國立臺灣大學理學院心理學研究所

碩士論文

Graduate Institute of Psychology

College of Science

National Taiwan University

Master Thesis

評估精神分裂症動物模式之 *Akt1* 基因缺損小鼠在五擇一序列反應時程作業的注意力功能

Evaluation of Attentive Functions in Akt1 Mouse Model of Schizophrenia: Using the Five-Choice Serial Reaction Time Task (5-CSRTT)

陳雅珊

Ya-Shan Chen

指導教授：賴文崧 博士

Advisor: Wen-Sung Lai, Ph.D.

中華民國 101 年 7 月

July, 2012

致謝

終於結束了 5-CSRTT 這個很簡單但要完成卻很不簡單的實驗，謝謝所有幫助過我的人，我才能完成這個研究。感謝我的指導教授賴文崧老師，非常辛苦的指導，我才能順利完成這個研究。謝謝賴老師一路包容我所犯的錯誤，並耐心的教導我做研究的能力。論文口試期間，感謝李季湜老師、邱麗珠老師、梁庚辰老師、劉亞平老師給予的建議與疏漏處的指正，使得本論文更臻完備。

感謝一直陪伴著我進行實驗的實驗室人員。謝謝淑婌姐不時的給予我鼓勵；勁勳學長教我許多動物實驗的技術；怡文學姐和耀主學長協助我建立 5-CSRTT 實驗；如淳教我各種不同的動物行為作業；琬婷、欣如和家源陪我抒發情緒與面對困難；為竣提供我統計上的建議。也要感謝婉容、雅文、達中、陳靜、牧融、家豪、璟儀忍受我非常笨拙的指導，讓我得以從中學習如何教學。

另外實驗以外所學的電生理技術，則要謝謝禮銘學長教我完整的電生理實驗程序與技術；儀芳學姐協助解決電生理的問題；姿蘭學姐幫忙解答我對電生理的疑惑；文愷學長教我電生理儀器的操作。

而在研究所的學業中，謝謝共同奮鬥的同學。感謝盈琇、敏衡、洛婷、佩璇、陳柔一起在研究室熬夜準備考試與作業。謝謝柏邑、碩衡、祐嘉、令明、婉慈、駿叡、佩潔、令德、皖君、逸涵、士哲共同勉勵為各自的實驗努力。感謝大學兼研究所同學裕翔、承瑾一同分享回憶以及為彼此加油、鼓勵。

由於實驗的關係，每次回家的時間總是非常短暫，常常是回家後的隔天就得再回學校做實驗，無法多陪陪家人以及協助家裡的事情，感謝家人的包容，一路陪伴我成長。謝謝妹妹鼓勵我推甄研究所，我才有機會完成學業；弟弟協助家裡發生的事務，讓我可以安心的進行研究；爸爸、媽媽辛苦的工作與鼓勵，我才能專心的唸完研究所；爺爺、奶奶以及叔叔的支持，讓我可以順利的完成學業。

最後要感謝所有在此實驗犧牲奉獻的小鼠。

摘要

精神分裂症是一種嚴重且多因子的精神疾患，並且具有高度的遺傳性。從人類遺傳學以及動物研究中，有愈來愈多的結果顯示 *AKT1* 基因可能參與了精神分裂症的致病歷程。在眾多精神分裂的臨床症狀上，精神分裂症患者具有明顯的認知功能異常，特別是注意力功能的缺損。連續操作測驗(continuous performance test)是常用於測量精神分裂症患者注意力功能的測驗，無論是精神分裂症患者或是與其有高血緣關係的親屬，普遍可觀察到他們在這測驗中具有較差的注意力表現。在動物研究中，五擇一序列反應時程作業(five-choice serial reaction time task)是個類似連續操作測驗的注意力作業，近年來廣泛用於評估大鼠或是小鼠的注意力表現。相較於針對人類所進行精神疾病遺傳連鎖研究的侷限性，基因突變小鼠的模式更能直接探究精神分裂症候選基因（如 *Akt1* 基因）的生物功能以及它在精神分裂症致病上之因果關係。本論文旨在探討 *AKT1* 是否參與精神分裂症的注意力功能，以 *Akt1* 基因缺損小鼠為模式，觀察牠們在五擇一序列反應時程作業中，是否表現與精神分裂症類似的注意力功能缺失。實驗採用的 *Akt1* 同型合子、異型合子與野生控制組之雄性小鼠，皆在改良式五擇一序列反應時程作業的儀器中進行注意力表現的評估。實驗程序可以概分成學習階段與測驗階段，小鼠接受一系列每日的訓練學習階段，直到連續三天在刺激燈亮 2 秒的情況下，達到大於 80 %準確率、小於 20 %遺漏率的基本學習標準，接著才能進入測驗階段。測驗階段包含四種需要更多注意力功能的測驗：降低刺激燈亮時間、改變等待刺激燈亮時間、增加刺激燈的亮度、以及加入聲音的干擾。實驗結果顯示，*Akt1* 同型合子之小鼠在學習的第一階段持續地表現出較高的遺漏率。當給予足夠的訓練達到基本的學習標準後，*Akt1* 基因缺損小鼠則與野生控制組小鼠皆具有相同的行為表現。在測驗階段的四個測試中，只有在增加刺激燈之亮度與聲音干擾的測驗中，*Akt1* 基因缺損小鼠表現出不一致的衝動行為。本論文結果顯示 *Akt1* 基因缺損會影響小鼠注意力功能的表現，特別是學習的初期以及需要更多注意力投注的作業

中。由此可以推測在病人身上 *AKT1* 的缺損會造成與精神分裂症相關之注意力功能的異常，進而影響其他認知功能的表現。

關鍵詞：精神分裂症、*Akt1*、注意力、連續操作測驗、五擇一序列反應時程作業。

Evaluation of Attentive Functions in Akt1 Mouse Model of Schizophrenia: Using the Five-Choice Serial Reaction Time Task (5-CSRTT)

Ya-Shan Chen

Abstract

Schizophrenia is a severe and multifactor psychiatric disorder with a strong genetic component, and accumulating evidence from human genetics and animal studies suggest that *AKT1* gene might play a role in the pathogenesis of schizophrenia. Schizophrenia contains heterogeneous clinical symptoms marked by significant cognitive impairments especially attentive dysfunction. Attention deficits measured by the continuous performance test (CPT), the most popular clinical-based measure in schizophrenia research, is commonly observed among schizophrenia patients and those at genetic risk for the disease. The five-choice serial reaction time task (5-CSRTT) has been considered to analogue to the CPT and increasingly used to assess attentive functions in rats and mice. As a mutant mouse model is a simple and relatively straightforward approach for determining the causal relationships and biological functions of *AKT1* in schizophrenia, this thesis aims to discover the involvement of *AKT1* in attetional functions of schizophrenia through investigating whether *Akt1*deficiency in mice results in attentive impairments by using the 5-CSRTT. In this study, male *Akt1* homozygous (HOM) and heterozygous (HET)

mutant mice and their wild-type (WT) littermates were tested in a modified version of the 5-CSRTT. Mice were trained in a sequence of daily learning sessions until they reached the baseline criteria with ≥ 80% accuracy and ≤ 20% omissions in the condition with 2-sec stimulus durations for three consecutive days. After reaching these criteria, each subject went over a sequence of four test conditions, which required more attentive load, including manipulations of stimulus duration, inter-trial-interval, stimulus brightness, and white noise distractors. Behavioral analysis indicated that *Akt1* HOM mice have abnormal attention in the initially learning stage of the 5-CSRTT. After reaching preset criteria, *Akt1*-mutant mice showed normal baseline performances to enter those following tests. Both tests of brightness and white noise distractor inconsistently induced impulsive behavior instead of attention-related responses in *Akt1*-mutant mice. Our findings suggest that *AKT1* may participate in attentive functions of schizophrenia.

Keywords: schizophrenia, Akt1, attention, continuous performance test, five-choice serial reaction time task.

Table of Contents

Tables and Figures

Abbreviation

5-CSRTT	five-choice serial reaction time task
6-OHDA	6-hydroxydopamine
ADHD	attention deficit hyperactivity disorder
AKT1	v-akt murine thymoma viral oncogene homolog 1
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
cAMP	cyclic adenosine monophosphate
CPT	continuous performance test
CTMP	C-terminal modulator protein
DAT1	dopamine transporter
DRD2	dopamine receptor 2
DRD4	dopamine receptor 4
DISC1	disrupted in schizophrenia 1
DNA-PK	DNA-dependent protein kinase
GABA	γ-aminobutyric acid
GPCR	G protein-coupled receptor
GRK	G protein-coupled receptor kinase
GSK3	glycogen synthase kinase 3
HET	heterozygous
HM	hydrophobic motif
HOM	homozygous
HVA	homovanillic acid
ILK1	integrin-linked kinase-1
ITI	intertrial interval
LH	limited hold

MATRICS	Measurement and Treatment Research to Improve Cognition in Schizophrenia
MCCB	MATRICS Consensus Cognitive Battery
mGluR	metabotropic glutamate receptor
mTORC2	mammalian target of rapamycin complex 2
NIMH	National Institute of Mental Health
NMDA	N-methyl-D-aspartate
NRG1	neuregulin 1
PCP	phencyclidine
PCR	polymerase chain reaction
PDK1	3-phosphoinoitide-dependent protein kinase 1
PFC	prefrontal cortex
PH	pleckstrin homology
PHLPP	pleckstrin homology domain and leucine rich repeat protein phosphatase
PI3K	phosphoinositide 3-kinase
PIP3	phosphatidylinositol-3,4,5-triphosphate
PKB	protein kinase B
PP2A	protein phosphatase 2A
PPI	prepulse inhibition
PTEN	phosphatase and tensin homolog
SD	stimulus duration
TO	time-out
WT	wild-type

Chapter 1

General Introduction

1. Overview of schizophrenia

Schizophrenia is characterized by symptoms that profoundly alter affect, behavior, and cognition, particularly the pattern or form of thought as its name meaning splitting of the mind (Kosslyn & Rosenberg, 2011). Schizophrenia mostly often manifests in late adolescence and early adulthood, and approximately 1 % of the population world-wide develops schizophrenia during their lifetime (Kulhara & Chakrabarti, 2001; Tandon, Nasrallah, & Keshavan, 2009). Schizophrenia is presumed as heterogeneity with multiple factors contributing to disease generation, and there is no essentially consensus criterion that must be met the diagnosis of schizophrenia. Meta-analyses of schizophrenic populations have demonstrated the clustering of symptoms into at least three distinct symptom domains in schizophrenia: (1) Positive symptoms: an excess or distortion of normal functions, including hallucinations, delusions, thought disorder, and paranoia. (2) Negative symptoms: a lessening or loss of normal functions, including anhedonia, social withdrawal, and thought poverty. (3) Cognitive dysfunctions include inattention, impairment of working memory, and deficits of executive functions (Tamminga & Holcomb, 2005). According to their clinical symptoms, the Diagnostic and Statistical Manual of Mental

Disorders (2000) specifies five subtypes of schizophrenia: paranoid, disorganized, catatonic, undifferentiated, and residual types (DSM-IV-TR, 2000). From human and animal studies, various disease-associated physiological alterations have been noted in morphology (e.g., enlarged lateral ventricles) and neurochemistry (e.g., dopamine and glutamate). The etiology of schizophrenia is still controversy, and is considered as the combination of environmental and genetic factors.

2. Cognitive symptoms of schizophrenia

2.1. Impaired cognitive functions in schizophrenia

Cognitive impairment is considered the core feature of schizophrenia and strongly influences quality of life and function in people with this illness (Elvevag & Goldberg, 2000). As a core feature, cognitive deficits are not the result of the symptoms of schizophrenia or of the antipsychotic treatment of the illness (Green, 2006). Additionally, cognitive dysfunctions in schizophrenia often pre-date the development of the illness (O'Carroll, 2000) and tend to be more severe and more independent of symptomatic state than other neuropsychiatric disorders (Gold, 2004). Therefore, cognitive impairment has emerged as a critical target in schizophrenia therapeutics (Barnett et al., 2010). The National Institute of Mental Health's (NIMH's) Measurement and Treatment Research to Improve Cognition in Schizophrenia

(MATRICS) identified some key cognitive domains and developed the MATRICS Consensus Cognitive Battery (MCCB) to select corresponsive tests in the clinic for schizophrenia to promote novel cognitive treatments. As represented in Table 1.1, these candidate cognitive domains for schizophrenia are speed of processing, attention/vigilance, working memory, verbal learning and memory, visual learning and memory, reasoning and problem solving, verbal comprehension, and social cognition (Nuechterlein et al., 2004). The MCCB further selected 10 tests covering above seven domains of cognitive function (e.g., the continuous performance test for attention/vigilance) for use in clinical trials of schizophrenia (Nuechterlein et al., 2008).

2.2. The attentive deficiency in schizophrenic patients

Attention impairment as identified in the MATRICS is commonly observed among schizophrenic patients and those at genetic risk for the disease (Cornblatt & Keilp, 1994). It is considered as an endophenotype of schizophrenia with association with this illness, state independent, heritability, and higher rate in unaffected relatives than in the general population (Chen & Faraone, 2000; Gur et al., 2007). The continuous performance test (CPT), which includes measures of vigilance, response inhibition, and signal detection, is among the most popular objective measures of

attentive functions in the clinic for its high test-retest reliability and the absence of a ceiling effect (Nuechterlein et al., 2008). In the CPT, subjects are required to visually monitor a series of letters presented continuously and then follow demanded rules to response to a rare target, like the letter ‘‘X’’, and to withhold responding to all other letters (Beck, Bransome, Mirsky, Rosvold, & Sarason, 1956). The performance indices of the CPT have evolved from the hit rate or false alarm rates alone to indices derived from signal detection theory: sensitivity (d’, the variable of interest in studies on attention) refers to an individual’s ability to discriminate target stimuli from non-target stimuli; whereas, the response criterion (lnβ) measures a subject’s tendency either to overrespond or underrespond (Abdi, 2010; Chen & Faraone, 2000). Schizophrenic patients and subjects at risk for schizophrenia are characterized by lower d’s than controls, and the lower the d’, the poorer the processing capacity (Cornblatt & Keilp, 1994).

3. The neurochemistry of schizophrenia

3.1. Clues from the treatments of schizophrenia

Schizophrenia involves abnormal neurotransmitter levels, and most treatments are based upon regulating neurotransmitters. The primal biological theories of schizophrenia were established in the 1950s through the discovery of the

antipsychotic effect of chlorpromazine, the first antipsychotic drug. Conventionally, antipsychotics can be classified into 2 major groups: typical (first-generation) agents (e.g., chlorpromazine and haloperidol) and atypical (second-generation) agents (e.g., clozapine and risperidone). Typical agents are well known for effectively reducing positive symptoms, but are only minimally effective for negative and cognitive symptoms. As typical agents are associated with serious treatment burdens, including extrapyramidal side effects (e.g., muscle stiffness, akathisia, tremors) and tardive dyskinesia (i.e., a neurological syndrome involving involuntary movements in the extremities, such as fingers, toes, or oral-facial region), atypical agents were introduced in the late 1960s. Evidence suggests that atypical agents have a more favorable side-effect profile and beneficial effects on cognitive function compared with typical agents, although some of these apparent benefits can be achieved by the relatively high dosages of typical agents used in many studies (Tandon, 2011). The pharmacologic property shared by all available antipsychotics is blockade of the dopamine D2 receptor either through direct or secondary blockade. Better effects of atypical antipsychotics are the result of combination of dopamine and other neurotransmitters (e.g., serotonin, glutamate, and γ-aminobutyric acid). Through the usage of clinical antipsychotics for the treatment of schizophrenia, numerous neurotransmitter systems are revealed. Given the importance of dopamine D2

blockades in the mitigation of positive symptoms, the potential role of dopaminergic system in the pathogenesis of schizophrenia are described in further details below.

3.2. The dopamine system and dopamine hypothesis of schizophrenia

3.2.1. The dopamine system and dopamine receptors

Dopaminergic neurons in the brain form four major projecting pathways which mainly originate in the midbrain (Iversen & Iversen, 2007; Remington, Agid, & Foussias, 2011). These four projecting pathways are depicted in Figure 1.1 and described as below. The first, neurons of the nigrostriatal pathway originate in the substantia nigra and terminate in the neostriatum (i.e., the caudate nucleus and the putamen). The second, neurons of the mesolimbic pathway originate in the verntral tegmental area and terminate in the limbic system, including the nucleus accumbens, amygdale, and hippocampus. The third, neurons of the mesocortical pathway also originate in the verntral tegmental area and terminate in the prefrontal cortex. Lastly, neurons of the tuberoinfundibular pathway originate in the arcuate nucleus and terminate in the pituitary gland.

There are five subtypes of mammalian dopamine receptors and they are grouped into two classes, with the D1-class receptor composed of the D1 and D5 receptor subtypes, and the D2-class receptor composed of the D2, D3, and D4 receptor

subtypes. All of the dopamine receptors are G protein-coupled receptors (GPCRs) and their biological activities have been predominantly associated with the regulation of cyclic adenosine monophosphate (cAMP) cascades. Activating D1-class receptors increase the cAMP activity which D2-class receptors inhibit it (Neve, Seamans, & Trantham-Davidson, 2004).

3.2.2. The dopamine hypothesis

The dopamine hypothesis of schizophrenia was initiated in the 1950s through the discovery of antipsychotic drugs. These drugs were initially found that they blocked monoamine receptors in animals, and further researches revealed that there antipsychotics mainly blocked dopamine D2 receptors. Further evidences from postmortem studies and *in vivo* positron tomographic data indicated the density of D2 receptors were elevated in schizophrenic patients (Seeman, 1987). Furthermore, the antagonists of dopaminergic transmission, such as resperpine, relieved psychotic symptoms of schizophrenia (Seeman, 1987). In contrast, agents increasing dopaminergic transmission, such as amphetamine, provoked psychotic symptoms of schizophrenia. Thus the overactivation of dopamine pathway in schizophrenia was subsequently proposed as primal version of dopamine hypothesis of schizophrenia (Seeman, 1987).

However, Clozapine, another antipsychotic that effectively alleviate refractory symptoms in schizophrenic patients, displays lower binding affinities of dopamine receptors than other antipsychotics. *In vivo* electrophysiological recordings in animals and the measurement of plasma homovanillic acid (HVA), a metabolite of dopamine, also revealed that antipsychotics acted by reducing dopaminergic activities in mesolimbic dopamine neurons (Davis, Kahn, Ko, & Davidson, 1991). Higher concentrations of dopamine and HVA were also reported in various subcortical regions of post-mortem brains from schizophrenic patients (Davis et al., 1991). However, negative symptoms of schizophrenia were associated with low dopamine activity in the prefrontal cortex, and animal and human studies indicated that prefrontal dopamine neurons inhibited subcortical dopamine activities (Davis et al., 1991). These inconsistent findings suggested that dopamine seemed not to be universally elevated in the brain of schizophrenic patients. Therefore, it was proposed that schizophrenia might be characterized by abnormally low tonic activity of ascending dopamine neurons to the cortex and subsequently high activity in the subcortical dopamine nuclei, which could explain the concurrent presence of negative and positive symptoms in schizophrenic patients (Davis et al., 1991). Accordingly, negative symptoms of schizophrenia were resulted from frontal hypodopaminergia, and positive symptoms were resulted from striatal hyperdopaminergia. This version

can be considered as an updated version of dopamine hypothesis of schizophrenia (Davis et al., 1991).

To date, a newly updated version of dopamine hypothesis of schizophrenia was further proposed. This updated version provided a conceptualized framework to link dopamine dysfunction to real clinical expressions such as delusions and hallucinations (Kapur, 2003). Presumably, these psychoses observed in schizophrenic patients were induced by the dysregulation of dopamine transmission which resulted from stimulus-independent release of dopamine. This hyperdopaminergic state was termed "aberrant salience". Accordingly, in the psychotic patients, dopamine served as a potentional mediator to promote aberrant salience. Thus, delusions and hallucinations could be a state of aberrant salience that were driven and represented through an individual's top-down cognitive explanation. Negative symptoms could be resulted from dopamine dysregulation following increased noise in the system (Kapur, 2003; Kapur, Mizrahi, & Li, 2005). Besides, the dopaminergic disturbance was just a state that was abnormality associated with the dimension of psychosis in schizophrenia, and schizophrenia was the combination of other factors and not the dopamine dysfunction *per se*. Advances in neurochemical imaging further provided evidences to support that the dopamine dysregulation was controlled by the presynaptic dopamine (Howes & Kapur, 2009). Along the same line, additional evidence further support that

genetic factors and environmental risk factors are all involved in the alteration of dopaminergic pathways (Howes & Kapur, 2009). Therefore, these findings leaded to an updated version of dopamine hypothesis of psychosis in schizophrenia which suggested that multiple hits (e.g., genetics, environment, and their interactions) could result in presynaptic dopamine dysregulation and consequently cause a process of aberrant salience (Howes & Kapur, 2009).

3.3. The glutamate system and glutamate hypothesis of schizophrenia

In addition to dopamine and dopamine hypothesis of schizophrenia, glutamate and glutamate hypothesis have also drawn much attention and worthy of adding a few words.

3.3.1. The glutamate system

Glutamate is the major excitatory neurotransmitter in the central nervous system, and its receptors comprise two large families, including ionotropic and metabotropic glutamate receptors. Ionotropic glutamate receptors, which include NMDA (N-methyl-D-aspartate), kainate, and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) subtypes, contain associated ion channels gated by agonist binding. By contrast, metabotropic glutamate receptors (mGluRs) belong to the large superfamily of G protein-coupled receptors,

which modulate neurotransmission by activating G protein-coupled synaptic transduction mechanisms (Nestler, Hyman, & Malenka, 2009). There are eight subtypes of mGluRs that are divided into three subgroups: Group I ($mGluR_1$ and $mGluR_5$), group II ($mGluR_2$ and $mGluR_3$), and group III ($mGluR_4$, $mGluR_6$, $mGluR_7$, and $mGluR_8$) mGluRs (Platt, 2007).

3.3.2. The glutamate hypothesis of schizophrenia

The glutamate hypothesis of schizophrenia was based on the low cerebrospinal fluid glutamate levels in patients with schizophrenia (Kim, Kornhuber, Schmid-Burgk & Holzmüller, 1980) and expanded to other subtypes of glutamatergic receptors. Blocking NMDA receptor through phencyclidine (PCP) or ketamine produces schizophrenia-like symptoms, including all the three symptom clusters, in healthy individuals and profoundly exacerbates preexisting symptoms in patients with schizophrenia (Coyle, 2006; Javitt & Zukin, 1991). Thus, hypofunction of NMDA receptor-mediated neurotransmission is considered to be another etiological conceptualization of schizophrenia (Field, Walker, & Conn, 2011; Stahl, 2007). Postmortem studies also showed alterations of binding, transcription, and subunit protein expression of glutamate receptor in the prefrontal cortex, thalamus, and hippocampus of schizophrenia (Clinton & Meador-Woodruff, 2004). Besides, similar

alternations of dopaminergic and GABAergic (γ-aminobutyric acid; synthesized from glutamate) transmission in schizophrenia are found in normal volunteers and animals treated with NMDA receptor antagonists, hence both the impaired dopaminergic regulation and the impaired GABAergic neurotransmission in schizophrenia are implied the downstream of a primary deficit in NMDA function (Benes, McSparren, Bird, SanGiovanni, & Vincent, 1991; Javitt, 2010).

On the other hand, a series of orthosteric agonists for group II mGluRs have been identified (e.g., LY354740, LY379268, LY404039, MGS0008, and MGS0028) (Fell, McKinzie, Monn, & Svensson, 2012). These agents attenuate many of the behavioral and neurochemical effects of the most common psychotomimetic drugs used to model psychosis (e.g., non-competitive NMDA receptor antagonists, dopamine agonists, and serotonergic hallucinogens) in a variety of animal models (Moghaddam & Adams, 1998; Rorick-Kehn et al., 2007). However, studies of group II mGluR expression and functions in the brains of patients with schizophrenia have not revealed consistent changes compared to the brains of normal subjects (Corti et al., 2007; Gonzalez-Maeso et al., 2008; Gupta et al., 2005). These suggest that the pathophysiological mechanism underlying schizophrenia may involve the alternation of group II mGluRs.

4. The etiology of schizophrenia

The etiology of schizophrenia remains much unclear. Roughly, it can be divided into environmental risk factors and genetic factors.

4.1. Environmental risk factors of schizophrenia

Variations in the occurrence of schizophrenia by spatial and temporal distributions are indicative of environmental etiologies (Brown, 2011). Epidemiological researches in spatial distributions have revealed high rates of schizophrenia in developing countries, individuals living in large cities, and immigrant populations (van Os, Rutten, & Poulton, 2008). The epidemiological research in temporal distributions has consistent found that subjects born during the winter and early spring have an increased risk of schizophrenia, and subjects born during the autumn months have a declined in schizophrenia risk (Brown, 2011). These environmental risk factors of schizophrenia can be further classified into biological and social factors (Mueser & McGurk, 2004). Biological environmental factors in the fetal life concern maternal pregnancy complications, prenatal maternal infection, paternal age, and prenatal exposure to chemical agents; in the early life concern abnormal rearing environment, and childhood trauma; in the middle childhood/adolescence concern abnormal urban environments, cannabis using, migration, stressful life events, and traumatic brain injury (Sullivan, 2005; van Os et

al., 2008). Social environmental factors concern the social fragmentation, the social capital, and the social deprivation (Tost & Meyer-Lindenberg, 2012). The causal relationships between these environmental risk factors and schizophrenia need further investigation.

4.2. Genetics of schizophrenia

Twin, family, and adoption studies around the world indicate a familial basis for schizophrenia and point to a genetic influence for this disorder (Calkins & Iacono, 2003). Pattern of familial heritability of schizophrenia suggests that risk of developing schizophrenia is increased among closer relatives. The concordance rate of schizophrenia for monozygotic twins, with 100 % identical genome, is approximately 50 %. In the case of dizygotic twins, with 50 % shared genome, 17 % is this chance. The first-degree biological relatives with schizophrenia have about 10 % chance of having the diagnosis whereas the risk for second-degree relatives is about 5 % (Gottesman, 1991). From a multigroup twin model, the heritability of schizophrenia is 73 % to 90 % (Sullivan, Kendler, & Neale, 2003). Adoption and cross fostering studies are also in favor of a large genetic contribution to risk for schizophrenia (Mulle, 2012). Genetic analysis through linkage studies, association studies, candidate gene studies, copy number variants studies, and de novo mutations studies have

revealed many schizophrenia susceptibility loci and genes (e.g., *AKT1*, *NRG1*, *DISC1*) (Mulle, 2012; Sullivan, 2005). However, the precise role of these candidate genes in the involvement of schizophrenia remains unclear. It is thought to be a pool of genes that increases the vulnerability of this illness (Austin, 2005).

5. What is AKT1?

5.1. AKT1 - a schizophrenia candidate gene

Among these schizophrenia susceptibility genes, *AKT1* appears to be a promising candidate. Emamian and colleagues (2004) first identified that variants of *AKT1* gene, located on human chromosome 14q32.32, are associated with schizophrenia and implicated *AKT1* as a schizophrenia susceptibility gene. The genetic association was found between an *AKT1* haplotype and schizophrenia in 268 affected families. Protein extracts from lymphocyte-derived cell lines from these schizophrenic patients showed 68 % lower of AKT1 proteins than in controls. From postmortem brain tissue of individuals with schizophrenia, AKT1 protein levels were also lower in the frontal cortex and hippocampus compared with controls (Emamian et al., 2004). Further evidence from the Japanese population (Ikeda et al., 2004), European sib-pair families (Schwab et al., 2005), the Iranian population (Bajestan et al., 2006), the Chinese population (Xu et al., 2007), Irish families (Thiselton et al., 2008), and the British

population (Mathur, Law, Megson, Shaw, & Wei, 2010) have also supported the association of *AKT1* gene with schizophrenia. Individuals carrying genetic variants of *AKT1* had increased the hippocampal activation under a memory encoding and retrieval task (Tan et al., 2011). Besides, *AKT1* was further associated with attention and other cognitive functions (e.g., executive function, processing speed verbal learning, and memory) in schizophrenia (Blasi et al., 2011; Ohi et al., 2011; Pietiläinen et al., 2009; Tan et al., 2008).

5.2. AKT and the structure of AKT

AKT1 belongs to the AKT family. The cellular *AKT* gene is the homolog of the v-*akt* oncogene transduced by AKT8, an acute transforming retrovirus (Staal, Hartley, & Rowe, 1977), and encodes the cytoplasmic serine-threonine protein kinase AKT in mice (Bellacosa, Testa, Staal, & Tsichlis, 1991; Bellacosa et al., 1993). AKT is related to protein kinase A and C (PKA/PKC), hence it is also termed as protein kinase B (PKB) (Coffer & Woodgett, 1991; Jones, Jakubowicz, Pitossi, Maurer, & Hemmings, 1991). The AKT kinase family contains three homologous isoforms, AKT1 (PKBα), AKT2 (PKBβ), and AKT3 (PKBγ), all encoded by distinct genes localized on different chromosomes (Franke, 2008). All AKT isoforms share similar signaling pathways and structures: an N-terminal pleckstrin homology (PH) domain; a central

kinase catalytic domain; a C-terminal extension containing a regulatory hydrophobic motif (HM) (Kumar & Madison, 2005) (see Figure 1.2).

5.3. The activation and deactivation of AKT

As depicted in Figure 1.2, the AKT activity is induced by growth factor receptor-mediated signaling cascades that elicit the production of PIP3 (phosphatidylinositol-3,4,5-triphosphates) by PI3K (phosphoinositide 3-kinase) (Franke, 2008). AKT is maintained in an inactive state by the interaction of its PH and kinase domains in the cytoplasm, and this conformation is recruited to the plasma membrane and relaxed upon binding of PIP3 to the PH domain (Calleja et al., 2007). AKT is subsequently activated through phosphorylation by the intracellular kinases PDK1 (3-phosphoinoitide-dependent protein kinase 1) at a threonine residue in its kinase domain (Thr308 in AKT1, Thr309 in AKT2, and Thr305 in AKT3), and phosphorylation at a serine residue in the HM domain (Ser473 in AKT1, Ser474 in AKT2, and Ser472 in AKT3) (Freyberg, Ferrando, & Javitch, 2010; Kumar & Madison, 2005). Phosphorylation of the serine residue is the key step in the activation of AKT because it stabilizes the active conformation state (Yang et al., 2002). mTORC2 (mammalian target of rapamycin complex 2) is the main kinase activity that through phosphorylation of the serine residue, and other kinases such as DNA-PK

(DNA-dependent protein kinase) and ILK1 (integrin-linked kinase-1) are also capable of phosphorylating at the serine residue (Franke, 2008). Once activated, AKT phosphorylates a number of its downstream molecules (e.g., glycogen synthase kinase 3, GSK3) to control an array of diverse functions including cell growth, survival, proliferation and metabolism (Manning & Cantley, 2007). AKT can be inactive indirectly through dephosphorylating PIP3 by the PTEN (phosphatase and tensin homolog) which results in reducing production of PIP3. Besides, AKT also can be inactive directly through dephosphorylating the serine residue and/or the threonine residue by PP2A (protein phosphatase 2A) and PHLPP (PH domain and leucine rich repeat protein phosphatases) (Franke, 2008), and directly through inhibiting phosphorylation by CTMP (C-terminal modulator protein) (Maira et al., 2001).

5.4. The involvement of AKT in dopamine signaling

Decreased AKT1 levels are correlated with increased its downstream GSK3 activity in schizophrenic patients and this suggests that alterations in AKT-GSK3 signaling contribute to the pathogenesis of schizophrenia (Emamian et al., 2004). Both a reduction of AKT activity and a concomitant activation of GSK3 were found in dopamine transporter-knockout mice, and increased dopamine neurotransmission also resulted in inactivation of AKT and concomitant activation of GSK3 (Beaulieu et

al., 2004). Besides, using SCH23390 and raclopride (i.e., D1 and D2 dopamine receptor antagonists), haloperidol (a typical antipsychotic), or mice lacking different subtypes of dopamine receptors showed that AKT and GSK3 are regulated by D2-class receptors in these mice (Beaulieu, 2011). Therefore, it was suggested that the activation of dopamine D2-class receptors led to changes of AKT-GSK3 pathway through a cAMP-independent pathway (Beaulieu, Del'guidice, Sotnikova, Lemasson, & Gainetdinov, 2011). As presented in Figure 1.3, activation of dopamine D2 receptor by dopamine leads to receptor phosphorylation by a GPCR kinase (GRK) following the recruitment of β-arrestin 2, a scaffolding protein, to the membrane. AKT is also recruited along with PP2A to form the Akt-β-arrestin 2-PP2A complex, and PP2A consequently dephosphorylates and inactivates AKT resulting in increased GSK3 activity (Beaulieu, Gainetdinov, & Caron, 2009). These studies indicate the involvement of AKT in dopamine signaling and dopamine-dependent activities.

6. Using animal models to study schizophrenia

6.1. Advantages of animal models

Animal models can offer an efficient and alterative platform to monitor disease progression than in humans and offer the opportunity to perform invasive monitoring of structural and molecular changes that underlie the cause of the disease, and test

novel therapeutics not possible in patients (Jones, Watson, & Fone, 2011). An attempt has been made to map brain areas in rodents that are responsible for functions played by homologous parts of the human (Gainetdinov, Mohn, & Caron, 2001), and the high level of genomic homology between rodents and humans justifies the use of animals as a model of human disorder (Stubbs, 1996). Although animal models arose from their obvious inability to model hallmark features of schizophrenia, such as delusions and hallucinations, a combined approach that begins at the behavioral level and culminates at the cellular, molecular, or genetic levels can provide an effective approach to identify the psychopathology in human (Arguello & Gogos, 2006). All available animal models of schizophrenia could fit into the following four different induction categories: developmental, drug-induced, lesion, or genetic manipulation. Besides, these useful animal models should have the appropriate triad of face (symptom homology), construct (replicate the theoretical neurobiological rationale and pathology) and predictive (show the expected pharmacological response, or lack of it, to treatment by known antipsychotics and potential new adjunct therapies yet to be developed) validity to schizophrenia (Jones et al., 2011). Schizophrenia-related gene mutant rodents have achieved construct validity for schizophrenia (Nestler, & Hyman, 2010), hence it will afford a better understanding details of pathogenesis of schizophrenia through allowing for the identification of early mutational effects, the

study of their developmental progression, and providing an opportunity to understand how genetic, molecular, or environmental associations leading to this disease (Powell & Miyakawa, 2006).

6.2. Taking advantage of Akt1 mutant mice as an animal model of schizophrenia

The expression of Akt1 proteins in *Akt1*-mutant mouse brains, including the whole cortex, prefrontal cortex, hippocampus, cerebellum, and striatum, occurred in a gene-dosage-dependent manner. Accordingly, *Akt1* heterozygous (HET) mice had similarly decreased AKT1 expressions level as schizophrenic patients (Emamian et al., 2004), and *Akt1* homozygous (HOM) mice expressed no Akt1 protein in comparison to wild-type (WT) controls (Chen et al., 2012). In comparison to WT mice, HET mice were found no significant difference in the basal levels of dopamine and its metabolites (e.g., L-3,4-dihydroxyphenylalanine, dopamine, 3,4-dihydroxyphenylacetic acid, and homovanillic acid) in their frontal cortex, striatum, midbrain, somatosensory cortex, and hippocampus (Chen et al., 2012). Besides, protein levels of tyrosine hydroxylase (an enzyme in dopamine biosynthesis) and dopamine D2 receptors were revealed no difference between WT and HOM mice in their PFC (Lai et al., 2006). Extracellular dopamine and its metabolite 3,4-dihydroxyphenylacetic acid in both PFC and striatum were also found no

difference between WT and HOM mice (Lai et al., 2006). On the other hand, HOM mice are born smaller with reduction body weight than their WT littermates and have approximately 40 % increased neonatal mortality (Cho, 2001; Yang et al., 2004). Normal spontaneous locomotor activity has been revealed in HOM mice under the open field task (Chen & Lai, 2011; Lai et al., 2006). Both male and female HOM mice also displayed normal performances in the dark/light transition test (for bright light-induced anxiety), the elevated plus maze (for anxiety-like behaviors), the auditory trace fear conditioning (for auditory learning and associative learning), and the passive avoidance task (for hippocampus-related learning and memory) (Chen, Chen, & Lai, 2011; Chen & Lai, 2011). But HOM mice exhibited impairments in the recall of spatial memory in the Y-maze, mild impairment in the acquisition of Morris water maze, and severely impairment in the relearning of a new platform location in the maze (Chen et al., 2011). Furthermore, female HOM mice showed longer duration of immobility in the tail suspension test (for depressive-like behaviors), and exhibited deficient sensorimotor gating function in the prepulse inhibition task (Chen & Lai, 2011), which is also impaired in individuals with schizophrenia (Braff, Geyer, & Swerdlow, 2001). In addition, male HOM mice exhibit a deficiency in the prepulse inhibition task under the treatment of amphetamine (Emamian et al., 2004). Besides, a prefrontal cortex-dependent cognitive function, working memory, is abnormal in male

HOM mice under neurochemical challenge (i.e., quinpirole, SKF38393, guanfacine, and scopolamine) of three schizophrenia-related neurotransmitter systems, and male HOM mice are found alterations expression of prefrontal cortex genes controlling neuron functions (Lai et al., 2006). From a reward prediction error of dopamine-dependent decision making study, where subjects with schizophrenia showed the abnormality (Gradin et al., 2011; Murray et al., 2008), Male HET mice displayed a relatively efficient method of updating reward information from the environment (Chen *et al.*, 2012). These results may suggest that *AKT1* is involved in cognitive functions of schizophrenia and that schizophrenia-like cognitive functions can be suitably modeled in *Akt1*-mutant mice.

6.3. The use of 5-choice serial reaction time task to measure attentive functions in rodents

Attention, a cognitive function, is not unique in human and it can also be directly assessed in animals. The 5-choice serial reaction time task (5-CSRTT) is now among the most widely used tests of attentive functions in rodents, and has been applied to model attentive dysfunction in schizophrenia (Chudasama & Robbins, 2004). It has been developed and extensively validated by the common neural circuits and neurochemical modulation shared across species (Carli, Robbins, Evenden, & Everitt,

1983; Chudasama & Robbins, 2004; Robbins, 2002). The 5-CSRTT contains high face, predictive, and construct validity for CPT in human (Young, Powell, Risbrough, Marston, & Geyer, 2009) and is analogous to Leonard's five-choice serial reaction time task used in human (Leonard, 1959; cited in Wilkinson, 1963). The 5-CSRTT is initially developed for assessing attention in rats (Carli, Robbins, Evenden, & Everitt, 1983) and recently its procedures are greatly modified and performed in mice (Sanchez-Roige, Peña-Oliver, & Stephens, 2011). This task essentially tests the ability of the rodent to sustain spatial attention divided among a number of locations over a large number of trials, and consequently they have to detect a brief visual stimulus presented pseudorandomly across several spatial locations in a five-hole box (Bari, Dalley, & Robbins, 2008; Robbins, 2002). Analyzing the behavior of rodents' performed in the 5-CSRTT produces a variety of measures, including accuracy, correct responses, incorrect responses, omissions, premature responses, perseverative responses, as well as several response latency indices (Robbins, 2002). As presented in Table 1.2, each measurement is corresponding to different cognitive functions (i.e., attention, impulsivity responses, processing speed, motivation, and cognitive flexibility) and attentive functions are major linked to accuracy and omission behaviors (Robbins, 2002; Young et al., 2009). Due to diversely cognitive functions measured at a time, taking into consideration of all measures in the 5-CSRTT provides

precisely attentive functions without other cognitive effects.

7. The objective of this study

From above literature reviews, attentive impairments measured by the CPT are a cognitive deficits commonly observed in schizophrenic patients. We also have learned that the schizophrenia candidate gene, *AKT1*, encodes AKT1 protein which belongs to AKT family modulating a great diversity of cellular functions. In addition to be modulated by PI3K, AKT1 is a downstream mediator of dopamine D2 receptors which are mainly focused on the dopamine hypothesis of psychosis in schizophrenia. The *Akt1*-mutant mouse has been found abnormities in schizophrenia-like and dopamine-related cognitions; hence it is a suitable animal model to study cognitive functions of schizophrenia. Therefore, in order to examine whether *AKT1* participates in attentive function of schizophrenia, the 5-CSRTT which paralleled to the CPT in human was applied to understand attentive function in the Akt1 mouse model of schizophrenia.

In this study, *Akt1* HOM mice, *Akt1* HET mice, and their WT littermates were trained and tested in the 5-CSRTT. The experimental design and purpose of each experiment are listed in Table 1.3. Animals' attentive functions were revealed under both learning and testing conditions of the 5-CSRTT. We first examined whether they

had different attentive performances during the beginning of training. After they reached preset testing criteria, their baseline performances were analyzed to confirm that they had learned the task and they were ready for follow-up behavioral tests. Four different testing conditions were conducted through modulating parameters of the 5-CSRTT procedure to examine whether there were genotypic differences on their behavioral performances under difficult or high attentive load conditions. The details of behavioral procedure were described in the following chapter.

Chapter 2

Materials and Methods

1. Animals

All male *Akt1* homozygous mice (HOM, $n = 19$), *Akt1* heterozygous mice (HET, $n = 14$), and their wild-type littermates (WT, $n = 11$) used in this study were generated from *Akt1* heterozygous breeding pairs in C57BL/6J genetic background ($n > 10$) and genotyped using PCR analysis of mouse-tail DNA, as described previously (Cho, 2001). After weaning, animals were housed in groups of maximum 5 per cage with food and water available *ad libitum* in polysulfone individually ventilated cages (Alternative Design Manufacturing & Supply, Arkansas, AR, USA) within the animal rooms in the Psychology Department, National Taiwan University. All animals were 3-4 month-old at the beginning of experiments. Animals were handled at least 1 week before the behavioral experiments, and behavioral experiments were conducted during the dark phase at least half an hour after dark/light cycle (lights off at 8:00 A.M.) began. Animals were brought to the behavioral room 60 min before experiments. All animal procedures were performed according to protocols approved by the appropriate Animal Care and Use Committees established by the National Taiwan University.

2. Experimental apparatus

Behavioral apparatus were two custom-built 5-aperture operant chambers (31.8 L × 25.8 W × 29.1 H cm^3; Coulbourn Instruments, Whitehall, PA, USA) under red lighting condition (11.4 lux). Each chamber had a stainless-steel grid floor, aluminum front and back modular walls, aluminum top with a hole (4 cm diameter) in the center, and clear acrylic sides. Five 1.5 cm diameter and 4 cm deep stimulus-response apertures were spaced 3 cm apart, 1 cm above the grid floor, and centered on the front, curved wall of the chamber. Each stimulus-response aperture contained three pair of white light-emitting diode (LED) lights to generate a light stimulus and a photocell sensor to signal nose poke responses. The food magazine was located in the low center of the back wall of the chamber with a yellow LED light fitted in the magazine as a cue of nose poke responses, and was spanned horizontally by a photocell sensor to signal nose poke responses. Above the food magazine was a reward deliver to dispense food pellets (20 mg chocolate sucrose reward tablet; TestDiet, 5-TUT, Richmond, IN, USA). A 3 W house light was mounted above the food magazine. The Graphic State 3.03 (Coulbourn Instruments, Whitehall, PA, USA) was used to perform on-line control of this apparatus and data collection. Speaker to generate white noise (Coulbourn Instruments, Whitehall, PA, USA) was also used in some behavioral experiments.

3. Experimental procedures

3.1. Food restriction schedule

Animals subjected to behavioral experiments were housed individually in the animal room and their baseline body weights were recorded after a habituation period of minimally 1 week. Animals were food-restricted by maintaining a restricted diet and kept at 80-85% of free-feeding body weight throughout the behavioral experiments with daily weighed. According to their weights, food was put daily in their home cages at least an hour after they finished experiment. Water was available *ad libitum* in their home cages throughout the behavioral experiments.

3.2. Locomotor activity before 5-CSRTT

Before the beginning the 5-CSRTT procedure, animals underwent two locomotor activity tasks. On the first day, each animal was placed in a clean transparent PVC cage (47.2 L × 25.3 W × 21 H cm^3) containing a thin layer of clean bedding for 30 min. Animals were allowed to explore this new arena and their distance movements were recorded and analyzed by the EthoVision tracking system (Noldus, Wageningen, the Netherlands). On the second day, their locomotion was individually monitored in the 5-CSRTT apparatus. The 5-CSRTT apparatus was divided into three areas (i.e., aperture, middle, and magazine) as depicted in Figure 2.1. Each mouse was allowed

to explore and habituate this new experimental environment for 30 min. Their movements were recorded by videos, and analyzed off-line by a video tracking system, TopScan 2.0 (Cleaver System Incorporated, Reston, VA, USA). Their travel distances or duration in these 2 tasks were used as a measurement of locomotor activity.

3.3. 5-CSRTT procedures

Animals were trained and tested in a modified version of the 5-CSRTT procedure (Bari, Dalley, & Robbins, 2008; Debruin, Fransen, Duytschaever, Grantham, & Megens, 2006; Humby, Wilkinson, & Dawson, 2005) and final trial sequences were represented in Figure 2.2. Animals conducted sessions of 25 min duration with 1 session per day and 6 days per week for approximately 3 months. The 5-CSRTT procedure consisted of 3 sequential phases, including shaping, learning, and testing phases.

3.3.1. 5-CSRTT shaping phase

Mice were first trained to operate the 5-CSRTT apparatus by a series of shaping stages. In each stage, each mouse was required to reach shaping criteria in 25 min, and then they moved to next shaping stages. Under stage 1~3, a trial started with the illumination of the house light, and ended after animals collected their earned reward

pellets following a new trial started automatically. Stage 1: Animals were required accumulating 10 nose pokes into either stimulus-response apertures or the food magazine. Each nose poke was followed by the delivery of a reward pellet and the illumination of the magazine light. Stage 2: Each mouse was required accumulating 5 nose pokes into one of the 5 stimulus-response apertures. Nose poke into the food magazine was still followed by the delivery of a reward. But after accumulating 5 nose pokes into the food magazine, no reward was delivered from the food magazine if the animal kept performing nose pokes into the food magazine. Stage 3: Each mouse was required to accumulate 10 nose pokes into one of the 5 stimulus-response apertures, and nose poking into the food magazine was no longer followed by any delivery of a reward. Each nose poke into stimulus-response apertures was followed by the delivery of a reward pellet and the illumination of the magazine light. Stage 4: A trial started with the illumination of the house light, and then animals had to wait an intertrial interval (ITI) of 5 sec for the illumination of light stimuli. After the ITI, one of the 5 light stimuli illuminated for a stimulus duration (SD) of 32 sec. Animals were required to accumulate 15 nose pokes into the illuminated aperture within the SD or during the following 2 sec fixed limited hold (LH) after the light stimuli extinguished. Each nose poke into the illuminated aperture within the SD or LH was followed by the delivery of a reward pellet and the illumination of the magazine light. Each trial

ended after animals collected their earned reward pellets or after the LH duration and then a well-prepared new trial started automatically. Stage 5: Daily sessions started with the illumination of the house light and the magazine light, and a trial started after mice nose poked into the magazine. One of the 5 light stimuli illuminated for a SD of 32 sec after the 5-sec ITI. Each mouse that nose poked into the illuminated aperture within the SD or the 2 sec LH earned a reward pellet followed the illumination of the magazine light. Animals had 5 sec to eat their food pellet after collecting it. Each mouse nose poked into the non-illuminated aperture within the SD was signaled a punishment by a 5 sec time-out (TO) with 1 sec successive on and off of the house light. Trials ended after the 5 sec for eating their earned reward pellets or after the LH and TO duration and then a new trial started automatically. Each mouse was required to accumulate 15 completed trials for 2 consecutive daily sections and then animals underwent the training phase.

3.3.2. 5-CSRTT learning phase

Each session began with the illumination of the house light and the magazine light. A nose poke into the magazine initiated a trial and extinguished the magazine light. A fixed ITI of 5 sec preceded the illumination of stimulus-response apertures. After the ITI, one of the 5 light stimuli illuminated during the SD. The animal had to

respond with a nose poke into the illuminated aperture within the SD or during the following fixed 2 sec LH period after the light stimuli extinguished. A nose poke into the illuminated aperture resulted in the delivery of a reward food pellet following the illumination of the magazine light. The magazine light remained on until the animal collected its reward pellet and then the animal had fixed 5 sec duration to eat this pellet. A trial was finished after this 5 sec duration ended following a well-prepared new trial. The behavioral sequence described above was considered as a *correct response* and recorded as a correct trial. If the animal did not follow such behavioral sequence on any trial, the trial was considered as an improper trial and the response was recorded as an improper response. There were 4 types of improper responses and they were described as below. A *premature response* was recorded if the mouse did not nose poke into one of the 5 stimulus-response apertures during the ITI, before stimulus illuminated. An *incorrect response* was recorded if the mouse nose poked into one of the non-illuminated stimulus-response apertures during the SD or the LH period. An *omission response* was recorded if the mouse did not perform any nose poke into one of the 5 stimulus-response apertures during the SD or the LH period. A *perseverative response* was recorded if the mouse repeatedly nose poked into one of the 5 stimulus-response apertures, whether it illuminated or not, during the period of collecting reward pellet. Immediately after an improper response, a punishment was

signaled by a fixed 5 sec TO with 1 sec successive on and off of the house light. An improper trial finished after the TO period following a well-prepared new trial. The general parameters of the 5-CSRTT were set as ITI = 5 sec, TO = 5 sec, and LH = 2 sec. The learning phase of the 5-CSRTT was divided into 4 learning stages based on a scheduled stepped descending sequence of SD at 16, 8, 4 and finally 2 sec. Each mouse was required to reach the criteria (≥ 30 trials, ≥ 70% accuracy, ≤ 20% omission) for 2 consecutive days in first three learning stages (SD = 16, 8, and 4 sec) before passing to the next training stage. Each mouse was considered to have acquired the task when they meet the baseline performances (≥ 30 trials, ≥ 80% accuracy, ≤ 20% omission) for 3 consecutive days in the final stage of SD = 2 sec and then the mice underwent the testing phase. The accuracy of responding expressed as a percentage was calculated using the following formula:

$$\text{Accuracy } \% = \frac{\text{Correct responses}}{\text{Correct responses} + \text{Incorrect responses}} \times 100\ \%$$

The percentage of other behavioral measures (including correct, incorrect, omissions, premature, and perseverative responses) were calculated along with the following formula:

$$\text{Behavioral responses}\% = \frac{\text{Behavioral responses}}{\text{Total completed trials}} \times 100\%$$

The latency (in second) to correct nose poke after stimulus occurred (i.e., correct latency), and the latency (in second) to collect earned food pellets (i.e., reward latency)

were also calculated. According to our preliminary data, mice spent longer time to learn the final learning stage (SD = 2 s). Thus a mouse that did not reach the learning criteria for up to 18 days (i.e., 3 weeks of training; under SD = 16, 8, 4 s stages) and 24 days (under SD = 2 s stage) during the 4 learning stages was considered that it could not acquire this task. Consequently, the mouse was excluded from this experiment. Daily sessions were accumulated until animals finished required criteria in one learning stage.

3.3.3. 5-CSRTT testing phase

Once baseline performance (≥ 30 trials, ≥ 80% accuracy, and ≤ 20% omission for 3 consecutive days of SD = 2 s) had been established in each mouse, the testing phase began. The testing phase consisted of 4 tests in which the difficulty of the 5-CSRTT was managed by manipulations of the 5-CSRTT parameters. Each mouse was sequentially tested in one of the 4 tests once it reached the baseline performance. Each test lasted for 25 min or 50 trials whichever completed first. Each of the 4 tests consisted of 2 testing conditions that were applied in a pseudo-random order from trial to trial and they were described as below. (1) *The alteration of the ITI duration*: The duration of the ITI was either 2 or 8 seconds. (2) *The alteration of the SD duration*: The duration of the SD was reduced to either 1 or 0.5 second. (3) *The*

alteration of stimulus brightness: The brightness of the light stimuli was either relatively lighter (i.e., enhanced approximately three times the illumination of daily training) or relatively darker (i.e., the same brightness of daily training). (4) *The distracting test*: A distractor condition (i.e., a 100-dB, 0.5-sec on and off white noise) or no distractor condition were used and played during the duration of the SD. After finishing one test, each mouse was re-trained to the baseline performance for 3 consecutive days and tested again until finished the 4 tests. A mouse that did not reach the baseline performance for up to 24 days before the 4 tests was also excluded from this experiment. The behavioral measures during the 4 tests were the same as described above in the learning phase.

4. Data analysis

Behavioral data were first evaluated the homogeneity of variances by the Levene statistic. If variances of behavioral data were equal, they were analyzed using the one-way analysis of variance (ANOVA). *F*-values reaching significant difference ($p < .05$) were evaluated further by *post hoc* analysis of a conservative test, the Scheffé method. If variances of behavioral data were not equal, they were also analyzed using the one-way ANOVA. But their *F*-values were adjusted by the Welch test. *F*-values reaching significant difference ($p < .05$) were evaluated further by the Games-Howell

post hoc test. We expected that each test differed from their baseline condition and the 2 test conditions of each test differed from each other, hence we also used *a priori* comparisons and adjusted the critical *p*-value by the Bonferroni correction. The Chi-squared test (χ^2) was also used in some behavioral data where appropriate. All statistical analyses were conducted by the SPSS 17.0 for Windows (Chicago, IL, USA). All behavioral data are depicted as means ± standard error of the mean (SEM).

Chapter 3

Results

1. Observed genotypic distribution of offsprings from *Akt1* heterozygous breeding pairs

Genomic DNA isolated from animal's tail was submitted to PCR (polymerase chain reaction) to distinguish wild-type and recombinant alleles, and the 3 genotypes of animals were detected by PCR genotyping with agarose gel electrophoresis (Figure 3.1). As represented in Table 3.1, fewer than expected HOM mice were generated from *Akt1* HET breeding pairs when they were examined 3-4 weeks after birth. A total of 700 mice were generated from this study and male mice of the 3 genotypes were used. The sex ratios among each genotype were not much different. Both male and female HOM mice appeared with the same lower than expected Mendelian frequency.

2. Measurement of spontaneous locomotor activity in a new cage and the 5-CSRTT apparatus

In a new cage, no significantly genotypic difference was found in the total moving distance ($F(2, 41) = 0.0004$, $p = .9996$; Figure 3.2). In the 5-CSRTT apparatus, the time which animals in the 3 groups spent in the 3 areas was compared by the one-way ANOVA ($F(2, 15) = 68.88$, $p = .00000003$ for WT; $F(2, 12) = 59.51$, p

= .0000006 for HET; $F(2, 21) = 61.08$, $p = .000000002$ for HOM) and then the Scheffé *post hoc* test (all $p < .05$). All mice significantly spent most time in the area of magazine and less time in the area of middle at the 5-CSRTT apparatus (see Figure 3.3). No significantly genotypic difference was found in the total time spent in the areas of aperture ($F(2, 16) = 0.18$, $p = .83$), middle ($F(2, 16) = 0.40$, $p = .68$), or magazine ($F(2, 16) = 0.17$, $p = .85$).

3. Behavioral performance in the 5-CSRTT learning phase

The learning phase consisted of 4 learning stages (i.e., SD = 16, 8, 4, and 2 sec). Based upon the preset criteria, the passing rate and number of accumulated daily sessions in each learning stage were used as indexes to compare the overall genotypic differences during the 4 learning phases. Besides, special emphasis was given to the first learning stage (i.e., SD = 16 s) because it represented an initial stage of learning and it was not affect by the passing rate at each learning stage. The behavioral performance during the last three consecutive days of the last learning stage (i.e., SD = 2 s) was averaged and used as behavioral baseline for testing phase.

3.1. Passing rate in each learning stage

The total number of animals used in each learning stage and their passing rate were summarized in Table 3.2. The passing rate was calculated as the total number of

animals that reached the preset criteria in each learning stage divided by the total number of animals that were used in each learning stage (e.g., 11 / 11 for WT in the phase of SD = 16 s, 10 / 11 for WT in the phase of SD = 8 s and so forth). A marginally genotypic difference was found in the final learning stage (i.e., SD = 2 s, $\chi_2^2 = 5.299$, $p = .071$). No significantly genotypic difference was found in the other 3 learning stages and their χ_2^2 values were 4.49 ($p = .11$) for SD = 16 s, 2.89 ($p = .24$) for SD = 8 s, and 2.44 ($p = .30$) for SD = 4 s, respectively.

3.2. Accumulated daily sessions in each learning stage

In average, each mouse spent at least 7 accumulated daily sessions (i.e., 7 days) to complete a learning phase. As depicted in Figure 3.4, the one-way ANOVA revealed that there was no genotypic difference among the 3 groups in their accumulated daily sessions ($F(2, 39) = 1.42$, $p = .25$ for SD = 16 s; $F(2, 38) = 2.04$, $p = .14$ for SD = 8 s; $F(2, 36) = 0.35$, $p = .70$ for SD = 4 s; $F(2, 27) = 0.25$, $p = .78$ for SD = 2 s).

3.3. Learning performances during the first learning stage (SD = 16 s)

Since the minimum learning days required for all animals was 2 days, their behavioral performances on the first 2 days of the first learning stage were calculated

as their learning ability. Daily behavioral performances were averaged from the first day that animals participated in this stage to the final day that they reached the preset criteria.

3.3.1. Behavioral performances on the first and second day of SD = 16 s

On the first day, most animals started to learn this task, except that two HOM mice did not perform a correct nose poke or collect any reward, and one HET mice did not collect reward. The Levene test revealed equal variances in all behavioral measurements except premature responses. Thus the Welch test was used to adjust the *F* value for the premature response. As depicted in Figure 3.5 (the left side of each panel), significantly genotypic differences were found in the omission responses ($F(2, 41) = 6.16$, $p = .005$), premature responses ($F(2, 19.78) = 4.97$, $p = .018$), and latency to correct nose poke ($F(2, 39) = 6.97$, $p = .003$). The Scheffé *post hoc* test further revealed that HOM mice displayed significantly higher omission responses compared with WT mice ($p = .012$) and HET mice ($p = .037$). HOM mice also had significantly longer latency to correct nose poke compared with HET mice ($p = .003$) and marginally longer latency to correct nose poke compared with WT mice ($p = .080$). The Games-Howell *post hoc* test revealed that HOM mice showed significantly lower premature responses compared with WT mice ($p = .048$) and marginally lower premature responses compared with HET mice ($p = .092$). There was no significantly

genotypic difference in the accuracy responses ($F(2, 41) = 0.46$, $p = .64$), perseverative responses ($F(2, 41) = 0.06$, $p = .94$), and latency to collect reward ($F(2, 38) = 1.93$, $p = .16$).

On the second day, as depicted in Figure 3.5 (the right side of each panel), significantly genotypic differences were revealed in omission responses ($F(2, 41) = 5.73$, $p = .006$), perseverative responses ($F(2, 41) = 4.55$, $p = .016$), and latency to correct nose poke ($F(2, 41) = 3.38$, $p = .044$). The Scheffé *post hoc* test further revealed significantly higher omission responses in HOM mice compared with WT mice ($p = .017$) and HET mice ($p = .040$). HOM mice also showed significantly lower perseverative responses compared with WT mice ($p = .019$). The latency to correct nose poke in HOM mice was marginally higher than the one in HET mice ($p = .092$). No significant difference among the 3 groups was found in accuracy responses ($F(2, 41) = 2.32$, $p = .11$), premature responses ($F(2, 41) = 1.84$, $p = .17$), and latency to collect reward ($F(2, 41) = 2.01$, $p = .15$).

3.3.2. Averaged daily behavioral performance in the first learning stage (SD = 16 s)

As shown in Figure 3.6, among our measurements, the one-way ANOVA revealed that there were significantly genotypic differences in the omission response ($F(2, 39) = 6.25$, $p = .004$) and the latency to correct nose poke ($F(2, 39) = 4.48$, p

= .018). The Scheffé *post hoc* test further revealed that mice in the HOM group had significantly higher omission responses compared with the WT group (p = .043) and HET group (p = .011). HOM mice also had significantly longer latency to correct nose poke compared with HET mice (p = .022). In contrast to omission responses, no significant difference among the 3 groups was found in accuracy responses (F(2, 39) = 2.52, p = .09), premature responses (F(2, 39) = 0.20, p = .82), perseverative responses (F(2, 39) = 0.83, p = .44), and latency to collect reward (F(2, 39) = 0.93, p = .40).

3.4. Baseline performances in the last learning stage (SD = 2 s)

As depicted in Figure 3.7, no significantly genotypic difference was found in accuracy responses (F(2, 27) = 0.14, p = .87), omission responses (F(2, 27) = 0.06, p = .94), premature responses (F(2, 27) = 2.30, p = .12), perseverative responses (F(2, 27) = 0.05, p = .95), latency to correct nose poke (F(2, 27) = 0.18, p = .84), and latency to collect reward (F(2, 27) = 0.76, p = .48).

3.5. Summary of 5-CSRTT learning phase

WT, HET, and HOM mice were not significantly different in their pass rate and daily accumulated sessions. But in the first learning stage (i.e., SD = 16 s), HOM

mice consistently displayed higher omission responses on the first 2 days and in their averaged daily performances. HOM mice also showed lower premature responses, lower perseverative responses, and longer latency to correct nose poke on the first and second days of the first learning stage. Once mice in each group learned the task and reached the preset criteria in the last learning stage (i.e., SD = 2 s), no significant genotypic difference was found in their behavioral baseline. These mice were further tested in the testing phase of the 5-CSRTT.

4. Behavioral performance in the 5-CSRTT testing phase

The testing phase consisted of 4 tests (i.e., alternation of ITI, SD, brightness, and white noise distractor). Behavioral performances of WT mice between baseline and testing day were first used to evaluate the efficacy of each test. We expected that each test differed from their baseline condition and the 2 test conditions of each test differed from each other, hence we used *a priori* comparisons and adjusted the critical *p* value by the Bonferroni correction. Behavioral measurements for the 3 groups were analyzed to compare the overall genotypic differences in these 4 tests.

4.1. Behavioral performances in the 1st test (ITI = 2 and 8 s) of testing phase

4.1.1. Behavioral performances for WT mice in the 1st test (ITI = 2 and 8 s)

We conducted three *a priori* comparisons by paired t-test, thus the adjusted

critical *p* value was 0.0167. As depicted in Figure 3.8, in the ITI = 2 s condition, WT mice only showed significant longer latency to correct nose poke than their baseline performances ($t(6) = 5.07$, $p = .0022$). Compared with the baseline condition, there was no significant difference in accuracy response ($t(6) = -0.54$, $p = .61$, see Figure 3.8A), omission responses ($t(6) = 1.52$, $p = .18$, see Figure 3.8B), premature responses ($t(6) = -3.11$, $p = .021$, see Figure 3.8C), perseverative responses ($t(6) = -1.20$, $p = .28$, see Figure 3.8D), and latency to collect reward ($t(6) = -3.02$, $p = .023$, see Figure 3.8F) of WT mice under the ITI = 2 s condition. In the ITI = 8 s condition, significantly higher premature responses was found in WT mice compared with their performances in the baseline ($t(6) = 3.89$, $p = .0080$) and the ITI = 2 s condition ($t(6) = -4.48$, $p = .0042$). Accuracy responses ($t(6) = -1.39$, $p = .21$), omission responses ($t(6) = 0.87$, $p = .42$), perseverative responses ($t(6) = -1.46$, $p = .19$), latency to correct nose poke ($t(6) = 2.53$, $p = .044$), and latency to collect reward ($t(6) = -3.02$, $p = .023$) were showed no significant difference in WT mice between the ITI = 8 s condition and the baseline condition. WT mice also showed no significant difference in accuracy responses ($t(6) = 1.10$, $p = .31$), omission responses ($t(6) = 0.83$, $p = .44$), perseverative responses ($t(6) = -0.33$, $p = .75$), latency to correct nose poke ($t(6) = 0.98$, $p = .37$), and latency to collect reward ($t(6) = -0.86$, $p = .42$) between these 2 test conditions.

4.1.2. Genotypic comparisons in the 1st test (ITI = 2 and 8 s)

In the baseline condition, as depicted in Figure 3.9 (the left side of each panel), no significant difference among the 3 groups was found in accuracy responses ($F(2, 27) = 0.14$, $p = .87$, see Figure 3.9A), omission responses ($F(2, 27) = 0.06$, $p = .94$, see Figure 3.9B), premature responses ($F(2, 27) = 2.30$, $p = .12$, see Figure 3.9C), perseverative responses ($F(2, 27) = 0.05$, $p = .95$, see Figure 3.9D), latency to correct nose poke ($F(2, 27) = 0.18$, $p = .84$, see Figure 3.9E), and latency to collect reward ($F(2, 27) = 0.76$, $p = .48$, see Figure 3.9F). In the ITI = 2 s condition, as depicted in Figure 3.9 (the middle part of each panel), no significant genotypic difference was found in accuracy responses ($F(2, 27) = 0.22$, $p = .81$, see Figure 3.9A), omission responses ($F(2, 27) = 0.14$, $p = .87$, see Figure 3.9B), premature responses ($F(2, 27) = 0.30$, $p = .74$, see Figure 3.9C), perseverative responses ($F(2, 27) = 0.55$, $p = .58$, see Figure 3.9D), latency to correct nose poke ($F(2, 27) = 1.57$, $p = .23$, see Figure 3.9E), and latency to collect reward ($F(2, 27) = 1.17$, $p = .33$, see Figure 3.9F). In the ITI = 8 s condition, the Levene test revealed that the assumption of equal variances was violated in the accuracy response, thus the Welch test was used to adjust the F value in this response. As depicted in Figure 3.9 (the right side of each panel), there was no significant difference among the 3 groups in accuracy responses ($F(2, 13.43) = 0.57$, $p = .58$, see Figure 3.9A), omission responses ($F(2, 27) = 1.50$, $p = .24$, see Figure

3.9B), premature responses ($F(2, 27) = 0.05$, $p = .95$, see Figure 3.9C), perseverative responses ($F(2, 27) = 0.02$, $p = .98$, see Figure 3.9D), latency to correct nose poke ($F(2, 27) = 0.22$, $p = .80$, see Figure 3.9E), and latency to collect reward ($F(2, 27) = 0.66$, $p = .53$, see Figure 3.9F) in the ITI = 8 s condition.

4.2. Behavioral performances in the 2nd test (SD = 1 and 0.5 s) of testing phase

4.2.1. Behavioral performances for WT mice in the 2nd test (SD = 1 and 0.5 s)

Since we conducted three *a priori* comparisons by paired t-test, the adjusted critical *p* value was 0.0167 for accuracy, omission, premature, and perseverative responses. As depicted in Figure 3.10, in the SD = 1 s condition, WT mice displayed significantly higher omission responses compared with their baseline performances ($t(6) = 5.13$, $p = .0022$, see Figure 3.10B). No significant difference was revealed in accuracy ($t(6) = -2.47$, $p = .049$, see Figure 3.10A), premature ($t(6) = 1.13$, $p = .30$, see Figure 3.10C), and perseverative ($t(6) = 0.08$, $p = .94$, see Figure 3.10D) responses of WT mice between the SD = 1 s condition and the baseline condition.

In the SD = 0.5 s condition, significantly higher omission ($t(6) = 4.51$, $p = .0040$, see Figure 3.10B) and premature responses ($t(6) = 3.46$, $p = .013$, see Figure 3.10C) were found in WT mice compared with their baseline performances. WT mice also spent significantly less time to correct nose poke in the SD = 0.5 s condition than in

the SD = 1 s condition ($t(6) = 3.33$, $p = .016$, see Figure 3.10E). There was no significant difference in accuracy ($t(6) = -3.01$, $p = .024$, see Figure 3.10A) and perseverative ($t(6) = -7.01$, $p = .50$, see Figure 3.10D) responses of WT mice between the SD = 0.5 s condition and the baseline condition. Besides, no significant difference was revealed in accuracy responses ($t(6) = 1.75$, $p = .13$, see Figure 3.10A), omission responses ($t(6) = -1.26$, $p = .26$, see Figure 3.10B), premature responses ($t(6) = -0.41$, $p = .69$, see Figure 3.10C), perseverative responses ($t(6) = 0.51$, $p = .63$, see Figure 3.10D), and latency to collect reward ($t(6) = -0.67$, $p = .53$, see Figure 3.10F) of WT mice between the 2 test conditions.

4.2.2. Genotypic comparisons in the 2nd test (SD = 1 and 0.5 s)

In the baseline condition, the Levene test revealed equal variances in all behavioral measurements except omission responses; consequently, the Welch test was used to adjust the F value in the omission response. As depicted in Figure 3.11A-D, no significant difference among the 3 groups was found in the accuracy ($F(2, 24) = 2.33$, $p = .12$), omission ($F(2, 15.49) = 1.83$, $p = .19$), premature ($F(2, 24) = 1.28$, $p = .30$), and perseverative ($F(2, 24) = 0.52$, $p = .60$) responses in the baseline condition. In the SD = 1 s condition, as depicted in Figure 3.11A-F, no significant genotypic difference was found in accuracy responses ($F(2, 24) = 0.69$, $p = .51$),

omission responses ($F(2, 24) = 1.60$, $p = .22$), premature responses ($F(2, 24) = 0.35$, $p = .71$), perseverative responses ($F(2, 24) = 0.12$, $p = .88$), latency to correct nose poke ($F(2, 24) = 0.63$, $p = .54$), and latency to collect reward ($F(2, 24) = 0.04$, $p = .96$). In the SD = 0.5 condition, the assumption of equal variances had been violated in the latency to collect reward by the Levene test, thus the Welch test was used to adjust the *F* value in this response. Again, there was no significantly genotypic difference in the accuracy responses ($F(2, 24) = 0.52$, $p = .60$), omission responses ($F(2, 24) = 0.03$, $p = .97$), premature responses ($F(2, 24) = 0.15$, $p = .86$), perseverative responses ($F(2, 24) = 0.48$, $p = .62$) responses, latency to correct nose poke ($F(2, 24) = 1.26$, $p = .30$), and latency to collect reward ($F(2, 10.64) = 0.44$, $p = .66$) in the SD = 0.5 condition as shown in Figure 3.11A-F.

4.3. Behavioral performances in the 3rd test (brightness) of testing phase

4.3.1. Behavioral performances for WT mice in the 3rd test (brightness)

We used paired t-test to conduct three *a priori* comparisons, thus the adjusted critical *p* value was 0.0167 for accuracy, omission, premature, and perseverative responses. As depicted in Figure 3.12, only latency to correct nose poke ($t(5) = -2.59$, $p = .049$) in WT mice was found significantly shorter in the relatively darker condition than in the relatively lighter condition. In the relatively lighter condition, as

shown in Figure 3.12A-D, WT mice showed no significant difference in accuracy ($t(5) = 0.56$, $p = .60$), omission ($t(5) = 0.20$, $p = .85$), premature ($t(5) = 0.55$, $p = .60$), and perseverative responses ($t(5) = 0.21$, $p = .84$) compared with their baseline responses. In the relatively darker condition, WT mice also showed no significant difference in accuracy ($t(5) = 0.87$, $p = .42$), omission ($t(5) = 0.15$, $p = .89$), premature ($t(5) = -0.86$, $p = .43$), and perseverative responses ($t(5) = -1.55$, $p = .18$) compared with their baseline responses. There was no significant difference in accuracy responses ($t(5) = 0.23$, $p = .83$), omission responses ($t(5) = -0.02$, $p = .98$), premature responses ($t(5) = -1.58$, $p = .17$), perseverative responses ($t(5) = -1.85$, $p = .12$) responses, and latency to collect reward ($t(5) = -1.14$, $p = .31$) of WT mice between the 2 test conditions.

4.3.2. Genotypic comparisons in the 3rd test (brightness)

As showed in Figure 3.13A-D (left side of each panel), in the baseline condition, no significantly genotypic difference was found in accuracy ($F(2, 23) = 0.73$, $p = .49$), omission ($F(2, 23) = 2.95$, $p = .07$), premature ($F(2, 23) = 2.34$, $p = .12$), and perseverative ($F(2, 23) = 1.04$, $p = .37$) responses. In the relatively lighter condition (see Figure 3.13A-F), accuracy responses ($F(2, 23) = 0.69$, $p = .51$), omission responses ($F(2, 23) = 0.21$, $p = .81$), premature responses ($F(2, 23) = 1.26$, $p = .30$), perseverative responses ($F(2, 23) = 0.41$, $p = .67$), latency to correct nose poke ($F(2,$

23) = 1.21, p = .32), and latency to collect reward (F(2, 23) = 2.16, p = .14) were also found no significantly genotypic difference.

In the relatively darker condition (see Figure 3.13A-F, right side of each panel), the assumption of equal variances was violated in premature responses and latency to collect reward by the Levene test, thus the Welch test was used to adjust the F value in these two responses. Significantly genotypic differences were revealed in perseverative responses (F(2, 23) = 6.16, p = .0072, see Figure 3.13D) and latency to collect reward (F(2, 12.30) = 4.26, p = .039, see Figure 3.13F) in the relatively darker condition. The Scheffé *post hoc* analyses further revealed that HOM mice had significantly higher perseverative responses compared with WT (p = .011) and HET (p = .047) mice; the Games-Howell *post hoc* test revealed that only HET mice showed marginally longer latency to collect reward compared with WT mice (p = .0698). No significantly genotypic difference was found in accuracy responses (F(2, 23) = 0.54, p = .59), omission responses (F(2, 23) = 1.17, p = .33), premature responses (F(2, 11.97) = 3.13, p = .08), and latency to correct nose poke (F(2, 23) = 0.74, p = .49).

4.4. Behavioral performances in the 4th test (white noise distractor) of testing phase

4.4.1. Behavioral performances for WT mice in the 4th test (white noise distractor)

Since we used paired t-test to conduct three *a priori* comparisons, the adjusted

critical *p* value was 0.0167 for accuracy, omission, premature, and perseverative responses. As represented in Figure 3.14A-D, in the no distractor condition, WT mice displayed no significant difference in accuracy ($t(5) = -0.78$, $p = .47$), omission ($t(5) = 2.58$, $p = .0495$), premature ($t(5) = -2.80$, $p = .038$), and perseverative ($t(5) = -0.42$, $p = .69$) responses compared with their baseline behavior. As depicted in Figure 3.14B, WT mice had significantly higher omission response in the distracting condition compared with their baseline ($t(5) = 4.34$, $p = .0074$) and no distractor condition ($t(5) = -3.81$, $p = .013$). No significant difference was found between the distracting condition and baseline in the accuracy ($t(5) = -0.52$, $p = .63$), premature ($t(5) = -1.02$, $p = .36$), and perserverative ($t(5) = -1.60$, $p = .17$) responses. There was also no significant difference in accuracy responses ($t(5) = 0.13$, $p = .90$), premature responses ($t(5) = -0.45$, $p = .67$), perseverative ($t(5) = 0.45$, $p = .67$) responses, latency to correct nose poke ($t(5) = -0.29$, $p = .78$), and latency to collect reward ($t(5) = -0.03$, $p = .98$) of WT mice between the 2 test conditions.

4.4.2. Genotypic comparisons in the 4th test (white noise distractor)

As depicted in Figure 3.15A-D (left side of each panel), in the baseline condition, there was no significantly genotypic difference in accuracy ($F(2, 23) = 0.32$, $p = .73$), omission ($F(2, 23) = 0.92$, $p = .41$), premature ($F(2, 23) = 1.62$, $p = .22$), and

perseverative ($F(2, 23) = 0.59$, $p = .23$) responses. In the no distractor condition, the Levene test revealed equal variances in all behavioral performances except accuracy responses and latency to collect reward; hence, the Welch test was used to adjust the F value in these two responses. As shown in Figure 3.15A-F, no significant difference was found in the accuracy responses ($F(2, 10.25) = 0.78$, $p = .48$), omission responses ($F(2, 23) = 0.72$, $p = .50$), premature responses ($F(2, 23) = 2.63$, $p = .09$), perseverative responses ($F(2, 23) = 0.52$, $p = .60$), latency to correct nose poke ($F(2, 23) = 0.77$, $p = .47$) and latency to collect reward ($F(2, 9.31) = 0.05$, $p = .95$) in the no distractor condition.

In the distracting condition, the Levene test revealed that the assumption of equal variances was violated in the premature response. Therefore, the Welch test was used to adjust the F value in this response and revealed significantly genotypic differences in it ($F(2, 14.21) = 4.198$, $p = .037$). Further the Games-Howell *post hoc* test found that HET mice displayed significantly higher premature response than WT mice ($p = .024$, see Figure 15C). As shown in Figure 3.15A-F (right side of each panel), no significant difference was found in the accuracy responses ($F(2, 23) = 0.06$, $p = .94$), omission responses ($F(2, 23) = 0.21$, $p = .81$), perseverative responses ($F(2, 23) = 0.37$, $p = .70$), latency to correct nose poke ($F(2, 23) = 0.13$, $p = .88$), and latency to collect reward ($F(2, 23) = 0.28$, $p = .76$).

4.5. Summary of 5-CSRTT testing phase

The behavioral performances of WT mice in the 4 tests were summarized in Table 3.3. Compared with baseline, WT mice behaved differently in the 2 test conditions. Such results indicated that each test was different from the baseline condition and the 2 test conditions of each test were different from each other. A summary of animals' behavioral responses in each test was shown in Table 3.4. No significant difference among the 3 groups was found in the baseline of each test. Genotypic comparisons in the testing stage showed that *Akt1*-mutant mice had abnormal premature percentage in the test of brightness and aberrant perseverative responses in the test of white noise distractor.

Chapter 4

General Discussion

In this study, we used the 5-CSRTT to investigate the causal relationship between *Akt1* and attentive functions using *Akt1*-mutant mice. Mice first learned to operate the 5-CSRTT and subsequently went through testing conditions with highly attentive demands. Before conducting the 5-CSRTT procedure, we confirmed that *Akt1*-defient mice had normal motor activity as their wild-type controls. In the 5-CSRTT learning phase, all animals showed similar learning ability to acquire this task, and *Akt1*-mutant mice also showed normal baseline performances after acquired this task. But genotype-specific alterations in omission responses were consistently found in HOM mice in the beginning of the learning stage (SD = 16 s), especially on the first two days of learning. In the 5-CSRTT testing phase, instead of having impaired attention-related response, HOM mice exhibited alteration of impulsive responses in the tests of brightness and white noise distractor. Our findings mainly indicated that *Akt1* HOM mice displayed impaired attentive functions in the acquisition of the 5-CSRTT and *AKT1*deficiency might be involved in the attentive functions of schizophrenia. The precise role of *AKT1* in attention of schizophrenia is worth further studying and it would be interesting to apply antipsychotic drugs to see whether this genotypic alteration of attention could be rescued in the future study. The details were

further discussed below.

Advantages and disadvantages of our 5-CSRTT procedures

The major drawback of the 5-CSRTT is the extensive training needed to reach a stable baseline performance (Levin, Bushnell, & Rezvani, 2011). Although C57BL/6 mice can reach a stable baseline of attentive performance in the SD = 1 s condition of the 5-CSRTT, it requires approximately 4 months training (Hoyle, Genn, Fernandes, & Stolerman, 2006; Patel, Stolerman, Asherson, & Sluyter, 2006). In order to shorten the training days and prevent overtraining in our experiment, 18-day (for SD = 16, 8, 4 s) and 24-day (for SD = 2 s) training days were applied as maximum training days for each learning stage. Consequently, some mice were eliminated from the 3 groups because they could not reach the preset criteria. But the total training days were reduced to approximately 2 months. In this study, we also modified some parameters of the 5-CSRTT procedures in the testing stage to manipulate the difficulty of the tests. These variable manipulations may more or less increase the unpredictability of stimulus; hence, animals need to further pay attention to this task (i.e., increase attentive demands). To the best of our knowledge, none of mouse studies has applied variably alternations of SD, brightness, and distractor in one test as we did in this study (Sanchez-Roige et al., 2011). If mice conduct one test condition in a test, they

may improve their performance after extensive trials. Thus, this test could not reflect real attentive functions of mice. Additionally, we observed that WT mice in our first test (ITI = 2 and 8 s) displayed alternations in premature responses which was similar to the previous results in mice (Debruin, Fransen, Duytschaever, Grantham, & Megens, 2006; Relkovic et al., 2010; Walker, Peña-Oliver & Stephens, 2011). We found that our manipulations were successes as indicated by the differential behavioral responses WT mice between their baseline and the testing conditions. Therefore, our 5-CSRTT procedure can be applied to other lines of mutant mice to evaluate their attentive function.

The involvement of Akt1 in attentive functions

As the expression of Akt1 protein in mutant mouse brains occurred in a gene-dosage-dependent manner (Chen et al., 2012), we persistently observed a gene-dosage-dependent alternation of omission responses in *Akt1*-mutant mice during the learning stage of SD = 16 s. Accordingly, HOM mice showed significantly higher omission responses compared with the other 2 groups, especially in the first two days and in their averaged daily performances. Although *Akt1* HOM mice also displayed alterations in their premature responses, perseverative responses, and latency to collect reward on the first 2 days of the learning stage of SD = 16 s, these indexes did

not continually detected in this phase. These inconsistently abnormal performances might be the result which animals adjusted their responses to performing the 5-CSRTT.

There are several possible explanations to explain the higher omission responses found in HOM mice under their learning stage. Omission responses were defined as no response during either the SD or the LH period, hence increased omission responses might be generated from either no interest in this 5-CSRTT, motor impairments, or not attending to the stimulus (Young et al., 2009). Accuracy was the proportion of correct responses over total correct plus incorrect responses. The over 50 % of accuracy in the stage of SD = 16 s suggested that all animals not only learned how to earn a reward but also motivated to response for rewards. Besides, from the index of latency to collect reward in the stage of SD = 16 s, this appeared to imply that all animals from different groups had similar motivation to get rewards in the 5-CSRTT. Furthermore, our HOM mice in the present study exhibited normal basic locomotion as reported previously (Chen & Lai, 2011; Lai et al., 2006) and showed normal latency to correct nose poke. Additionally, no significant difference revealed in both accumulated sessions and pass rate under each learning stage implied that all animals can learn this task with appropriate training days. Thus, these findings are in favor of HOM mice have normal learning ability and motor functions. Taken together,

it is more likely that the higher omission responses in HOM mice resulted from not attending to the stimulus which suggests that *Akt1* HOM mice have dysfunctions in attention. Accordingly, the inconsistency on longer latency to correct nose poke in HOM mice during the learning stage of SD = 16 s may be the outcome of increased omission responses. As a number of parameters of the 5-CSRTT have been validated to relate to human CPT and attentive functions (Young et al., 2009), it is expected to observe some attentive dysfunction in schizophrenia patients with *AKT1* deficiency.

The involvement of Akt1 in inhibitory control

In the testing phase of this study, significantly genotypic differences were found in behavior of inhibitory control (i.e., premature and perseverative responses) under the tests of brightness and distraction rather than their attention-related behavior. Both responses require inhibition of the impulsivity to nose poke while the stimulus light was extinguished. In the test of brightness, it is relatively more difficult for the animals to detect the stimulus in the relatively darker condition compared with the relatively lighter condition. Consequently, a gene-dosage-dependent alternation of perseverative responses was revealed in the relatively darker condition, and HOM mice had the highest perseverative responses. This finding seems to suggest that *Akt1* might play a role in inhibitory control. However, HET but not HOM mice showed

significantly higher premature responses in the distracting condition of the white noise distracting test. Besides, HOM mice showed opposite alternations of perseverative responses in the learning stage of SD = 16 s. It is of interest to further investigate the precise role of *Akt1* in inhibitory control and its underlying mechanism. Further studies are highly needed.

The potential role of AKT1 and dopaminergic system in the regulation of attentive functions

As described above in the introduction, convergent evidence indicates that AKT1 is a signaling intermediate downstream from the dopamine D2 receptor (Beaulieu, 2011) and *AKT1* has some impact on dopamine-dependent cognitive functions of schizophrenia from human and animal studies (Chen et al., 2012; Chen & Lai, 2011; Lai et al., 2006; Tan et al., 2008). Poor attentive performances in the CPT were also reported in healthy subjects with genetic variations of *AKT1* and dopamine receptor 2 (*DRD2*) (Blasi et al., 2011). Therefore, *AKT1* deficiency may result in the impairment of attentive function and the risk of developing schizophrenia through its interaction with dopamine signaling.

Dopaminergic system has been found to participate in some components of attention (Posner & Rothbart, 2007). Accordingly, dopamine might be involved in the

modulation of conflicts in planning, decision making, error detection, and overcoming habitual actions for attention, which require networks in the target areas of the ventral tegmental dopamine system, particularly the anterior cingulated cortex, the lateral prefrontal cortex, and the basal ganglia. In addition, a high dose of haloperidol (a typical antipsychotic antagonizing dopamine D2 receptor) was reported to impair attentive functions of the CPT in normal subjects (Saeedi, Remington, & Christensen, 2006). Risperidone (an atypical antipsychotic antagonizing dopamine and serotonin receptor) was found to improve attentive deficits of the CPT in schizophrenia and schizoaffective disorder (Houthoofd, Morrens, & Sabbe, 2008). On the same line, amphetamine (increase synaptic dopamine concentrations), a psychostimulant, was reported to increase attentive responses of the CPT in normal individuals. Methylphenidate (a norepinephrine-dopamine reuptake inhibitor), a common treatment of attention deficit hyperactivity disorder (ADHD), increases attentive responses of the CPT in both normal individuals and patients with ADHD (Koelega, 1993; Lawrence et al., 2005; O'Toole, Abramowitz, Morris, & Dulcan, 1997). Furthermore, the dopamine transporter (*DAT1*), the dopamine receptor 4 (*DRD4*), and the *DRD2*, dopamine-related genetic polymorphisms were also associated with the performance of attention under the CPT in ADHD patients (Kieling, Roman, Doyle, Hutz, & Rohde, 2006; Kollins et al., 2008; Loo et al., 2003). These findings suggested

that increasing dopaminergic functions resulted in increased attentive functions, and decreasing dopaminergic functions led to decrease attentive functions in the CPT. Human studies indicate the importance of dopamine and its receptors in the regulation of attentive functions and the attentive symptoms of ADHD.

In addition, manipulations of dopamine functions in the medial prefrontal cortex (mPFC) (a projecting site of dopaminergic neurons in the midbrain) through infusing dopamine receptor agents or 6-hydroxydopamine (6-OHDA) depletions in rats indicated the involvement of mPFC in the modulation of accuracy responses rather than omission and other responses in the 5-CSRTT (Granon et al., 2000; Robbins, 2002). The depletion of dopamine using 6-OHDA in both ventral and dorsal striatum (projecting sites of dopaminergic neurons) in well-trained rats resulted in higher omission responses and longer latency to correct nose poke in the 5-CSRTT (Baunez & Robbins, 1999; Cole & Robbins, 1989). Besides, injections of both dopamine D1 (i.e., SCH23390) and D2 (i.e., sulpiride) receptor antagonists into the nucleus accumbens (in the ventral striatum) increased omission responses, reduced accuracy responses, and increased latency to correct nose poke of the 5-CSRTT in well-trained rats (Pezze, Dalley, & Robbins, 2006). These studies suggested that modulating dopaminergic functions in dopamine-related brain area (e.g., PFC and striatum) led to distinct attentive performances (e.g., accuracy and omission) of the

5-CSRTT. Although we did not conduct any pharmacological challenges in the present study, the behavioral pattern that we observed in HOM mice under the first learning stage (i.e., SD = 16 s) was somewhat similar to these findings with modulation of dopaminergic functions in the striatum. Given the involvement of Akt1 in dopamine signaling and the importance of dopaminergic system in the modulation of attentive functions in animals and ADHD patients, the potential role of AKT1 in the regulation of attentive function in schizophrenic patients is worth further investigating.

The potential role of AKT1 and the frontal cortex in the regulation of attentive functions

Akt1-deficient mice, which mimic the reduction of AKT1 proteins in the prefrontal cortex of schizophrenia patients (Chen et al., 2012; Emamian et al., 2004), were revealed to alter the expression of genes controlling neuron functions in prefrontal cortex (PFC), the dendritic architecture in the PFC, and PFC-dependent cognitive functions (Lai et al., 2006). Similar findings were also reported in a human study. When subjects were performing the CPT, the frontal cortex was activated in the normal subjects but not in the schizophrenic patients (Cohen, Nordahl, Semple, Andreason, & Pickar, 1998; MacDonald et al., 2005). Thus alternations of AKT1 may

result in dysfunctions of the frontal cortex and then lead up to poor attentive performances in schizophrenia.

Cognitive symptoms of schizophrenia was considered a result in frontal hypometabolism (Weinberger, 1988), and the frontal cortex correlated consistently with the tasks requiring planning, executive control, decision making, working memory and attention in patients with schizophrenia (Antonova, Sharma, Morris, & Kumari, 2004; Miller & Cohen, 2001). The frontal cortex was involved in attentive network controlling attention (Corbetta & Shulman, 2002), particularly the association between the right dorsolateral PFC and attention (Antonova, Sharma, Morris, & Kumari, 2004). The homolog of the dorsolateral PFC in primates is the medial PFC in the rat according to anatomical and lesion studies (Uylings, Groenewegen, & Kolb, 2003). Rats with the medial PFC lesion showed higher omission responses, lower accuracy responses, higher perseverative responses, and longer latency to correct nose poke in the 5-CSRTT (Passetti, Chudasama, & Robbins, 2002). In our study, *Akt1* HOM mice under the first learning stage (i.e., SD = 16 s) showed higher omission responses and similar behavioral patterns to rats with the PFC lesion. These results from schizophrenic patients and our current study support the potential role of AKT1 in the PFC on the involvement of poor attentive responses in schizophrenia. The *AKT1* deficiency may result in the abnormality of dendritic

architecture in the PFC of schizophrenic patients as reported previouosly in *Akt1*-deficient mice (Lai et al., 2006) and consequently lead up to the impairment of attentive functions in patients. Further studies are needed to reveal how AKT1 affects functions of the PFC and then impairs attentive functions of schizophrenia.

Modeling schizophrenia-related attentive function in animals

Attention refers to a set of mechanisms allowing people to selectively perceive and respond to events that are relevant to their behavioral goals (Gazzaniga, 2009). James (1890) was the first to write about its multiplicity, and many researchers suggested that there were multiple components to attention since then. Posner and Boies (1971) divided human attention into three components: Alertness (also known as sustained attention, vigilance, or alerting) was the ability to increase and maintain response readiness in preparation for an impending stimulus; selectivity (also called orienting or scanning) was the ability to select specific information from among multiple sensory stimuli; processing capacity (also known as supervisory, selective, conflict resolution and focused attention) was the ability to handling more than two tasks simultaneously (Raz & Buhle, 2006). Both the CPT and the 5-CSRTT are required that subjects monitor displays over extended periods of time for the occasional occurrence of specified signals (Rosvold et al., 1956; Robbins, 2002).

Thus they are known for assessing the sustained attention.

Attentive dysfunctions often pre-date the development of schizophrenia (O'Carroll, 2000) and tend to be independent of symptomatic state (Gold, 2004). Attentive deficits also greatly compromise the patient's ability to function effectively in society, such as social problem solving and skill acquisition (Green, 1996). Besides, attention stands at the forefront of cognitive functions (Keeler & Robbins, 2011), thus it may affect other cognitive symptoms (e.g., learning, working memory, and problem solving) of schizophrenia (Nuechterlein et al., 2004). Therefore, it is important to discover the attentive function for understanding schizophrenia and animal models are necessary to understand the biological basis of this disorder. Compared with other animal models of schizophrenia (e.g., neurodevelopmental, pharmacological, and lesion models), genetic models could be used to more precisely mimic the nature state of schizophrenic patients (Jones et al., 2011).

In this thesis, we modeled schizophrenia-related attentive functions in *Akt1*-mutant mice. We suggested that AKT1 may affect attentive functions through its interaction with dopaminergic signaling or its modulation in the PFC. In addition to understand the mechanism of attention in schizophrenia, this model could further lead to the development of more effective therapy for schizophrenic patients, especially schizophrenic patients with genetic variants of *AKT1*. Besides, subjects with genetic

variants of *AKT1* can be screened by their attentive performances to discover those at the risk of developing schizophrenia. Consequently, the risk of developing schizophrenia will decrease when it is recognized and treated at the early stage.

Future research

The findings of this study open up several lines of future experiments. Since *Akt1* HOM mice have showed attentive impairments in this thesis, we may try some drugs to see whether the impaired omission responses can be rescued or not. Antipsychotics can improve attention in schizophrenic patients (Tandon, 2011), and some atypical antipsychotics (i.e., clozapine, amisulpride, risperidone, and olanzapine) are considered more effective than typical antipsychotics for cognitive functions in the clinic (Davis, Chen, & Glick, 2003). However, both raclopride (an antagonist on dopamine D2 receptors as the effect of typical antipsychotics) and clozapine could not meliorate PPI (a pre-attention task) deficits in *Akt1* HOM mice. But this deficit could be rescued by direct (SB216763) or indirect (8-OH-DPAT) inhibiting the function of GSK3 (a downstream substrate of AKT1) (Chen & Lai, 2011). Therefore, we may try these two drugs to see whether they can normalize the higher omission responses of *Akt1* HOM mice in the first 5-CSRTT learning stage of SD = 16 s. In addition, a psychostimulant methylphenidate is known to enhance attention in normal humans

(Koelega, 1993), and improve poor attention in subjects with ADHD (Lawrence et al., 2005; O'Toole et al., 1997). Methylphenidate could also improve or enhance attention by increasing accuracy, reducing omissions, and decreasing latency to correct nose poke in the 5-CSRTT (Bizarro, Patel, Murtagh, & Stolerman, 2004; Grottick, & Higgins, 2002). Consequently, we are also able to use methylphenidate to discover their effects on the higher omission response of *Akt1* HOM mice in this thesis.

For the confused premature and perseverative responses in *Akt1*-mutant mice under our 5-CSRTT testing phase, we need to apply other behavioral tasks for understanding the role of AKT1 in impulse control. Although both responses require inhibitory control also called executive functions, premature responses (i.e., nose poke before the stimulus light illuminates) are akin to impulsive behavior and perseverative responses (i.e., repeat nose poke after a correct nose poke) are similar to compulsive behavior (Robbins, 2002). A behavioral task contains a trial required inhibiting a motor response, such as the go/no-go task and the 5-choice CPT, may be suitable for responses of impulse control like premature response. Consequently, mice with more premature responses in the 5-CSRTT may also show more error responses in the no-go trial of the go/no-go task and the 5-choice CPT. The attentive set-shifting task may be proper for responses of impulse control like premature response since it require shifting attention to compound stimuli from one perceptual dimension (e.g.,

color) to another (e.g., odor) based on the reinforcement or feedback. Thus, mice with more perseverative responses in the 5-CSRTT may require more trials to shift their attention. However, as the concept of inhibitory control covers a wide range (Evenden, 1999), these tasks may display distinct dimensions of impulse control from the 5-CSRTT.

In order to link the biological function of AKT1 to attentive functions, we can try dopamine-related or PFC-related manipulations in *Akt1*-mutant mice. To see whether *Akt1*-mutant mice show dysfunctions in the PFC, we can record neuron activities in the PFC of *Akt1*-mutant mice while they are conducting the 5-CSRTT by implanting electrodes. Consequently, we are able to reveal different firing patterns when mice conduct distinct responses and compared these patterns among these 3 groups of mice. For revealing the interaction between AKT1 and dopamine, we can infuse dopamine receptor agents into dopamine-related brain areas (e.g., striatum, frontal cortex) of *Akt1*-mutant mice to see their effects in the performance of the 5-CSRTT. These may help us to understand the precise role of *AKT1* in attentive functions of schizophrenia.

Table 1. 1

The MATRICS Consensus Cognitive Battery

Test	**Cognitive domain**
Trail Making Test, Part A	Speed of processing
Brief Assessment of Cognition in Schizophrenia, symbol coding subtest	Speed of processing
Hopkins Verbal Learning Test-Revised, immdiated recall (three learning trials only)	Verbal learning
Wechsler Memory Scale, 3rd ed., spatial span subtest	Working memory (nonverbal)
Letter-Number Span test	Working memory (verbal)
Neuropsychological Assessment Battery, mazes subtest	Reasoning and problem solving
Brief Visuospatial Memory Test-Revised	Visual learning
Category fluency test, animal naming	Speed of processing
Mayer-Salovery-Caruso Emotional Intelligence Test, managing emotions branch	Social cognition
Continuous Performance Test, Identical Pairs version	Attention/vigilance

Note. Adapted from "The MATRICS Consensus Cognitive Battery, part 1: test selection, reliability, and validity," by K. H. Nuechterlein, M. F. Green, R. S. Kern, L. E. Baade, D. M. Barch, J. D. Cohen, ...S. R. Marder, 2008, *The American Journal of Psychiatry, 165*, 211.

Table 1. 2

Measures and their Interpretations from the 5-CSRTT

Measures	**Related cognitive domain**
Accuracy response	Attention (selective and sustained)
Omission response	Sustained attention, motivation, motoric effects
Premature response	Impulsivity, motivation
Perseverative response	Impulsivity, cognitive flexibility
Incorrect response	Attention
Latency to correct nose poke	Processing speed
Latency to collect reward	Motivation, cognitive flexibility
Latency to incorrect nose poke	Processing speed

Note. Adapted from "Using the MATRICS to guide development of a preclinical cognitive test battery for research in schizophrenia," by J. W. Young, S. B. Powell, V. Risbrough, H. M. Marston, & M. A. Geyer, 2009, *Pharmacology & Therapeutics, 122*, 155.

Table 1. 3

Summary of Experiments in this Thesis

Experiment	**Condition**	**Purpose**
I. Locomotion	Day 1: In a new cage	Estimate basic behavior
	Day 2: In the 5-CSRTT apparatus	Examination of behavioral preference
II. 5-CSRTT shaping phase		Direct to manipulate the 5-CSRTT
III. 5-CSRTT learning phase	Stage 1: Stimulus duration (SD) = 16 s	Investigate initial behavioral performances
	Stage 2: SD = 8 s	Train to reach baseline responses
	Stage 3: SD = 4 s	Train to reach baseline responses
	Stage 4: SD = 2 s	Evaluate baseline performances
IV. 5-CSRTT testing phase	Test 1: Intertrial interval (ITI) = 2 and 8 s	Estimate behavioral responses with higher attentive demand
	Test 2: SD = 1 and 0.5 s	
	Test 3: Brightness	
	Test 4: White noise distractor	

Table 3. 1

Genotypic Distribution of Offsprings from Akt1 Heterozygous Breeding Pairs

	WT	HET	HOM	Total
Male	14.4 % (101)	32.1 % (225)	4.1 % (29)	50.7 % (355)
Female	17.3 % (121)	29.4 % (206)	2.6 % (18)	49.3 % (345)
Total	31.7 % (222)	61.6 % (431)	6.7 % (47)	100 % (700)

Table 3. 2

Total Number of Animals in 4 Stages of the 5-CSRTT Learning Phase

# of animals	Genotype	SD = 16 s	SD = 8 s	SD = 4 s	SD = 2 s
Pass	WT	11	10	10	7
	HET	12	12	12	12
	HOM	19	19	17	11
Non-pass	WT	0	1	0	3
	HET	2	0	0	0
	HOM	0	0	2	6

Table 3. 3

Behavioral Performance of Wild-Type Mice in 4 Tests of the 5-CSRTT Testing Phase

Behavior	ITI = 2 & 8 s			SD = 1 & 0.5 s			Brightness			Distracting noise		
	Baseline	ITI = 2 s	ITI = 8 s	Baseline	SD = 1 s	SD = 0.5 s	Baseline	Lighter	Darker	Baseline	No	Distractor
Accuracy	96.29 ± 0.73	95.09 ± 2.02	89.64 ± 4.77	94.45 ± 1.24	84.94 ± 4.21	65.41 ± 9.29	94.07 ± 1.24	95.35 ± 2.13	96.02 ± 2.19	95.86 ± 1.32	93.45 ± 3.10	92.35 ± 6.54
Omission	14.31 ± 1.35	20.29 ± 3.66	17.08 ± 2.23	14.81 ± 0.52	33.47 ± 3.44 *	39.64 ± 5.53 *	11.50 ± 1.44	12.08 ± 2.92	12.00 ± 3.10	11.05 ± 1.47	17.47 ± 3.26	44.71 ± 7.47 *#
Premature	6.68 ± 2.15	0 ± 0	24.50 ± 5.48 *#	6.97 ± 2.97	11.04 ± 4.30	12.32 ± 3.70 *	5.08 ± 1.50	7.33 ± 3.78	3.33 ± 1.61	5.51 ± 1.48	2.26 ± 1.04	3.33 ± 1.61
Perseveration	8.76 ± 1.65	5.24 ± 2.74	6.25 ± 1.70	7.58 ± 1.27	7.79 ± 2.72	6.36 ± 1.96	7.57 ± 2.70	8.03 ± 2.72	2.18 ± 1.53	5.66 ± 1.22	4.67 ± 2.17	3.68 ± 0.78
Correct latency	1.08 ± 0.05	1.32 ± 0.07 *	1.25 ± 0.08		0.82 ± 0.04	0.67 ± 0.05 #		1.14 ± 0.04	0.95 ± 0.09 #		1.10 ± 0.07	1.15 ± 0.19
Reward latency	2.10 ± 0.23	1.47 ± 0.08	1.49 ± 0.09		1.61 ± 0.12	1.68 ± 0.13		1.37 ± 0.04	1.32 ± 0.05		1.69 ± 0.31	1.69 ± 0.27

Note. The 5-CSRTT testing phase consisted of 4 tests (i.e., ITI = 2 and 8 s, SD = 1 and 0.5 s, brightness, and distracting noise; top row). 4 behavioral performances (in percentage) and 2 response latencies (in second) were recorded (i.e., accuracy, omission, premature, perseveration, latency to correct nose poke, and latency to collect reward; left column). Data were displayed as mean ± SEM. *: $p < .05$ vs. baseline; #: $p < .05$ vs. middle column of each test.

Table 3. 4

Behavioral Performance of All Animals in 4 Tests of the 5-CSRTT Testing Phase

Behavior	ITI = 2 & 8 s			SD = 1 & 0.5 s			Brightness			Distracting noise		
Genotype	Baseline	ITI = 2 s	ITI = 8 s	Baseline	SD = 1 s	SD = 0.5 s	Baseline	Lighter	Darker	Baseline	No	Distractor
Accuracy												
WT	96.29 ± 0.73	95.09 ± 2.02	89.64 ± 4.77	94.45 ± 1.24	84.94 ± 4.21	65.41 ± 9.29	94.07 ± 1.24	95.35 ± 2.13	96.02 ± 2.19	95.86 ± 1.32	93.45 ± 3.10	92.35 ± 6.54
HET	96.40 ± 0.83	96.65 ± 1.10	94.64 ± 1.90	96.61 ± 0.61	89.95 ± 2.89	71.76 ± 5.81	95.86 ± 0.82	96.30 ± 1.33	98.16 ± 0.99	96.80 ± 0.71	97.57 ± 0.89	94.03 ± 2.33
HOM	96.87 ± 0.74	96.30 ± 1.81	95.22 ± 1.82	97.01 ± 0.79	89.60 ± 2.37	76.24 ± 6.04	95.13 ± 1.11	93.64 ± 1.87	97.10 ± 1.56	96.80 ± 0.72	96.98 ± 1.30	92.51 ± 4.28
Omission												
WT	14.31 ± 1.35	20.29 ± 3.66	17.08 ± 2.23	14.81 ± 0.52	33.47 ± 3.44	39.64 ± 5.53	11.50 ± 1.44	12.08 ± 2.92	12.00 ± 3.10	11.05 ± 1.47	17.47 ± 3.26	44.71 ± 7.47
HET	14.13 ± 0.66	19.50 ± 4.82	12.04 ± 1.75	13.36 ± 0.86	25.75 ± 2.91	41.43 ± 4.67	13.58 ± 0.92	14.67 ± 2.27	18.33 ± 2.63	12.45 ± 0.82	11.14 ± 2.88	39.00 ± 3.31
HOM	14.55 ± 0.82	22.55 ± 4.12	19.37 ± 4.54	13.27 ± 0.77	24.19 ± 4.61	41.17 ± 4.35	10.30 ± 0.80	14.61 ± 3.58	16.92 ± 2.69	13.11 ± 0.76	12.16 ± 4.69	40.34 ± 8.45
Premature												
WT	6.68 ± 2.15	0 ± 0	24.50 ± 5.48	6.97 ± 2.97	11.04 ± 4.30	12.32 ± 3.70	5.08 ± 1.50	7.33 ± 3.78	3.33 ± 1.61	5.51 ± 1.48	2.26 ± 1.04	3.33 ± 1.61
HET	10.77 ± 2.05	0.33 ± 0.33	24.34 ± 4.51	8.03 ± 1.91	14.12 ± 5.09	16.00 ± 5.26	8.22 ± 1.29	5.67 ± 1.34	8.00 ± 2.46	5.44 ± 0.90	7.70 ± 1.66	12.33 ± 2.58 *
HOM	5.28 ± 1.74	0.36 ± 0.36	22.46 ± 3.93	3.61 ± 0.93	9.04 ± 2.23	13.00 ± 5.54	4.81 ± 1.03	2.59 ± 1.35	1.59 ± 0.78	3.24 ± 0.65	5.22 ± 1.54	5.60 ± 1.28
Perseveration												
WT	8.76 ± 1.65	5.24 ± 2.74	6.25 ± 1.70	7.58 ± 1.27	7.79 ± 2.72	6.36 ± 1.96	7.57 ± 2.70	8.03 ± 2.72	2.18 ± 1.53	5.66 ± 1.22	4.67 ± 2.17	3.68 ± 0.78
HET	9.07 ± 1.41	7.73 ± 1.91	6.88 ± 2.24	10.35 ± 1.88	9.26 ± 2.09	4.36 ± 1.04	9.20 ± 1.48	10.33 ± 2.48	5.67 ± 1.94	9.74 ± 1.72	6.36 ± 2.53	3.33 ± 1.08
HOM	8.47 ± 1.44	9.00 ± 2.51	6.55 ± 1.89	8.94 ± 2.20	9.54 ± 2.72	6.08 ± 2.19	12.07 ± 2.40	7.43 ± 2.16	12.86 ± 2.10 *#	10.30 ± 1.88	10.57 ± 6.03	5.10 ± 2.36
Correct latency												
WT	1.08 ± 0.05	1.32 ± 0.07	1.25 ± 0.08		0.82 ± 0.04	0.67 ± 0.05		1.14 ± 0.04	0.95 ± 0.09		1.10 ± 0.07	1.15 ± 0.19
HET	1.12 ± 0.04	1.25 ± 0.05	1.20 ± 0.06		0.87 ± 0.04	0.65 ± 0.04		1.00 ± 0.06	1.07 ± 0.07		1.03 ± 0.04	1.19 ± 0.13
HOM	1.09 ± 0.05	1.41 ± 0.08	1.18 ± 0.08		0.81 ± 0.03	0.78 ± 0.10		1.02 ± 0.07	0.98 ± 0.06		0.99 ± 0.07	1.26 ± 0.15
Reward latency												
WT	2.10 ± 0.23	1.47 ± 0.08	1.49 ± 0.09		1.61 ± 0.12	1.68 ± 0.13		1.37 ± 0.04	1.32 ± 0.05		1.69 ± 0.31	1.69 ± 0.27
HET	2.03 ± 0.07	1.60 ± 0.06	1.77 ± 0.12		1.63 ± 0.06	1.64 ± 0.07		1.52 ± 0.05	1.48 ± 0.05$		1.59 ± 0.05	1.80 ± 0.11
HOM	2.27 ± 0.18	1.84 ± 0.25	2.23 ± 0.67		1.59 ± 0.10	2.28 ± 0.67		1.76 ± 0.21	1.90 ± 0.28		1.58 ± 0.10	1.67 ± 0.06

Note. The 5-CSRTT testing phase contained 4 tests (i.e., ITI = 2 and 8 s, SD = 1 and 0.5 s, brightness, and distracting noise; top row). 4 behavioral performances (in percentage) and 2 response latencies (in second) were recorded (i.e., accuracy, omission, premature, perseveration, latency to correct nose poke, and latency to collect reward; left column). Data were displayed as mean ± SEM. WT: wild-type control; HET: *Akt1* heterozygous; HOM: *Akt1* homozygous; *: $p < .05$ vs. WT; #: $p < .05$ vs. HET; $: trend to $p < .05$ vs. WT.

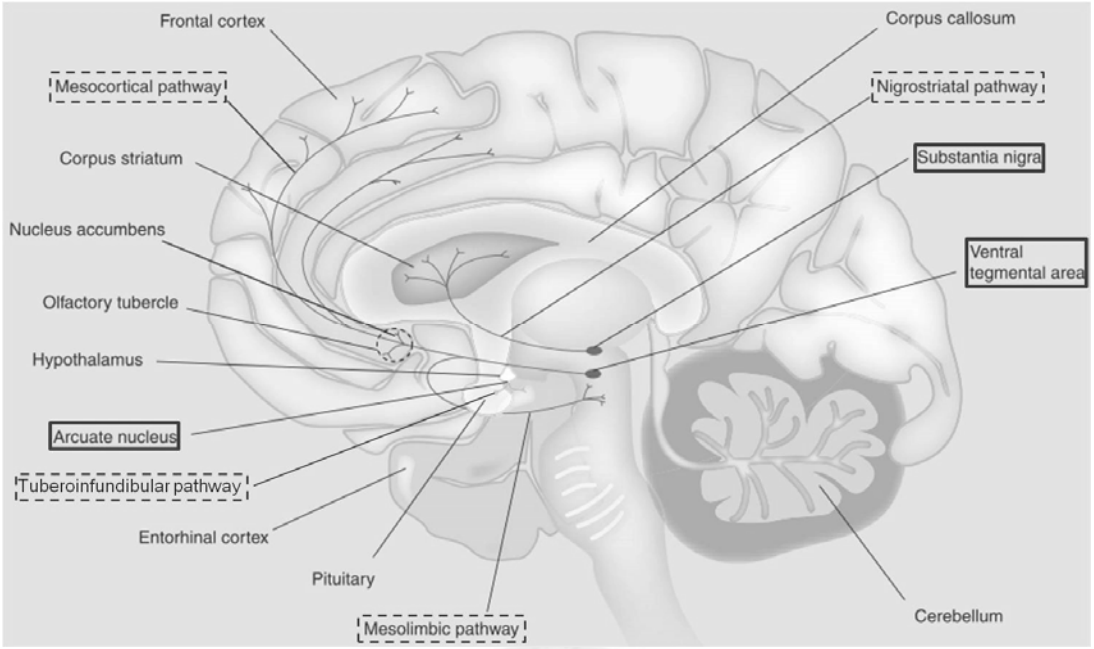

Figure 1. 1. Dopamine pathways in human brain

Note. Dopamine contains nigrostriatal, mesolimbic, mesocortical, and tuberoinfundibular pathways. Adapted from "Schizophrenia as a disorder of too little dopamine: implications for symptoms and treatment," by G. Remington, O. Agid, & G. Foussias, 2011, *Expert Review of Neurotherapeutics, 11*, 590.

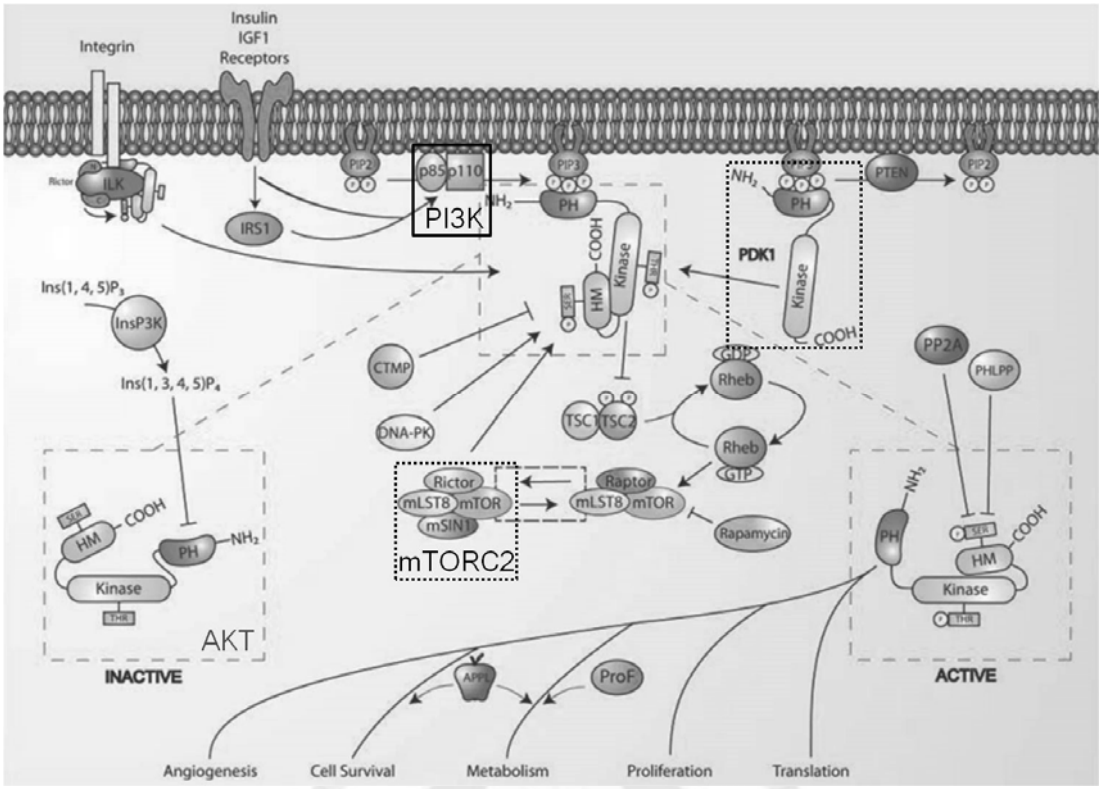

Figure 1. 2. Signaling pathways of AKT

Note. This figure illustrates the PI3K-mediated AKT signal transduction, and following biological functions of active AKT. Adapted from "PI3K/Akt: getting it right matters," by T. F. Franke, 2008, *Oncogene*, *27*, 6477.

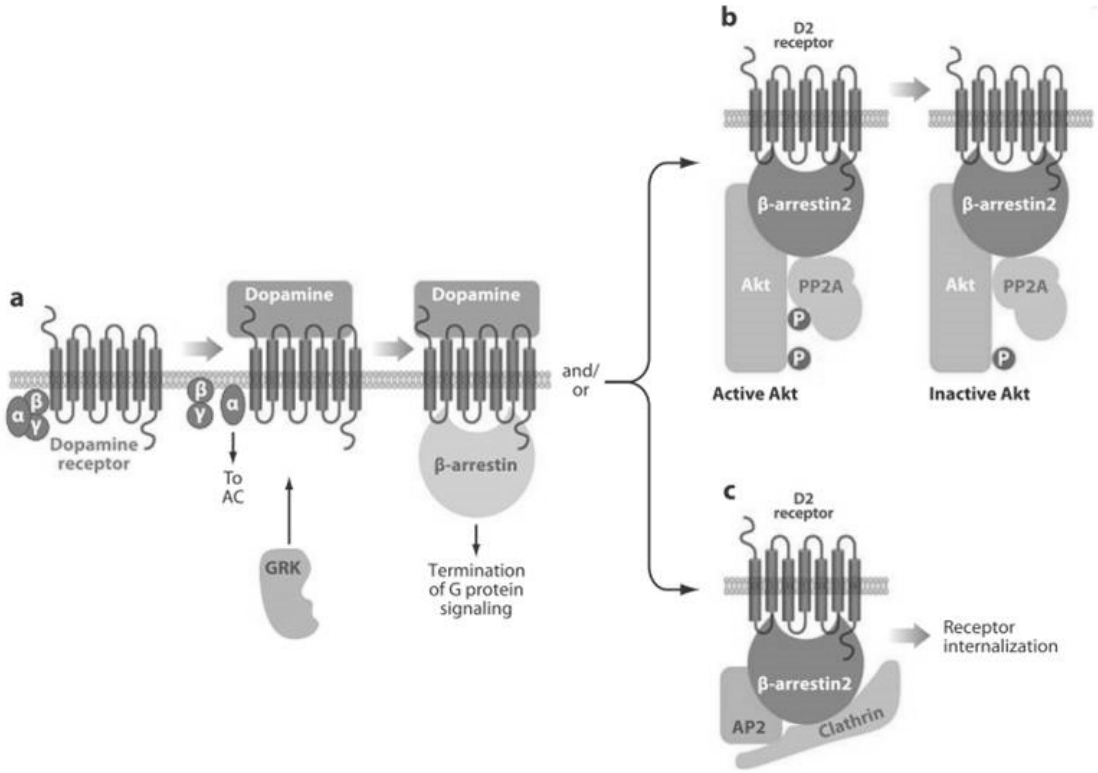

Figure 1. 3. AKT as a downstream regulator of dopamine D2 receptor

Note. (a) Activation of dopamine D2 receptor by dopamine leads to receptor phosphorylation by a GRK following the recruitment of β-arrestin 2. (b) AKT is recruited along with PP2A to form the Akt:β-arrestin 2:PP2A complex, and PP2A consequently dephosphorylates and inactivates AKT. (c) Recruitment of β-arrestin 2 results in dopamine D2 receptor internalization. Adopted from "Akt/GSK3 Signaling in the Action of Psychotropic Drugs," by J. M. Beaulieu, R. R. Gainetdinov, & M. G. Caron, 2009, *Annual Review of Pharmacology and Toxicology, 49*, 331.

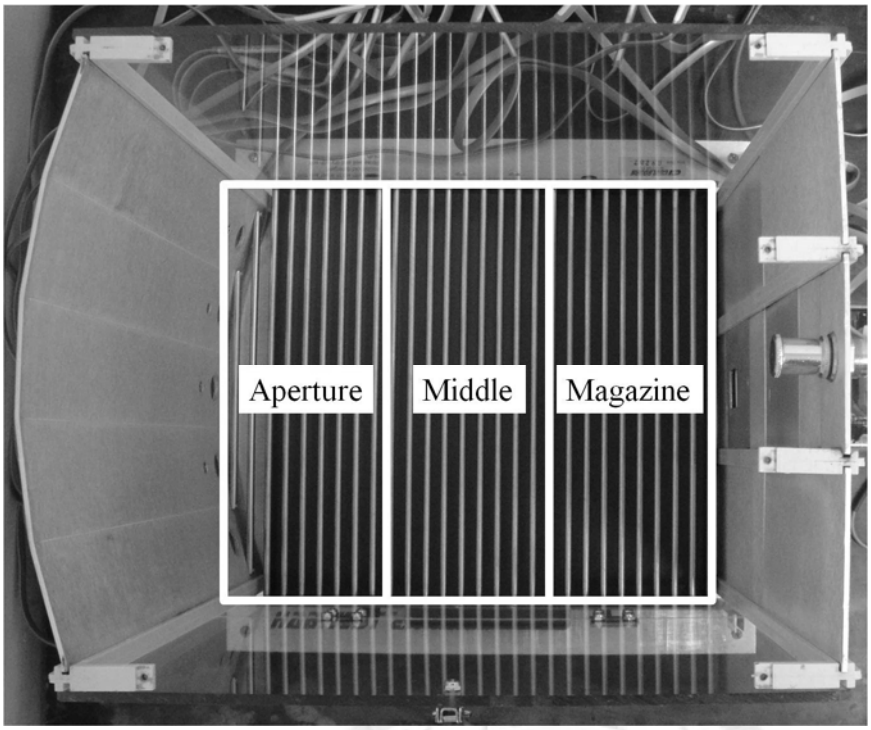

Figure 2. 1. Three areas of the 5-CSRTT apparatus

Note. The 5-CSRTT apparatus is equally divided into three areas as named by their nearby states, the aperture (left) area, the middle (middle) area, and the magazine (right) area, to separately record animals' locomotor activities.

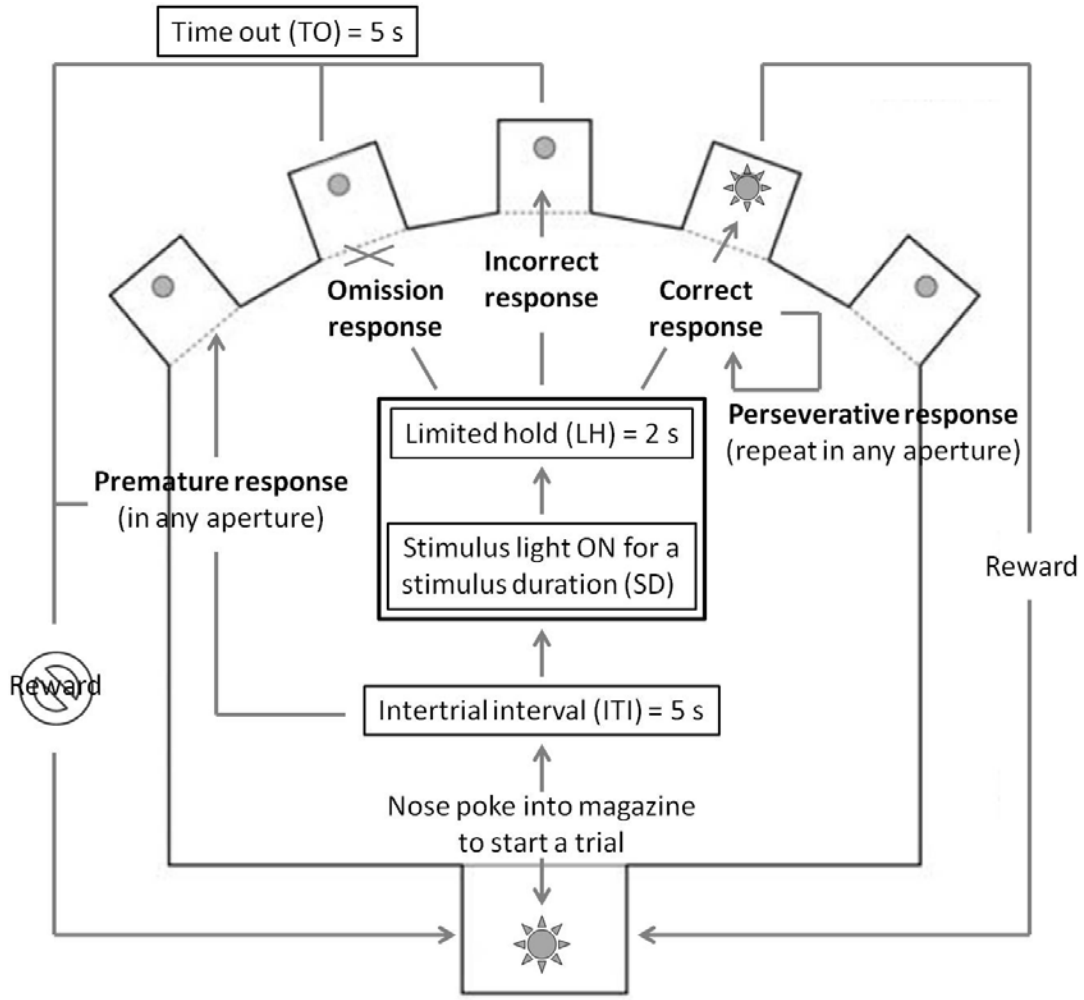

Figure 2. 2. Trial sequences of the 5-CSRTT

Note. Mice has to nose poke into the food magazine to initiate a trial. A 5 sec intertrial interval (ITI) then preceded the illumination of stimulus-response apertures, and a brief light stimulus is illuminated for a stimulus duration (SD) in one of five apertures. The animal has to respond with a nose poke into the illuminated aperture within either the SD or the following 2 sec limited hold (LH) period to earn a reward (correct response) and then it has 5 sec to eat the food pellet. If the mouse responds before the light stimulus (premature response), a 5 sec time out (TO) period is introduced where the house light is successive on and off and no food reward is delivered. During both the SD or the LH period, if mice conduct no response (omission response) or respond to an adjacent non-illuminated aperture (incorrect response), a 5 sec TO period is then followed. Besides, a repeatedly nose poke into one of 5 apertures during the period of collecting reward (perseverative response) results in a subsequent 5 sec

TO period. After eating earned reward or at the end of the TO period, a nose poke into the food magazine starts a new well-prepared trial. The parameters (including ITI, SD, LH, and TO) which can be manipulated in the 5-CSRTT are circumscribed with squares. Adapted from "Prefrontal executive and cognitive functions in rodents: neural and neurochemical substrates," by J. Dalley, R. Cardinal, & T. Robbins, 2004, *Neuroscience and Biobehavioral Reviews, 28*, 775; "Measuring impulsivity in mice: the five-choice serial reaction time task," by S. Sanchez-Roige, Y. Peña-Oliver, & D. N. Stephens, 2012, *Psychopharmacology, 219*, 255.

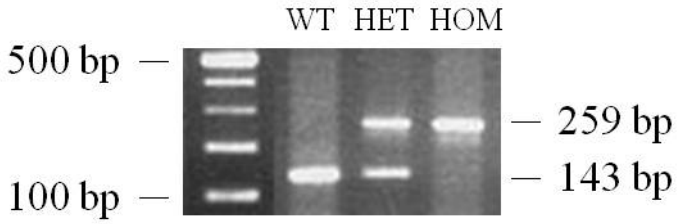

Figure 3. 1. PCR genotyping results

Note. PCR genotyping with 3 primers was used and PCR products were analyzed by agarose gel electrophoresis to detect the 3 genotypes. The DNA of Wild-type (WT) mice formed a band at 143 base pairs (bp), heterozygous (HET) mice formed distinct bands at 143 bp and 259 bp, and homozygous (HOM) mice formed a band at 259 bp.

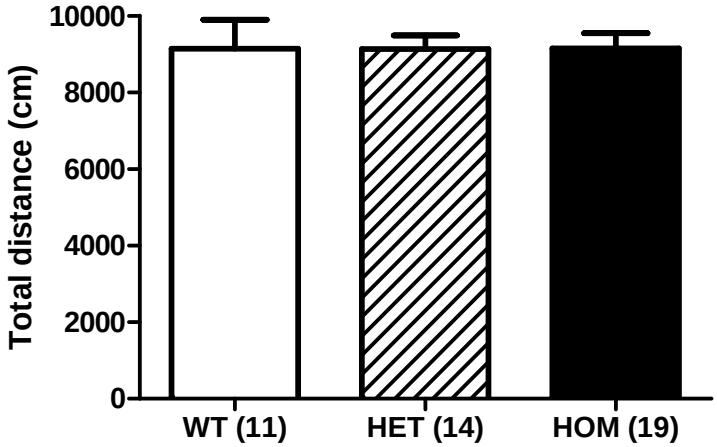

Figure 3. 2. Total moving distance in a new cage

Note. Animals' free moving distance in a new cage was recorded as their locomotion. No significant difference was detected among wild-type (WT), heterozygous (HET), and homozygous (HOM) mice in the total moving distance. This figure was depicted as mean + SEM. White bar: WT; bar with oblique line: HET; black bar: HOM; parentheses: total number of subjects.

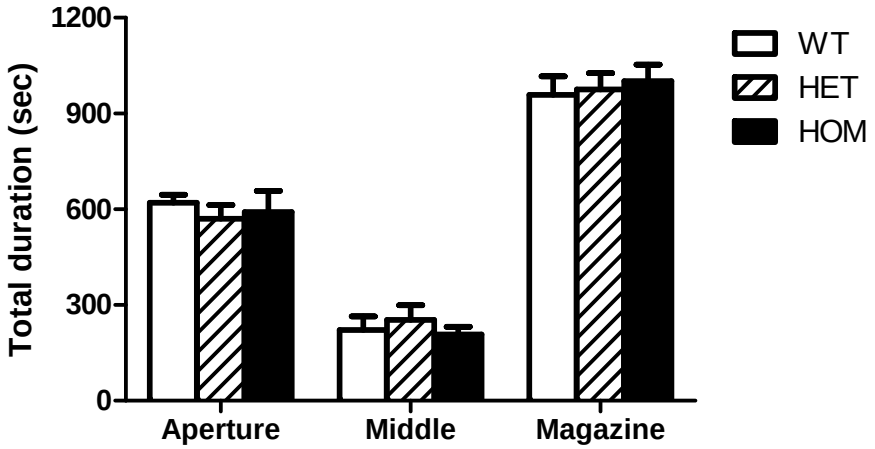

Figure 3. 3. Total duration in three areas of the 5-CSRTT apparatus

Note. The 5-CSRTT apparatus was equally divided into aperture (left), middle (middle), and magazine (right) areas to record animals' total duration as their locomotion. No significantly genotypic difference was found in the total duration of these three areas. Wild-type (WT), heterozygous (HET), and homozygous (HOM) spent most time in the magazine area and less time in the middle area. The figure was depicted as mean + SEM. White bar: WT; bar with oblique line: HET; black bar: HOM.

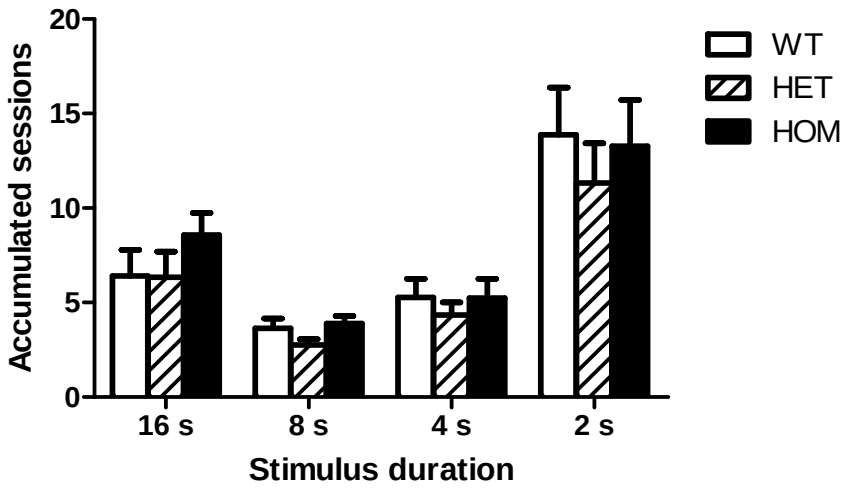

Figure 3. 4. Daily accumulated sessions in 4 stages of 5-CSRTT learning phase

Note. The 5-CSRTT learning phase comprised 4 stages (stimulus duration = 16, 8, 4, and 2 sec). Accumulated sessions in all learning phases were found no significant differences among wild-type (WT), heterozygous (HET), and homozygous (HOM) mice. This figure was represented as mean + SEM. White bar: WT; bar with oblique line: HET; black bar: HOM.

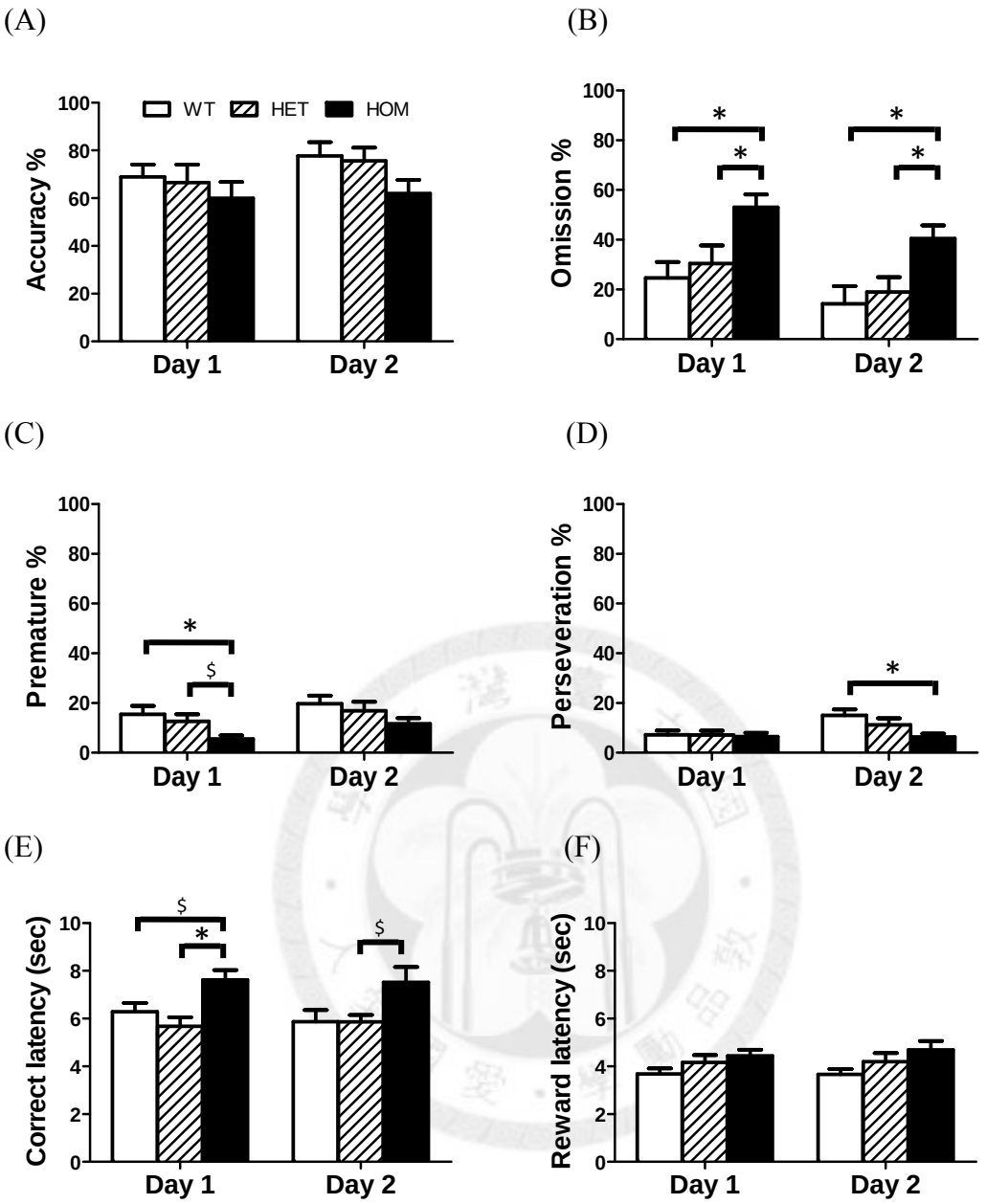

Figure 3. 5. Behavioral performance on the first and second day during the first learning stage of SD = 16 s

Note. (A) Accuracy, (B) omission, (C) premature, and (D) perseverative responses were represented as percentage. (E) Latency to correct nose poke and (F) latency to collect reward were illustrated as second. On the first day (the left side of each panel), homozygous (HOM) mice showed significantly higher omission responses, significantly lower premature responses, and marginally longer latency to correct nose poke than wild-type (WT). HOM mice also displayed significantly higher omission responses, marginally lower premature

responses, and significantly longer latency to correct nose poke than heterozygous (HET) mice. On the second day (the right side of each panel), HOM mice showed significantly higher omission and lower perseverative responses than WT mice. HOM mice also displayed significantly higher omission responses and marginally longer latency to correct nose poke than HET mice. These figures were depicted as mean + SEM. *: $p < .05$; $: trend to $p < .05$; white bar: WT; bar with oblique line: HET; black bar: HOM.

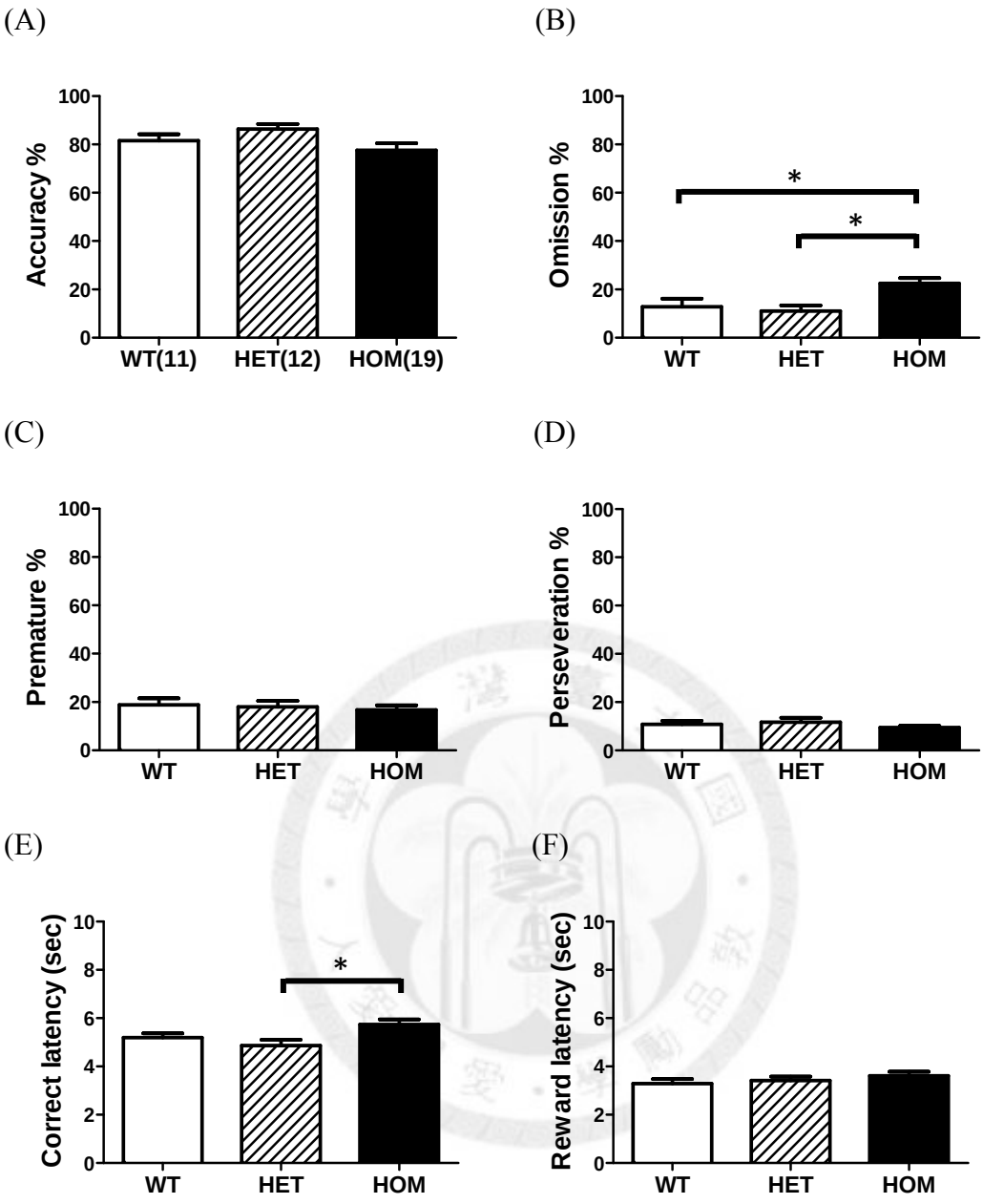

Figure 3. 6. Averaged daily behavioral performance in the first learning stage of SD = 16 s

Note. (A) Accuracy, (B) omission, (C) premature, and (D) perseverative responses were represented as percentage. (E) Latency to correct nose poke and (F) latency to collect reward were showed as second. Homozygous (HOM) mice displayed higher omission responses compared with both wild-type (WT) and heterozygous (HET) mice, and also showed longer latency to correct nose poke compared with HET mice. The figures were depicted as mean + SEM. *: $p < .05$; white bar: WT; bar with oblique line: HET; black bar: HOM; parentheses: total number of subjects.

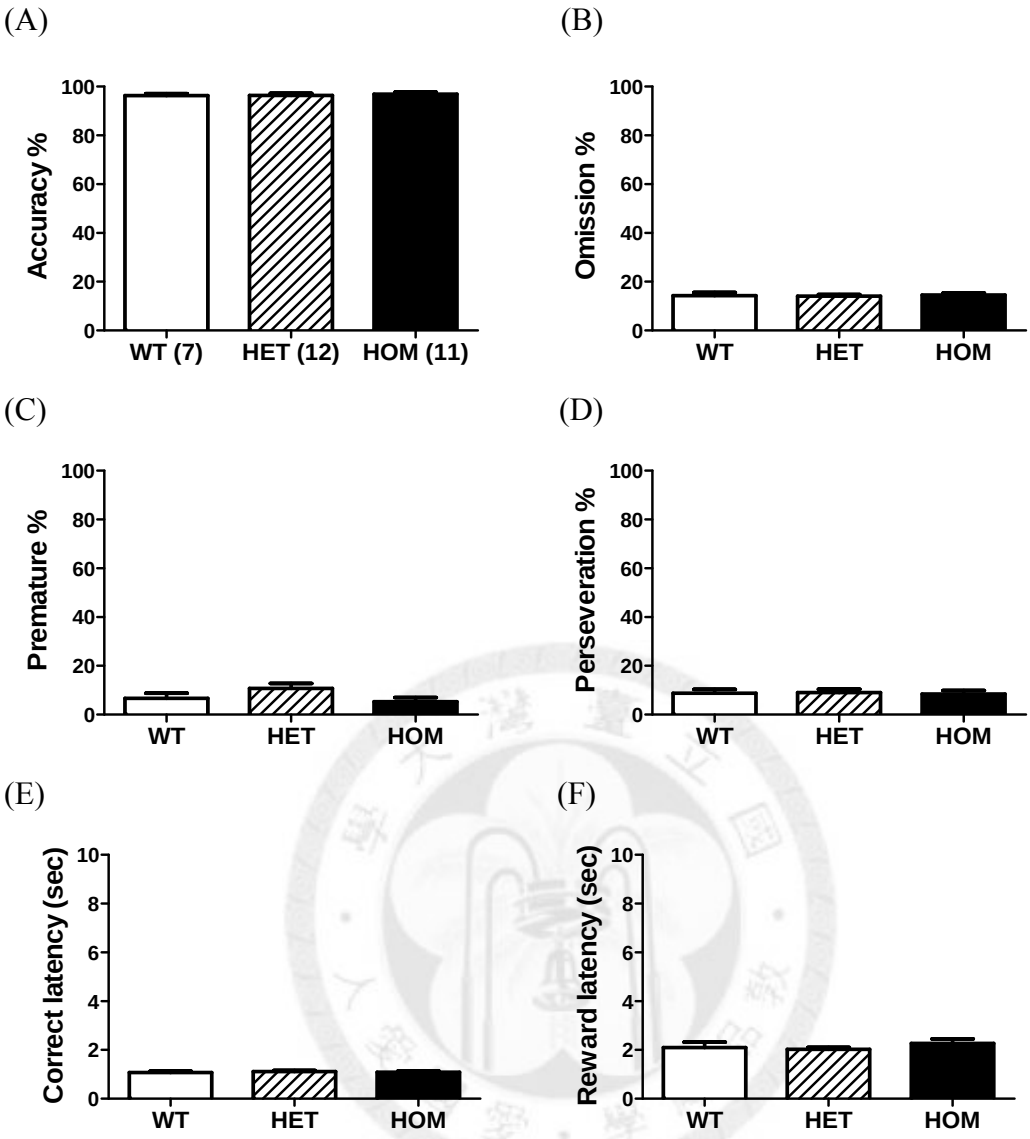

Figure 3. 7. Baseline behavioral performances in the last learning stage of SD = 2 s

Note. (A) Accuracy, (B) omission, (C) premature, and (D) perseverative responses were represented as percentage. (E) Latency to correct nose poke and (F) latency to collect reward were illustrated as second. No significant difference was found among wild-type (WT), heterozygous (HET), and homozygous (HOM) mice in all baseline behavioral performances. These figures were depicted as mean + SEM. White bar: WT; bar with oblique line: HET; black bar: HOM; parentheses: total number of subjects.

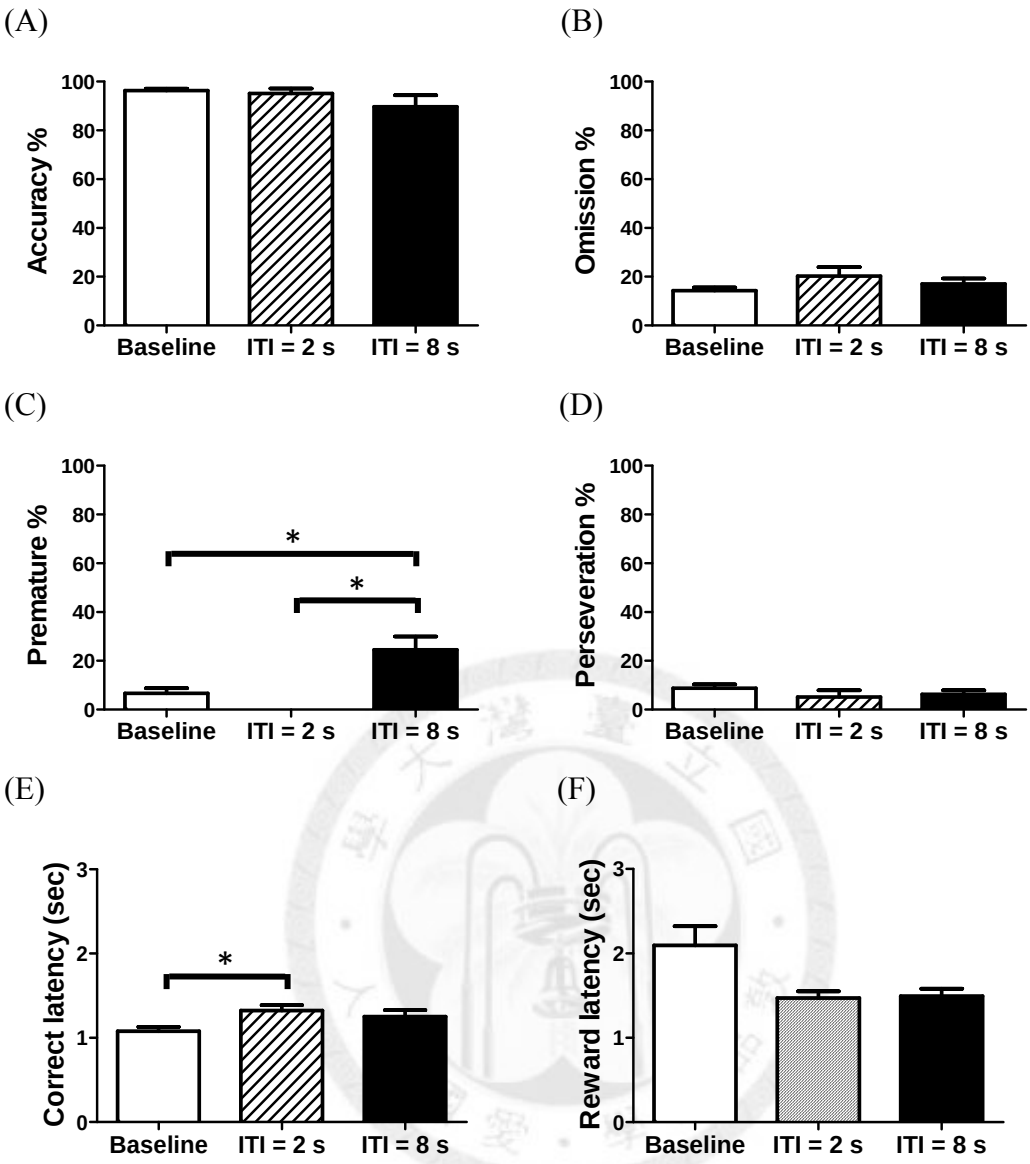

Figure 3. 8. Behavioral performance of wild-type (WT) mice in the 1st test (ITI = 2 and 8 s)

Note. (A) Accuracy, (B) omission, (C) premature, and (D) perseverative responses were represented as percentage. (E) Latency to correct nose poke and (F) latency to collect reward were displayed as second. WT mice showed longer latency to correct nose poke in the ITI = 2 s condition than in the baseline condition. In the ITI = 8 s condition, WT mice had higher premature responses compared with the ITI = 2 s and baseline condition. These figures were depicted as mean + SEM. *: $p < .05$; white bar: baseline condition; bar with oblique line: ITI = 2 s condition; black bar: ITI = 8 s condition.

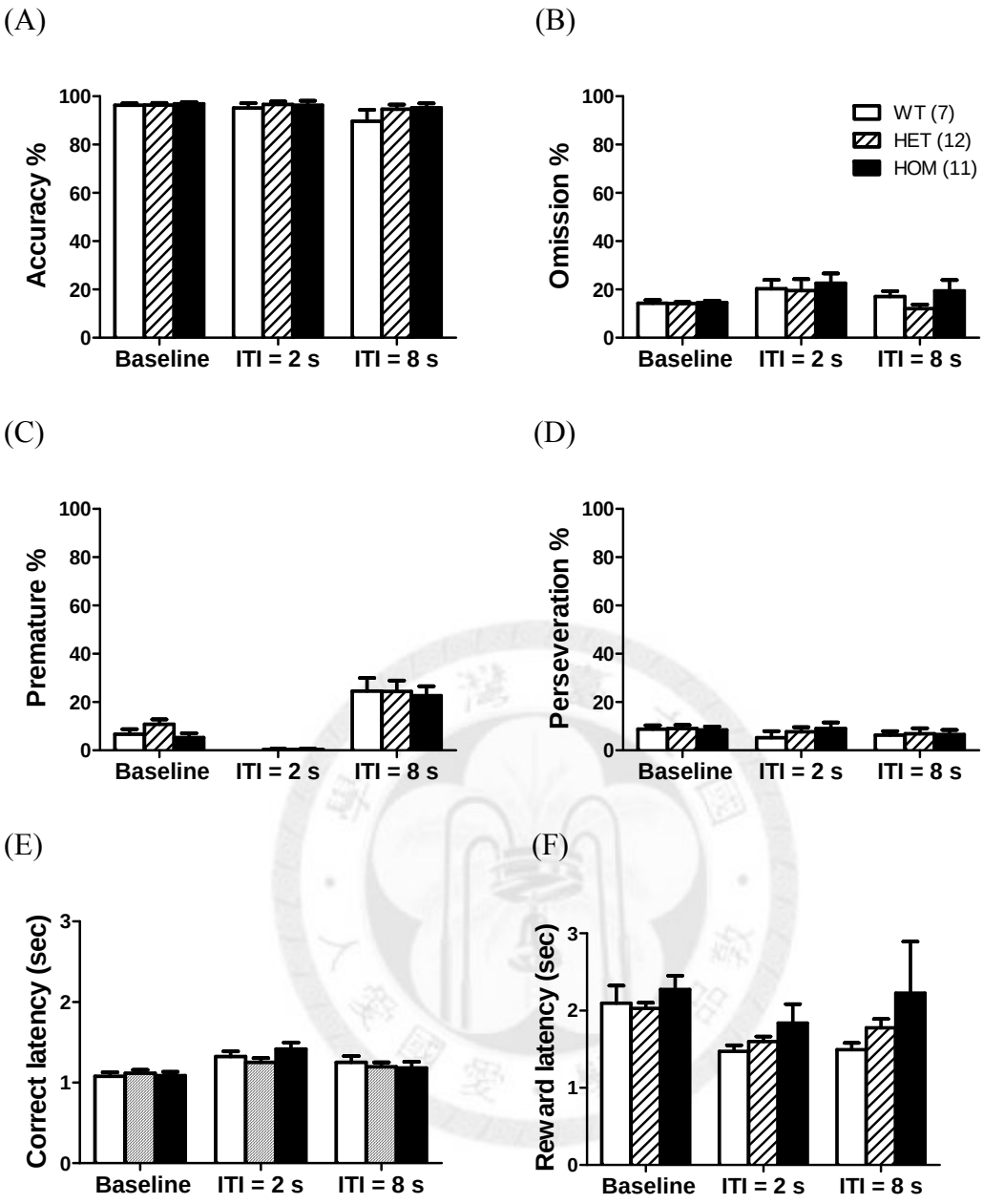

Figure 3. 9. Behavioral performance of all animals in the 1st test (ITI = 2 and 8 s)

Note. (A) Accuracy, (B) omission, (C) premature, and (D) perseverative responses were represented as percentage. (E) Latency to correct nose poke and (F) latency to collect reward were illustrated as second. *Akt1* wild-type (WT), heterozygous (HET), and homozygous (HOM) mice showed no significant differences in all behavioral performances among the baseline condition, the condition of ITI = 2 s, and the condition of ITI = 8 s. These figures were depicted as mean + SEM. White bar: WT; bar with oblique line: HET; black bar: HOM; parentheses: total number of subjects.

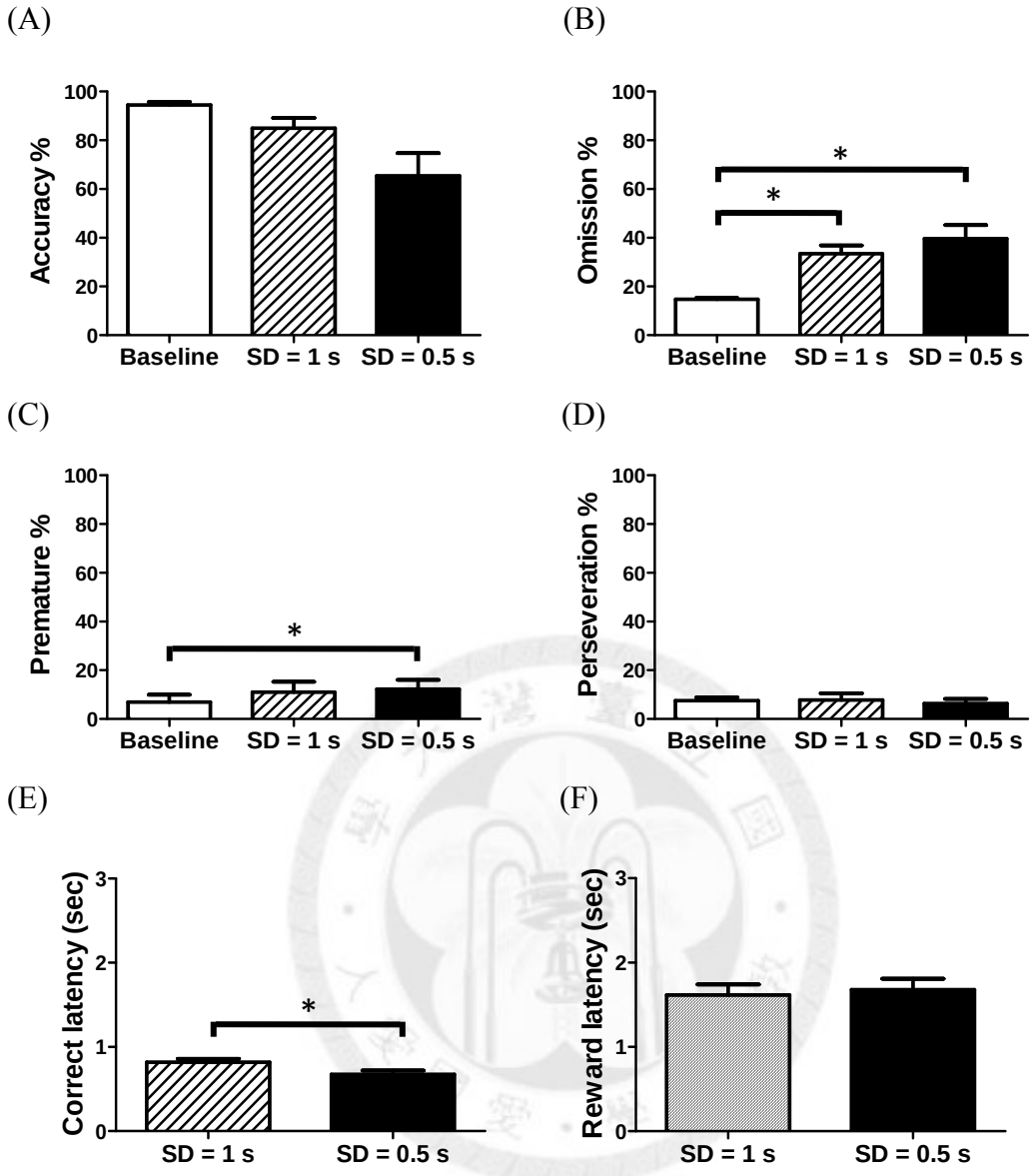

Figure 3. 10. Behavioral performance of wild-type (WT) mice in the 2nd test (SD = 1 and 0.5 s)

Note. (A) Accuracy, (B) omission, (C) premature, and (D) perseverative responses were represented as percentage. (E) Latency to correct nose poke and (F) latency to collect reward were showed as second. WT mice showed higher omission responses in both SD = 1 s and SD = 0.5 s conditions than in the baseline condition. WT mice also displayed higher premature responses in the SD = 0.5 s condition than in the baseline condition. Shorter latency to correct nose poke was found in WT mice under the SD = 0.5 s condition than under the SD = 1 s condition. These figures were depicted as mean + SEM. *: $p < .05$; white bar: baseline condition; bar with oblique line: SD = 1 s condition; black bar: SD = 0.5 s condition.

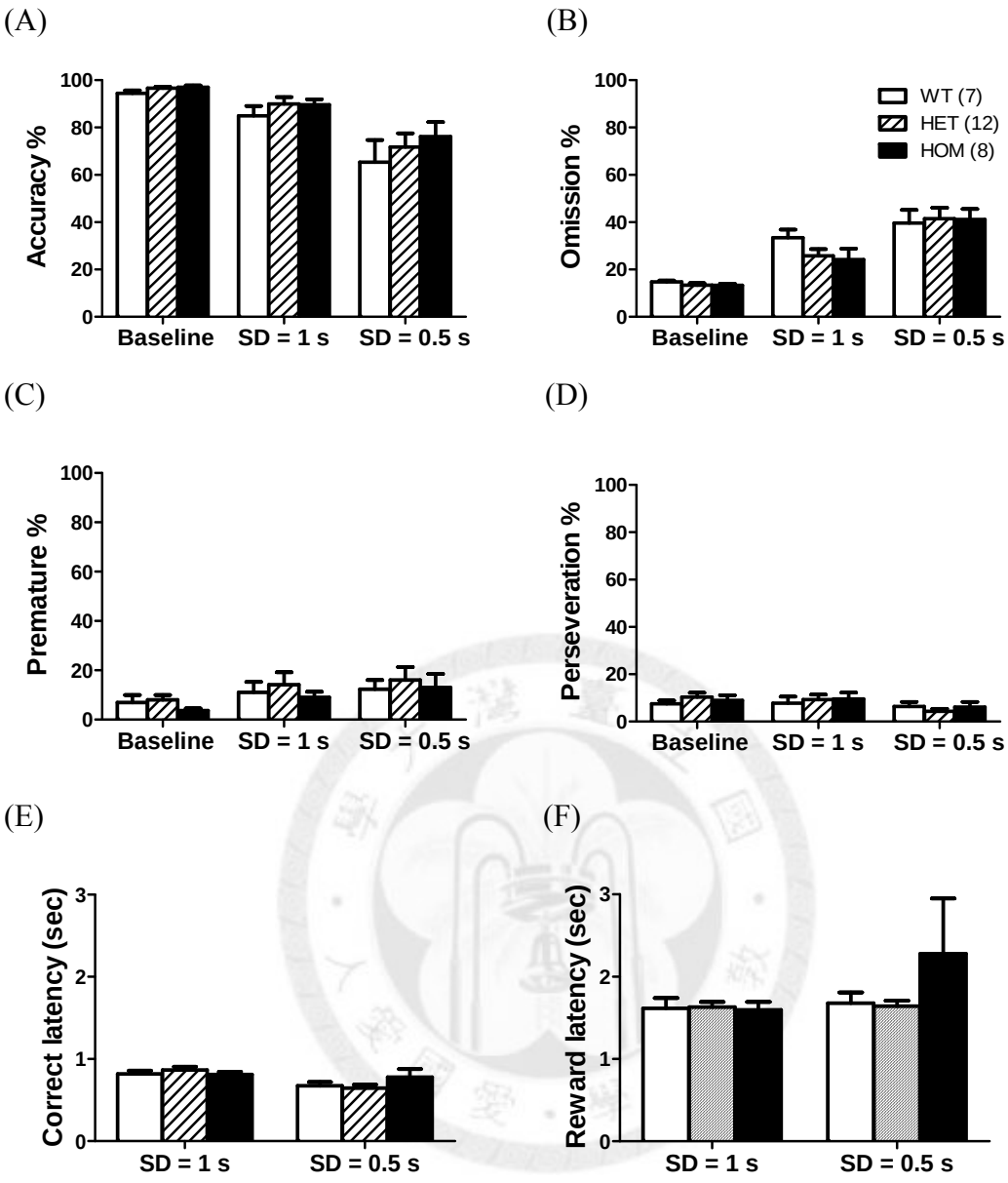

Figure 3. 11. Behavioral performance of all animals in the 2nd test (SD = 1 and 0.5 s)

Note. (A) Accuracy, (B) omission, (C) premature, and (D) perseverative responses were represented as percentage. (G) Latency to correct nose poke and (H) latency to collect reward were showed as second. *Akt1* wild-type (WT), heterozygous (HET), and homozygous (HOM) mice displayed no significant differences in all behavioral performances among the baseline, SD = 1 s, and SD = 0.5 s conditions. These figures were depicted as mean + SEM. White bar: WT; bar with oblique line: HET; black bar: HOM; parentheses: total number of subjects.

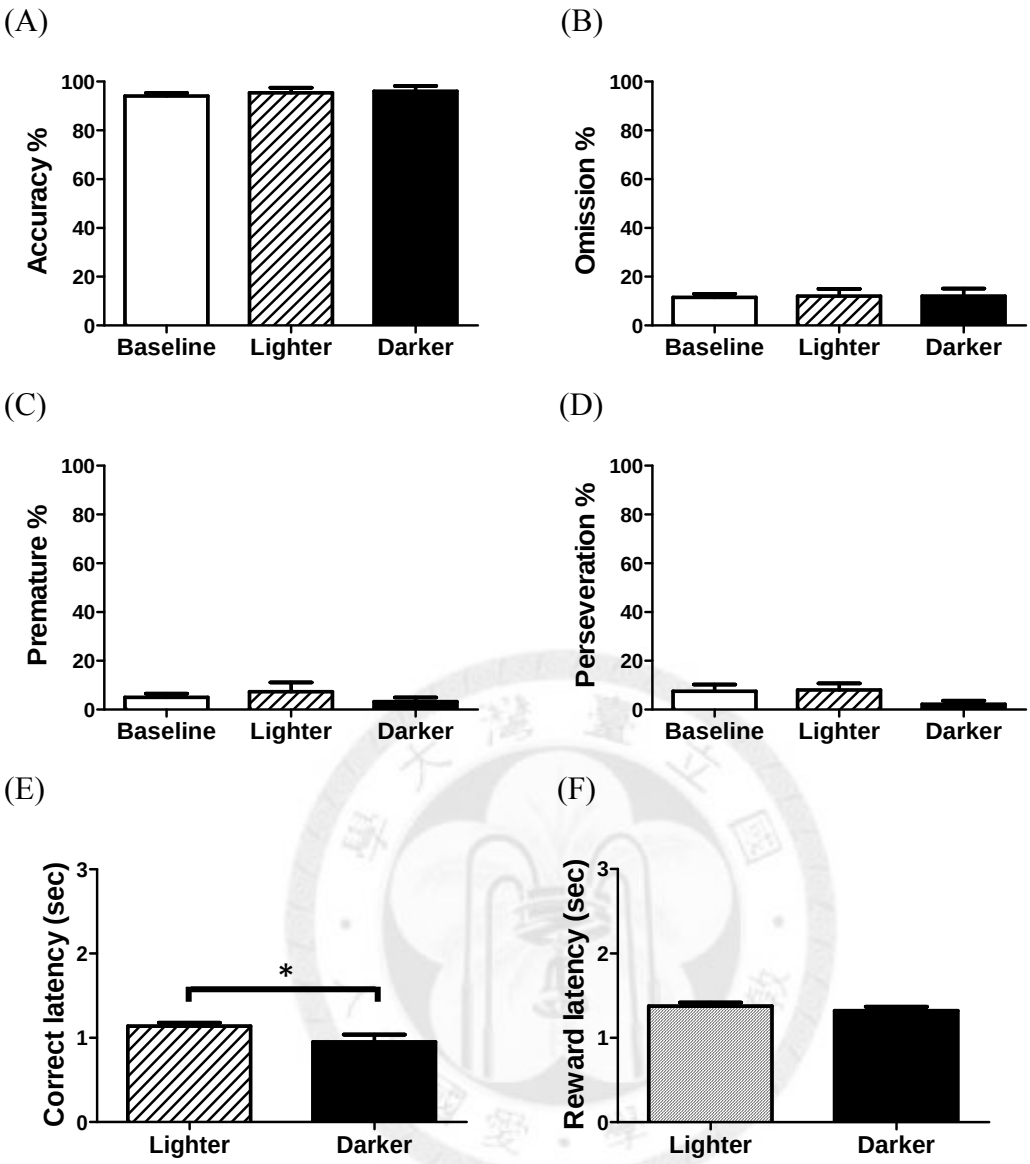

Figure 3. 12. Behavioral performance of wild-type (WT) mice in the 3rd test (brightness)

Note. (A) Accuracy, (B) omission, (C) premature, and (D) perseverative responses were represented as percentage. (E) Latency to correct nose poke and (F) latency to collect reward were illustrated as second. WT mice only showed shorter latency to correct nose poke in the relatively darker condition than in the relatively lighter condition. These figures were depicted as mean + SEM. *: $p < .05$; white bar: baseline condition; bar with oblique line: relatively lighter condition; black bar: relatively darker condition.

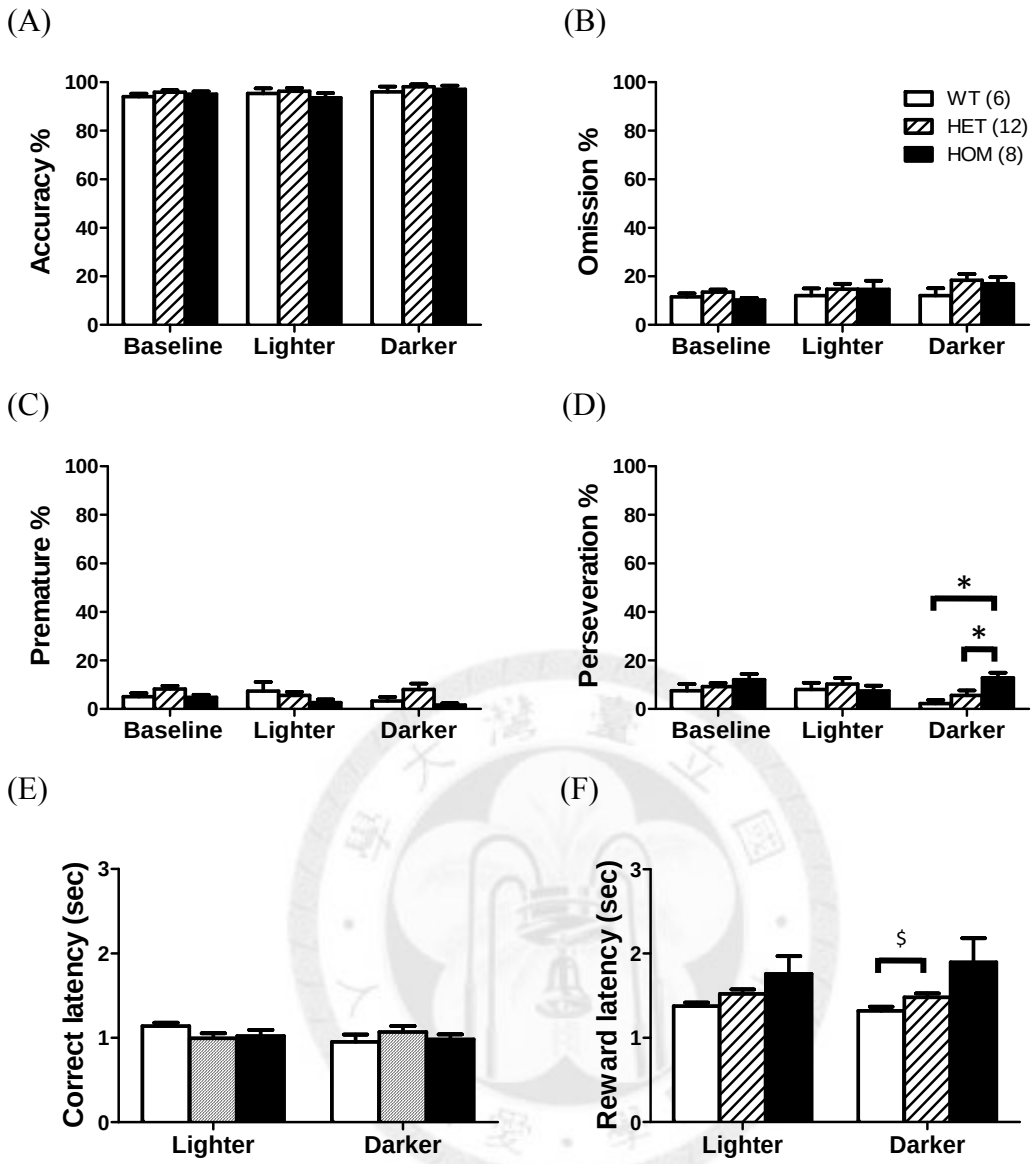

Figure 3. 13. Behavioral performance of all animals in the 3rd test (brightness)

Note. (A) Accuracy, (B) omission, (C) premature, and (D) perseverative responses were represented as percentage. (E) Latency to correct nose poke and (F) latency to collect reward were showed as second. Significantly genotypic differences were only found in the relatively darker condition. *Akt1* homozygous (HOM) mice displayed higher perseverative responses compared with wild-type (WT) and heterozygous (HET) mice. HET mice showed slightly longer latency to collect reward than WT mice. No genotypic difference was found in the baseline and relatively lighter conditions. These figures were depicted as mean + SEM. *: $p < .05$; $: trend to $p < .05$; white bar: WT; bar with oblique line: HET; black bar: HOM; parentheses: total number of subjects.

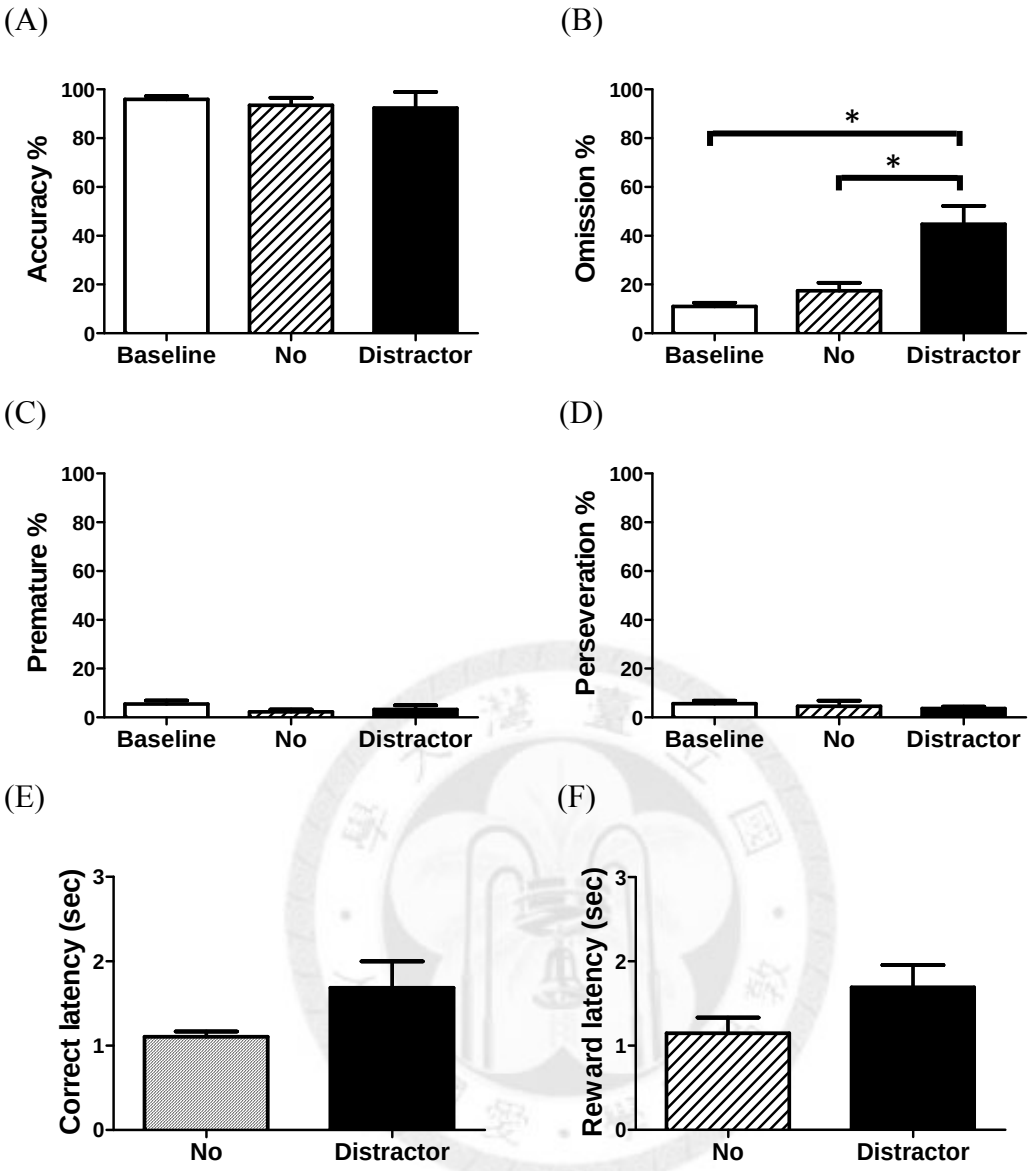

Figure 3. 14. Behavioral performance of wild-type (WT) mice in the 4th test (white noise distractor)

Note. (A) Accuracy, (B) omission, (C) premature, and (D) perseverative responses were represented as percentage. (E) Latency to correct nose poke and (F) latency to collect reward. WT mice only showed higher omission responses in the distracting condition compared with the baseline and the no distractor conditions. These figures were depicted as mean + SEM. *: $p < .05$; white bar: baseline condition; bar with oblique line: no distractor condition; black bar: distracting condition.

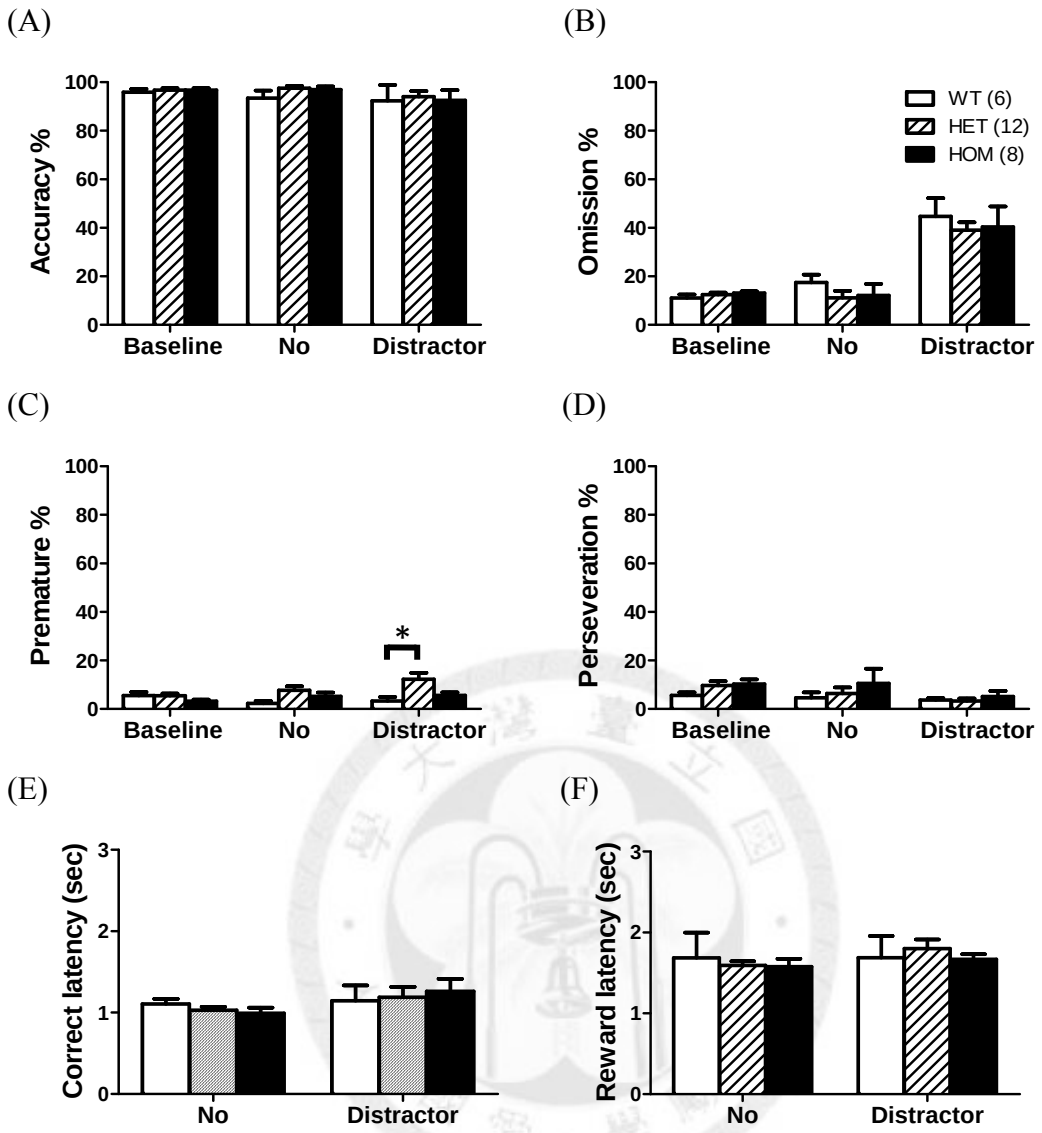

Figure 3. 15. Behavioral performance of all animals in the 4th test (white noise distractor)

Note. (A) Accuracy, (B) omission, (C) premature, and (D) perseverative responses were depicted as percentage. (E) Latency to correct nose poke and (F) latency to collect reward were represented as second. Only *Akt1* heterozygous (HET) showed higher premature responses compared with wild-type (WT) mice in the distracting condition. These figures were depicted as mean + SEM. *: $p < .05$; white bar: WT; bar with oblique line: HET; black bar: HOM; parentheses: total number of subjects.

Reference

Abdi, H. (2010). Signal detection theory (SDT). In P. L. Peterson, E. Baker, & B. McGaw (Eds.), *International encyclopedia of education* (3rd ed., Vol. 7, pp. 407-410). New York: Elsevier.

American Psychiatric Association (2000). *Diagnostic and statistical manual of mental disorders* (4th ed., text revision). Washington, DC: Author.

Antonova, E., Sharma, T., Morris, R., & Kumari, V. (2004). The relationship between brain structure and neurocognition in schizophrenia: A selective review. *Schizophrenia Research, 70*, 117-145.

Arguello, P. A., & Gogos, J. A. (2006). Modeling madness in mice: One piece at a time. *Neuron, 52*, 179-196.

Austin, J. (2005). Schizophrenia: An Update and Review. *Journal of Genetic Counseling, 14*, 329-340.

Bajestan, S. N., Sabouri, A. H., Nakamura, M., Takashima, H., Keikhaee, M. R., Behdani, F., ...Osame, M. (2006). Association of AKT1 haplotype with the risk of schizophrenia in Iranian population. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics, 141B*, 383-386.

Bari, A., Dalley, J. W., & Robbins, T. W. (2008). The application of the 5-choice serial reaction time task for the assessment of visual attentive processes and impulse

control in rats. *Nature Protocols, 3*, 759-767.

Barnett, J. H., Robbins, T. W., Leeson, V. C., Sahakian, B. J., Joyce, E. M., & Blackwell, A. D. (2010). Assessing cognitive function in clinical trials of schizophrenia. *Neuroscience and Biobehavioral Reviews, 34*, 1161-1177.

Baunez, C., & Robbins, T. W. (1999). Effects of dopamine depletion of the dorsal striatum and further interaction with subthalamic nucleus lesions in an attentive task in rats. *Neuroscience, 92*, 1343-1356.

Beaulieu, J. M., Del'guidice, T., Sotnikova, T. D., Lemasson, M., & Gainetdinov, R. R. (2011). Beyond cAMP: The Regulation of Akt and GSK3 by Dopamine Receptors. *Frontiers in Molecular Neuroscience, 4*(38).

Beaulieu, J. M., Gainetdinov, R. R., & Caron, M. G. (2009). Akt/GSK3 Signaling in the Action of Psychotropic Drugs. *Annual Review of Pharmacology and Toxicology, 49*, 327-347.

Beaulieu, J. M., Sotnikova, T. D., Yao, W. D., Kockeritz, L., Woodgett, J. R., Gainetdinov, R. R., & Caron, M. G. (2004). Lithium antagonizes dopamine-dependent behaviors mediated by an AKT/glycogen synthase kinase 3 signaling cascade. *Proceedings of the National Academy of Sciences of the United States of America, 101*, 5099-5104.

Beaulieu, J. M. (2011). A role for Akt and glycogen synthase kinase-3 as integrators

of dopamine and serotonin neurotransmission in mental health. *Journal of Psychiatry & Neuroscience, 37*, 7-16.

Beck, L. H., Bransome, E. D., Jr., Mirsky, A. F., Rosvold, H. E., & Sarason, I. (1956). A continuous performance test of brain damage. *Journal of Consulting Psychology, 20*, 343-350.

Bellacosa, A., Franke, T. F., Gonzalez-Portal, M. E., Datta, K., Taguchi, T., Gardner, J., ...Tsichlis, P. N. (1993). Structure, expression and chromosomal mapping of c-akt: Relationship to v-akt and its implications. *Oncogene, 8*, 745–754.

Bellacosa, A., Testa, J. R., Staal, S. P., & Tsichlis, P. N. (1991). A retroviral oncogene, akt, encoding a serine-threonine kinase containing an SH2-like region. *Science, 254*, 274-277.

Benes, F. M., McSparren, J., Bird, E. D., SanGiovanni, J. P., & Vincent, S. L. (1991). Deficits in small interneurons in prefrontal and cingulate cortices of schizophrenic and schizoaffective patients. *Archives of General Psychiatry, 48*, 996-1001.

Braff, D. L., Geyer, M. A., & Swerdlow, N. R. (2001). Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies . *Psychopharmacology, 156*, 234-258.

Brown, A. S. (2011). The environment and susceptibility to schizophrenia. *Progress in*

Neurobiology, 93, 23-58.

Bizarro, L., Patel, S., Murtagh, C., & Stolerman, I. P. (2004). Differential effects of psychomotor stimulants on attentive performance in rats: Nicotine, amphetamine, caffeine and methylphenidate. *Behavioural Pharmacology, 15*, 195-206.

Blasi, G., Napolitano, F., Ursini, G., Taurisano, P., Romano, R., Caforio, G., ... Bertolino, A. (2011). DRD2/AKT1 interaction on D2 c-AMP independent signaling, attentive processing, and response to olanzapine treatment in schizophrenia. *Proceedings of the National Academy of Sciences, 108*, 1158-1163.

Calkins, M. E., & Iacono, W. G. (2003). *Schizophrenia* (2nd ed., pp. 1402-1405). New York: Thomson-Gale.

Calleja, V., Alcor, D., Laguerre, M., Park, J., Vojnovic, B., Hemmings, B. A., ... Larijani, B. (2007). Intramolecular and intermolecular interactions of protein kinase B define its activation in vivo. *PLoS Biology, 5*, e95.

Carli, M., Robbins, T. W., Evenden, J. L., & Everitt, B. J. (1983). Effects of lesions to ascending noradrenergic neurones on performance of a 5-choice serial reaction task in rats: Implications for theories of dorsal noradrenergic bundle function based on selective attention and arousal. *Behavioural Brain Research, 9*,

361-380.

Chen, W. J., & Faraone, S. V. (2000). Sustained attention deficits as markers of genetic susceptibility to schizophrenia. *American Journal of Medical Genetics, 97*, 52-57.

Chen, Y. C., Chen, Y. W., Hsu, Y. F., Chang, W. T., Hsiao, C. K., Min, M. Y., & Lai, W. S. (2012). Akt1 deficiency modulates reward learning and reward prediction error in mice. *Genes, Brain and Behavior, 11*, 157-169.

Chen, Y. W., Chen, Y. S., & Lai, W. S. (2011, November). *The deficiency of AKT1 results in the reduction of hippocampus-related cognitive performance and hippocampal neuromorphology in mice.* Poster session presented at the meeting of the Society for Neuroscience, Washington, DC

Chen, Y. W., & Lai, W. S. (2011). Behavioral phenotyping of v-akt murine thymoma viral oncogene homolog 1-deficient mice reveals a sex-specific prepulse inhibition deficit in females that can be partially alleviated by glycogen synthase kinase-3 inhibitors but not by antipsychotics. *Neuroscience, 174*, 178-189.

Cho, H. (2001). Akt1/PKBalpha is required for normal growth but dispensable for maintenance of glucose homeostasis in mice. *Journal of Biological Chemistry, 276*, 38349-38352.

Chudasama, Y., & Robbins, T. W. (2004). Psychopharmacological approaches to

modulating attention in the five-choice serial reaction time task: Implications for schizophrenia. *Psychopharmacology, 174*, 86–98.

Clinton, S. M., & Meador-Woodruff, J. H. (2004). Abnormalities of the NMDA Receptor and Associated Intracellular Molecules in the Thalamus in Schizophrenia and Bipolar Disorder. *Neuropsychopharmacology, 29*, 1353-1362.

Coffer, P. J., & Woodgett, J. R. (1991). Molecular cloning and characterisation of a novel putative protein-serine kinase related to the cAMP-dependent and protein kinase C families. *European Journal of Biochemistry, 201*, 475-481.

Cohen, R. M., Nordahl, T. E., Semple, W. E., Andreason, P., & Pickar, D. (1998). Abnormalities in the distributed network of sustained attention predict neuroleptic treatment response in schizophrenia. *Neuropsychopharmacology, 19*, 36-47.

Cole, B. J., & Robbins, T. W. (1989). Effects of 6-hydroxydopamine lesions of the nucleus accumbens septi on performance of a 5-choice serial reaction time task in rats: Implications for theories of selective attention and arousal. *Behavioural Brain Research, 33*, 165-179.

Corbetta, M., & Shulman, G. L. (2002). Control of goal-directed and stimulus-driven attention in the brain. *Nature Reviews Neuroscience, 3*, 201-215.

Cornblatt, B. A., & Keilp, J. G. (1994). Impaired attention, genetics, and the

pathophysiology of schizophrenia. *Schizophrenia Bulletins, 20*, 31-46.

Corti, C., Crepaldi, L., Mion, S., Roth, A. L., Xuereb, J. H., & Ferraguti, F. (2007). Altered dimerization of metabotropic glutamate receptor 3 in schizophrenia. *Biological Psychiatry, 62*, 747-755.

Coyle, J. T. (2006). Glutamate and schizophrenia: Beyond the dopamine hypothesis. *Cellular and Molecular Neurobiology, 26*, 365-384.

Dalley, J., Cardinal, R., & Robbins, T. (2004). Prefrontal executive and cognitive functions in rodents: Neural and neurochemical substrates. *Neuroscience and Biobehavioral Reviews, 28*, 771-784.

Davis, K. L., Kahn, R. S., Ko, G., & Davidson, M. (1991). Dopamine in schizophrenia: A review and reconceptualization. *American Journal of Psychiatry, 148*, 1474-1486.

Davis, J. M., Chen, N., & Glick, I. D. (2003). A meta-analysis of the efficacy of second-generation antipsychotics. *Archives of General Psychiatry, 60*, 553-564.

Debruin, N., Fransen, F., Duytschaever, H., Grantham, C., & Megens, A. (2006). Attentive performance of (C57BL/6J×129Sv)F2 mice in the five-choice serial reaction time task. *Physiology & Behavior, 89*, 692-703.

Emamian, E. S., Hall, D., Birnbaum, M. J. Karayiorgou, M., & Gogos, J. A. (2004). Convergent evidence for impaired AKT1-GSK3β signaling in schizophrenia.

Nature Genetics, 36, 131-137.

Elvevag, B., & Goldberg, T. E. (2000). Cognitive impairment in schizophrenia is the core of the disorder. *Critical Reviews in Neurobiology, 14*, 1-21.

Evenden, J. L. (1999). Varieties of impulsivity. *Psychopharmacology, 146*, 348-361.

Fell, M. J., McKinzie, D. L., Monn, J. A., & Svensson, K. A. (2012). Group II metabotropic glutamate receptor agonists and positive allosteric modulators as novel treatments for schizophrenia. *Neuropharmacology, 62*, 1473-1483.

Field, J. R, Walker, A. G, & Conn, P. J. (2011). Targeting glutamate synapses in schizophrenia. *Trends in Molecular Medicine, 17*, 689-698.

Franke, T. F. (2008). PI3K/Akt: getting it right matters. *Oncogene, 27*, 6473-6488.

Freyberg, Z., Ferrando, S. J., & Javitch, J. A. (2010). Roles of the Akt/GSK-3 and Wnt signaling pathways in schizophrenia and antipsychotic drug action. *The American Journal of Psychiatry, 167*, 388-396.

Gainetdinov, R. R., Mohn, A. R., & Caron, M. G. (2001). Genetic animal models: Focus on schizophrenia. *TRENDS in Neurosciences, 24*, 527-533.

Gazzaniga, M. S. (2009). *The cognitive neurosciences* (4th ed., p. 219). Cambridge, Massachusetts Institute of Technology: MIT Press.

Gold, J. M. (2004). Cognitive deficits as treatment targets in schizophrenia. *Schizophrenia Research, 72*, 21-28.

Gonzalez-Maeso, J., Ang, R. L., Yuen, T., Chan, P., Weisstaub, N. V., Lopez-Gimenez, J. F., ...Sealfon, S. C. (2008). Identification of a serotonin/glutamate receptor complex implicated in psychosis. *Nature, 452*, 93-97.

Gottesman, I. I. (1991). *Schizophrenia genesis: The origins of madness*. New York: Freeman.

Gradin, V. B., Kumar, P., Waiter, G., Ahearn, T., Stickle, C., Milders, M., ...Steele, J. D. (2011). Expected value and prediction error abnormalities in depression and schizophrenia. *Brain, 134*, 1751-1764.

Granon, S., Passetti, F., Thomas, K. L., Dalley, J. W., Everitt, B. J., & Robbins, T. W. (2000). Enhanced and impaired attentive performance after infusion of D1 dopaminergic receptor agents into rat prefrontal cortex. *The Journal of Neuroscience, 20*, 1208-1215.

Green, M. F. (1996). What are the functional consequences of neurocognitive deficits in schizophrenia? *The American Journal of Psychiatry, 153*, 321-330.

Green, M. F. (2006). Cognitive impairment and functional outcome in schizophrenia and bipolar disorder. *Journal of Clinical Psychiatry, 67*(Supplement 9), 3-8.

Grottick, A. J., & Higgins, G. A. (2002). Assessing a vigilance decrement in aged rats: Effects of pre-feeding, task manipulation, and psychostimulants. *Psychopharmacology, 164*, 33-41.

Gupta, D. S., McCullumsmith, R. E., Beneyto, M., Haroutunian, V., Davis, K. L., & Meador-Woodruff, J. H. (2005). Metabotropic glutamate receptor protein expression in the prefrontal cortex and striatum in schizophrenia. *Synapse, 57*, 123-131.

Gur, R. E., Calkins, M. E., Gur, R. C., Horan, W. P., Nuechterlein, K. H., Seidman, L. J., & Stone, W. S. (2007). The Consortium on the Genetics of Schizophrenia: Neurocognitive endophenotypes. *Schizophrenia Bulletin, 33*, 49-68.

Houthoofd, S. A., Morrens, M., & Sabbe, B. G. (2008). Cognitive and psychomotor effects of risperidone in schizophrenia and schizoaffective disorder. *Clinical Therapeutics, 30*, 1565-1589.

Howes, O. D., & Kapur, S. (2009). The dopamine hypothesis of schizophrenia: Version III-the final common pathway. *Schizophrenia Bulletin, 35*, 549-562.

Hoyle, E., Genn, R. F., Fernandes, C., & Stolerman, I. P. (2006). Impaired performance of alpha7 nicotinic receptor knockout mice in the five-choice serial reaction time task. *Psychopharmacology, 189*, 211-223.

Humby, T., Wilkinson, L. S., & Dawson, G. R. (2005). Assaying aspects of attention and impulse control in mice using the 5-choice serial reaction time task. *Current Protocols in Neuroscience,* 8.5H.1-8.5H.15.

Ikeda, M., Iwata, N., Suzuki, T., Kitajima, T., Yamanouchi, Y., Kinoshita, Y., ...Ozaki,

N. (2004). Association of AKT1 with schizophrenia confirmed in a Japanese population. *Biological Psychiatry, 56*, 698-700.

Iversen, S. D., & Iversen, L. L. (2007). Dopamine: 50 years in perspective. *Trends in Neurosciences, 30*, 188-193.

James, W. (1890). *The principles of psychology* (vol. 1, pp. 403-404). New York: Henry Holt.

Javitt, D. C., & Zukin, S. R. (1991). Recent advances in the phencyclidine model of schizophrenia. *The American Journal of Psychiatry, 148*, 1301-1308.

Javitt, D.C. (2010). Glutamatergic theories of schizophrenia. *Israel Journal of Psychiatry and Related Sciences, 47*, 4-16.

Jones, C. A., Watson, D. J., & Fone, K. C. (2011). Animal models of schizophrenia. *British Journal of Pharmacology, 164*, 1162-1194.

Jones, P. F., Jakubowicz, T., Pitossi, F. J., Maurer, F., & Hemmings, B.A. (1991). Molecular cloning and identification of a serine/threonine protein kinase of the second-messenger subfamily. *Proceedings of the National Academy of Sciences of the United States of America, 88*, 4171-4175.

Kapur, S. (2003). Psychosis as a state of aberrant salience: A framework linking biology, phenomenology, and pharmacology in schizophrenia. *The American Journal of Psychiatry, 160*, 13-23.

Kapur, S., Mizrahi, R., & Li, M. (2005). From dopamine to salience to psychosis-linking biology, pharmacology and phenomenology of psychosis. *Schizophrenia Research, 79*, 59-68.

Keeler, J. F., & Robbins, T. W. (2011). Translating cognition from animals to humans. *Biochemical Pharmacology, 81*, 1356-1366.

Kieling, C., Roman, T., Doyle, A. E., Hutz, M. H., & Rohde, L. A. (2006). Association between DRD4 gene and performance of children with ADHD in a test of sustained attention. *Biological Psychiatry, 60*, 1163-1165.

Kim, J. S., Kornhuber, H. H., Schmid-Burgk, W., & Holzmüller, B. (1980). Low cerebrospinal fluid glutamate in schizophrenic patients and a new hypothesis on schizophrenia. *Neuroscience Letters, 20*, 379-382.

Koelega, H. S. (1993). Stimulant drugs and vigilance performance: A review. *Psychopharmacology, 111*, 1-16.

Kollins, S. H., Anastopoulos, A. D., Lachiewicz, A. M., FitzGerald, D., Morrissey-Kane, E., Garrett, M. E., ...Ashley-Koch, A. E. (2008). SNPs in dopamine D2 receptor gene (DRD2) and norepinephrine transporter gene (NET) are associated with continuous performance task (CPT) phenotypes in ADHD children and their families. *American Journal of Medical Genetics Part B (Neuropsychiatric Genetics), 147B*, 1580–1588.

Kosslyn, S. M., & Rosenberg, R. S. (2011). *Introducing psychology* (4th ed., pp. 422-423). New Jersey: Pearson Education, Incorporation.

Kulhara, P., & Chakrabarti, S. (2001). Culture and schizophrenia and other psychotic disorders. *Psychiatric Clinics of North America, 24*, 449-464.

Kumar, C. C., & Madison, V. (2005). AKT crystal structure and AKT-specific inhibitors. *Oncogene, 24*, 7493-7501.

Lai, W. S., Xu, B., Westphal, K. G. C., Paterlini, M., Olivier, B., Pavlidis, P., ...Gogos, J. A. (2006). Akt1 deficiency affects neuronal morphology and predisposes to abnormalities in prefrontal cortex functioning. *Proceedings of the National Academy of Sciences, 103*, 16906-16911.

Lawrence, C. A., Barry, R. J., Clarke, A. R., Johnstone, S. J., McCarthy, R., Selikowitz, M., & Broyd, S. J. (2005). Methylphenidate effects in attention deficit/hyperactivity disorder: Electrodermal and ERP measures during a continuous performance task. *Psychopharmacology, 183*, 81-91.

Levin, E. D., Bushnell, P. J., & Rezvani, A. H. (2011). Attention-modulating effects of cognitive enhancers. *Pharmacology Biochemistry and Behavior, 99*, 146-154.

Loo, S. K., Specter, E., Smolen, A., Hopfer, C., Teale, P. D., & Reite, M. L. (2003). Functional effects of the DAT1 polymorphism on EEG measures in ADHD. *Journal of the American Academy of Child and Adolescent Psychiatry, 42*,

986-993.

MacDonald, A. W. 3rd, Carter, C. S., Kerns, J. G., Ursu, S., Barch, D. M., Holmes, A. J., ...Cohen, J. D. (2005). Specificity of prefrontal dysfunction and context processing deficits to schizophrenia in never-medicated patients with first-episode psychosis. *The American Journal of Psychiatry, 162*, 475-484.

Maira, S. M., Galetic, I., Brazil, D. P., Kaech, S., Ingley, E., Thelen, M., & Hemmings , B. A. (2001). Carboxyl-terminal modulator protein (CTMP), a negative regulator of PKB/Akt and v-Akt at the plasma membrane. *Science, 294*, 374-380.

Manning, B. D., & Cantley, L. C. (2007). AKT/PKB signaling: Navigating downstream. *Cell, 129*, 1261-1274.

Mathur, A., Law, M. H., Megson, I. L., Shaw, D. J., & Wei, J. (2010).Genetic association of the AKT1 gene with schizophrenia in a British population. *Psychiatric genetics, 20*, 118-122.

Miller, E. K., & Cohen, J. D. (2001). An integrative theory of prefrontal cortex function. *Annual Review of Neuroscience, 24*, 167-202.

Moghaddam, B., & Adams, B. W. (1998). Reversal of phencyclidine effects by a group II metabotropic glutamate receptor agonist in rats. *Science, 281*, 1349-1352.

Mueser, K. T., & McGurk, S. R. (2004). Schizophrenia. *Lancet, 363*, 2063-2072.

Mulle, J. G. (2012). Schizophrenia genetics: Progress, at last. *Current Opinion in Genetics & Development, 22*, 1-7.

Murray, G. K., Corlett, P. R., Clark, L., Pessiglione, M., Blackwell, A. D., Honey, G., ...Fletcher, P. C. (2008). Substantia nigra/ventral tegmental reward prediction error disruption in psychosis. *Molecular Psychiatry, 13*, 267-276.

Nestler, E. J., Hyman, S. E., & Malenka, R. C. (2009). *Molecular neuropharmacology : A foundation for clinical neuroscience* (2nd ed., pp. 148-151). New York: McGraw-Hill Medical.

Nestler, E. J., & Hyman, S. E. (2010). Animal models of neuropsychiatric disorders. *Nature Neuroscience, 13*, 1161-1169.

Neve, K. A., Seamans, J. K., & Trantham-Davidson, H. (2004). Dopamine receptor signaling. *Journal of Receptors and Signal Transduction Research, 24*, 165-205.

Nuechterlein, K. H., Barch, D. M., Gold, J. M., Goldberg, T. E., Green, M. F., & Heaton, R. K. (2004). Identification of separable cognitive factors in schizophrenia. *Schizophrenia Research, 72*, 29-39.

Nuechterlein, K. H., Green, M. F., Kern, R. S., Baade, L. E., Barch, D. M., Cohen, J. D., ...Marder, S. R. (2008). The MATRICS Consensus Cognitive Battery, part 1: Test selection, reliability, and validity. *The American Journal of Psychiatry, 165*, 203-213.

O'Carroll, R. (2000). Cognitive impairment in schizophrenia. *Advances in Psychiatric Treatment, 6*, 161-168.

Ohi, K., Hashimoto, R., Yasuda, Y., Fukumoto, M., Nemoto, K., Ohnishi, T., ...Takeda , M. (2011). The AKT1 gene is associated with attention and brain morphology in schizophrenia. *World Journal of Biological Psychiatry*. Early Online, 1-14. doi:10.3109/15622975.2011.591826.

O'Toole, K., Abramowitz, A., Morris, R., & Dulcan, M. (1997). Effects of methylphenidate on attention and nonverbal learning in children with attention-deficit hyperactivity disorder. *Journal of the American Academy of Child & Adolescent Psychiatry, 36*, 531-538.

Passetti, F., Chudasama, Y., & Robbins, T. W. (2002). The frontal cortex of the rat and visual attentive performance: Dissociable functions of distinct medial prefrontal subregions. *Cerebral Cortex, 12*, 1254-1268.

Patel, S., Stolerman, I., Asherson, P., & Sluyter, F. (2006). Attentive performance of C57BL/6 and DBA/2 mice in the 5-choice serial reaction time task. *Behavioural Brain Research, 170*, 197-203.

Pezze, M. -A., Dalley, J. W., & Robbins, T. W. (2006). Differential Roles of Dopamine D1 and D2 Receptors in the Nucleus Accumbens in Attentive Performance on the Five-Choice Serial Reaction Time Task.

Neuropsychopharmacology, 32, 273-283.

Pietiläinen, O. P., Paunio, T., Loukola, A., Tuulio-Henriksson, A., Kieseppä, T., Thompson, P., ...Peltonen, L. (2009). Association of AKT1 with verbal learning, verbal memory, and regional cortical gray matter density in twins. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics, 150B*, 683-692.

Platt, S. R. (2007). The role of glutamate in central nervous system health and disease --a review. *The Veterinary Journal, 173*, 278-286.

Posner, M. I., & Boies, S. J. (1971). Components of attention. *Psychological Review, 78*, 391-408.

Posner, M. I., & Rothbart, M. K. (2007). Research on attention networks as a model for the integration of psychological science. *Annual Review of Psychology, 58*, 1-23.

Powell, C., & Miyakawa, T. (2006). Schizophrenia-relevant behavioral testing in rodent models: A uniquely human disorder? *Biological Psychiatry, 59*, 1198-1207.

Raz, A., & Buhle, J. (2006). Typologies of attentional networks. *Nature Reviews Neuroscience, 7*, 367-379.

Relkovic, D., Doe, C. M., Humby, T., Johnstone, K. A., Resnick, J. L., Holland, A. J., ...Isles, A. R. (2010). Behavioural and cognitive abnormalities in an

imprinting centre deletion mouse model for Prader-Willi syndrome. *European Journal of Neuroscience, 31*, 156-164.

Remington, G., Agid, O., & Foussias, G. (2011) Schizophrenia as a disorder of too little dopamine: Implications for symptoms and treatment. *Expert Review of Neurotherapeutics, 11*, 589-607.

Robbins, T. (2002). The 5-choice serial reaction time task: behavioural pharmacology and functional neurochemistry. *Psychopharmacology, 163*, 362-380.

Rorick-Kehn, L. M., Johnson, B. G., Knitowski, K. M., Salhoff, C. R., Witkin, J. M., Perry, K. W., ...Schoepp, D. D. (2007). In vivo pharmacological characterization of the structurally novel, potent, selective mGlu2/3 receptor agonist LY404039 in animal models of psychiatric disorders. *Psychopharmacology, 193*, 121-136.

Saeedi, H., Remington, G., & Christensen, B. K. (2006). Impact of haloperidol, a dopamine D2 antagonist, on cognition and mood. *Schizophrenia Research, 85*, 222-231.

Sanchez-Roige, S., Peña-Oliver, Y., & Stephens, D. N. (2012). Measuring impulsivity in mice: The five-choice serial reaction time task. *Psychopharmacology, 219*, 253-270.

Schwab, S. G., Hoefgen, B., Hanses, C., Hassenbach, M. B., Albus, M., Lerer, B., ... Wildenauer, D.B. (2005). Further evidence for association of variants in the

AKT1 gene with schizophrenia in a sample of European sib-pair families. *Biological Psychiatry, 58*, 446-450.

Seeman, P. (1987). Dopamine receptors and the dopamine hypothesis of schizophrenia. *Synapse, 1*, 133-152.

Staal, S. P., Hartley, J. W., & Rowe, W. P. (1977). Isolation of transforming murine leukemia viruses from mice with a high incidence of spontaneous lymphoma. *Proceedings of the National Academy of Sciences of the United States of America, 74*, 3065–3067.

Stahl, S. M. (2007). Beyond the dopamine hypothesis to the NMDA glutamate receptor hypofunction hypothesis of schizophrenia. *CNS Spectrums, 12*, 265-268.

Stubbs, L. (1996). How closely related are mice and humans? What percentage of genes are the same? *Human Genome News, 8*, 16.

Sullivan, P. F., Kendler, K. S., & Neale, M. C. (2003). Schizophrenia as a complex trait: Evidence from a meta-analysis of twin studies. *Archives of General Psychiatry, 60*, 1187-1192.

Sullivan, P. F. (2005). The genetics of schizophrenia. *PLoS Medicine, 2*, e212.

Tan, H. Y., Chen, A. G., Chen, Q., Browne, L. B., Verchinski, B., Kolachana, B., ... Weinberger, D. R. (2011). Epistatic interactions of AKT1 on human medial temporal lobe biology and pharmacogenetic implications. *Molecular Psychiatry*,

1-11.

Tan, H. Y., Nicodemus, K. K., Chen, Q., Li, Z., Brooke, J. K., Honea, R, ...Weinberger , D. R. (2008). Genetic variation in AKT1 is linked to dopamine-associated prefrontal cortical structure and function in humans. *Journal of Clinical Investigation, 118*, 2200-2208.

Tandon, R. (2011). Antipsychotics in the Treatment of Schizophrenia. *The Journal of Clinical Psychiatry, 72*(supplement 1), 4-8.

Tandon, R., Nasrallah, H. A., & Keshavan, M. S. (2009). Schizophrenia, "just the facts" 4. Clinical features and conceptualization. *Schizophrenia Research, 110*, 1-23.

Tamminga, C. A., & Holcomb, H. H. (2005). Phenotype of schizophrenia: A review and formulation. *Molecular Psychiatry, 10*, 27-39.

Thiselton, D. L., Vladimirov, V.I., Kuo, P. H., McClay, J., Wormley, B., Fanous, A., ... Riley, B. P. (2008). AKT1 is associated with schizophrenia across multiple symptom dimensions in the Irish study of high density schizophrenia families. *Biological Psychiatry, 63*, 449-457.

Tost, H., & Meyer-Lindenberg, A. (2012). Puzzling over schizophrenia: Schizophrenia, social environment and the brain. *Nature Medicine, 18*, 211-213.

Uylings, H. B., Groenewegen, H. J., & Kolb, B. (2003). Do rats have a prefrontal

cortex? *Behavioural Brain Research, 146*, 3-17.

van Os, J., Rutten, B. P., & Poulton, R. (2008). Gene-environment interactions in schizophrenia: Review of epidemiological findings and future directions. *Schizophrenia Bulletin, 34*, 1066-1082.

Walker, S. E., Peña-Oliver, Y., & Stephens, D. N. (2011). Learning not to be impulsive : Disruption by experience of alcohol withdrawal. *Psychopharmacology*, 217, 433-442.

Weinberger, D. R. (1988). Schizophrenia and the frontal lobe. *Trends in Neurosciences, 11*, 367-370.

Wilkinson, R. T. (1963). Interaction of noise with knowledge of results and sleep deprivation. *Journal of Experimental Psychology, 66*, 332-337.

Xu, M. Q., Xing, Q. H., Zheng, Y. L., Li, S., Gao, J. J., He, G., ...He, L. (2007). Association of AKT1 gene polymorphisms with risk of schizophrenia and with response to antipsychotics in the Chinese population. *The Journal of clinical psychiatry, 68*, 1358-1367.

Yang, J., Cron, P., Good, V. M., Thompson, V., Hemmings, B. A., & Barford, D. (2002). Crystal structure of an activated Akt/protein kinase B ternary complex with GSK3-peptide and AMP-PNP. *Nature Structural Biology, 9*, 940-944.

Yang, Z. Z., Tschopp, O., Baudry, A., Dummler, B., Hynx, D., & Hemmings, B. A.

(2004). Physiological functions of protein kinase B/Akt. *Biochemical Society Transactions, 32*, 350-354.

Young, J. W., Powell, S. B., Risbrough, V., Marston, H. M., & Geyer, M. A. (2009). Using the MATRICS to guide development of a preclinical cognitive test battery for research in schizophrenia. *Pharmacology & Therapeutics, 122*, 150-202.

Appendix

A Modification of the 5-CSRTT to Reexamine the Behavioral Performance of *Akt1* Heterozygous Mice in an Automatic Version of Dynamic Foraging Task

Introduction

AKT1 (also known as PKBα) is encoded by the *AKT1* gene and belongs to a serine-threonine protein kinase of the AKT family. AKT is involved in multiple biological processes and diverse transduction pathways including angiogenesis, cell survival, metabolism, proliferation, and translation (Franke, 2008). AKT is also a downstream mediator of dopamine D2 receptor, and AKT regulates dopamine signaling cascades through its interactions with β-arrestin 2 and PP2A which is distinct from a cAMP-dependent signaling traditionally associated with the dopamine D1-class receptors (Beaulieu, 2011). Mice with *Akt1* deficiency showed normal basal levels of dopamine and its metabolites in mouse brains, but Akt1 protein was reduced in their prefrontal cortex (PFC) and other brain areas (Chen et al., 2012). As AKT1 is highly correlated with dopamine and the PFC, it is of great interest to figure out its interaction with dopamine and the PFC. Accumulated studies suggest that AKT1 are involved in dopamine- and PFC-related cognitive function (Chen et al., 2012; Chen & Lai, 2011; Lai et al., 2006; Tan et al., 2008). In a cognition-related dynamic foraging T-maze experiment, *Akt1* heterozygous (HET) mice showed aberrant behavior (Chen

et al., 2012). This dynamic foraging task was a two-alternative forced-choice task with one arm presenting a reward at a high rate, and the other presenting at a low rate. Additionally, each mouse needed to learn this rule and chose the high-reward rate arm based on trial and error, and the probability were reversed after they learned the rule.

The five-choice serial reaction task (5-CSRTT) is common used to assess attentive performances in rats and mice (Carli et al., 1983; Humby, Laird, Davies, & Wilkinson, 1999). A great benefit of the 5-CSRTT is that it provides information on distinct cognitive functions including attention, impulsivity, processing speed, motoric effects, motivation, and cognitive flexibility (Robbins, 2002; Young et al., 2009). Besides, the 5-CSRTT procedure can be extensively manipulated to increase or decrease the difficulty of this task (Jones & Higgins, 1995; Sanchez-Roige et al., 2011), such as alternation of stimulus brightness or reducing five choices to one choice (Dalley, Theobald, Eagle, Passetti, & Robbins, 2002), depending on the requirement of experiments. Based on the Iowa gambling task in human, the 5-CSRTT apparatus was further applied for gambling behavior as a novel rat gambling task (Zeeb, Robbins, & Winstanley, 2009). As numerous variations could be modulated in the 5-CSRTT, we modified it into a novel dynamic foraging task of two-choice. To investigate whether this 5-CSRTT can be further modified to an automatic vision of dynamic foraging task to evaluate reward learning in a

trial-by-trial basis, *Akt1* HET mice and their wild-type littermates were used in this experiment. We observed their behavioral performances in different testing conditions of this novel dynamic foraging task, and examined whether *Akt1* HET mice showed similar aberrant behavior in this task as we found previously in the dynamic foraging T-maze task (Chen et al., 2012).

Materials and methods

Animals

Male *Akt1* heterozygous mice (HET, $n = 9$) and their wild-type littermates (WT, $n = 9$) used in this study were generated from *Akt1* heterozygous breeding pairs in C57BL/6J genetic background ($n > 10$) and genotyped using PCR analysis of mouse-tail DNA as described previously (Chen et al., 2012; Cho, 2001). After weaning, animals were housed in groups of maximum 5 per cage with food and water available *ad libitum* in polysulfone individually ventilated cages (Alternative Design Manufacturing & Supply, Arkansas, AR, USA) within the animal rooms in the Psychology Department, National Taiwan University. All animals were 3-4 month-old at the beginning of experiments. Animals were handled at least 1 week before the behavioral experiments, and behavioral experiments were conducted during the dark phase at least half an hour after dark/light cycle (lights off at 8:00 A.M.) began. Animals were brought to the behavioral room 60 min before experiments. All animal procedures were performed according to protocols approved by the appropriate Animal Care and Use Committees established by the National Taiwan University.

Behavioral apparatus

Behavioral apparatus were two custom-built 5-aperture operant chambers (31.8 L

× 25.8 W × 29.1 H cm^3; Coulbourn Instruments, Whitehall, PA, USA) in a behavioral testing room under a red lighting condition (11.4 lux). Each chamber had a stainless-steel grid floor, aluminum front and back modular walls, aluminum top with a hole (4 cm diameter) in the center, and clear acrylic sides. Five 1.5 cm diameter and 4 cm deep stimulus-response apertures were spaced 3 cm apart, 1 cm above the grid floor, and centered on the front, curved wall of the chamber. Each stimulus-response aperture contained three pair of white light-emitting diode (LED) lights to generate a light stimulus and a photocell sensor to signal nose poke responses. The 3 apertures in the middle were covered by a white opaque acrylic (22 L × 15 W × 0.3 H cm^3) throughout the experiment and only the 2 apertures on the side of the curved wall of the chamber were used in this study. The food magazine was located in the low center of the back wall of the chamber with a yellow LED light fitted in the magazine as a cue of nose poke responses, and was spanned horizontally by a photocell sensor to signal nose poke responses. Above the food magazine was a reward deliver to dispense food pellets (20 mg chocolate sucrose reward tablet; TestDiet, 5-TUT, Richmond, IN, USA). A 3 W house light was mounted above the food magazine. The Graphic State 3.03 (Coulbourn Instruments, Whitehall, PA, USA) was used to perform on-line control of this apparatus and data collection.

Behavioral procedures

Animals were trained and tested in a 2-choice dynamic foraging task modified from the dynamic foraging task used in human and mice previously (Chen et al., 2012; Rutledge, Lazzaro, Lau, Myers, Gluck, & Glimcher, 2009). It was a two-alternative forced-choice task, and one of the alternative apertures presented a reward at a high rate, while independently, the probability of receiving a reward in the other aperture was low. Animals conducted a 1-hr daily session per day. The procedure consisted of a shaping phase and 5 sequential testing sections in a testing phase, including acquisition of 45% reward rate, reverse learning of 45% reward rate, relearning of 60% reward rate, reverse learning of 60% reward rate, and methamphetamine (MA) challenge.

Shaping phase. Mice were first trained to operate the experimental apparatus by a series of 5 shaping stages. In each stage, each mouse was required to reach shaping criteria in 1 hour, and then they could move to next stage. During the first 4 shaping stages, a trial started with the illumination of the house light, and ended after animals collected their reward pellets following a new trial started automatically. Besides, the food magazine illuminated to signal the delivery of a reward food pellet. Stage 1: Animals were required accumulating 10 nose pokes into either the 2 stimulus-response apertures or the food magazine, and each nose poke was followed

by the delivery of a reward pellet. Stage 2: Animals were still required to perform a nose poke into the food magazine followed by the delivery of a reward. But after accumulating 5 nose pokes into the food magazine, no reward was delivered from the food magazine if the animal kept performing nose pokes into the food magazine. Each mouse was required accumulating 5 nose pokes into one of the 2 apertures, and each nose poke into stimulus-response apertures or into the food magazine was followed by the delivery of a reward pellet. Stage 3: Each mouse was required accumulating 10 nose pokes into one of the 2 stimulus-response apertures, and nose poking into the food magazine was not followed by any delivery of a reward. Additionally, each nose poke into stimulus-response apertures was followed by the delivery of a reward. Stage 4: Animals were required accumulating 11 nose pokes into the 2 stimulus-response apertures to show their preference for left or right stimulus-response apertures, and each nose poke into apertures was followed by the delivery of a reward pellet. Stage 5: A trial started with the illumination of the house light, and then mice had to wait an intertrial interval (ITI) of 5 sec for the illumination of stimulus-response apertures. The 2 apertures subsequently illuminated, and animals were required to nose poke into one of the illuminated apertures. One aperture contained 75 % chance of delivery of rewards and the other aperture contained 25 % chance of delivery of rewards depending on each mouse's preference (e.g., if a mouse preferred the left aperture in

stage 4, the aperture with 75 % chance of reward delivery would be located in the opposite site, the right aperture). Each nose poke into the illuminated aperture was followed by either the delivery of a reward or no any reward and both of them were followed by the illumination of the food magazine. Each trial ended either after animals collected their earned reward pellets or after animals nose poked into the illuminated food magazine. This session comprised 20 trials, and animals could freely learn the rule in the first 10 trials by trial and error. In the following 10 trials, each mouse was required to accumulate 6 nose pokes into the aperture that contained 75 % chance of reward delivery, and then it could pass the shaping phase and move on to the testing phase.

Testing phase. The testing phase consisted of 5 sequential testing sections, including acquisition of 45% reward rate, reverse learning of 45% reward rate, relearning of 60% reward rate, reverse learning of 60% reward rate, and methamphetamine (MA) challenge. The 1st and 2nd sections contained the reward rate of 45 % and 15 % in one of the 2 stimulus-response apertures. The 3rd and 4th sections had the reward rate of 60 % and 20 % in one of the 2 stimulus-response apertures. The last section contained the reward rate of 60 % and 20 % with a daily challenge of MA (1 mg/kg, i.p.; administrated 15 min prior to the daily training). The location of high and low reward aperture was switched back and forth one day after each mouse

completed preset criteria in each section. On each day, each animal underwent an hour daily session or minimum 3 to maximum 6 blocks (a block consisted of 10 trials). Daily session began with the illumination of house and magazine lights. A nose poke into the magazine initiated a trial and extinguished the magazine light. A fixed ITI of 5 sec preceded the illumination of stimulus-response apertures. The 2 stimulus-response apertures subsequently illuminated after the ITI, and animals were required nose poking into one of the illuminated apertures. Each nose poke into the illuminated aperture was followed by either the delivery of a reward pellet or no any reward, and both of them were subsequently followed by the illumination of food magazine. Each trial ended after animals collected earned reward pellets or after animals nose poked into the illuminated food magazine. Each mouse discovered these rules and chose the high reward rate aperture by trial and error. The criterion of accomplishing each section was accumulating choice of the high reward rate aperture for at least 70% accuracy in 3 consecutive blocks. Once the criterion was achieved, each mouse moved on to the next section on the next testing days and the reward rates of the 2 apertures were switched. Accumulated trials, choice results, and latency both to response to the illuminated apertures and to reach the food magazine were recorded trial by trial by computer software during daily training.

Data analysis

Behavioral data were first evaluated the homogeneity of variances by Levene's test. If variances of behavioral data were equal, they were analyzed using Student's t-tests. If variances of behavioral data were unequal, these heterogeneous data were analyzed using Mann-Whitney *U* test (i.e., a non-parametric method). *P* values of < 0.05 were considered statistically significant. All statistical analyses were conducted by the SPSS 17.0 for Windows (Chicago, IL, USA). All behavioral data are depicted as means ± standard error of the mean (SEM).

Results

Levene test revealed equal variances in total number of cumulated trials in all testing sections except the sections of 45 % reverse learning and MA challenge, and data of these two sections were analyzed by the Mann-Whitney *U* test. For cumulated trials, as depicted in Figure 5.1, both WT and HET mice took about 300 trials to acquire the 45% and 60% reward learning sections. There is no significant difference between the two groups ($t(16) = 1.56$, $p = .14$ for 45 %; $t(16) = 1.54$, $p = .14$ for 60 %). After each transition, there is a trend that HET mice spent fewer trials to achieve the criteria compared with WT mice in the two reverse learning sections. However, there is no significant difference between the 2 groups ($U = 29$ and $p = .31$ for 45 % reverse learning; $t(16) = 0.51$ and $p = .62$ for 60 % reverse learning). Genotypic differences were only found in the section with MA challenge in which HET mice spent significantly fewer trials to achieve the criteria compared with WT mice ($U = 17$, $p = .038$).

Discussion

Although we did not find significant differences in either learning or reverse learning sections, the behavioral pattern of HET mice in this study are in line with a previous study done by colleagues in our laboratory (Chen et al., 2012). HET mice also required significantly fewer trials to reach the criteria compared with WT mice in MA challenge section. From this pilot study, these results suggest that the 5-CSRTT can be modified into an automatic dynamic foraging task to study higher cognitive functions (e.g., reward prediction error or decision making) in mice.

In the dynamic foraging T-maze task, the experimenter needed to carry a mouse back to the start site of the T-maze after every trial ended as described in a previous study done by our lab (Chen et al., 2012). Therefore, the duration between trial and trial may be difficult to be controlled. The reaction time of mice is also hard to be recorded simultaneously since the experimenter also needs to monitor the process of this task. It is somewhat difficult for the experimenter to get better control of the time between the start and the end of each trial. In contrast, in this modified 5-CSRTT, all process is monitored and controlled automatically by a computer and a software, thus the unnecessary error between trial and trial can be reduced. We can not only simultaneously and correctly record the reaction time of mice, but also can divide the reaction time based on their behavior. One possible drawback may be that the time for

each trial is controlled by the subject rather than by the experimenter. Thus, animals may explore this apparatus and then increase the experimental duration. As the modified 5-CSRTT is an automatic version of a dynamic foraging task, we can further use the recorded data to analyze performance of a mouse trial by trial. As we demonstrated nicely in a previous study from our laboratory to estimate parameters for reward prediction error using a standard Q-learning model (Chen et al., 2012), we can also apply electrodes to record neuronal activities in the target regions of the mouse brain while they are performing this task. Therefore, the modified 5-CSRTT seems to be better and contain more applications than the dynamic foraging T-maze task. Thus, this revision is less labor-intensive and time consumption. It is highly recommended to apply this automatic version of task to study higher cognitive functions in mice.

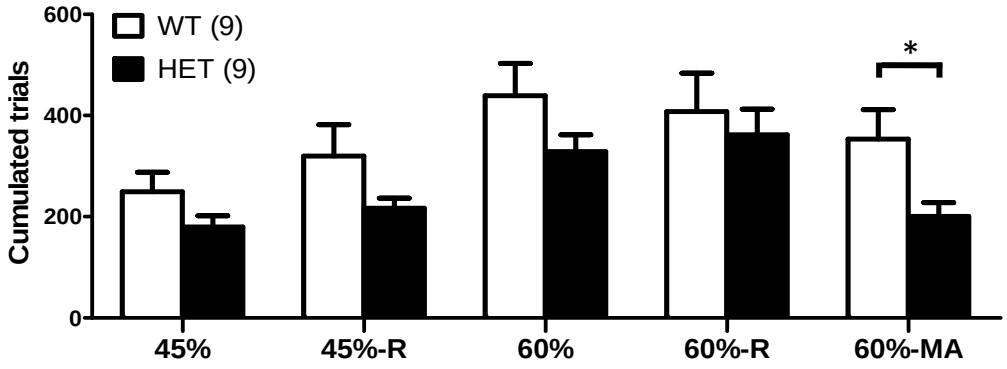

Figure 5. 1. Cumulated trials in each section of the 2-choice dynamic foraging task

Note. Total cumulated trials (mean + SEM) to achieve the preset criterion in the acquisition, reverse learning (R) and MA challenge sections. In MA challenge section, *Akt1* HET mice spent significantly fewer trials than WT mice. *: $p < .05$; 45 %: the condition of 45 % and 15 %; 60 %: the condition of 60 % and 20 %; parentheses: number of subjects.

Reference

Carli, M., Robbins, T. W., Evenden, J. L., & Everitt, B. J. (1983). Effects of lesions to ascending noradrenergic neurones on performance of a 5-choice serial reaction task in rats; implications for theories of dorsal noradrenergic bundle function based on selective attention and arousal. *Behavioural Brain Research, 9*, 361-380.

Chen, Y. C., Chen, Y. W., Hsu, Y. F., Chang, W. T., Hsiao, C. K., Min, M. Y., & Lai, W. S. (2012). Akt1 deficiency modulates reward learning and reward prediction error in mice. *Genes, Brain and Behavior, 11*, 157-169.

Chen, Y. W., & Lai, W. S. (2011). Behavioral phenotyping of v-akt murine thymoma viral oncogene homolog 1-deficient mice reveals a sex-specific prepulse inhibition deficit in females that can be partially alleviated by glycogen synthase kinase-3 inhibitors but not by antipsychotics. *Neuroscience, 174*, 178-189.

Dalley, J. W., Theobald, D. E., Eagle, D. M., Passetti, F., & Robbins, T. W. (2002). Deficits in impulse control associated with tonically-elevated serotonergic function in rat prefrontal cortex. *Neuropsychopharmacology, 26*, 716-728.

Emamian, E. S., Hall, D., Birnbaum, M. J. Karayiorgou, M., & Gogos, J. A. (2004). Convergent evidence for impaired AKT1-GSK3β signaling in schizophrenia. *Nature Genetics, 36*, 131-137.

Humby, T., Laird, F. M., Davies, W., & Wilkinson, L. S (1999). Visuospatial attentive functioning in mice: interactions between cholinergic manipulations and genotype. *European Journal of Neuroscience, 11*, 2813-2823.

Jones, D. N., & Higgins, G. A. (1995). Effect of scopolamine on visual attention in rats. *Psychopharmacology, 120*, 142-149.

Lai, W. S., Xu, B., Westphal, K. G. C., Paterlini, M., Olivier, B., Pavlidis, P., ...Gogos, J. A. (2006). Akt1 deficiency affects neuronal morphology and predisposes to abnormalities in prefrontal cortex functioning. *Proceedings of the National Academy of Sciences, 103*, 16906-16911.

Robbins, T. (2002). The 5-choice serial reaction time task: Behavioural pharmacology and functional neurochemistry. *Psychopharmacology, 163*, 362-380.

Rutledge, R. B., Lazzaro, S. C., Lau, B., Myers, C. E., Gluck, M. A., & Glimcher, P. W. (2009). Dopaminergic drugs modulate learning rates and perseveration in Parkinson's patients in a dynamic foraging task. *The Journal of Neuroscience, 29* , 15104-15114.

Sanchez-Roige, S., Peña-Oliver, Y., & Stephens, D. N. (2011). Measuring impulsivity in mice: The five-choice serial reaction time task. *Psychopharmacology, 219*, 253-270.

Tan, H. Y., Nicodemus, K. K., Chen, Q., Li, Z., Brooke, J. K., Honea, R, ...Weinberger

, D. R. (2008). Genetic variation in AKT1 is linked to dopamine-associated prefrontal cortical structure and function in humans. *Journal of Clinical Investigation, 118*, 2200-2208.

Young, J. W., Powell, S. B., Risbrough, V., Marston, H. M., & Geyer, M. A. (2009). Using the MATRICS to guide development of a preclinical cognitive test battery for research in schizophrenia. *Pharmacology & Therapeutics, 122*, 150-202.

Zeeb, F. D., Robbins, T. W., & Winstanley, C. A. (2009). Serotonergic and dopaminergic modulation of gambling behavior as assessed using a novel rat gambling task. *Neuropsychopharmacology, 34*, 2329-2343.