<0.0001$) in the absence of Group x Time interaction (RM 2-way ANOVA: $F(7,126)=1.21$; $p=0.300$). Simultaneously, the locomotor activity of both groups gradually decreased (figure 7B), as justified by the significant effect of Time on specific locomotor activity parameter (RM 2-way ANOVA: $F(7,98)=9.43$; $p<0.0001$) without any Group x Time interaction (RM 2-way ANOVA: $F(7,98)=0.49$; $p=0.838$). Eventually, no convincing evidence of altered response (table 2) was found over acquisition through correct touch, incorrect touch or magazine latencies (unpaired two-tailed tests, respectively: $t(18)=1.705$, $p=0.105$; $t(18)=1.904$, $p=0.073$; $t(18)=0.90$, $p=0.378$). Together, these results supported a similar acquisition of the dPAL task in both groups before hippocampal/sham lesions.

Mice underwent surgery and after recovery, were retested in the same assay (figure 7). Data were then compared between groups over time (final block of acquisition *vs* post-acquisition retrieval sessions). The free-training period following operations later provoked a robust diminution of the recall performance in both groups (figure 7A), which was proved by a main Time effect observed on % of correct responses (RM 2-way ANOVA: $F(1,18)=105.00$; $p<0.0001$). However, an overall effect of Group and a Time x Group interaction on accuracy (RM 2-way ANOVA, respectively: $F(1,18)=16.02$, $p=0.0008$; $F(1,18)=6.23$; $p=0.022$) indicated dissociable changes between groups. Indeed, and contrary to last sessions of acquisition (Bonferroni's *post-hoc* test: $t(36)=0.99$; $p=0.653$), performance of mice bearing post-training

HPC lesions (around chance levels) was significantly lower than those of sham controls during retrieval assessment (Bonferroni's *post-hoc* test: t(36)=4.58; p=0.0001). Similar observations (figure 7B) stemmed from the specific locomotor activity parameter (RM 2-way ANOVA: group effect: F(1,14)=32.59, p<0.0001; time effect: F(1,14)=18.52, p=0.0007; group x time interaction: F(1,14)=40.82, p<0.0001). Unlike the last acquisition block (Bonferroni's *post-hoc* test: t(28)=0.12, p=0.99), a specific rise of locomotion appeared in post-training HPC lesioned mice during recall sessions in comparison with sham controls (Bonferroni's *post-hoc* test: t(28)=8.49, p<0.0001). Globally, these results suggested that unlike sham controls, post-training hippocampal lesioned mice were unable to remember the learned rule during recall sessions and displayed a characteristic increase in locomotor activity.

4) Discussion

This study investigated the selective effects of HPC or DS lesions in different cognitive tasks adapted to mice in touchscreen boxes. Contrary to pre-training DS lesions, whole HPC lesions spared the acquisition of two instrumental paradigms in which access to the appetitive reward was conditioned by the assimilation of visuo-spatial (dPAL task) or visuo-motor (VMCL task) associations. Both types of lesions impaired the memory performance in the T-maze continuous alternation task (experiment A). Moreover, in experiment B, post-training hippocampal lesions disturbed memory retrieval performance during the recall of the dPAL task. In agreement with previous studies (Deacon et al., 2002; Goddyn et al., 2006), HPC lesioned mice were found to be hyperactive, whereas DS lesioned mice showed activity levels similar to controls. This last finding strengthened the idea that the observed cognitive deficits were not biased by motoric lesion effects (Featherstone et al., 2005).

Based on the association of object or word pairings (desRosiers and Ivison, 1988; Duchek et al., 1991), early human versions of the dPAL task have long served as remarkable neuropsychological tools to characterize a selective deficit of visuo-spatial memory in patients with medial temporal lobe dysfunctions, e.g. Alzheimer's disease. The preferential use of a visuo-spatial version of the task with stimuli being displayed on a touchscreen device and associated with specific locations similarly showed hippocampal sensitivity in humans (Swainson et al., 2001; de Rover et al., 2011). In addition, it widened perspectives with respect to the possibility to translate the paradigm in animals (Taffe et al., 2004; Talpos et al., 2009; Bartko et al., 2011). A majority of animal studies have ruled in favor of the requirement of the HPC during acquisition of various PAL tasks, in particular when one of the

two pieces of information to be associated is spatial by nature (Gilbert and Kesner, 2002; Langston et al., 2010). Therefore, our finding emphasizing that only DS lesions affected the acquisition of the dPAL touchscreen task in mice might be seen as intriguing. However, the participation of the HPC to appetitive forms of instrumental learning remains largely controversial when it pertains to the elaboration of long-term memories. Whilst rodents with HPC lesions are clearly impaired in studies involving working memories (McTighe et al., 2009; Talpos et al., 2010), they are generally found to learn other paradigms (Corbit and Balleine, 2000; Balleine et al., 2009). This last conclusion similarly arose in this study concerning the VMCL task as HPC lesions had no effect on the acquisition of this paradigm in mice, which was consistent with a previous study performed in HPC-lesioned rats assessed in a prior version of the VMCL task utilizing slow *vs* fast flashing lights as discriminative stimuli (Marston et al., 1993). Moreover, according to the testing conditions and/or required adaptations, a similar cognitive task can depend upon the integrity of different brain areas across species. For instance, the eight-pair concurrent discrimination task is hippocampal-dependent in humans but striatal-dependent in monkeys (Broadbent et al., 2007). In this context, it is conceivable that adapting the dPAL touchscreen task from Humans (1 session; 6-8 patterns for a maximal of 56 trials; no reward) to rodents (40 sessions; 3 patterns for a total of 1440 trials; liquid reward) could result in a paradigm in which flexible choices required an alternative strategy and were mediated by neural substrates other than the HPC.

The DS constitutes a good candidate to arbitrate cognitive processes supporting incentive learning, decision-making and action control (Balleine et al., 2007; Devan et al., 2011; Hart et al., 2014). In agreement with the heterogeneous connectivity of this brain structure (McGeorge et Faull, 1989; Groenewegen & Berendse, 1994), recent research has emphasized the existence of two distinct forms of learning that depend upon different DS subregions in rodents. The DMS, or associative striatum, thus encodes the causal association of specific responses and outcomes (R-O), guiding the expression of goal-directed actions. In this model, the value of the outcome is permanently estimated and strongly conditions the maintenance of the action control, as demonstrated by the sensitivity to outcome devaluation and contingency degradation. By contrast, habit learning involves the DLS, or sensorimotor striatum, and results from an exclusive focus on the associations linking stimuli and responses (S-R); consequently, this type of learning is insensitive to the aforementioned manipulations (Balleine et al., 2009; Balleine and O'Doherty, 2010). In this context, should one attribute behavioral impairments observed in touchscreen tasks to the dysfunction of one of the DS subregions, or could they rather reflect the effects of the global

DS lesion? We propose that the acquisition of the dPAL task is likely to be supported by the DMS in mice, while the DLS would solely drive the learning of the VMCL task, as previously described in rats (Reading et al., 1991). These assumptions are backed up by behavioral evidence in this study. First, the acquisition profile of sham controls in the dPAL and VMCL tasks suggests that these two paradigms can certainly be solved via the use of R-O and S-R associations, respectively (Devan et al., 2011): on one hand, the dPAL task is slowly acquired, with the final performance still being susceptible to increase after 40 sessions; one the other hand, the VMCL task is quickly acquired and final performance cannot be improved any more, even if the training period is extended. Second, although DS lesions disrupt the acquisition of both dPAL and VMCL paradigms, no memory interference is reported when controls are successively assessed in the two tasks (Delotterie et al., 2014). Third, preliminary data from few mice of this study exhibiting bilateral lesions restricted to the DMS indicate that they acquired the VMCL task normally, but were unable to learn the dPAL task (data not shown). One direction to verify this hypothesis could be to assess the acquisition of these tasks either following bilateral lesions of DMS or DLS regions, or corresponding cortical afferents (prelimbic or infralimbic areas of the medial prefrontal cortex). Indeed, as dorsal subregions are embedded in distinct corticostriatal loops, goal-directed and habitual forms of learning should be selectively disturbed after their direct or indirect loss of function (Coutureau and Kilcross, 2003; Ostlund and Balleine, 2005). Another approach might consist in examining devaluation effects following the administration of lithium chloride after acquisition of these tasks in normal mice: if the dPAL task is DMS-dependent, animals' performance should selectively and quickly decrease after outcome devaluation in this task; if the VMCL task is DLS-dependent, mice should carry on performing accurately.

Early promoted after the discovery of place cells, the role of the HPC in spatial learning and navigation has been established on many occasions in rodents (O'Keefe and Dostrovsky, 1971; Paul et al., 2009). Yet, hippocampal functions are not constrained to that unique role. Accordingly, it was abundantly expected and then confirmed to find a deficit in our hippocampectomized mice in the T-maze continuous alternation paradigm, an exploratory task especially designed to detect subtle dysfunctions of this brain area (Gerlai, 1998; Spowart-Manning et al., 2004). More prominent was the fact that mice with DS lesions were also impaired in the same task. As no retention interval separated successive free-choice trials, the important drop of alternation rates following HPC or DS lesions could not simply result from the poor use of extra-maze spatial cues (Lalonde, 2002). More likely, HPC lesions profoundly disrupted working memory processes (Roberts et al., 1962; Deacon et al., 2002) whereas DS lesions hindered the coordination of egocentric responses (Divac et al., 1975).

In addition to the different already addressed in experiment A, this study also provided complementary evidence that despite its striatal-dependent acquisition, the post-training retrieval (5 weeks) of the dPAL task implicated the hippocampus in mice. This last original finding seemed in line with the fact that post-acquisition intra-hippocampal or systemic injections of cognitive enhancers/impairers strongly influence the performance of rats prior trained in the dPAL task (Talpos et al., 2009; Talpos et al., 2014).

We have presently adduced weighty arguments in favor of the commitment of two brain regions, the HPC and the DS, to different cognitive processes in mice. More precisely, results obtained in touchscreen devices have allowed positioning functional roles in mice for the DLS in habit learning via incremental S-R associations in the acquisition of the VMCL task, for the DMS in goal-directed learning via flexible R-O associations and the HPC in relational memory during the acquisition or post-acquisition phases of the dPAL task, respectively. Thus, notwithstanding the difficulty to properly translate behavioral paradigms across species (herein illustrated by the eloquent example of the dPAL task), touchscreen assays may represent valuable tools for the future detection of cognitive impairments in translational animal models of human neurodegenerative and neuropsychiatric disorders (Hadj-Bouziane et al., 2012; Griffiths et al., 2014). Schizophrenia so far represents the most documented pathology (Bussey et al., 2012; Bussey et al., 2013), as seemingly demonstrated by recent publications stressing parallel impairments of goal-directed learning function in patients (Gold et al., 2008; Morris et al., 2014) and transgenic mouse models of the disease (Coba et al., 2012; Nithianantharajah et al., 2013).

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1. Tables and figures

Table 1. Stereotaxic coordinates and injected volumes used to induce bilateral lesions of the hippocampus or dorsal striatum.

Site names	AP (mm)	ML (mm)	DV (mm)	Volume (nL)
HPC1/5	- 2.0	± 1.2	- 1.8	100
HPC2/6	- 2.5	± 2.2	- 1.9	100
HPC3/7	- 3.0	± 3.2	- 3.0	125
HPC4/8	- 3.0	± 3.2	- 4.0	125
DS1/3	+ 0.3	± 1.7	- 3.1	300
DS2/4	+ 0.3	± 2.4	- 3.1	300

All coordinates were calculated after determination of the Bregma point.

AP: Antero-Posterior; ML: Medio-Lateral; DV: Dorso-Ventral axes; HPC: Hippocampus; DS: Dorsal Striatum.

Table 2. Mean values of the different latencies measured over acquisition of touchscreen tasks

Touchscreen paradigm	dPAL task (experiment A)		
Group	CTL (s)	ITL (s)	ML (s)
HPC Sham	2.56 ± 0.13	2.64 ± 0.12	1.51 ± 0.03
HPC Les	1.79 ± 0.16	1.75 ± 0.13	1.23 ± 0.08
DS Sham	3.20 ± 0.39	3.17 ± 0.38	1.65 ± 0.13
DS Les	5.54 ± 0.77*	5.38 ± 0.72*	2.70 ± 0.20*
Touchscreen paradigm	VMCL task (experiment A)		
Group	CTL (s)	ITL (s)	ML (s)
HPC Sham	0.87 ± 0.10	0.32 ± 0.06	1.16 ± 0.03
HPC Les	0.69 ± 0.06	0.27 ± 0.05	0.87 ± 0.06
DS Sham	1.03 ± 0.09	0.49 ± 0.05	1.35 ± 0.18
DS Les	1.12 ± 0.10	0.89 ± 0.12*	1.45 ± 0.10
Touchscreen paradigm	dPAL task (experiment B) - acquisition		
Group	CTL (s)	ITL (s)	ML (s)
Post-training HPC Sham	3.17 ± 0.38	3.23 ± 0.37	1.45 ± 0.04
Post-training HPC Les	2.48 ± 0.14	2.49 ± 0.12	1.38 ± 0.06

CTL: Correct Touch Latency ; ITL: Incorrect Touch Latency;

ML: Magazine Latency

All parameters are expressed as mean values ± SEM.

* $p<0.05$ vs all other groups

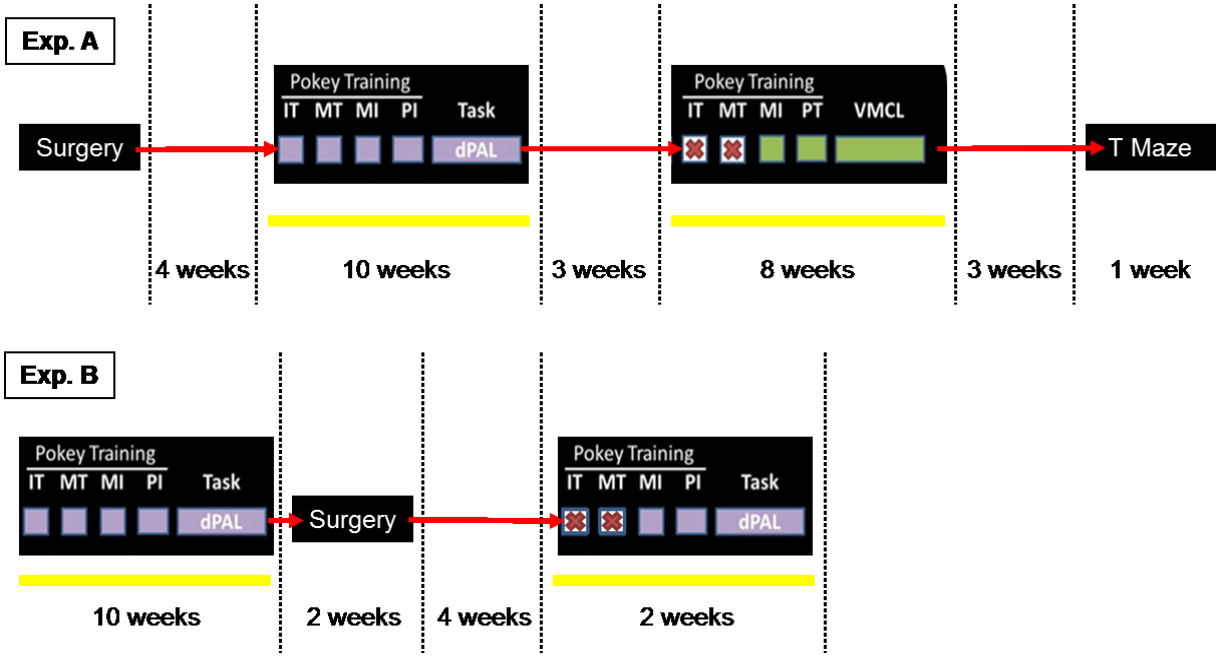

Figure 1. General design of behavioral procedures. In experiment A, hippocampal and dorso-striatal lesioned mice and their sham-operated littermates were successively assessed in two touchscreen paradigms (dPAL and VMCL tasks) and the T-Maze forced continuous alternation task (TCAT). Upon completion of a cognitive assay, mice were offered three weeks of free access to the food. This allowed avoiding an excessive time spent under food-restriction. In experiment B, animals were first trained in the dPAL task, then lesioned in the hippocampus and later retested in the same paradigm. After surgery, mice were always given 4 weeks of recovery. Yellow bars indicate the testing phases during which animals were subject to food-deprivation. IT: Initial Touch; MT: Must Touch; MI: Must Initiate; PI: Punish Incorrect; PT: Pre-Training.

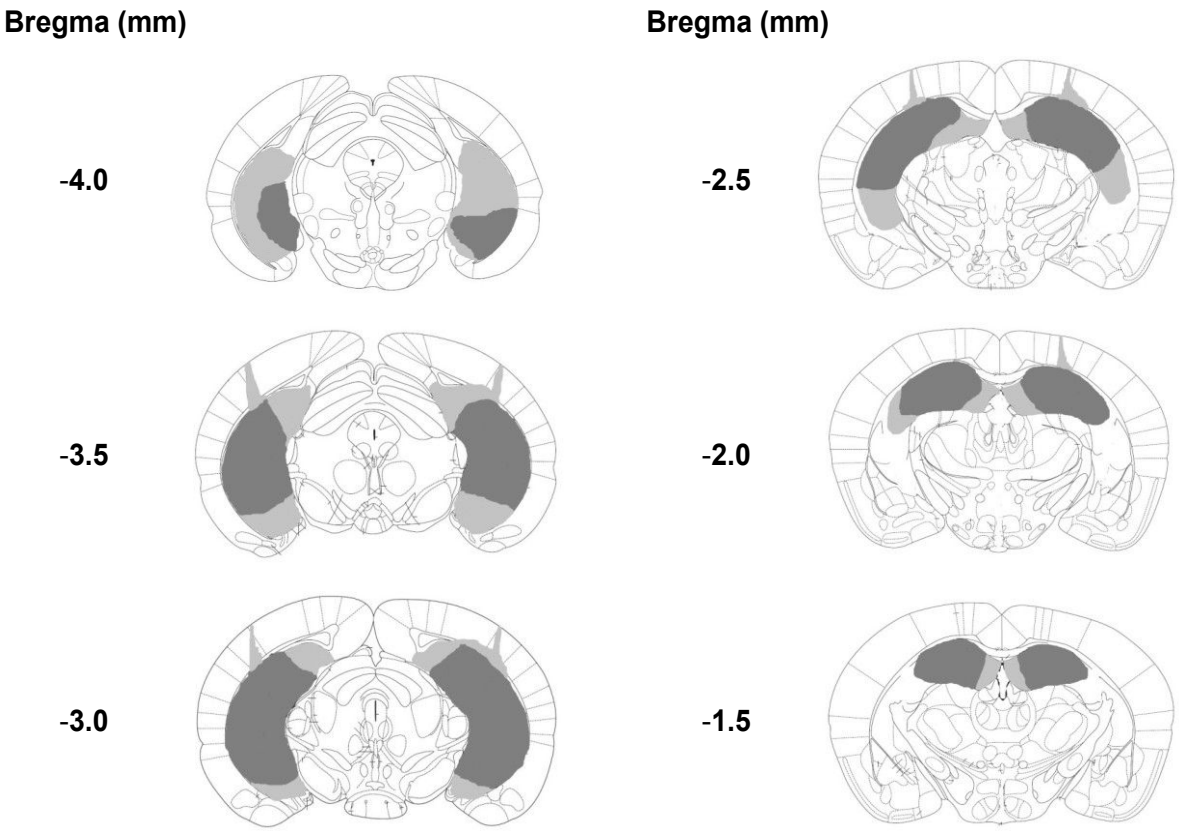

Figure 2. Reconstruction of the minimal and maximal extents of excitotoxic lesions performed in the hippocampus (experiments A and B) in mice. Dark grey shading corresponds to the smallest lesion, while light and dark greys indicate the largest lesion.

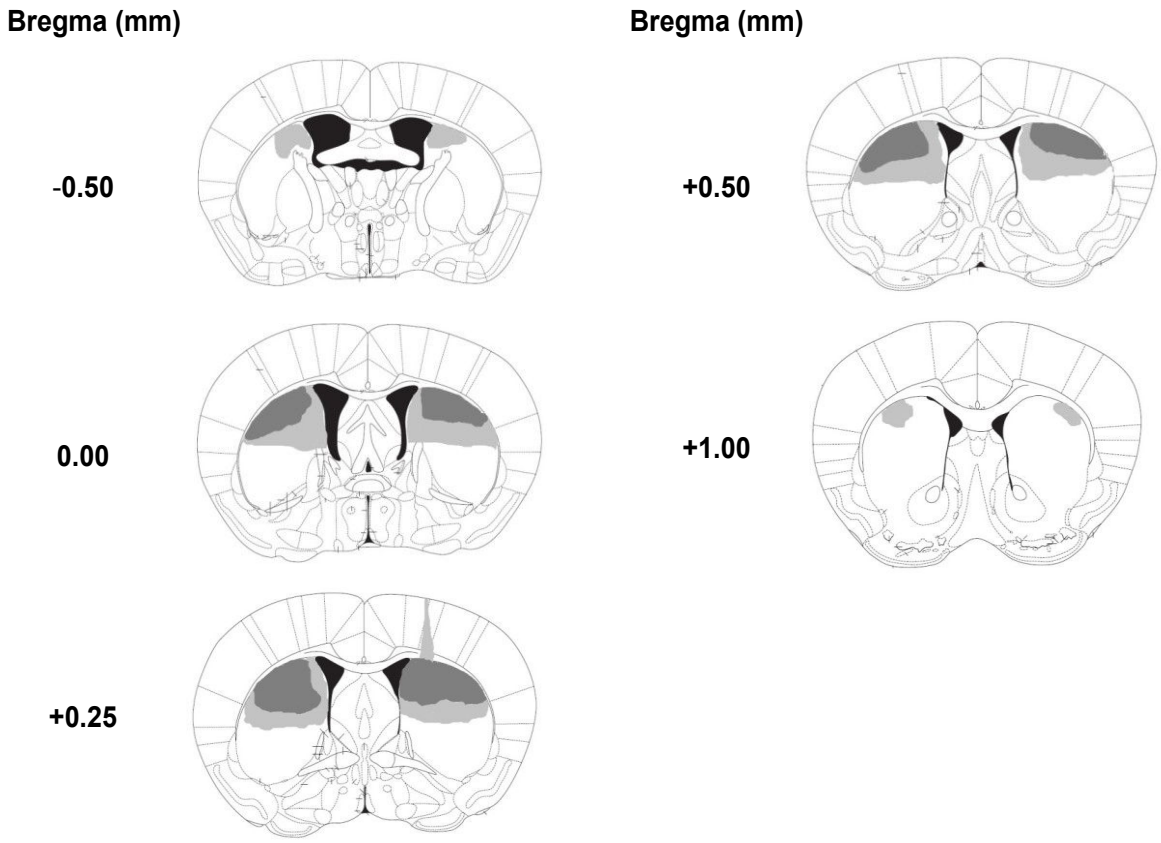

Figure 3. Reconstruction of the minimal and maximal extents of excitotoxic lesions performed in the dorsal striatum (experiment A) in mice. Dark grey shading corresponds to the smallest lesion, while light and dark greys indicate the largest lesion. In black, lateral ventricles.

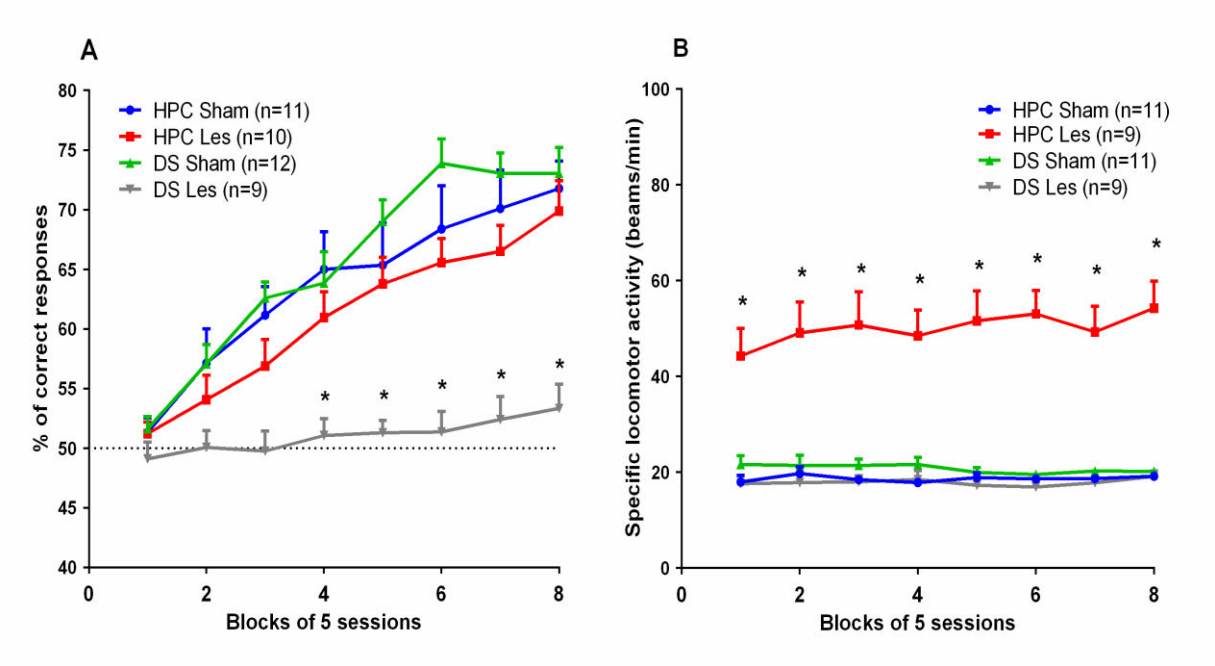

Figure 4. Acquisition performance measured through accuracy (A) and specific locomotor activity (B) in hippocampal or dorso-striatal lesioned/sham mice in the touchscreen dPAL task (experiment A). * p<0.05 *vs* all other groups.

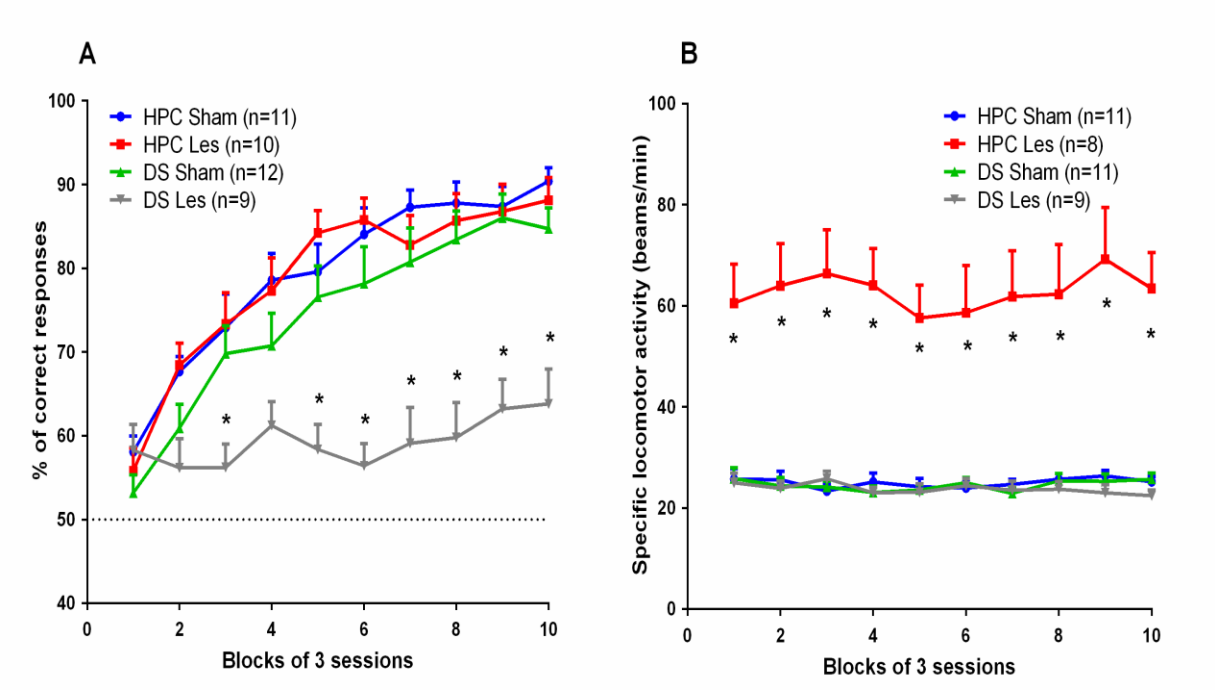

Figure 5. Acquisition performance measured through accuracy (A) and specific locomotor activity (B) in hippocampal or dorso-striatal lesioned/sham mice in the touchscreen VMCL task (experiment A). * p<0.05 *vs* all other groups.

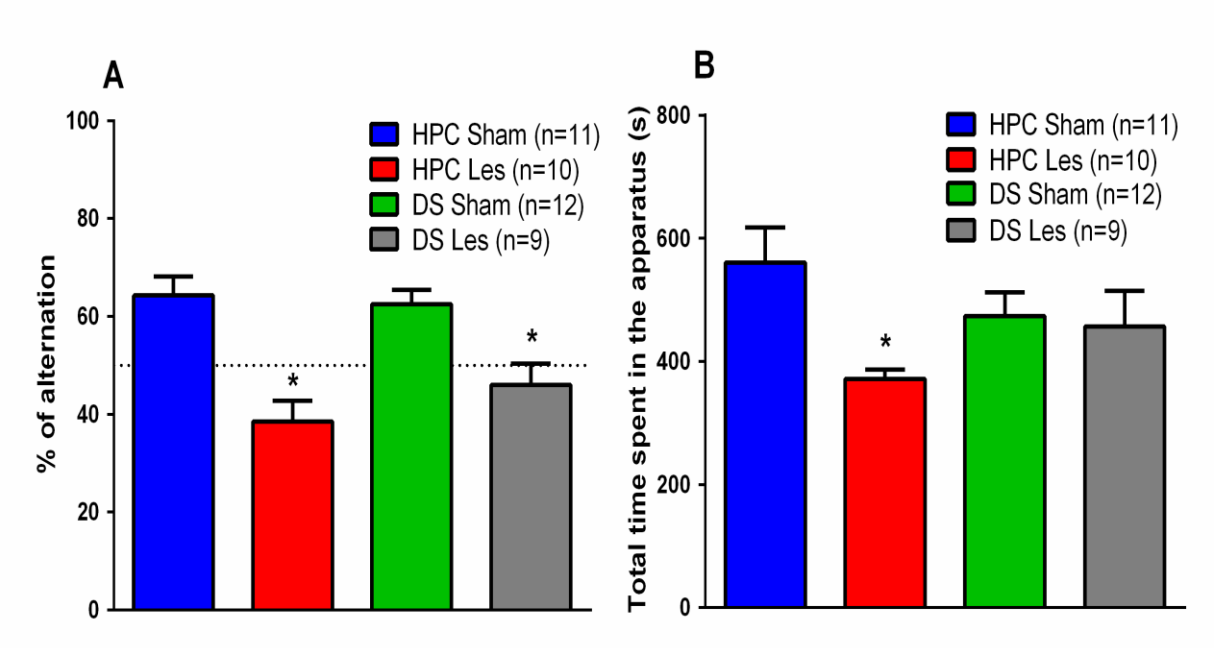

Figure 6. Percentage of alternation (A) and total time spent in the T-Maze (B) in hippocampal or dorso-striatal lesioned/sham mice assessed in the continuous alternation task (experiment A). * p<0.05 *vs* the corresponding sham group.

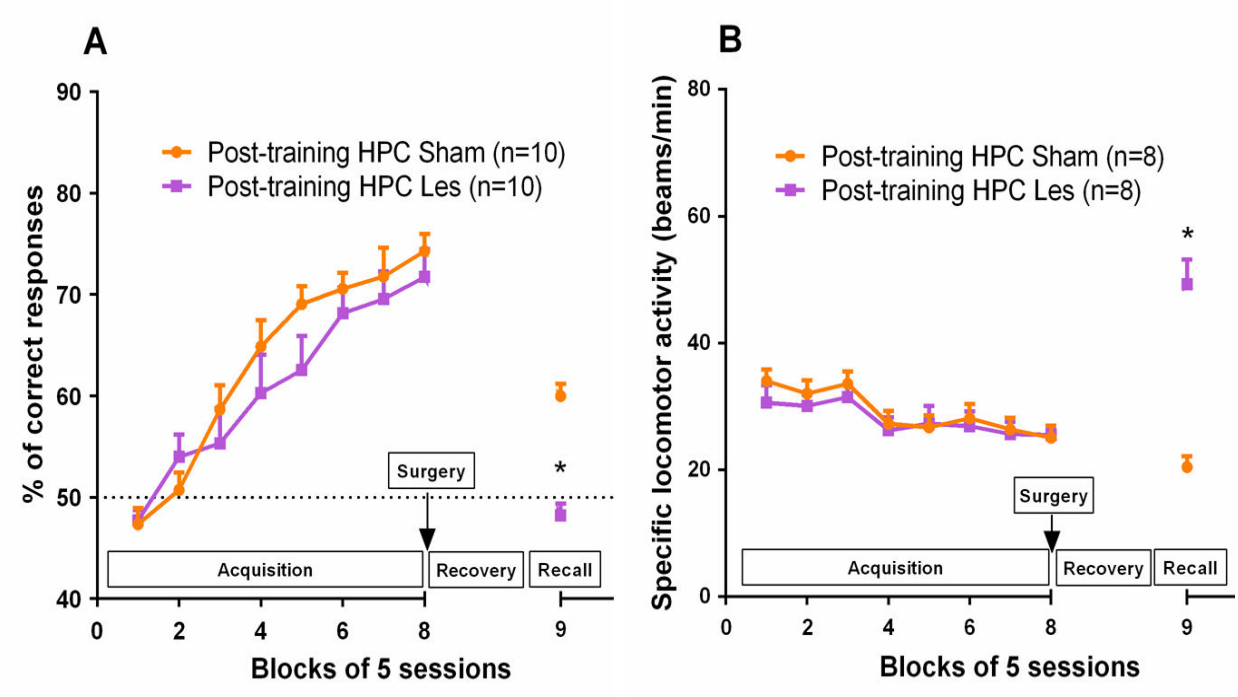

Figure 7. Acquisition and recall performance measured through accuracy (A) and specific locomotor activity (B) in post-trained hippocampal lesioned/sham mice in the touchscreen dPAL task (experiment B). * p<0.05 *vs* sham controls.

❖ Complementary results 1

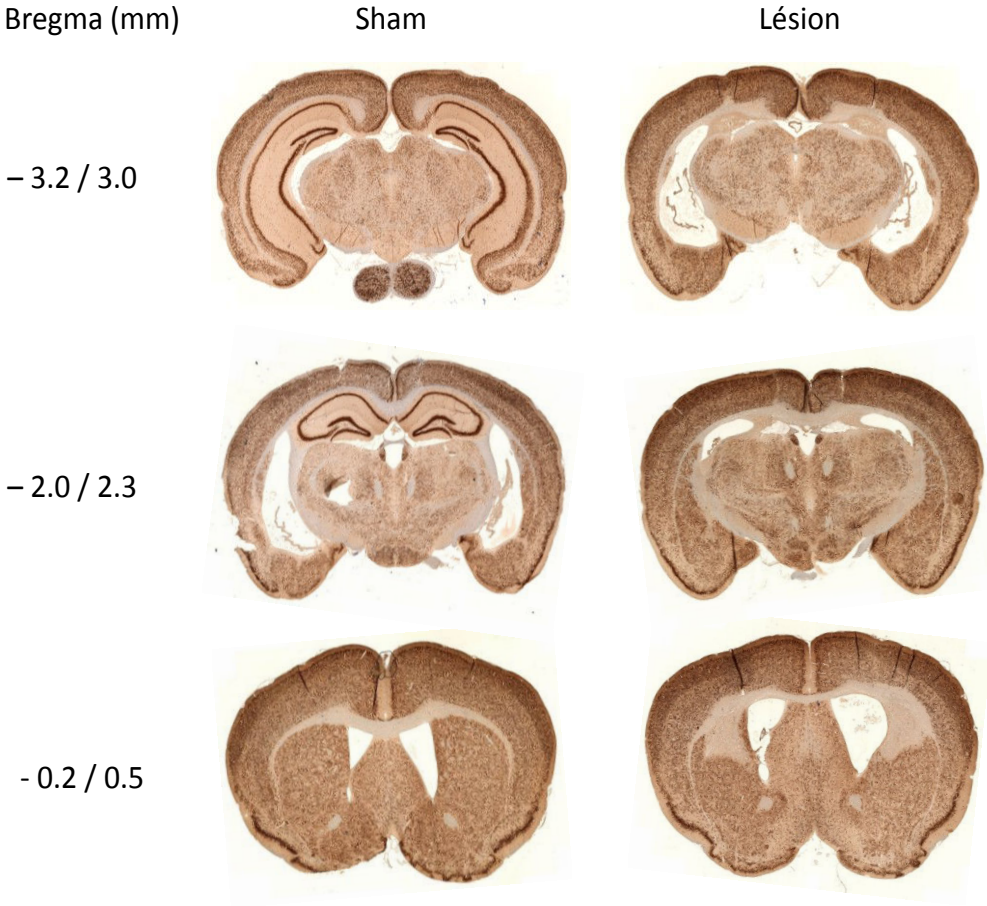

Figure 31. Localization of hippocampal (ventral hippocampus, upper panel; dorsal hippocampus, middle panel) or dorso-striatal (lower panel) lesions in coronal slices of mouse brain following NeuN immunostaining.

❖ Complementary results 2

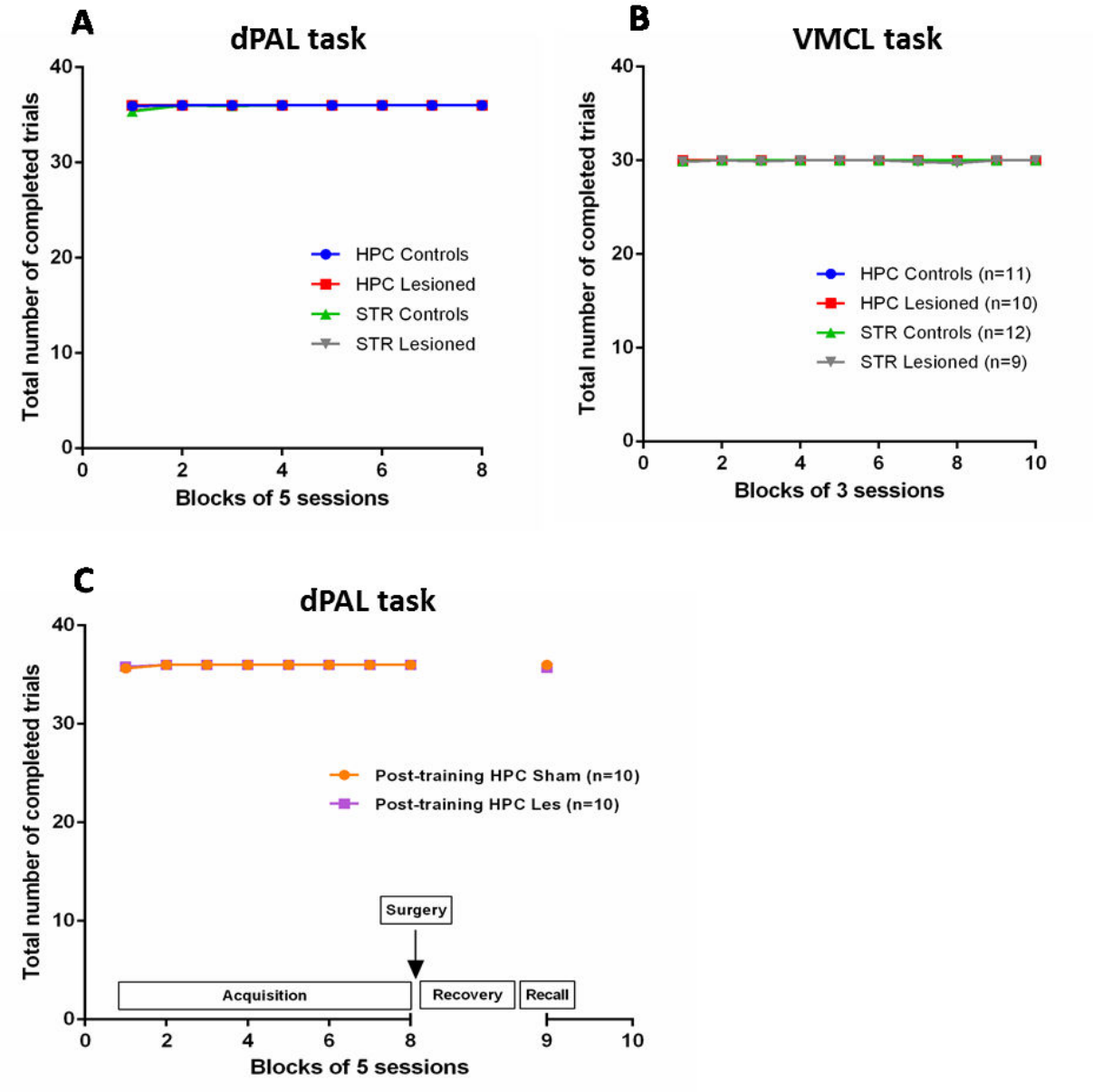

Figure 32. Total number of completed trials measured in hippocampal or dorso-striatal lesioned/sham mice assessed in the dPAL or VMCL tasks. Lesions occurred before training in experiment A (panels A and B) or post-training in experiment B (panel C). Because all animals achieved their daily session, no statistical analysis was conducted.

❖ Intermediary discussion

An overview of the main results cumulated throughout these two experiments is presented below (**Tables 16-17**). After a short summary, we will sequentially focus on cognitive aspects of each task.

Global outcomes

The hyperactive profile following chemically-induced lesions of the hippocampus (**Figure 31**) had been frequently reported in rodents (Deacon et al., 2002; Goddyn et al., 2006). Likewise, our HPC lesioned animals were between 2 and 3 times more active than other groups in touchscreen devices. Moreover, they achieved the total number of trials quicker than their corresponding sham group in the forced alternation task. In parallel, mice bearing DS lesions (**Figure 31**) presented levels of activity comparable to sham controls, as indicated by their specific locomotor activity in touchscreen tasks and total time spent to achieve the total number of trials in the T-Maze. Results were therefore consistent with previous lesion studies showing no major motor changes after partial (Featherstone and McDonald, 2005; Yin et al., 2005b) or full DS lesions (Neill et al., 1974; Lee et al., 2008). Besides, none of the two types of lesion resulted in the loss of motivation in appetitive tasks (**Figure 32**). Thus, DS and HPC lesioned mice readily completed the total number of trials imposed by the different paradigms.

Experiment A: DS recruitment during acquisition of the dPAL and VMCL tasks in mice

Severe learning impairments (dPAL: 50-55 % of accuracy after 40 sessions; VMCL: 60-65 % of accuracy after 30 sessions) were observed in touchscreen paradigms following DS, but not HPC lesions. These effects were strengthened by the significant increase of recorded latencies in DS lesioned mice. *A priori*, the finding related to the dPAL task was the most puzzling, as the task had been showed to be hippocampal-dependent in Humans (de Rover et al., 2011). Similarly, studies using a PAL task in rats trained in a cheeseboard maze apparatus had reported the sensitivity of hippocampal lesions (Gilbert and Kesner, 2002; Langston et al., 2010). However, as already evoked in chapter 6, efforts made to adapt the PAL assay from Humans to rodents comprised fundamental changes favoring the preferential requirement of DS regions. First, animals were rewarded after each correct response during their training. For this purpose, reinforcement learning occurred in operant

EXPERIMENT A	Accuracy	Locomotor activity	Total N° of completed trials	Latencies
dPAL task	HPC = DS ↘	HPC ↗ DS =	HPC = DS =	HPC = DS ↗ (CTL, ITL, ML)
VMCL task	HPC = DS ↘	HPC ↗ DS =	HPC = DS =	HPC = DS ↗ (ITL)
T Maze	HPC ↘↘ DS ↘	HPC ↗ DS =	HPC = DS =	n/d

EXPERIMENT B	Accuracy	Locomotor activity	Total N° of completed trials	Latencies
dPAL task (Acquisition)	**=**	**=**	**=**	**=**
dPAL task (Recall)	Sham ↘ HPC ↘↘	Sham = HPC ↗	=	n/d

Tables 16-17. Summary of the principal results obtained in the different cognitive tasks (dPAL and VMCL touchscreen tasks, forced alternation task) during lesion studies. n/d: not determined; =: no difference between lesioned mice and their corresponding sham group; ↗: significant increase; ↘: significant decrease; ↘↘: massive decrease; CTL: Correct Touch Latency; ITL: Incorrect Touch Latency; ML: Magazine Latency.

chambers, an environment sometimes associated with short-term (McTighe et al., 2009; Talpos et al., 2010) but more rarely with long-term memory deficits in HPC lesioned animals (Marston et al., 1993; Corbit and Balleine, 2000; Balleine et al., 2009). Conversely, DS lesions were often followed by cognitive disturbances paradigms assessing instrumental conditioning (Dunnett et al., 2012). Second, if the number of object-place associations (max. 3 in rodents *vs* 8 in Humans) to learn was voluntarily decreased in the rodent version of the task to attenuate the difficulty of its human counterpart, every trial systematically started with the presentation of 2 stimuli, the “rewarded” one and a distractor. Importantly, this distractor was not a neutral stimulus but consisted of 1 of the 2 other objects displayed in a wrong location. The resulting cognitive demand was thus elevated and certainly contributed to the slow, progressive learning of the task. As for the VMCL task, our data concluded to the selective effect of DS lesions, which was in agreement with early studies led in rats underlining the functional role of the DLS in a preliminary version of the task (Reading et al., 1991; Marston et al., 1993).

Although a new serie of experiments could have been implemented to validate the specific functions of DS subregions through more circumscribed lesions or outcome devaluation studies, converging lines of evidence were sufficient to dissociate cognitive processes reflecting goal-directed and habit forms of learning (Balleine and Dickinson, 1998; Yin and Knowlton, 2006; Balleine et al., 2007; Balleine et al., 2009), respectively via the integrity of the DMS in the dPAL paradigm or the DLS in the VMCL task. Several elements tallied with that hypothesis. In our optimization and lesion studies, the 2 tasks possessed distinguishable patterns of acquisition (flexible choices resulting in a slow acquisition in the dPAL task, rigid choices resulting in a quick acquisition in the VMCL task). Both paradigms could be combined in a battery of tasks without any risk of interaction, whereas in the same time, DS lesions disrupted the acquisition of both tasks. Furthermore, some of our animals (excluded for the main studies presented above) with bilateral lesions restricted to the DMS exhibited a momentous impairment in the dPAL task which contrasted with their preserved capacities in the VMCL task.

Experiment A: HPC and DS lesions differently impair alternation in the T-maze

To validate the deleterious effects of HPC lesions in a more classical cognitive assay, HPC and DS sham and lesioned mice were then assessed in a forced alternation task evaluating working memory (Lalonde, 2002). In agreement with the selected behavioral procedure (Spowart-Manning and van der Staay, 2004), HPC lesioned animals performed under the

chance level in this exploratory task (40 % of alternation *vs* 60-65 % in HPC sham controls). This might have been due to the development of perseverative behaviors following neophobia (Mitchell et al., 1993; Gerlai, 1998). In any event, this effect did not result in a strong preference for one of the goal arms. Another deficit was uncovered following DS lesions in the same task (50 % of alternation *vs* 60-65 % in DS sham controls), which was rather imputable to the disturbance of the egocentric repository and led to random choices to enter left or right goal arms. Prior lesion studies conducted in rats had given rise to conflicting results (Divac et al., 1975; Thullier et al., 1996).

Experiment B: the HPC is engaged during post-acquisition recall of the dPAL task

Our results in experiment A proved that the HPC was not necessary for the acquisition of the dPAL task in mice. Yet, in a previous publication (Talpos et al., 2009), this structure had been showed to be involved in rats as intra-hippocampal delivery of glutamatergic antagonists (MK-801 or CNQX) significantly decreased the post-acquisition accuracy performance. Given the similarity of the behavioral procedure in both species, it was highly valuable to explore the effects of post-training HPC lesions during recall sessions in mice. During acquisition, both groups learned the dPAL task in a similar manner (around 70 % of accuracy after 40 sessions). Nevertheless, in comparison with sham lesions, post-training HPC lesions provoked a stronger decrease of the memory performance during recall sessions (chance level: 50 % of accuracy against 60 % in sham controls). This result originally emphasized the crucial role of the HPC in system consolidation processes (Frankland and Bontempi, 2005) in an appetitive task initially dependent upon the integrity of the DS.

Conclusion and perspectives

Introduced two decades ago for the first time in rodents, the touchscreen-based methodology has recently drawn more and more attention due to the multiplicity of proposed paradigms and their claimed translational potential with related computerized neuropsychological tasks (CANTAB) used to detect cognitive impairments in Humans. Under these circumstances, the primary purpose of this thesis work was to evaluate to which extent human and animal data could coincide, notably in the physiopathological framework of Alzheimer's Disease.

To achieve this objective, we first selected three different touchscreen tasks thought to target distinct cognitive processes. Learning patterns of several groups of young C57BL/6JRj mice trained in dPAL (object in place memory), VMCL (habit learning) and/or PVD (visual memory and executive functions) appetitive tasks were recorded. These early experiments taught us important lessons. Training conditions allowed significant improvements over time in all tasks. However, as exemplified by the new stimuli utilized in the PVD task, the variation of a single parameter, such as the size of objects displayed on the screen (Lines *vs* Ring), was sufficient to cause noticeable changes pertaining to the preferential choice of certain stimuli. Another point of interest concerned the successive evaluation of similar animals in different paradigms. Building a touchscreen test battery could lead to memory interferences (sPAL after VMCL task), presumably when similar neural substrates were repeatedly implicated throughout tasks of increasing difficulty. In this regard, and given the unsuccessful association of VMCL and PVD tasks in subsequent assays, it would be worthwhile creating a database allowing new investigators to estimate the possibility to cumulate some of these various assays consecutively. Indeed, albeit warmly recommended (Bussey et al., 2012), very few studies have so far appealed for longitudinal experimental designs (Nithianantharajah et al., 2013; Romberg et al., 2013) with the touchscreen technology. In this respect, such a tool could notably help avoiding future data misinterpretations.

Once testing conditions had been defined, we initiated the behavioral characterization of the Tg2576 murine line. As previously detailed, contrary to their WT littermates, TG mice modeled hallmarks of Alzheimer's Disease pathology. They carried out the mutated allel of the amyloid peptide precursor (APP) human gene, which caused a massive production of Aß during their life. This entailed the progressive appearance of neuropathological, behavioral and synaptic abnormalities in these mice. From a cognitive point of view, hippocampal-dependent forms of learning, for instance, were known to be impaired in those animals as early as 4-6 months of age. During pilot studies, we demonstrated that training conditions in touchscreen devices were suitable for the assessment of young and aged WT animals of the

Tg2576 line. Moreover, we identified two categories of individuals that met exclusion criteria (Yassine et al., 2013): on one hand, blind mice carrying a *rd* mutation resulting in early-life retinal degeneration; on the other hand, mice manifesting exacerbated forms of stereotypies (circling or wall-climbing behaviors). In spite of these precautions, following experiments did not stress any cognitive impairment in touchscreen tasks in young or aged TG mice. Although the absence of deficit was sometimes expected (preserved procedural memory in the VMCL task) or likely due to task interactions (PVD after VMCL task, no possible interpretation), the main and conflicting finding unmasked similar performances in young and aged animals of both genotypes in the dPAL task.

We then hypothesized that certain adaptations could account for the lack of translation of that task between Humans and mice. In particular, we wanted to investigate whether the extensive amount of training and presence of the reward (Nadel and Hardt, 2011) could lead to the differential involvement of neural substrates in the latter species. Accordingly, we compared the effects of HPC or DS fiber-sparing excitotoxic lesions on the acquisition of dPAL and VMCL tasks. Only DS lesions were found to disrupt the acquisition of both dPAL and VMCL tasks. Conversely, HPC lesions had no cognitive effect but increased the locomotor activity during acquisition. In contrast, post-acquisition HPC lesions displayed a substantial hyperactivity accompanied by a significant recall deficit, suggesting a late role of this structure limited to consolidation processes. These diverse effects indicated that the dPAL task was not translational as it has most probably distinct neural substrates in Humans and mice. In that context, the use of the Tg2576 line or pharmacological studies (Talpos et al., 2009; Talpos et al., 2014) was straightaway restricted to post-acquisition sessions only.

Even so, the interest for the dPAL task and to a lesser extent, the VMCL task, still remains as the identification of their respective neural substrates paradoxically opens new perspectives. Indeed, if further functional dissociations between the different subparts of the DS (DMS *vs* DLS) or their corresponding afferences (prelimbic or infralimbic cortices) constitutes a further direction to dissociate both paradigms, some evidence (learning profiles, absence of task interaction, behavior of mice with DMS-limited lesions) already convey the idea that in mice, the dPAL DMS-dependent task allows assessing goal-directed learning whereas the VMCL DLS-dependent task evaluates habit learning. Furthermore, in many neurodegenerative and neuropsychiatric disorders, the subtle balance between these 2 processes is susceptible to be affected. For example, apathy, that can be defined as a reduction of goal-directed learning in patients (Levy, 2012), is observed in various pathological conditions comprising schizophrenia (Gold et al., 2008; Sitnikova et al., 2009),

depression (Griffiths et al., 2014), drug addiction (Everitt and Robbins, 2005), Alzheimer's Disease (Onyike et al., 2007), stress and certain forms of anxiety (Gillan et al., 2011; Schwabe and Wolf, 2011), while habit learning is decreased in Parkinson's Disease (Redgrave et al., 2010; Hadj-Bouziane et al., 2012) but see (de Wit et al., 2011). On this matter, two recent publications have highlighted a deficit in the dPAL task in mice carrying TNIK and Dlg2 mutations associated with the onset of schizophrenia or cognitive dysfunction in Humans (Coba et al., 2012; Nithianantharajah et al., 2013). Such touchscreen tasks could therefore allow assessing the validity of new animal models for costly brain diseases, not to mention the promising possibility to dissect the differential involvement of neural circuitries through optogenetic approaches.

French summary

Potentiel translationnel d'une méthodologie basée sur des écrans tactiles pour évaluer les capacités cognitives chez la souris

La recherche biomédicale doit actuellement faire face à de nombreux défis, notamment en ce qui concerne la prise en charge socio-économique, le traitement et le suivi de personnes atteintes par des maladies en tout genre. Conséquence directe de l'amélioration des conditions de vie de l'être humain, le vieillissement de la population mondiale s'accompagne de l'émergence massive de maladies neurodégénératives chez les personnes âgées. Ainsi, la maladie d'Alzheimer, qui affecte drastiquement les capacités mnésiques des malades jusqu'à la perte totale de leur autonomie, constitue un exemple particulièrement préoccupant. En effet, cette maladie touchera bientôt près d'un million de personnes en France. Or, il n'existe à ce jour aucun traitement efficace permettant d'empêcher l'apparition et l'évolution morbide des symptômes associés à cette pathologie. La recherche de nouveaux modèles animaux et de méthodes innovantes d'évaluation cognitive constitue donc un enjeu sans précédent pour le développement et la sélection de futurs traitements curatifs ou préventifs (Savonenko et al., 2012).

Classiquement, les modèles animaux des maladies neurodégénératives humaines sont caractérisés sur la base de réponses comportementales variées obtenues dans différents tests cognitifs et dans des environnements distincts pour chaque test. Par exemple, un animal peut être assigné à un environnement identique tout au long de l'apprentissage d'une tâche de conditionnement opérant et devoir appuyer sur une pédale pour obtenir un agent renforçateur (renforcement appétitif). Cela contraste fortement avec le labyrinthe aquatique de Morris, où ce même animal, introduit dans un environnement différent, doit apprendre à utiliser les indices spatiaux disponibles afin de se construire une représentation spatiale de l'emplacement de la plate-forme et de se servir de son souvenir pour l'atteindre (renforcement négatif). De telles différences rendent difficile, voire critiquable la comparaison entre les tests. De plus, elles compliquent considérablement la construction de batteries de tâches

complexes. Pour remédier à ces problèmes, un des courants de recherche les plus en vogue du moment implique l'utilisation d'un écran tactile (touchscreen) pour mesurer différents aspects des fonctions cognitives du rongeur dans un même environnement (Bussey et al., 1997; Bussey et al., 2012). En effet, les différents paradigmes déjà disponibles permettraient de s'intéresser à des domaines cognitifs distincts et ont en commun de tous s'appuyer sur la présentation de stimuli visuels sur un écran tactile. Par ailleurs, quelle que soit la tâche considérée, les réponses et leurs conséquences directes sont standardisées, puisque l'animal doit toucher un stimulus défini ou une partie spécifique de l'écran à l'aide de son museau (nose-poke) afin d'obtenir une récompense alimentaire (renforcement positif). Cette approche translationnelle, directement inspirée des batteries de tâches neuropsychologiques assistées par ordinateur chez l'Homme (Nithianantharajah et Grant, 2013), est cependant relativement récente et méritait d'être substantiellement approfondie.

Au cours de ce travail de thèse, nous nous sommes donc intéressés à la validation de cette méthode d'évaluation comportementale dans le cadre préclinique de l'étude de la maladie d'Alzheimer. Chez l'Homme et l'Animal, il est désormais clairement établi qu'il existe différentes formes de mémoire (Squire, 2004). De façon intéressante, la mémoire relationnelle et la mémoire procédurale, qui dépendent de l'intégrité de deux régions subcorticales distinctes, à savoir l'hippocampe et le striatum, respectivement, sont différemment affectées au cours du vieillissement normal et pathologique (Nilsson, 2003; Budson, 2009). Ainsi, les patients atteints de la maladie d'Alzheimer, bien que très vite incapables d'effectuer des tâches cognitives spatiales ou associatives, gardent très longtemps des performances comparables aux sujets âgés sains dans des tâches de mémoire procédurale. Au sein de l'éventail de tâches comportementales basées sur l'utilisation des touchscreens (Horner et al., 2013; Mar et al., 2013; Oomen et al., 2013), nous avons identifié et sélectionné deux tâches permettant d'évaluer *a priori* ces deux formes de mémoire, la tâche d'apprentissage d'associations de paires (PAL) et la tâche d'apprentissage visuo-moteur conditionnel (VMCL). Dans la première, l'animal doit apprendre à associer différents stimuli visuels avec des emplacements spécifiques. Dans la seconde, il doit suivre une règle conditionnelle du type : « Si le stimulus A

apparaît, touche la partie gauche de l'écran ; si le stimulus B apparaît, touche la partie droite ». Enfin, la tâche de discrimination visuelle de paires (PVD), qui consiste initialement à identifier lequel de deux stimuli visuels est associé à la récompense, a également été sélectivement choisie pour certains travaux. En effet, une fois la règle apprise, il est possible d'inverser les contingences associées aux stimuli. Ce changement de règle est pratique pour évaluer la flexibilité comportementale des animaux.

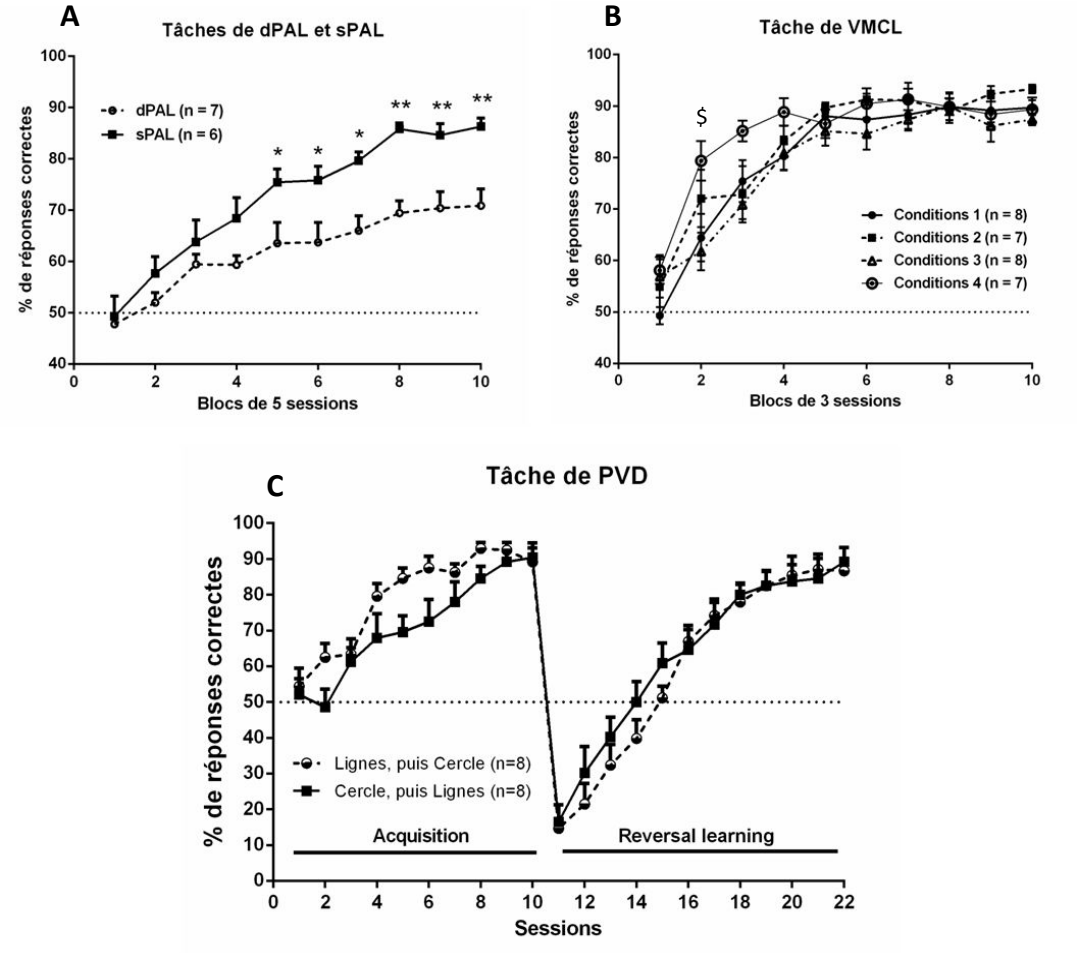

Figure 33. Acquisition des différentes tâches cognitives après optimisation des conditions d'entraînement chez la souris C57BL/6JRj : variantes de la tâche de PAL (graphique A), tâches d'apprentissage de VMCL (graphique B) et de PVD (graphique C). Toutes les tâches considérées se caractérisent par une amélioration significative de la performance au cours du temps. * p<0,05 et ** p<0,01 groupe sPAL *vs* groupe dPAL ; $ p<0,05 groupe 4 *vs* groupe 1 et p<0,01 groupe 4 *vs* groupe 3.

Dans un premier temps, nous avons cherché à optimiser les conditions d'entraînement (PVD, VMCL), à les reproduire (dPAL, d pour présentation de stimuli différents) et à étudier l'impact d'un changement intrinsèque à la règle d'apprentissage (sPAL, s pour présentation de stimuli similaires) en évaluant des souris jeunes non entraînées dans les différentes tâches évoquées. Nos premiers résultats ont démontré l'importance critique de certains paramètres comme la taille des stimuli. Nous sommes progressivement parvenus à définir des conditions d'entraînement permettant d'observer des apprentissages significatifs (**Figure 33**) dans les différentes tâches, notamment pour la première fois dans la tâche de VMCL chez la Souris (90 % de réponses correctes après 15-20 sessions), mais aussi dans la tâche de PVD (acquisition : 85-90 % de réponses correctes après 8-10 sessions ; règle inversée : 80 % de réponses correctes après 12-15 sessions) et dans les deux variantes de la tâche de PAL étudiées (dPAL et sPAL, respectivement 70 et 85 % de réponses correctes après 50 sessions).

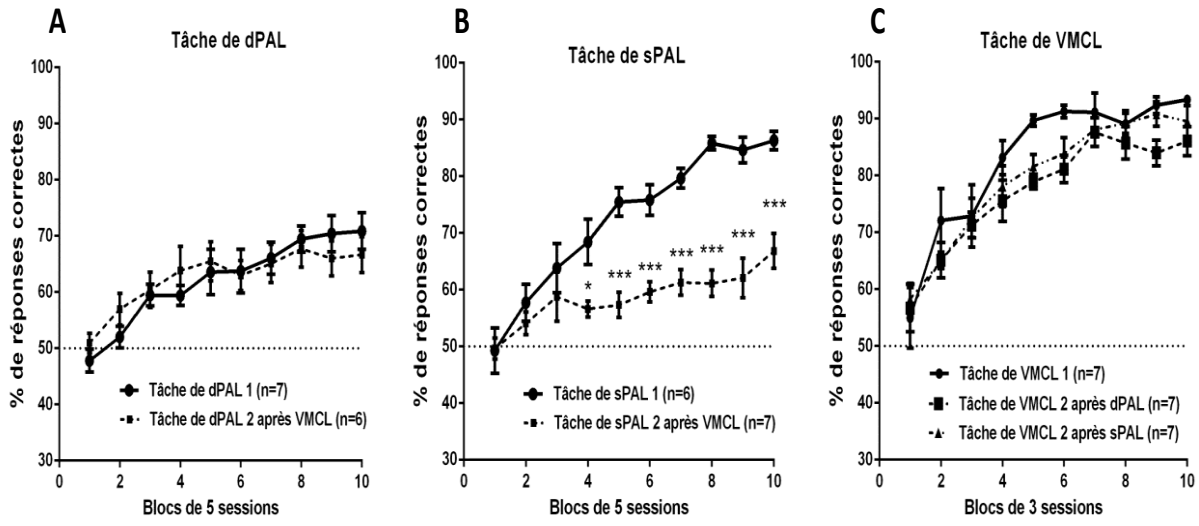

Figure 34. Effet d'une précédente expérience basée sur l'utilisation d'écrans tactiles sur l'acquisition d'une nouvelle tâche d'apprentissage. Courbes d'acquisition de souris naïves ou entraînées dans les tâches de dPAL (graphique A), sPAL (graphique B) ou VMCL (graphique C). Les tâches de VMCL et sPAL donnent lieu à des interférences proactives lorsqu'elles sont réalisées dans cet ordre, d'où le déficit d'acquisition observé dans la deuxième tâche. * $p<0,05$ et *** $p<0,001$ sPAL (tâche 2) vs sPAL (tâche 1).

En complément de ces premiers résultats, nous avons testé l'hypothèse selon laquelle il était possible de combiner de telles tâches sous forme d'une batterie de tâches successives (**Figure 34**). Si les animaux entraînés au préalable dans la tâche de VMCL pouvaient acquérir la tâche de dPAL à une vitesse similaire à celle d'animaux jamais entraînés, les animaux qui apprenaient la tâche de sPAL après la tâche de VMCL étaient beaucoup moins performants (65 % en fin d'apprentissage). De leur côté, les animaux entraînés dans les tâches de dPAL ou sPAL en premier lieu présentaient un faible retard d'acquisition lorsqu'ils étaient testés dans la tâche de VMCL (90 % après 20 sessions environ).

A la suite de cette étude méthodologique, nous avons poursuivi notre travail par la caractérisation comportementale de la lignée murine Tg2576, un modèle transgénique de la maladie d'Alzheimer (Hsiao et al., 1996), dans les tâches de dPAL, VMCL et PVD. Contrairement aux animaux contrôles, les animaux transgéniques portent un allèle muté du gène humain du précurseur du peptide amyloïde (APP) qui est responsable de la production massive de peptide amyloïde (Aß) au cours de la vie de l'animal. Chez ces souris, l'augmentation de la charge amyloïde, qui débute vers 7 mois, est suivie de l'accumulation de plaques amyloïdes vers 12 mois, notamment dans l'hippocampe, l'amygdale et le cortex (Kawarabayashi et al., 2001). En plus de ces changements histopathologiques, ces animaux présentent des déficits cognitifs, notamment dans les tâches relationnelles hippocampo-dépendantes et d'inversion de la règle cortico-dépendantes (Stewart et al., 2011; Webster et al., 2014). La tâche de dPAL était supposée dépendre de la mémoire relationnelle, et donc notamment de l'hippocampe, alors que la tâche de VMCL était supposée dépendre de la mémoire procédurale, et donc notamment du striatum. En parallèle, la tâche de PVD était censée évaluer les fonctions exécutives cortico-dépendantes. Nous nous attendions donc à ce que les animaux transgéniques soient uniquement déficitaires dans les tâches de dPAL et PVD. Nous avons testé des animaux jeunes (5 mois) dont la pathologie amyloïde est encore très débutante et des animaux plus âgés (12 mois) dont la pathologie amyloïde est déjà bien installée (**Figures 35 et 36**).

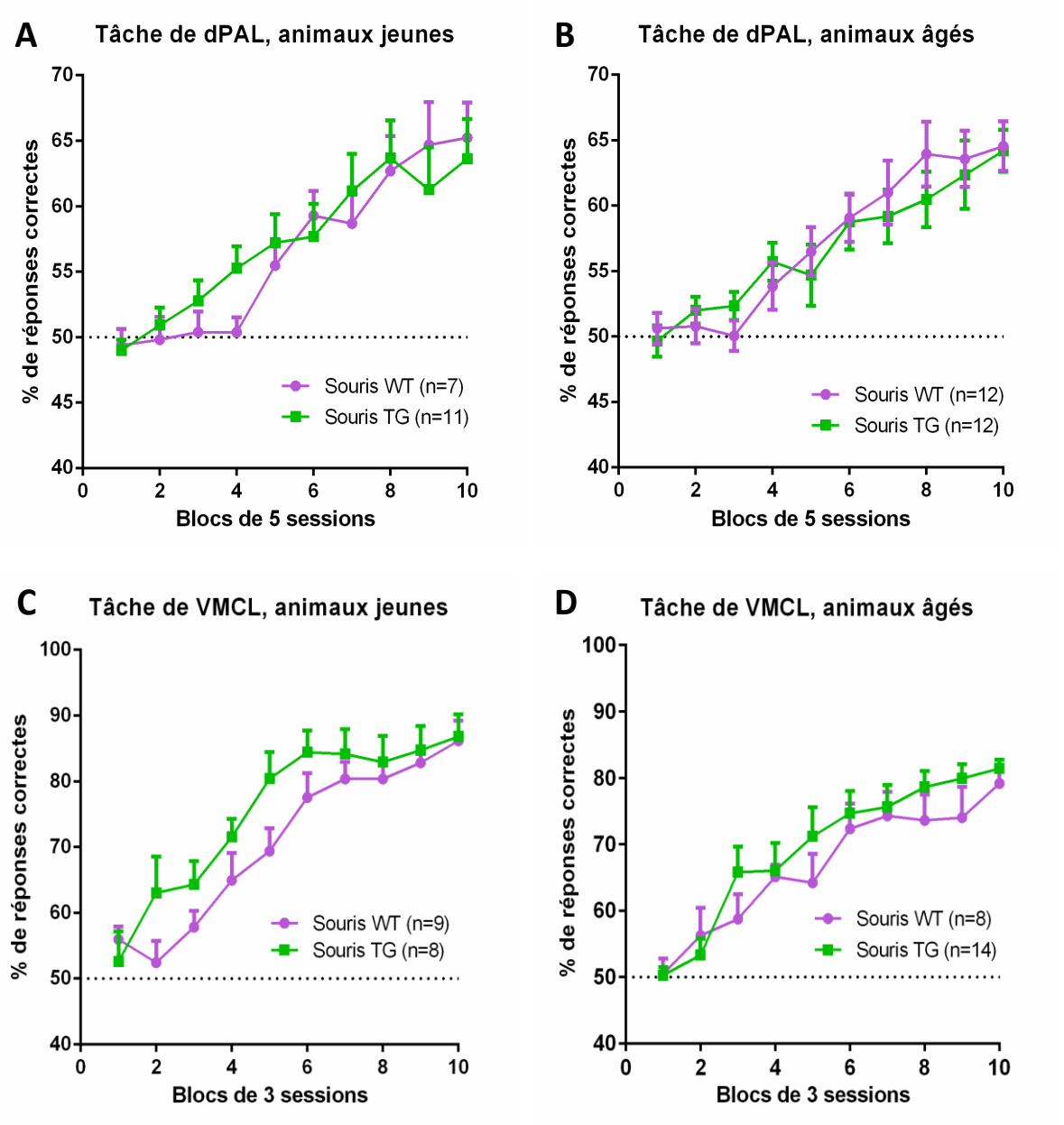

Figure 35. Acquisition des tâches d'apprentissage de dPAL (graphiques A et B) et VMCL (graphiques C et D) chez des souris jeunes (5-8 mois) ou âgées (12-15 mois) de la lignée transgénique Tg2576. Quelle que soit la charge amyloïde considérée, les animaux WT et TG apprennent les 2 tâches de façon comparable.

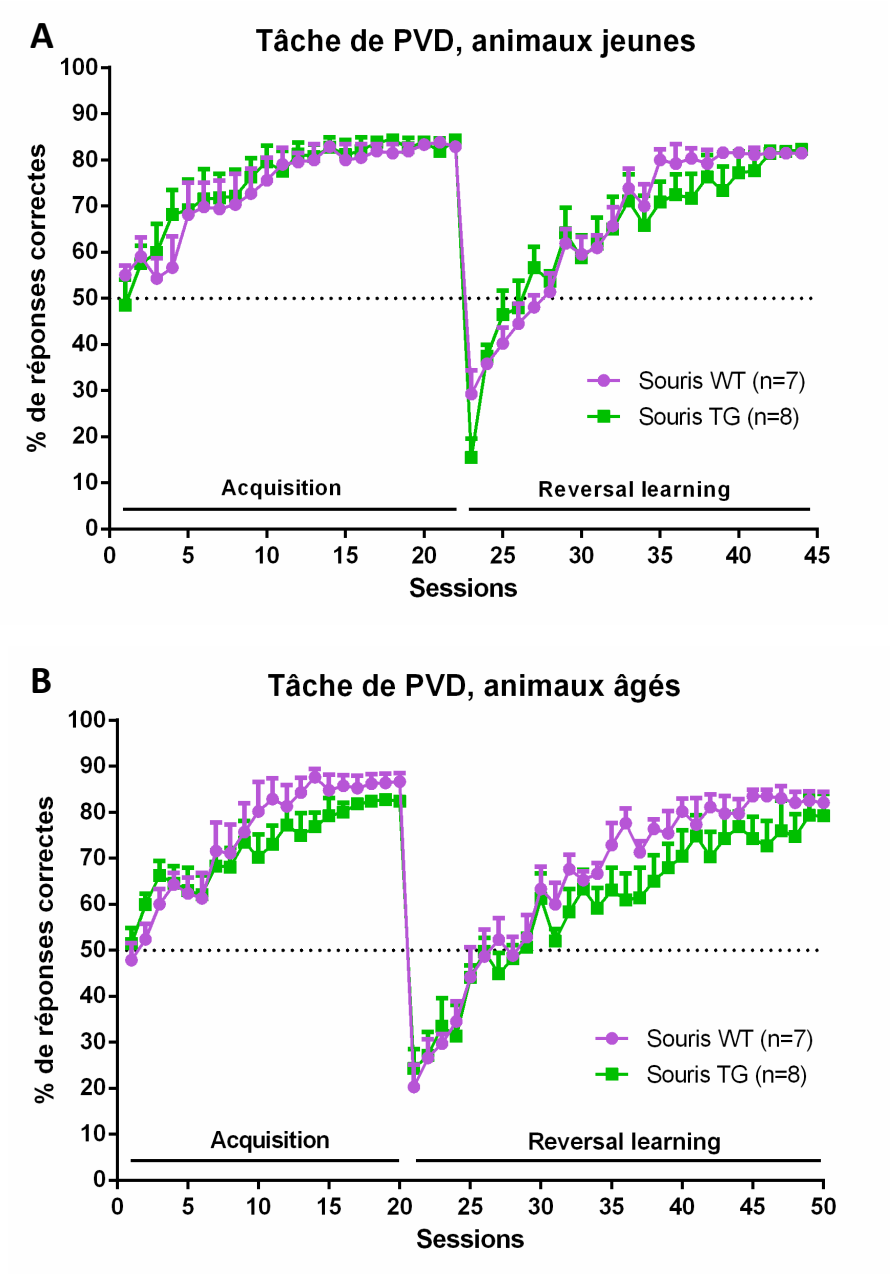

Figure 36. Acquisition de la tâche d'apprentissage de PVD (acquisition puis reversal learning) chez des souris jeunes (graphique A ; 7-9,5 mois) ou âgées (graphique B ; 14-16,5 mois) de la lignée transgénique Tg2576. Comme pour les tâches de dPAL et VMCL, aucune différence n'est appréciable entre les animaux WT et TG.

Ces animaux ont été évalués sur une période de 3-5 mois dans les différentes tâches. De façon surprenante, aucune de nos études n'a révélé de déficit majeur chez les animaux transgéniques par rapport aux animaux témoins, et ce même après avoir écarté les animaux qui présentaient une dégénérescence de la rétine (**Figure 37**; mutation *rd* identifiée par PCR) ou une stéréotypie invalidante.

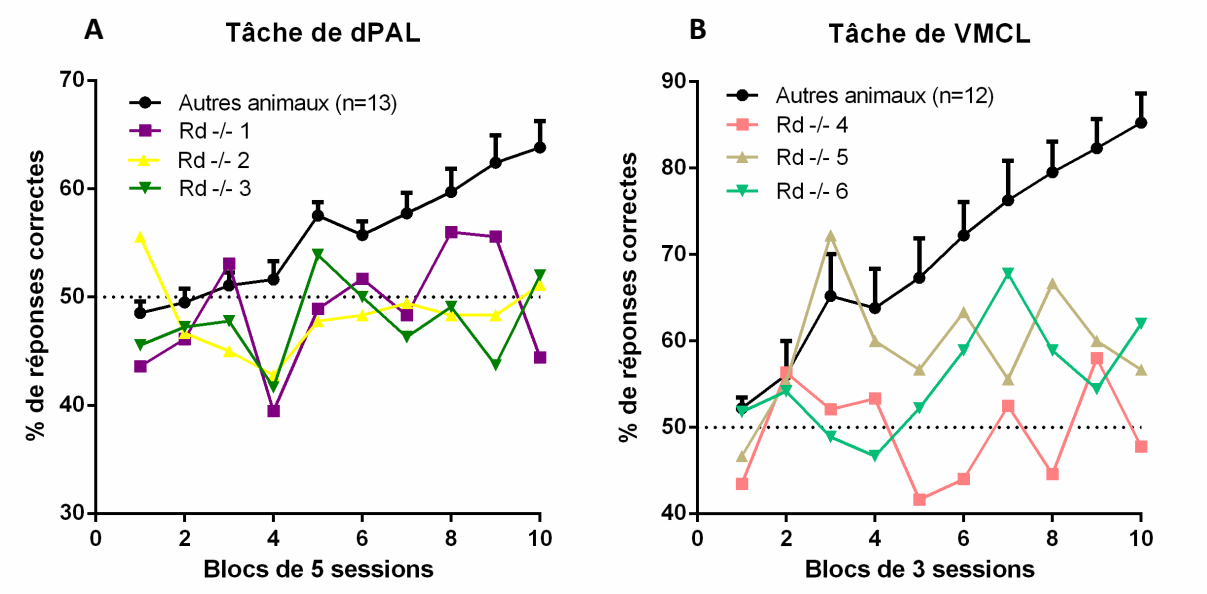

Figure 37. Acquisition des tâches d'apprentissage de dPAL (graphique A) et VMCL (graphique B) chez des souris WT de la lignée Tg2576 voyantes ou porteuses de la mutation *rd* responsable de dégénérescence rétinienne (*Rd -/-*). Comme en témoignent les fluctuations des courbes d'acquisition de ces dernières, les animaux aveugles ne parviennent pas à apprendre les différentes tâches s'appuyant sur la présentation de stimuli visuels apparaissant sur un écran tactile.

Pour pouvoir interpréter ces résultats en désaccord avec nos hypothèses de départ, nous avons décidé dans la troisième partie de notre travail de nous focaliser directement sur la nature des substrats neuronaux supposés intervenir lors de l'acquisition des tâches de dPAL et VMCL chez la souris jeune. La possibilité d'évaluer les mêmes animaux successivement dans ces deux tâches avait déjà été confirmée plus haut. Nous avons donc généré des animaux contrôles ou lésés au niveau de l'hippocampe ou du striatum dorsal après action d'un agent excitotoxique, le N-Methyl-D-Aspartate (Schwarcz et al., 1984). Nous les avons ensuite testés dans

cette batterie de tâches. Après vérification immunohistochimique (**Figure 38**) et exclusion des animaux ne présentant pas des lésions acceptables (mal placées, insuffisantes ou excessives en étendue), nous avons alors pu constater que seules les souris porteuses d'une lésion du striatum dorsal (**Figure 39**) présentaient un déficit d'acquisition des tâches de dPAL et VMCL (respectivement, 55 et 65 % de réponses correctes après 40 ou 30 sessions chez les animaux dorso-striato-lésés contre 70 et 85 % pour les autres groupes). Pourtant, en accord avec la littérature du domaine, les animaux hippocampo-lésés étaient hyperactifs, ce qui attestait de l'efficacité des lésions (**Figure 40**).

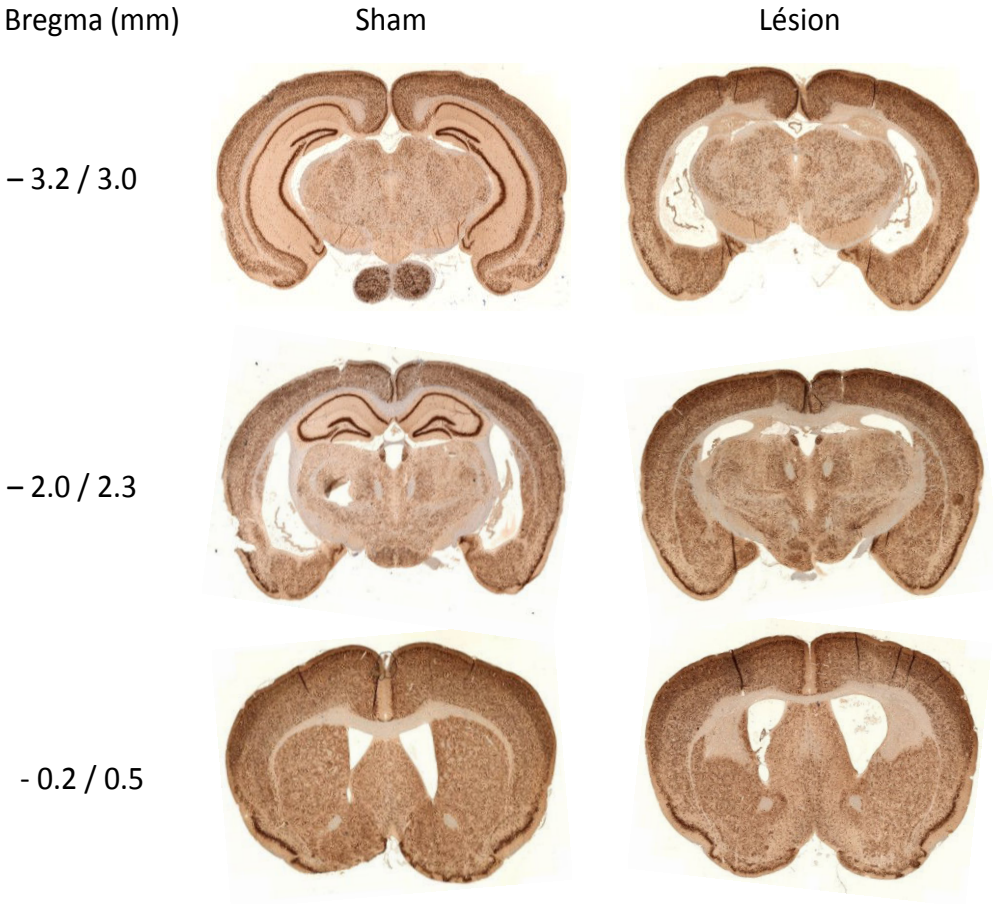

Figure 38. Localisation des lésions hippocampiques (hippocampe ventral, en haut; hippocampe dorsal, au centre) or dorso-striatales (en bas) visualisées sur des coupes coronales de cerveau de souris à la suite d'un immunomarquage NeuN.

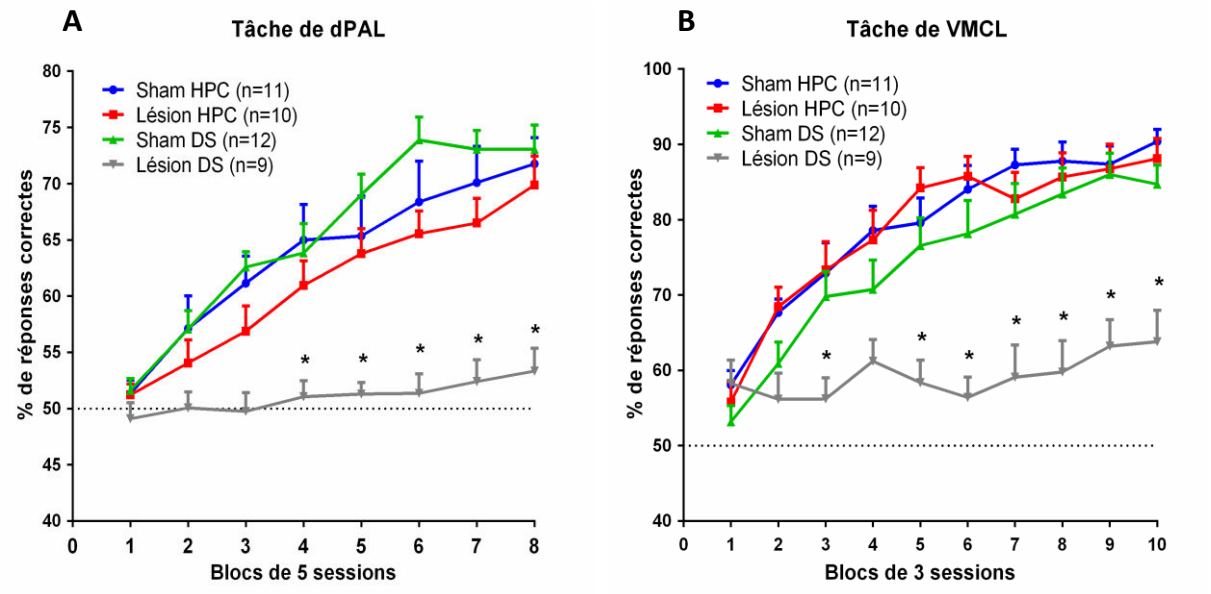

Figure 39. Acquisition des tâches d'apprentissage de dPAL (graphique A) et VMCL (graphique B) à la suite de lésions excitotoxiques de l'hippocampe ou du striatum dorsal effectuées chez des souris C57BL/6JRj jeunes. Seules les lésions dorso-striatales perturbent de façon significative l'apprentissage des animaux dans les paradigmes susmentionnés. * p<0,05 groupe Lésion DS *vs* les 3 autres groupes.

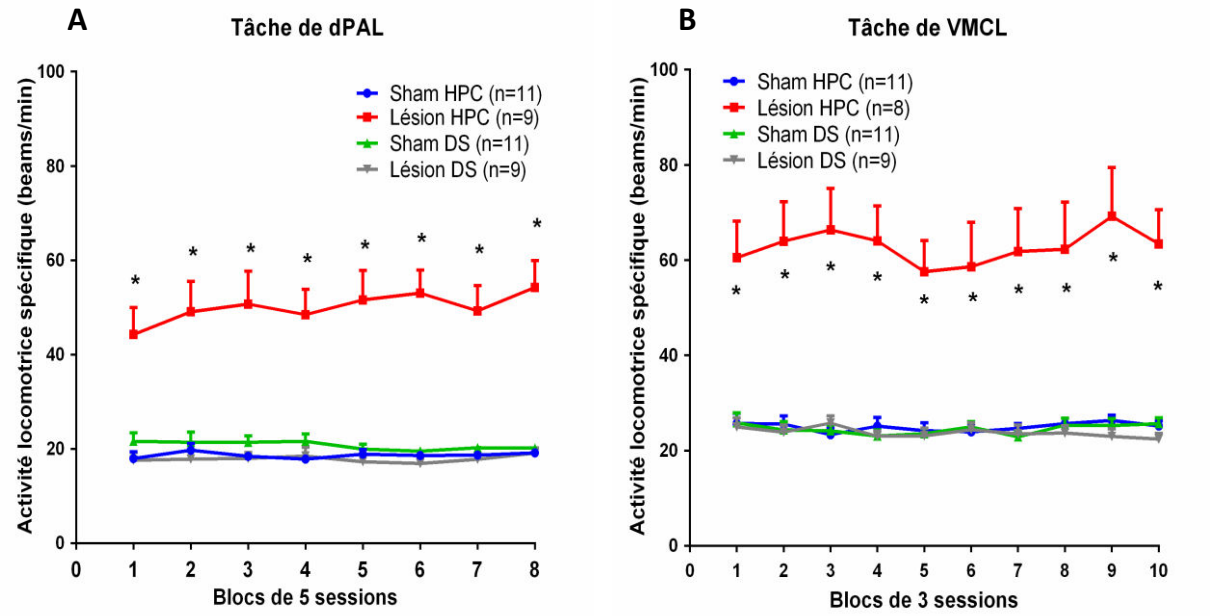

Figure 40. Mesures de l'activité locomotrice réalisées lors de l'acquisition des tâches d'apprentissage de dPAL (graphique A) et VMCL (graphique B) à la suite de lésions excitotoxiques de l'hippocampe ou du striatum dorsal effectuées chez des souris C57BL/6JRj jeunes. Par contraste avec les lésions

dorso-striatales, les lésions hippocampiques entraînent une augmentation substantielle de la locomotion. * p<0,05 groupe Lésion HPC *vs* les 3 autres groupes.

Etant donné que la tâche de dPAL possède une composante spatiale sur l'écran et qu'elle est sensible, après apprentissage, aux manipulations pharmacologiques ayant lieu dans l'hippocampe chez le rat (Talpos et al., 2009), nous avons finalement exploré l'effet de lésions hippocampiques réalisées après l'acquisition de la tâche de dPAL sur la récupération de l'information précédemment apprise (**Figure 41**). Lors des séances de rappel intervenant environ 4-5 semaines après l'intervention chirurgicale, les animaux témoins se souvenaient encore partiellement de la règle (60 % de réponses correctes) alors que les animaux hippocampo-lésés se comportaient comme des animaux naïfs (50 % de réponses correctes). De plus, ces derniers présentaient bien une hyperactivité locomotrice. Ces données montrent que la lésion de l'hippocampe affecte la mémoire à long terme pour la tâche de dPAL, indépendamment de ses effets sur l'activité locomotrice.

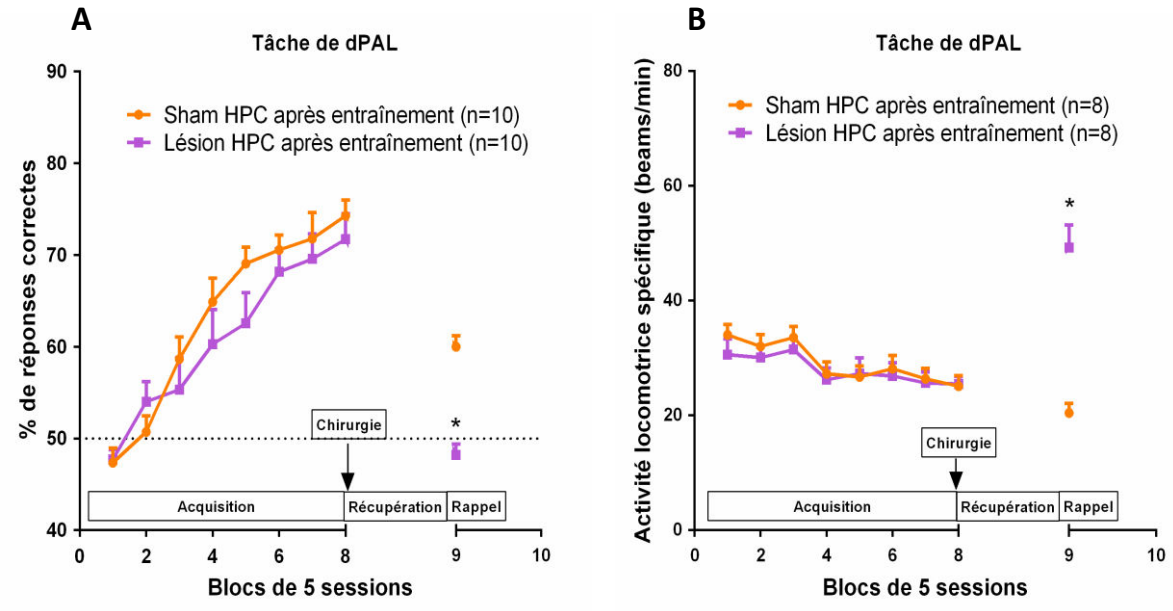

Figure 41. Mesures d'acquisition et de rappel chez des souris C57BL/6JRj hippocampo-lésées après entraînement dans la tâche de dPAL: pourcentage de réponses correctes (graphique A) et activité locomotrice (graphique B). L'acquisition de la tâche d'apprentissage et le pattern d'activité inhérent sont similaires chez les 2 groupes. Par contre, alors que les animaux Sham se souviennent encore partiellement de la règle d'apprentissage 5 semaines après la chirurgie, les animaux lésés au niveau

de l'hippocampe ne peuvent restituer l'information précédemment acquise. De plus, ils affichent une hyperactivité caractéristique. * p<0,05 groupe Lésion HPC *vs* groupe Sham HPC après entraînement.

En conclusion, les travaux réalisés dans le cadre de cette thèse nous ont tout d'abord permis de confirmer la possibilité de mesurer les performances cognitives chez la souris via cette méthode basée sur l'utilisation d'écrans tactiles. Bien qu'un écran tactile ne constitue pas un environnement éthologiquement reconnu, il s'avère que différents types de tâches peuvent être évalués dans ces chambres opérantes à l'occasion d'études transversales (une seule tâche) ou longitudinales (batterie de tâches). Les résultats obtenus avec les souris Tg2576 n'ont en revanche pas confirmé les données de la littérature obtenues précédemment dans des tâches classiques, notamment celles évaluant la mémoire spatiale, les tâches de PVD et dPAL ne permettant pas de différencier les animaux transgéniques de leurs congénères contrôles, quel que soit leur âge. Cependant, une approche lésionnelle de la tâche de dPAL nous a permis de montrer qu'alors que l'hippocampe est sollicité dès l'acquisition du paradigme chez l'Homme, cette même phase de l'apprentissage ne requiert pas l'hippocampe chez la Souris, celui-ci n'intervenant que bien plus tard, lors du rappel de l'information consolidée. La tâche de dPAL reste donc adaptée pour l'évaluation préclinique de composés potentiellement pro-cognitifs, à condition toutefois que leurs effets soient déterminés une fois la tâche acquise. Cette approche lésionnelle nous a également permis de démontrer que le striatum dorsal semble indispensable à l'acquisition des tâches de VMCL et de dPAL chez la Souris. Ceci pourrait s'avérer d'une grande utilité lors de la caractérisation future de modèles animaux de pathologies neuropsychiatriques ou neurodégénératives (schizophrénie, maladie de Parkinson) pour lesquelles un déséquilibre des formes d'apprentissage dépendant de l'intégrité de cette région (apprentissage dirigé par un but ou apprentissage d'une habitude) a déjà été mis en évidence.

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Translational potential of the touchscreen-based methodology to assess cognitive abilities in mice

Ce travail de thèse visait à préciser le potentiel translationnel d'une méthodologie innovante récemment adaptée à la Souris sur la base de tests neuropsychologiques utilisés en clinique humaine. Au sein d'une chambre opérante disposant d'un écran tactile (touchscreen), la présentation de stimuli visuels obéissant à une règle d'apprentissage prédéfinie permettait l'évaluation d'aspects cognitifs variés sur la base de performances comportementales. Après avoir optimisé 3 tâches (PAL, VMCL, PVD) ciblant différentes fonctions cognitives chez l'animal, nous avons montré que la construction d'une telle batterie de tests était sujette à caution, l'acquisition d'une première tâche pouvant parfois influencer la façon dont une seconde tâche pouvait être postérieurement apprise. Dans un deuxième temps, nous avons caractérisé sur le plan comportemental des souris de la lignée Tg2576, un modèle transgénique reconnu de la maladie d'Alzheimer. Néanmoins, ces animaux n'étaient jamais déficitaires en comparaison de leurs homologues non transgéniques dans l'ensemble des tâches susmentionnées, quelle que soit l'étendue de la neuropathologie (charge amyloïde). Pour tenter d'expliquer ces données, nous avons alors mis en œuvre une approche lésionnelle pour les tâches de dPAL et de VMCL. Alors que la lésion bilatérale hippocampique n'avait pas d'impact sur l'acquisition de ces paradigmes, les animaux lésés au niveau du striatum dorsal présentaient à chaque fois un important déficit d'acquisition. L'hippocampe était cependant impliqué dans une phase tardive de la tâche de dPAL, une lésion intervenant après l'entraînement ayant des conséquences majeures sur le rappel de la tâche chez la Souris. Nos résultats suggèrent qu'en dépit des efforts déployés pour s'assurer du caractère translationnel d'une tâche cognitive dans le paradigme du touchscreen, certaines adaptations inhérentes à chaque espèce influencent profondément les bases neurobiologiques associées. Ainsi, l'acquisition de la tâche de dPAL est hippocampo-dépendante chez l'Homme mais requiert l'intégrité du striatum dorsal chez la Souris. Malgré cela, de telles tâches restent d'un grand intérêt, notamment dans la perspective de l'étude des déficits cognitifs de formes d'apprentissages dirigées par un but pour cette dernière tâche.

Mots-clés: écran tactile, mémoire à long terme, fonctions exécutives, maladie d'Alzheimer, hippocampe, striatum dorsal, lésions excitotoxiques

This thesis work aimed to specify the translational potential of an innovative methodology latterly adapted in mice from neuropsychological tasks used in Humans. Within operant chambers individually equipped with a touchscreen, the presentation of visual stimuli obeying a determined learning rule permitted the evaluation of various cognitive aspects according to the behavioral performances. After the independent optimization of 3 assays (PAL, VMCL, PVD) taxing different cognitive functions in animals, we have showed that the construction of a battery of touchscreen tasks was sometimes limited by the proactive impact of a first learning on the subsequent acquisition of another task. We have then behaviorally characterized the Tg2576 murine line, a well-known transgenic model of Alzheimer's Disease. Unexpectedly, these animals performed similarly to their wild-type littermates in all touchscreen assays, whatever the extent of their pathology (amyloid load). In an attempt to explain these data, the differential effects of hippocampal or dorso-striatal lesions were then evaluated in dPAL and VMCL tasks. We observed that dorso-striatal lesions induced important deficits of acquisition in both paradigms, whereas hippocampal lesions had no effect. In contrast, a second group of animals that had been trained in the dPAL task before undergoing a hippocampal lesion showed no evidence of memory retrieval during recall sessions. Our results suggest that despite momentous efforts in order to ensure the translational feature of touchscreen cognitive tasks, certain adaptations inherent to each species deeply influence the nature of underlying neurobiological substrates. Thus, the dPAL task depends upon the integrity of the hippocampus in Humans but requires the dorsal striatum in mice. Notwithstanding these findings, such tasks remain of high interest, notably to study goal-directed forms of learning in the case of this task.

Keywords: Touchscreen tasks, long-term memory, executive functions, Alzheimer's Disease, hippocampus, dorsal striatum, excitotoxic lesions